From cell culture to population enquiry: Experimental, clinical and epidemiological studies on restenosis post coronary intervention

Van celkultuur naar populatie studie: Experimenteel, Klinisch, en epidemiologisch onderzoek naar restenose na coronair-interventie

Proefschrift

Ter verkrijging van de graad van doctor aan de Erasmus Universiteit Rotterdam op gezag van de rector magnificus

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Cover photograph: Left panels: Quantitative angiographic assessment of an eccentric left anterior descending coronary artery stenosis prior (upper panel), and post (lower panel), successful rotablator therapy. Right panels: Angiograms, prior (upper), during (middle), and post (lower) rotablator therapy with ultrasound images prior (upper) and after therapy (lower). Note the eccentric nature of the, mainly fibrous plaque, with deep seated calcification resulting in some acoustic shadowing, at the 5 o'clock position. Following rotablator therapy most of the plaque has been removed apart from the, previously, deep seated calcification (arrow).

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To my wife Jane, who supported me unreservedly through the long gestation period of this thesis and our children Sophia and Elizabeth from whom the time to finish this thesis was stolen

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Chapter 1

General overview

Percutaneous coronary intervention is widely used in the treatment of coronary artery disease but currently limited by restenosis in a proportion of cases. We investigated the restenosis problem using complimentary experimental, clinical and epidemiological approaches.

In the first part of this thesis laboratory models and techniques are evaluated which may provide an insight into the undelying pathological processes involved. For example extracellular matrix synthesis by vascular smooth muscle cells (vsmc) may be an important pathophysiological mechanism for restenosis. In chapter 2 we assessed the ability of vsmc to produce extracellular matrix in-vitro using vsmc cultivated from primary and restenotic tissue samples obtained, in-vivo, by directional coronary atherectomy. In chapter 3 we investigated the relationship between the clinical and histopathological substrate of tissue retrieved during directional coronary atherectomy and the proliferative and migratory potential of smooth muscle cells as judged from successful outgrowth during cell culture. Cell culture however, does not take into account the normal anatomical relationships of the different cellular components of the vessel wall or the cell to cell interactions which may modulate growth. Therefore in Chapter 4 we developed an organ culture of human coronary artery subjected to balloon angioplasty in order to investigate the cellular and molecular basis of intimal proliferation in a preparation which maintained the anatomical relationships of the vessel wall. In chapter 5 we used a serum free adaptation of the organ culture technique in conjunction with an in vivo porcine model of restenosis to assess whether the release of growth factors from cells intrinsic to the vessel wall is implicated in the development of restenosis. One of the ways it is hoped to reduce restenosis is with local drug delivery, perhaps with growth factor antagonists, by perfusion catheters. Pressure mediated trauma and duration of balloon inflation are however key limitations of these devices. In Chapter 6 we evaluated a new delivery catheter which promises to overcome both these restrictions.

Although experimental models can provide insight into the pathophysiology of restenosis they can never however replace the clinical situation. Intracoronary ultrasound imaging allows direct visualisation of the vessel wall in vivo. In the second part of this thesis we looked at what role this may play in our understanding of the pathophysiological mechanisms involved in the mechanism of acute luminal gain and subsequent restenosis after coronary intervention. Chapter 7 provides an introduction and a succinct overview of the subject whilst chapter 8 compares the luminal dimensions measured by intracoronary ultrasound with the hitherto gold standard, quantitative coronary angiography. Chapter 9 summarises our initial clinical experience with a new device combining intravascular ultrasound imaging with balloon angioplasty thus allowing on line imaging during the procedure and obviating the need for repeated catheter exchanges. In chapter 10 we used intracoronary ultrasound to assess the influence of vascular remodeling on the mechanisms of acute luminal gain and long term restenosis following both coronary balloon angioplasty and directional atherectomy.

In the third part of this thesis data from four major European and American trials were used to examine the influence of clinical angiographic and procedural variables on subsequent restenosis. Chapter 11 examines the role of cholesterol and cholesterol subfractions whilst chapter 12 examines the influence of past and present smoking habits on post angioplasty restenosis. Chapter 13 expands on some of our previous findings and assessess the role of local thrombus formation and incorporation on subsequent restenosis. Chapter 14 looks as total occlusion and what influence these lesions may have on subsequent restenosis whilst chapter 15 examines the role of favourable arterial remodeling on outcome after coronary angioplasty. Chapter 16 reviews critically the large multicentre trials on which the previous studies were based and defines ways in which some of their limitations may be minimised in future studies.

In the final part of this thesis we look at coronary stenting, the only technique demonstrated to date to reduce restenosis and summarise the current and future stent technology available to the interventional cardiologist in his fight against restenosis.

Part 1- Experimental

Chapter 2

Increased extracellular matrix synthesis by smooth muscle cells obtained from in-vivo restenotic lesions by directional coronary atherectomy

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Increased extracellular matrix synthesis by smooth-muscle cells obtained from in vivo restenotic lesions by directional coronary atherectomy

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Coronary angioplasty continues to be limited by a 30% restenosis rate within 6 months. Postmortem histopathologic studies suggest that the intimally directed migration and proliferation of vascular smooth-muscle cells (VSMC) may play a critical role in this problem. These studies, however, can provide only limited static information regarding a specific moment in the disease process. To overcome this limitation and to look at the underlying mechanisms involved, we used directional coronary atherectomy to percutaneously retrieve tissue from patients with clinically and angiographically documented coronary artery disease and examined the hypothesis that VSMC cultured from restenotic lesions differ in extracellular matrix synthesis from primary atherosclerotic cells. Our experimental strategy was first to culture the tissue segment and confirm the identity of the explant cells and then to compare extracellular matrix synthesis between primary and restenotic cells.

Methods. A total of 98 in vivo plaque specimens were obtained from 98 patients undergoing directional coronary atherectomy with a previously described standard technique.[1] Tissue specimens were cultured with standard explant techniques. Smooth-muscle cell outgrowth was assessed by light microscopy with morphologic criteria reinforced by electron microscopy and positive immunostaining against specific smooth-muscle cell α-actin. Extracellular matrix synthesis was assessed by studying the synthesis of collagen and sulfated aminoglycans, the principal components of the extracellular matrix. To determine collagen synthesis growth, arrested cells of passage 2 to 4 were incubated for 48 hours at 37° C in culture medium containing 2 μmol/L ascorbic acid and 10 $\mu Ci.ml^{-1}$ ^3H-proline (specific activity 23 $mCi.mg^{-1}$). To determine sulfated glycosaminoglycans synthesis growth, arrested cells were incubated for 48 hours at 37° C in culture medium containing 0.5 ml ^{35}S-sulfate (specific activity 20 $mCi.mg^{-1}$). Subsequent analyses were performed with previously described techniques.[1] Measurements are expressed as mean ± SEM. Mean values were compared by the Student's *t* test for unpaired data. Data were considered significant if $p < 0.05$.

Results. Initial cell outgrowth and successful secondary culture (up to seven serial passages) was achieved in 11 patients, 7 with primary and 4 with restenotic lesions. Cells started to grow out from explants after 4 to 8 days (Fig. 1), and confluent multilayer primary cultures from the 11 patients were established after 4 to 6 weeks. Subconfluent cultures took the form of a network of multilayered elongated cells, whereas in confluent multilayer structures the cells appeared as whorls, producing the "hill and valley" pattern typical of vascular smooth-muscle cells in culture (Fig. 1). Immunostaining of the cells for α-actin was positive and confirmed them to be of smooth-muscle origin. Electron microscopy revealed features consistent with the synthetic phenotype. Collagen synthesis, reflected by the incorporation of ^3H-proline, was significantly greater for cells of restenotic than primary origin (0.034 ± 0.002 vs 0.024 ± 0.001 nmol [^3H]-proline · μg total cell $protein^{-1}$, $n = 11$, $p < 0.05$) (Fig. 1). Similarly, production of sulfated aminoglycans as assessed by the incorporation of ^{35}S-sulfate was significantly greater for restenotic than primary cells (7.49 ± 0.58 vs 5.11 ± 0.48 nmol [^3H]-sulphate · μg total cell $protein^{-1}$, $n = 11$, $p < 0.05$) (Fig. 1).

Comments. Our study has demonstrated that restenotic cells synthesize significantly more collagen and sulfated aminoglycans than do primary cells, suggesting that extracellular matrix synthesis may play a significant role in postangioplasty restenosis. This finding is in contrast to our previous study,[1] in which we found that restenotic and primary cells synthesized more extracellular matrix pro-

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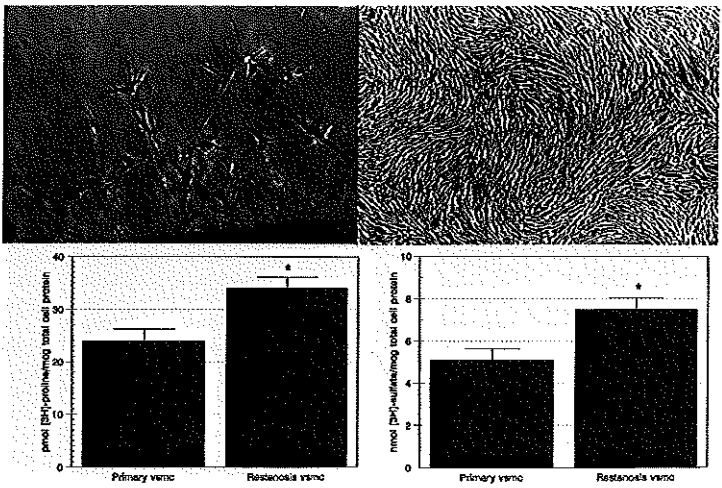

Fig. 1. *Upper left panel*, Fragment of retrieved atherectomy tissue in culture showing dense population of VSMC migrating out of explant (Original magnification ×60). *Upper right panel*, "Hill and valley" pattern typical of vascular smooth-muscle cells in culture. *Lower left panel*, Production of extracellular matrix collagen. *Lower right panel*, Production of extracellular matrix sulfated aminoglycans. $*p < 0.05$.

tein than did human umbilical vein cells, but no differences were seen between the two. This finding is likely to have occurred because of the significantly higher proliferation rate in restenotic cells during those experiments. In this study, in which we looked for differences in extracellular matrix synthesis during growth arrest, we found that restenotic cells synthesize significantly more extracellular matrix than do primary cells.

Because the difference in extracellular matrix synthesis between primary and restenotic cell lines occurred under identical culture conditions, we believe that the secretory behavior of the restenotic cells in vitro reflects a previous phenomenon of phenotypic modulation and selection in vivo rather than some effect of the culture process itself.[2] In support of this belief are histopathologic studies that suggest that smooth-muscle cells in both primary atherosclerotic and restenotic lesions are frequently of the synthetic phenotype. Additionally, coronary smooth-muscle cells in tissue excised at atherectomy have been recently shown to express messenger RNA for nonmuscle myosin heavy chain, which is associated with the synthetic smooth-muscle cell phenotype.[3]

Our data suggest that restenotic cells are fundamentally different from primary atherosclerotic cells in the production of extracellular matrix, in keeping with previous studies suggesting that restenotic cells differ from primary cells in terms of their migratory activity,[4] growth regulation,[1] and motility.[5] This finding may be a reflection of a more specialized function of the VSMC situated in the restenotic lesion, which may have been activated as a result of the angioplasty procedure itself in the response to injury hypothesis. Alternatively, it may signify a selected population of already activated VSMC in lesions that have subsequently undergone restenosis. The small numbers involved preclude us from commenting on which of the two mechanisms is most likely, but both are likely to involve the induction of growth factors such as transforming growth factor-β, the expression of which has been shown to be increased in restenotic lesions.[6] This finding is in keeping with our data and would suggest that the increased expression of transforming growth factor-β may result in increased production of extracellular matrix. This study has demonstrated that coronary smooth-muscle cells obtained from in vivo restenotic lesions synthesize significantly more extracellular matrix than do cells from primary lesions, suggesting that increased extracellular matrix production may play a fundamental role in restenosis after angioplasty.

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Chapter 3

Clinical and histological determinants of smooth muscle cell outgrowth in cultured atherectomy specimens: Importance of thrombus organisation.

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Clinical and histological determinants of smooth-muscle cell outgrowth in cultured atherectomy specimens: Importance of thrombus organization

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Background: Coronary atherectomy provides a unique opportunity to obtain plaque tissue from a wide variety of clinical syndromes. We investigated the relation between the clinical status and histopathological substrate of tissue retrieved during directional coronary atherectomy and the proliferative and migratory potential of smooth-muscle cells judged from successful outgrowth during cell culture.
Methods: After directional coronary atherectomy, tissue samples were examined macroscopically, divided into two equal pieces, and separately subjected to cell culture and histopathological study. Cell culture was performed using an explant technique. In-vitro smooth-muscle cell outgrowth was related to clinical and histological variables.
Results: Atherosclerotic tissue was obtained from 98 consecutive atherectomy procedures. Histological examination revealed a broad spectrum of appearances, ranging from complex atheroma containing dense fibrous tissue, calcium deposits, macrophages, and necrotic debris to neointimal proliferation and organized thrombi. Smooth-muscle cell outgrowth was observed in 43 of the 98 samples (44%). Although not affected by any of the clinical variables, cell outgrowth was influenced by histological variables, in particular the presence of organizing thrombi. Outgrowth was successful in eight out of 10 samples with thrombus (80%) and in only 35 out of 88 (40%) without ($P=0.03$).
Conclusion: The presence of organizing thrombi in the retrieved tissue facilitates smooth-muscle cell outgrowth and suggests an enhanced proliferative and migratory potential. These findings may be relevant to the understanding of neointimal proliferation in coronary syndromes where mural thrombosis is likely to occur.

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Keywords: atherosclerosis, directional coronary atherectomy, intimal proliferation, thrombus organization, smooth muscle, cell culture

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Smooth-muscle cell proliferation plays a key role in the development of typical and accelerated forms of atherosclerosis [1]. Cell culture of atheromatous tissue retrieved during directional coronary atherectomy allows us to study smooth-muscle cells from the pathological substrate of a variety of human coronary syndromes. In studying the pathobiology of coronary atheroma, the use of coronary atherectomy specimens for cell culture offers several distinct methodological advantages over other sources of atheromatous material, such as peripheral atherectomy, carotid endarterectomy, or animal models of atherosclerosis. Vascular smooth-muscle cells are embryologically derived from local mesoderm [2,3]. Those found in the coronary arteries are likely to have biological differences from those located in other vessels. Likewise, the development of the atheromatous plaque is strongly affected by local factors [4] that may influence cell populations, extracellular matrix composition, and plaque architecture. The use of human coronary material also makes it possible to avoid some of the pitfalls associated with animal models of atherosclerosis [5,6].

Nevertheless, cell culture studies using human coronary smooth-muscle cells have some technical limitations. Once in culture, these cells undergo a progressive phenotypic modulation that is time-dependent and enhanced by successive cell passages [7–9]. This process may facilitate the selection of cell clones with a higher proliferative capacity. Furthermore, although isolated cell studies provide information regarding cellular function and pathophysiological conditions, their extrapolation to the clinical situation is limited because they ignore the complex cell–cell and cell–extracellular-matrix interactions that modulate smooth-muscle cell growth *in vivo* [10].

In order to minimize these limitations, in the present study we used an explant culture technique [11,12]. This maintains a representative section of the atherosclerotic plaque in the culture medium, thus retaining the intrinsic distribution and anatomical relations of the participating cells, cell–cell interactions, the correct chemical configuration of the extracellular matrix, and the general in-vivo milieu of the atherosclerotic plaque. Only coronary material was used. The initial outgrowth of smooth-muscle cells was then used as a surrogate index for their in-vivo migratory and proliferative potential while still under the influence of other histological and humoral variables that were present in the atheromatous plaque at the time of atherectomy.

Methods

Percutaneous directional coronary atherectomy was performed on 98 lesions in 98 patients. Informed consent was obtained from all patients before the procedure, in accordance with the protocol approved by the Thoraxcenter Institutional Review Board. Atherectomy was performed using Simpson's Atherocath (Devices for Cardiovascular Intervention, Redwood City, CA, USA) and a conventional technique [13]. Multiple cuts in different sectors of the vessel were routinely performed. Under sterile conditions, the specimens were removed from the housing of the atherocatheter, washed with 0.9% saline, and placed in M199 HEPES buffered culture medium (Gibco Laboratories, USA) with antibiotics (100 IU/ml penicillin and 0.1 mg/ml streptomycin). They were immediately transferred to the laboratory where they were flushed with fresh culture medium and examined with a dissecting microscope. A representative section was then fixed in 3.6% buffered formalin for histopathological examination and the remainder placed in culture.

Clinical variables

A number of clinical variables were recorded for each patient. These included age, sex, previous myocardial infarction, stable or unstable angina pectoris, previous coronary intervention, and risk factors for coronary artery disease (hypercholesterolaemia, diabetes mellitus, cigarette smoking, hypertension, and family history of coronary artery disease). Unstable angina was defined as continuous or intermittent chest pain at rest requiring hospitalization, associated with electrocardiographic evidence of myocardial ischaemia and no increase in cardiac enzyme levels.

Tissue analysis

Specimens for histopathological study were routinely processed for light microscopy and stained with haematoxylin–azophloxin and Verhoeff–van Giesson stains. All specimens were reviewed independently by two observers, who were blinded to the clinical data. If they disagreed, the opinion of a third pathologist was sought and a consensus reached.

For the analysis of intimal constituents, the recommendations of the American Heart Association Medical/Scientific statement on the definitions of the intima of human arteries and of its atherosclerosis-prone regions were followed [14]. Fibrous tissue was classified as 'dense' when composed of acellular or poorly cellular connective tissue formed predominantly by dense collagen, and 'loose' when the tissue fragments showed moderate cellularity and collagen bundles separated by accumulations of extracellular matrix. Fibromuscular hyperplasia was defined as fibrous connective tissue showing a random orientation of spindle-shaped and stellate cells embedded in abundant extracellular matrix. Thrombus was identified as amorphous material, often in close apposition with atheromatous material, and frequently showing collections of leukocytes between layers of fibrin. Discrimination between fibrin and dense collagen was achieved using Verhoeff–van Giesson staining. The thrombus was regarded as organizing when infiltrated by cellular elements such as smooth-muscle cells or fibroblasts. Cholesterol crystal clefts, necrotic debris, and calcium deposits were recorded independently. The presence of macrophages was recorded only when these formed clusters or when they were present in unusually high numbers. Medial tissue was identified on the basis of a parallel arrangement of smooth-muscle cells, embedded in collagen, and frequently associated with a fragment of the internal or external elastic lamina. The adventitia was recognized by the presence of coarse bundles of dense collagen intermingled with elastin fibres, sometimes in association with fragments of the external elastic lamina and media.

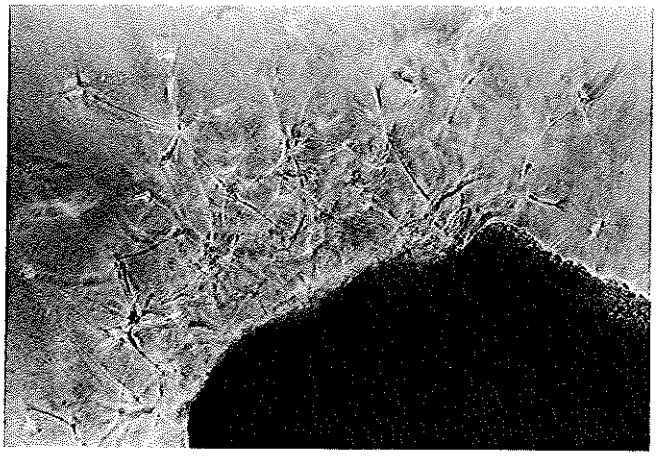

Fig. 1. Fragment of retrieved tissue examined under phase-contrast microscopy after 12 days of culture. A dense population of cells migrating out of the atherectomy explant is evident. These cells fulfil the morphological criteria of myofibroblasts discussed in detail in the text. (Original magnification ×60.)

Cell culture

The atheromatous tissue was cultured by a cell biologist (MdJ) blinded to the clinical data. An explant technique was used. Tissue explants were placed on human fibronectin-coated (10 μg/cm^2) glass cover slips in 2 cm^2 wells (Four-well plates, Nunc) and cultured in 300 μl culture medium [M199 with $NaHCO_3$ (Gibco Laboratories) supplemented with glutamine, 10% human serum, 10% fetal calf serum, 100 IU/ml penicillin and 0.1 mg/ml streptomycin, and mixed in a ratio of 1:1 with conditioned medium from established smooth-muscle cell lines actively growing in our laboratory]. Cultures were maintained in a CO_2 incubator at 37°C in a humidified atmosphere equilibrated with 5% (vol/vol) CO_2 in air. The culture medium was changed every 3–4 days. Smooth-muscle cell outgrowth was identified using inverted light microscopy and morphological criteria. These included a characteristic growth pattern of multiple layers of spindle-shaped or stellate cells showing stress fibres and lamellipodia (Fig. 1). These morphological criteria were reinforced by positive immunostaining for smooth-muscle-cell specific α-actin (DAKO, Denmark) with human skin fibroblasts as negative controls.

Statistical analysis

Mean values and standard deviations were calculated for all continuous variables. Comparison of mean values was made using the two-tailed unpaired t-test. Discrete variables were compared using the chi-squared test, and continuity correction was applied when indicated. $P<0.05$ was considered statistically significant.

Results

Clinical

Of the 98 patients in the study, 49 presented with stable and 47 with unstable angina pectoris. The remaining two were post-cardiac transplantation patients with cardiac allograph vasculopathy (Table 1). Twenty-four of the patients had a previous history of coronary intervention (14 of balloon angioplasty, six of stent implantation, three of atherectomy procedures, and one of excimer laser angioplasty) and had restenosis at the site of this intervention. The mean time interval between the previous revascularization procedure and the atherectomy was 147±108 days. The target lesion was located in the left anterior descending coronary artery in 62 patients, in the circumflex in 12, in the right coronary in 20, and in saphenous vein grafts in four. An average of 6±3 passes in different directions were made across each lesion.

In the study population, seven patients had a history of hypercholesterolaemia (serum cholesterol level ≥8 mmol/dl), 27 had systemic hypertension, 36 were smokers, and 18 had a family history of coronary artery disease; none of the patients had a history of diabetes mellitus. None of these risk factors appeared to influence smooth-muscle cell outgrowth (Table 1). Likewise, none of the other clinical variables discussed above could be related to enhanced cell outgrowth.

Histological

Thrombus was present in 10 out of 97 sections examined, predominantly in unstable angina patients: nine out of 49 (18%), compared with one out of 49 (2%) stable patients, $P=0.019$. Some degree of organization was present in all of the thrombotic specimens examined (Fig. 2). This ranged from the presence of endothelial-like cells in lacunar spaces or capillaries and the presence of scarce myofibroblasts infiltrating the thrombotic mass from the adjacent fibrous tissue, to infiltration by high numbers of myofibroblasts with the production of extracellular matrix. The thrombotic material appeared to be embedded in the fibrocellular tissue, suggesting that areas of fibrin and platelets derived from an episode of thrombosis or plaque haemorrhage were being integrated into the atheromatous plaque. In four cases,

Table 1. Clinical variables and outcome of smooth muscle cell culture.

	Successful cell culture	Failed cell culture	Significance (*P*)
Clinical variables			
Age (years, mean±SD)	57±10	57±11	NS
Previous MI	14/43	21/55	NS
Syndrome			
Stable angina	20/49 (41%)	29/49 (59%)	NS
Unstable angina	23/47 (49%)	24/47 (51%)	NS
Transplant vasculopathy	0/2	2/2	–
Risk factors for CAD			
Male sex	34/43 (79%)	46/55 (84%)	NS
Hypercholesterolemia	3/39 (8%)	4/59 (7%)	NS
Hypertension	10/40 (25%)	17/58 (29%)	NS
Smoking	17/42 (40%)	19/56 (34%)	NS
Family history of CAD	8/43 (19%)	10/55 (18%)	NS
Previous intervention	11/43 (26%)	13/55 (24%)	NS
Histological variables			
Neointimal hyperplasia	14/30 (47%)	29/68 (43%)	NS
Thrombus (organizing)	8/10 (80%)	35/88 (40%)	0.03
Dense fibrous tissue	32/79 (41%)	11/19 (58%)	NS
Loose fibrous tissue	10/16 (62%)	33/82 (40%)	NS
Cholesterol clefts	4/8 (50%)	39/90 (43%)	NS
Calcium deposits	13/27 (48%)	30/71 (42%)	NS
Necrotic debris	6/9 (67%)	37/89 (42%)	NS
Macrophage clusters	7/15 (47%)	36/83 (43%)	NS
Media	11/23 (48%)	32/75 (43%)	NS

MI, myocardial infarction; CAD, coronary artery disease.

this process was seen in conjunction with extensive fibromuscular proliferation.

Neointimal hyperplasia was observed in 31 samples, predominantly in restenotic (17 out of 24, 71%) rather than de-novo (14 out of 69, 20%) lesions ($P = 0.0001$).

In seven of the 31 samples, (22%), a neovascularization network was found in the interphase between neointimal hyperplasia and surrounding dense fibrous or loose fibrous tissue (four in primary and three in restenotic lesions). In secondary lesions,

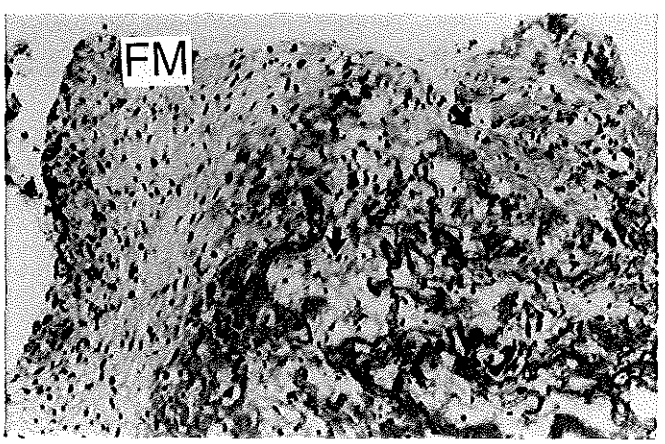

Fig. 2. Histological cross-section showing thrombus partially infiltrated by myofibroblasts (arrows) in close association with newly formed fibromuscular tissue (FM). (Original magnification ×30.)

neointimal proliferation showed identical characteristics irrespective of the nature of the previous intervention. Dense and loose fibrous tissue was found in 79 and 17 samples, respectively. Calcium deposits were observed in 27 samples. Macrophages were identified in 15 samples, predominantly in those with necrotic debris. Media and adventitia were found in 23 (23%) and 7 (7%) specimens, respectively.

Smooth-muscle cell outgrowth

Depending on the volume of the retrieved tissue, an average of 4.5 fragments (range 2–8), each measuring approximately 1 mm^3, were placed in culture. Cells started to grow out from explants after 4–14 days (Fig. 1), reaching a steady state within 4–6 weeks. The samples were discarded if no outgrowth was observed after 3–4 weeks. Despite the use of antibiotics in the culture medium, six of the cultured specimens developed infections and were discarded. The infections tended to develop after 3–4 weeks, by which time cell outgrowth should have occurred but did not.

Primary cell outgrowth was observed in 43 out of 98 samples (44%). When primary outgrowth occurred, light microscopy showed that the majority of cells tended to form multiple layers and were polygonal or spindle-shaped, with multiple stress fibres extending to lamellipodia. These appearances are characteristic of smooth-muscle cells. The cells were confirmed to be smooth-muscle cells using positive immunocytochemical staining with an α-actin-specific monoclonal antibody. In addition to smooth-muscle cells, a second cell type, oval in shape with eccentrically placed small indented nuclei, was identified in six cultures. Immunoperoxidase staining with macrophage-specific HAM 56 confirmed these cells to be macrophages; typically, they disappeared after 10–14 days of culture.

Cell outgrowth was not significantly influenced by any of the clinical variables recorded (Table 1), including the age or sex of the patient, coronary syndrome (stable or unstable angina), type of coronary lesion (de-novo or secondary), or risk factors for coronary artery disease (hypercholesterolaemia, hypertension, smoking, family history). Although cell culture failed in samples obtained from the two cardiac transplant patients, the small number involved precludes any conclusions about the statistical significance of this finding. Smooth-muscle cell outgrowth was significantly influenced, however, by the presence of organizing thrombus documented during histological examination. Smooth-muscle cell outgrowth was documented in eight out of 10 (80%) samples with and only 35 out of 88 (40%) without thrombus ($P = 0.03$, Table 1). None of the other histological variables analysed, including the presence of neointimal hyperplasia, fibrous tissue (dense or loose), lipid deposits, necrotic debris, macrophages, media, or adventitia, influenced cell outgrowth. Finally, there was no correlation between the number of explants used in each case and the outgrowth of smooth-muscle cells (4.5±1.6 and 4.5±1.9 explants in cases with successful and failed culture, respectively; NS).

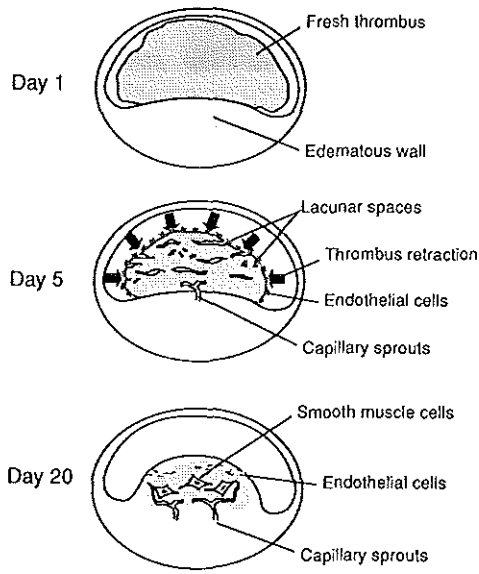

Fig. 3. The sequence of events leading to thrombus organization, based on observations in experimental animal models [15–17]. After the initial episode of thrombosis, a significant retraction of the thrombotic mass occurs, with the formation of lacunar spaces. These, like the thrombotic surface, become quickly endothelized. A granulation reaction with capillary ingrowth from the vessel wall occurs simultaneously. By day 20, smooth-muscle cells that may have migrated from the vascular wall during the granulation reaction or that are derived from circulating cells that have infiltrated the thrombus are seen along newly formed capillaries. Some of the endothelized lacunar spaces may give origin to multiluminal channels, which constitute a classic histopathological landmark of previous thrombotic recanalization.

Discussion

Atherectomy has facilitated not only the study of the histological constitution of the atheromatous plaque [13,18–20] but also the culture of smooth-muscle cells present in human atheroma [11,12,21–25]. Several groups [22,24], including ours [21], have reported improved cell outgrowth rates when an explant cell culture technique is used. The advantage of this method is that it minimizes the modifications of cell phenotype associated with enzymatic disper-

sion, prolonged culture, cell division, and successive cell passages [7–9], and therefore allows a better appreciation of the in-vivo proliferative and migratory potential of smooth-muscle cells. Since cell outgrowth occurs in the presence of the constituents of the atheromatous plaque present in the culture, the influence that the plaque milieu existing at the time of intervention has on the process of cell proliferation can be assessed.

Histological findings in both de-novo and restenotic lesions ranged broadly from neointimal hyperplasia to typical atheroma containing dense fibrous tissue, calcium deposits, macrophages, and necrotic debris. In agreement with previous communications based on the study of atherectomy specimens, neointimal proliferation was seen not only in the classic scenario of restenosis [1,26] but also in a substantial number of primary lesions [18,20,27]. Although the prevalent view on the development of fibromuscular proliferation is that it constitutes a non-specific response to various types of vessel-wall injury [1], the type of vascular insult responsible for its development in de-novo lesions remains unclear. Its occurrence in younger patients [20,27,28] suggests that it may differ from the classic sequence of events observed in the formation of atheroma [29], leading to a more aggressive form of atherosclerotic disease. Finally, we have found that organization is common in thrombotic atherectomy samples from patients with unstable angina [20], in agreement with recent work by Isner *et al.* [30], who used atherectomy specimens, but not with post-mortem studies, emphasizing the distinct advantages derived from the use of atherectomy samples in the in-vivo study of coronary syndromes.

Our explant culture success rate (44%) is low in comparison with other studies [12,24], although this must be the result of the sole use of coronary material. Cell culture from coronary atherectomy specimens is difficult, however, because of the small amounts of tissue involved, and yields significantly lower outgrowth success rates than peripheral tissue. When culture of both coronary and peripheral tissue has been attempted, a lower success rate and a longer time span until outgrowth has been observed in the coronary samples [12]. This observation was explained on the grounds that there were fewer coronary specimens and that their wet weight was lower [12]. However, it must be remembered that there are major differences in the histopathological characteristics of coronary and peripheral artery samples. The prevalence of thrombus in peripheral artery specimens obtained during directional atherectomy is as high as 61% [13], significantly higher than that found in the present and previous studies in the coronary arteries [19,20]. Although the relevance of this fact for cell culture is highlighted by the conclusions of the present study, it is unfortunate that none of the previous studies reported on the histological characteristics of the material used for culture, a limitation recently acknowledged in a report by Pickering *et al.* [24].

Our study is the first to consider the influence of a broad spectrum of histopathological features of retrieved tissue in addition to clinical features on cell outgrowth. Like Bauriedel and colleagues [12], we found that no clinical variables, including unstable angina and drug therapy, influence the outcome of plaque cultivation. This is reflected in clinical experience, which has shown little evidence that clinical factors influence the restenosis rate, and in which all therapeutic strategies have been singularly unsuccessful. Common sense dictates that clinical factors must operate through the histological milieu of the atherosclerotic plaque. In spite of the fact that human smooth-muscle cells cultured from restenotic lesions appear to migrate more rapidly than those from primary atheroma [12] and show accelerated growth curves [25], we did not find significant differences between the primary outgrowth of smooth-muscle cells from explants of restenotic and primary lesions. A possible reason for this discrepancy is that some of these studies used isolated smooth-muscle cells, obtained after several passages, and were free of the complex cell–cell and cell–extracellular-matrix interactions which modulate smooth-muscle cell proliferation and migration in the atherosclerotic plaque *in vivo* [10].

Evidence in the literature suggests an enhanced proliferative potential of smooth-muscle cells present in restenotic lesions. An improved smooth-muscle cell outgrowth from the injured vascular wall has been demonstrated by Grunwald *et al.* [31] in a rat model. Smooth-muscle cell outgrowth has been found to occur more rapidly in restenotic than in de-novo atherectomy specimens obtained in peripheral vessels [12,24], although the initial outgrowth was similar. In our study, cell outgrowth was not significantly different in explants from de-novo or restenotic lesions. There are a number of possible reasons for this. Our experiments were performed using only coronary atherectomy specimens, which, as discussed before, may differ substantially in their histological substrate from those obtained in peripheral vessels. The time interval from the former percutaneous intervention may also be of importance because evidence suggests that smooth-muscle cells experience a process of senescence during their migration to the neointima [32] and that their proliferation rate decreases after a period of time [33].

We found that smooth-muscle cells present in coronary atheroma where thrombotic organization is taking place had an enhanced proliferating potential. This may be related to three major factors. First, mural thrombus is rich in circulating elements, such as platelets, monocytes, and lymphocytes, which can secrete a number of vascular growth factors [1], promoting smooth-muscle cell proliferation. Thrombin and fibrin have both been shown to have chemotactic and mitogenic activity on vascular smooth-muscle cells [34], an effect that may be prolonged

after the incorporation of thrombus into the plaque. Thrombin may also act as a competence factor, stimulating the expression of growth factors, including platelet-derived growth factor, and their receptors [35,36], and thus help to perpetuate the activation of smooth-muscle cells. Any or all of these mechanisms may have been operating in our study, resulting in the increased migratory and proliferative activity of the smooth-muscle cells when surrounded by thrombus.

Second, the process of thrombus organization may have played a key role in the observed outgrowth of cells; since organization was taking place in all of our thrombotic specimens, we believe that our conclusions should be restricted to the presence of organizing thrombus. Growing evidence suggests that thrombus organization plays a key role in the development of neointimal hyperplasia after vascular injury [37–39,15]. It has been suggested that the smooth-muscle cells involved in this process are derived from circulating mononuclear cells rather than being of intimal or medial origin [15,16] (Fig. 3). Thrombus would serve as a biodegradable fibrin matrix colonized by circulating mononuclear cells that heal the injury site from the lumen side inwards, towards deeper vascular layers [15]. In this scenario the mononuclear cells that have colonized and started organizing the thrombus are self selected for their migratory and proliferative ability, providing an unexpected explanation for a previous report showing that smooth-muscle cells in organizing thrombi have a monoclonal origin [40]. Although the smooth-muscle cells cultured from thrombotic and non-thrombotic origin were morphologically indistinguishable, the above tentative scenario remains plausible.

Finally, a third explanation for our findings is that organizing thrombus may have facilitated cell outgrowth by optimizing cell transfer to the culture medium.

In our study we tried to reach a compromise between obtaining information from histopathology and from cell culture. As discussed above, meticulous inspection of the samples under the dissecting microscope was performed to ensure that the tissue fragments dedicated to the histological examination and cell culture samples were equally representative of the whole specimen. However, the possibility that the two pieces were significantly different cannot be ruled out. Atherosclerosis is a segmental disease process; in this regard our study shares the limitations of all histopathological studies using atherectomy specimens, in which conclusions are reached using fragmented samples of the arterial wall [26]. A second limitation is that pretreatment of the culture wells with fibronectin may have facilitated the transformation of smooth-muscle cells from the contractile to the synthetic phenotype [41], affecting explant outgrowth. We believe that this is unlikely to have occurred, however, because fibronectin is already present in serum and any additional effect that the fibronectin coating of the wells may have had is likely to have been constant in all specimens. Furthermore, poor adherence of atherosclerotic tissue to the culture medium occurs in the absence of fibronectin, and results in decreased explant success. The final potential source of error is variability in the area of contact between the explant tissue and the fibronectin. However, this error will probably have been randomly distributed among all the specimens studied, making it unlikely to account for the observed differences. We believe that the differences in cell outgrowth that we observed are a true reflection of the differing growth potentials of the cells present in the explants.

Our study emphasizes the research utility of clinically indicated directional coronary atherectomy and suggests that smooth-muscle cells present in atheromatous plaque where thrombotic organization is taking place have an enhanced migratory and proliferating potential. This supports the concept that plaque composition may influence the progression of atherosclerosis. It also suggests that monitoring in-vitro cell outgrowth may provide a means of assessing important biological features of the pathobiology of the atheromatous plaque.

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Chapter 4

Organ culture of human coronary artery following balloon angioplasty.

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Organ Culture of Human Coronary Artery Following Balloon Angioplasty

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Abstract

Intimal smooth muscle cell proliferation is the primary cause of restenosis following balloon angioplasty. Its underlying basis and progression remain unclear. The authors developed an organ culture of human coronary artery subjected to balloon angioplasty in order to investigate the cellular and molecular basis of intimal proliferation in a preparation that maintained the anatomic relationships of the vessel wall.

Artery segments obtained from the explanted hearts of transplant recipients were maintained at 37 °C in culture medium containing 30% fetal bovine serum for fourteen days. Balloon angioplasty produced partial endothelial denudation and medial smooth muscle cell damage, both of which tended to be reversed after fourteen days in culture. Transverse histologic sections of cultured artery showed the development of a new intima containing smooth muscle cells identified by immunocytochemistry with anti-α-actin. Labeling of cultures with [^{3}H] thymidine showed proliferating cells in the neointima.

The data demonstrate that intimal proliferation occurs in organ culture of human coronary artery subjected to balloon angioplasty. They also suggest the possibility that the smooth muscle cells in the neointimal layer are the result of both migration and proliferation.

Introduction

Coronary angioplasty is a technique widely used for the treatment of coronary artery disease.[14] The clinical benefits of the procedure are, however, tempered by a 30% restenosis rate within six months.[15] Clinical and experimental studies suggest that restenosis is due to the development of a neointima secondary to vascular smooth muscle cell migration and proliferation.[11,13,17]

Our current understanding of the problem is limited by the difficulty in studying the process in man. Histopathologic specimens obtained at post mortem, although valuable, provide static information regarding a specific moment in the course of the disease.[3] Isolated cells cultures do not take into account the normal anatomic relationships of the different cellular components of the vessel wall or the cell-to-cell interactions that may modulate growth.[4]

We sought, therefore, to establish whether intimal proliferation occurred in an organ culture of human coronary artery subjected to balloon angioplasty. Our strategy was first to assess the degree of injury produced by balloon angioplasty, determine whether tissue viability would be maintained in culture, and then detect, localize, and identify any proliferating cells.

Materials and Methods

Harvesting and tissue culture of coronary artery segments

Hearts were obtained from 8 male patients (mean age 33.5, range fifteen to forty-seven years) undergoing cardiac transplantation. Six patients had

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ischemic and 2 congestive cardiomyopathy. Routine premedication, anesthesia, and intraoperative heparinization were performed as previously described.[1] The heart, if possible, was explained with a cuff of ascending aorta to facilitate identification of the coronary ostiae, which were then cannulated and perfused with 0.9% sodium chloride solution to clear any blood. The coronaries were dissected free of surrounding tissue by use of no-touch technique[3] and transported to the laboratory within thirty minutes of explanation in Hepes buffered RPMI 1640 culture medium supplemented with penicillin (100 μg/mL) streptomycin (100 units/mL), gentamicin (2.5 μg/mL), amphotericin (2.5 μg/mL), glutamine (2mM), and sodium heparin (4 units/mL).

Coronary artery segments were subjected to balloon angioplasty twice by use of a 3mm balloon inflated to 10 atmospheres for sixty seconds. In one set of experiments the balloon was simply inflated and deflated while in the same position. In a separate set of experiments the balloon was moved slightly backward and forward within the vessel while inflated. Following angioplasty the vessel was cultured by previously described standard techniques.[2,18] Briefly the vessel was opened and longitudinally, and, with minuten pins, the intimal surface was pinned uppermost onto a polyester gauze resting on set sylgard resin in the base of a Petri dish. Vessel segments were washed several times with medium and then maintained in culture medium containing sodium bicarbonate (2.0 g/L) in place of Hepes, and 30% fetal calf serum at 37 °C in a humidified atmosphere with 5% (v/v) CO_2 in air. The culture medium was replaced every two to three days. Cultures were pulse labeled with 1 Ci/mL [^{3}H]-thymidine (concentration 25 Ci/mmol) for the last twenty-four hours of the fourteen-day culture period.

The measurement of adenosine nucleotides, DNA and total [^{3}H]-thymidine incorporation were performed as previously described.[2,18]

Electron and light microscopy

Artery segments were fixed in 10% buffered formalin, processed, and paraffin embedded. Transverse sections (4 m) were stained with hematoxylin/eosin and Alcian blue/Miller's elastic/Van Gieson for routine histologic examination.

Immunostaining of paraffin sections was performed with monoclonal anti-α-actin antibody to identify smooth muscle cells. Deparaffinized sections were incubated for thirty minutes with 1:200 dilution of α-actin antisera following initial incubations with methanol/hydrogen peroxide to inhibit endogenous peroxidase activity and with normal rabbit serum to inhibit nonspecific binding. Following two washes in Tris-buffered saline, biotinylated secondary antisera was added for thirty minutes. Sections were washed twice more and streptavidin/biotin complex was added for thirty minutes. Finally, diaminobenzidine was used to visualize the staining, and sections were counterstained with Mayer's hematoxylin.

Autoradiography was performed to localize any cell proliferation occurring in culture. Unstained 3 μm sections were deparaffinized, rehydrated through alcohol to water, dipped in K2 nuclear emulsion, and exposed for two weeks at 4 °C. Sections were then developed, fixed, and poststained with orcein.

Endothelial morphology was studied by means en face scanning electron microscopy (SEM). Vessel segments were fixed in 3% glutaraldehyde for twenty-four hours and then transferred to sodium cacodylate buffer. Dehydrated specimens were then critical point dried in liquid CO_2, sputter coated with gold, and observed in a scanning electron microscope.

Statistical methods

Values are shown as mean ± standard error of the mean. Data were considered significant if $P < 0.05$ using the Student's t test for unpaired data.

Results

Endothelial morphology

SEM of freshly isolated vessels showed a morphologically intact and almost continuous endothelial monolayer in the central area with a more variable morphology, including areas of endothelial denudation at the cut edges. Segments of vessel in which the balloon was moved backward and forward while inflated showed areas of substantial endothelial denudation exposing subendothelial structures (Figure 1b). Segments of vessel where the balloon was simply inflated in position showed a confluent endothelial monolayer with only occasional breaks (Figure 1c). After fourteen days in culture the endothelial monolayer was still intact but

TABLE I
Purine Metabolites and DNA Concentrations in Freshly Isolated Coronary and Coronary Subjected to Balloon Angioplasty, Before and After Culture

Vessel	Freshly Isolated	Angioplasty	Angioplasty
Days in culture	0	0	14
Number of observations	14	8	6
ATP concentration (nmol·g wet wt)	240 (34)	103 (25)*	210 (38)*
ATP ADP ratio	2.11 (0.17)	1.39 (0.24)*	2.53 (0.40)*
DNA concentration (μg·mg wet wt)	0.44 (0.03)	0.38 (0.04)	0.45 (0.06)

*$P < 0.05$ vs freshly isolated; *$P < 0.05$ vs day 0 angioplasty

the cells appeared to be slightly swollen. Fine filamentous projections referred to as lamellipodia were seen between cells (Figure 1d).

Tissue viability

Measurements of total adenosine triphosphate (ATP) concentration and ATP/adenosine diphosphate (ADP) ratio (calculated from total ADP) were performed to quantify the viability of the predominant cell type, the smooth muscle cells before and after culture. Balloon angioplasty resulted in a 60% fall in ATP concentration and a 35% fall in the ATP/ADP ratio as compared with freshly isolated vessel, suggesting that the procedure causes substantial injury to the medial smooth muscle cells (Table I). This damage was, however, fully reversed during the culture period as shown by the increase in ATP concentration and ATP/ADP ratio to values similar to those of freshly isolated coronary (Table I). The DNA concentration, which gives a measure of total cell numbers, did not alter significantly during the fourteen day culture period (Table I).

Cell proliferation

Histologic examination of freshly isolated segments of coronary artery showed a convoluted internal elastic lamina with a thickened intimal layer. In patients with ischemic cardiomyopathy plaque formation was also seen. The medial layer, enclosed by the internal and external elastic laminae, was characterized by the presence of abundant elastic fibers and axial and longitudinally arranged smooth muscle layers.

Balloon angioplasty resulted in disruption of the vessel wall with tears and fissures extending from the intima into the media (Figure 2a). In segments of vessels cultured for fourteen days, a neointimal layer was visible above the preexisting intima characterized by a layer of cells in a loose matrix (Figure 2b, 2c). This neointimal layer (mean thickness 47 ± 12 μm) did not develop over atherosclerotic plaque. Cell proliferation as assessed by [^{3}H]-thymidine incorporation occurred during culture (841 ± 294 dpm/μg DNA).

Immunocytochemistry with a monoclonal antibody to α-actin was performed to identify and localize vascular smooth muscle cells. In sections of uncultured coronaries most of the medial and intimal cells stained positively for α-actin. Following fourteen days in culture, positive staining was still observed in the media and intima but also throughout the neointimal layer (data not shown). Autoradiography of transverse sections showed cells that had incorporated [^{3}H]-thymidine predominantly in the neointimal layer, with a few dividing cells in the media (Figure 2d).

Discussion

The results from this study suggest that balloon angioplasty produces severe medial and endothelial damage that partly recovers after fourteen days in culture. A neointima develops during the culture period with proliferating cells identified as vascular smooth muscle cells by immunohistochemistry.

Previous morphologic studies have documented that balloon angioplasty results in substantial vessel wall damage with plaque fracture and medial as well

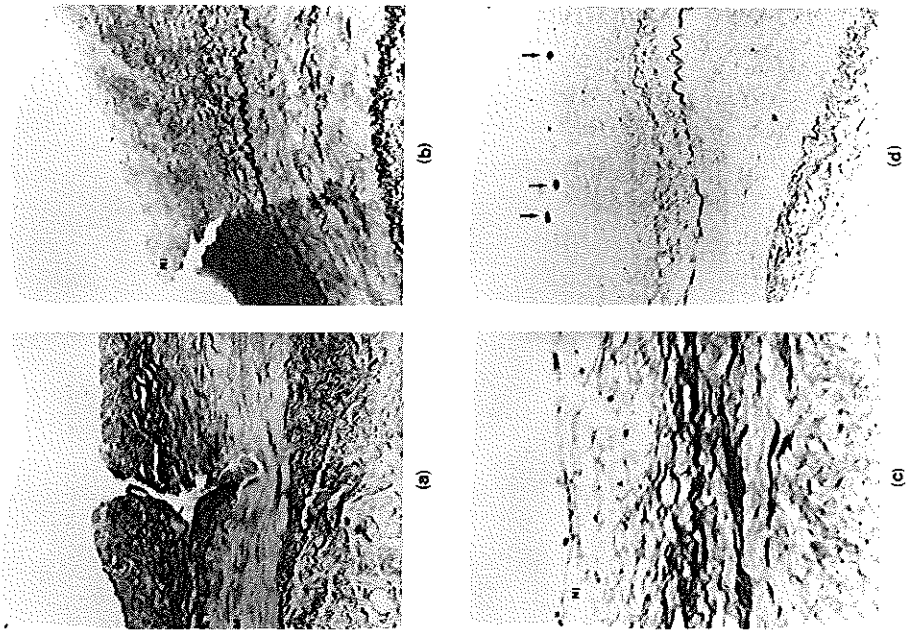

Fig. 1 Transverse histologic sections of human coronary artery before and after culture. (a) Angioplasty vessel day 0 stained with Gadsdon's modified trichrome stain. Note the preexisting intimal thickening (I) above the internal elastic lamina (IEL). Vessel damage induced by angioplasty (arrow) extends into the media (M) (x25). (b) Angioplasty vessel day 14 stained with Gadsdon's modified trichrome stain. Note the neointimal formation (NI) over the preexisting intimal thickening (I) and the relatively few cells surrounded by a large amount of extracellular matrix (x64). (d) Autoradiography of angioplasty vessel at day 14. Note that [^{3}H]-thymidine-labeled cells are present in the neointimal layer (arrows) but not in the medial or adventitial layers (x37).

as intimal tears.[3,16] These findings were confirmed in our study with the use of quantitative biochemical markers. Balloon angioplasty resulted in more than a 60% reduction in ATP concentration as compared with control vessel, but this damage was fully reversed during the culture period as shown by the doubling in ATP concentration. The ATP/A DP ratio also increased after culture to values above those seen in fresh vessels. This may be a characteristic of cultured vascular smooth muscle cells or may represent transformation of cells from the contractile into the synthetic phenotype as previously shown in organ culture of human saphenous vein[2,18] and internal mammary artery.[10]

SEM demonstrated that balloon angioplasty produces a variable degree of endothelial disruption with exposure of the underlying basement membrane. Following culture, endothelial regrowth occurs in areas with limited damage with lamellipodia-like filaments seen spanning the exposed surface between endothelial cells. Endothelial regrowth does not, however, take place in areas with extensive denudation.

A new intimal layer developed after fourteen days in culture. This was clearly identifiable from the preexisting intima owing to its relatively low level of elastin staining. The neointima was confined almost entirely to segments of nearly normal vessel wall. This finding is very similar to the clinical situation after balloon angioplasty where the neointimal proliferation occurs over the normal segment of the vessel wall and only later spreads to cover the atherosclerotic plaque.[5] The majority of the cells in the neointima were identified by immunocytochemistry as vascular smooth muscle cells consistent with human postmortem studies of restenosis.[3,6,13]

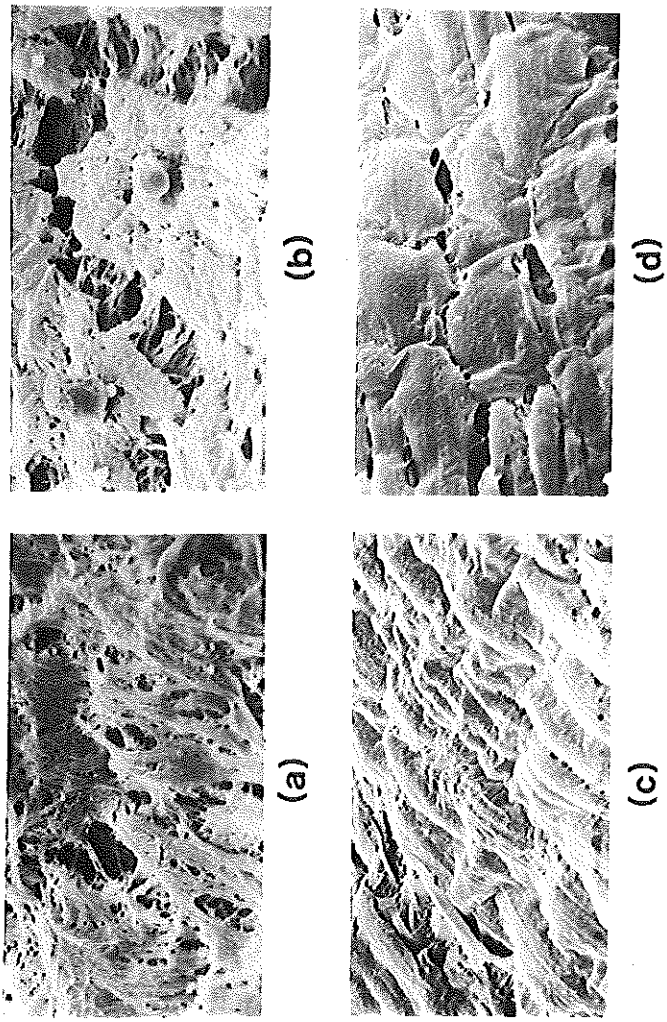

Fig. 2 Scanning electron microscopy of the intimal surface of human coronary artery, before and after culture (x1250). (a) Angioplasty vessel day 0. Note the extensive endothelial denudation, exposing subendothelial structure and the basal membrane. (b) Angioplasty vessel day 14. Some regrowth of endothelium has taken place, but there is still fairly extensive denudation. (c) Angioplasty vessel day 0. Note the well-preserved endothelium with only occasional gaps between cells. (d) Angioplasty vessel day 14. Note the occasional breaks in the endothelial monolayer spanner by fine filamentous projections (x2500).

These cells are likely to have originated from vascular smooth muscle cells in either the preexisting intima or the vessel media. The smooth muscle cells may have been intimally directed as a result of a concentration gradient of exogenous growth factors in the culture medium. This is unlikely, however, for the vessel segments were completely immersed in culture medium. Previous organ culture studies have suggested that intimal proliferation. depends on the presence of endothelium, which suggests that an endothelium-derived chemoattractant may be released during culture.[2,12] Further evidence from cell cultures suggests that growing endothelial cells release factors able to stimulate smooth muscle cell proliferation in vitro.[5] The injury produced by balloon angioplasty may therefore lead to increased synthesis of growth factors from damaged cells or from activated regenerating endothelial cells.

Autoradiography at fourteen days showed most of the proliferating cells to be in the neointimal layer with few dividing cells in the media. The low rate of smooth muscle cell proliferation in the tunica media is likely to be the result of constraints on proliferation by cell-cell interactions.[4] Once cells have migrated into the intima they are free of any such interactions and are more likely to proliferate. The lack of cell interaction may also be responsible for the large amount of extracellular matrix produced by the neointimal cells.

The organ culture system described here has certain limitations, in particular the variability in intimal thickening and plaque lesion both between and within vessels. This makes any direct quantitative comparison between angioplasty and freshly isolated vessel difficult. The high concentration of serum required to maintain the vessel in culture also prevents us from assessing the differential role of endogenous mitogens, which may play an important role in the development of intimal proliferation.

Conclusions

Nonetheless the model allows us to use segments of human coronary artery in which the cells maintain their normal anatomic relationships, in contact with the extracellular matrix. This interaction can therefore be studied in controlled conditions and both migration and proliferation can be monitored. The system may also be readily adapted to evaluate the role of factors such as lipoprotein concentration and monocyte adhesion and migration into the tissue, which are known to influence restenosis.

The recent development of serum-free organ culture techniques[5] should also allow us to investigate the role of growth factors regulating intimal proliferation. Furthermore, since intimal smooth muscle cell proliferation occurs within a relatively short time span, the model may be useful in evaluating therapeutic agents for the suppression of intimal proliferation.

Acknowledgments

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Chapter 5

Growth factor activity from porcine coronary artery following balloon angioplasty

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Submitted for publication

Abstract

The long term efficacy of coronary angioplasty remains limited by restenosis secondary to intimal vascular smooth muscle cell (vsmc) proliferation. The release of growth factors from cells intrinsic to the vessel wall has been implicated in the development of restenosis despite the lack of direct evidence.

To test this hypothesis, pig coronary arteries explanted 4 weeks after angioplasty (3mm balloon, 10 atmospheres, 60 seconds) were cultured for 24 hours in serum-free media supplemented with [^{3}H]-thymidine. Growth factor activity was then evaluated in the conditioned media using a Swiss 3T3 fibroblast bioassay.

Tissue viability assessed by adenosine triphosphate concentration (nmol/g wet wt) remained high during culture (267±21 [SEM], 0 hour vs 265±32, 24 hours) and cell proliferation occurred as judged by total [^{3}H]-thymidine incorporation (1326±226 dpm/mg wet wt). Transverse histological sections of angioplasty sites revealed the presence of a neointimal layer, with a mean thickness of 92.82±27.95 m ($n=9$, $p<0.001$ vs freshly isolated coronary). Autoradiography showed proliferating cells in the neointima identified as smooth muscle cells using a monoclonal antibody to α-actin.

Conditioned media from the angioplasty vessel caused a 6 fold increase in the proliferation of Swiss 3T3 fibroblasts above that produced by basal culture media. This mitogenic activity was inhibited by x% with a polyclonal neutralising antibody to platelet-derived growth factor (PDGF). Reverse transcription polymerase chain reaction analysis (RT-PCR) and Northern blot analysis demonstrated expression of PDGF B chain in angioplasty vessel but not in freshly isolated vessel.

These data provide direct evidence for growth factor activity and PDGF-B gene expression by cells intrinsic to the angioplasty site. They also suggest that a PDGF-like protein may play a role in regulating smooth muscle cell proliferation in vivo.

Introduction

Percutaneous transluminal coronary angioplasty is an effective means of alleviating ischaemia in coronary artery disease but is currently limited by restenosis in 30-40% of cases (1, 2). Histopathologically restenosis is characterised by intimal thickening secondary to smooth muscle cell migration, proliferation and synthesis of extracellular matrix (3, 4, 5). The factors which control this process are incompletely understood but preliminary work using isolated cell cultures has identified and characterised a number of growth factors, including platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), transforming growth factor β (TGFβ) and insulin like growth factor 1 (IGF-1), all of which induce vascular smooth muscle cell migration and proliferation in vitro (6). There is however no direct evidence that any of these factors play a role in regulating vascular smooth muscle cell (vsmc) proliferation in vivo.

To test the hypothesis that balloon angioplasty induces growth factor production by cells intrinsic to the vessel wall, pig coronary arteries previously subjected to balloon angioplasty were cultured for 24 hours in serum-free basal media. Release of growth factor activity was then assessed by the ability of the conditioned media to stimulate the proliferation of quiescent 3T3 fibroblasts. Neutralising antibodies were employed to further characterise the nature of the growth promoting activity. To confirm that cells intrinsic to the angioplasty vessel were responsible for the growth promoting activity detected, growth factor messenger RNA expression was also investigated by reverse transcription polymerase chain reaction (RT PCR) using primers derived from human gene sequences.

Methods

Coronary angioplasty

Domestic pigs (Yorkshire white), intitially weighing 25-30 Kg were used in this study. The investigations were performed according to the national guide-lines for the care and use of laboratory animals and the study protocol was approved by the Home Office. After an overnight fast the animals were sedated with Ketamine 12-15 mg/kg, intubated with an endotracheal tube and ventilated with Halothane and Oxygen to maintain adequate anaesthesia. Their ECG and arterial blood pressure were monitored continuously. Each animal was prepared and draped in a sterile fashion. A right femoral cut down was performed and an 8F introducer sheath (Cordis Inc) placed in the femoral artery. Each animal then received systemic heparin (200 units/kg iv) and Lignocaine (1 mg/kg) therapy. The left main or right coronary artery was engaged using standard techniques with an 8 F coronary guide catheter (Cordis Inc).

After coronary arteriography and under fluroscopic guidance a 3mm balloon angioplasty catheter (Mini-profile, Usci-Bard) was advanced and positioned in the mid-coronary artery. The balloon size (3mm) was substantially larger than the pig coronaries which were typically 1.5-2.0mm in diameter so as to induce maximum injury. Using random assignment each of the two branches of the left system (LAD and circumflex) underwent angioplasty or served as control. Balloon angioplasty was performed using two inflations at 10 atmospheres for 60 seconds. Following the procedures patency of the vessels was confirmed arteriographically, the catheters withdrawn and the cut down site repaired using 5.0 prolene sutures. The animals were allowed to recover and sacrificed at 4 weeks. Following sedation and anaesthetic the chest was opened via a median sternotomy, a lethal dose of phenobarbitone administered and the inferior and superior venae cavae ligated. The aorta and pulmonary trunk were cross clamped and the heart explanted. The coronary vessels were washed by infusing normal saline at 100 mmHg into the aortic root until all effluent from the heart was clear. The vessels were then dissected out using a no touch technique (7) and transported to the laboratory in sterile transport medium (RPMI 1640 tissue culture medium containing 20mM Hepes Buffer, 4 IU/ml of sodium heparin, Glutamine (200mm), Penicillin (100 µg/ml), Streptomycin (100 units/ml), Gentamycin (10 µg/ml), and Amphotericin (250 µg/ml)

Culture procedure

Segments (1cm long) of control vessel and vessel which underwent coronary angioplasty were cleaned of adventitial tissue and maintained in serum free organ culture by an adaptation of the method described by Pedersen and Bowyer (8). Briefly the vessel was opened out longitudinally and pinned intimal surface uppermost onto a polyester mesh support resting on set sylgard resin in the base of a Petri dish. The vessel was washed several times and then maintained in culture medium (sterile RPMI 1640 tissue culture medium as above). The vessel was then rested for 30 minutes after which time the conditioned media was removed. Fresh basal culture media supplemented with [^{3}H] thymidine (1 µci/ml, Amersham International, Amersham, UK) was then added to the cultures which were maintained at 37°C in a humidified atmosphere with 5% (v/v) CO_2 in air for 24 hours. At the end of this period the conditioned medium was collected, aliquoted into sterile tubes and stored at -80°C. The vessel was washed three times with non sterile phosphate buffered saline and then divided into two sections. One was placed in formalin for histology and the other in liquid nitrogen for assessment of nucleotides. Measurement of purine metabolites, DNA concentration and thymidine incorporation were performed as previously described (9).

Morphological studies

Endothelial morphology was studied using en face scanning electron microscopy. Segments of vessel were fixed in 3% glutaraldehyde in cacodylate buffer. Dehydrated specimens were then critical point dried in liquid CO_2, splutter coared with gold particles and observed in a scanning electron microscope (Phillips 500). For transmission electron microscopy specimens were treated similarly. After fixation in 3% glutaraldehyde followed by post fixation in 1% aqueous osmium tetroxide the specimens were dehydrated in alcohol, transferred to polypropylene oxide and embedded in epoxy resin. Ultrathin (100nm) sections were stained with 50% alcoholic uranyl acetate and Reynold's lead citrate and observed using a Phillips 400 transmission electron microscope.
For light microscopy vessel segments were fixed in 10% buffered formalin, processed and paraffin embedded. 4μm transverse sections were stained with alcian blue/Miller's elastic /Van Gieson and the mean neointimal, intimal and medial thickness was determined from measurements taken at 20 equidistant points along the section length using an image analyser (Solitaire, Seescan, Cambridge, UK). The intima consisted of a monolayer of endothelial cells. The neointima was clearly visible as a new layer above the internal elastic lamina and was distinguished by the presence of closely packed cells in an abundance of elastic fibres.
Autoradiography was performed to localize and quantify cell proliferation. Unstained 3 μm sections were deparaffinised, rehydrated through alcohol to water, dipped in K2 nuclear emulsion and exposed for two weeks at 4°C as previously described (9, 10). The total number of neointimal and medial labelled cells were counted and related to the section length which was determined using a calibrated eye piece graticule. To identify the cells in the neointimal layer immunostaining of paraffin sections was performed using monoclonal anti-smooth muscle α-actin antibody as previously described (9, 10).

Cell proliferation assay

Swiss mouse 3T3 fibroblasts were used to assess the mitogenic activity of the coronary vessel conditioned media. Measurements were performed in triplicate and the mean value for each specimen calculated. Cells (Flow labs) were passaged twice weekly and maintained in Dubecco's modified Eagles medium (DMEM) with bicarbonate (2g/l) and supplemented with 10% foetal calf serum (JBio chemicals, France), penicillin (100μg/ml), Streptomycin (100 IU/ml), Fungizone (2.5μg/ml), Gentamycin (2.5μg/ml), and glutamine (2mM). Cells were plated at a density of 104 cells per well in a 96 well plate (Costar, UK) in DMEM supplemented with antibiotics and 10% foetal calf serum. After 24 hours the cells were made quiescent by incubation in medium containing 2% pig plasma derived serum for a further 2 days before the addition of test samples and standards. [^{3}H]-Thymidine (1μCi/ml, specific activity 25 Ci/mmol) was added to the cells

simultaneously with the test agents and its incorporation into DNA determined over a 20 hour interval. After exposure, the cell layers were washed three times with phosphate buffered saline (PBS) and fixed for 3 minutes with ice-cold 10% w/v Trichloroacetic acid (TCA). The TCA precipitable material was harvested by aspirating the media and solubilising the remaining TCA-insoluble material in 100µl of 1M sodium hydroxide overnight at 37°C. The solubilised material was added to 2ml of Scintillation fluid (Ultima Gold, Packard, UK) and radioactivity determined by liquid scintillation counting. The data are expressed as disintegrations per minute (DPM/well) and as percentage stimulation over basal culture media and compared to 10% serum.
In a separate series of experiments, a polyclonal antibody to platelet derived growth factor, neutralising all forms of PDGF (British Biotechnology, Oxford, UK) was added, at an initial concentration of 50, and then a final concentration of 100 µg/ml to the conditioned media. The results were compared to those of the same sample of media incubated with non-immune IgG (Sigma chemicals, Poole, UK) at the same concentration. Media was pre incubated with antibody or non-immune IgG for 1 hour at 37°C prior to the addition of the fibroblasts.

Expression of mRNA for PDGF

Reverse transcription polymerase chain reaction was used to assess mRNA levels for PDGF A and B chain in angioplasty vessels. Cellular RNA was isolated from snap-frozen segments of freshly isolated coronary artery and angioplasty vessel using a one-step phenol chloroform method as previously described (11). For RT-PCR, cellular RNA (1µg) was reverse transcribed in a 20µl reaction containing 1.25 U.ml-1 AMV reverse transcriptase and 2.5µM $MgCl_2$, 10mM Tris-HCL, pH 9.0, 50mM KCL, 0.1% Triton X-100 dNTPs (1 mM each), and RNasin ($1U.ml^{-1}$). RNA-cDNA hybrids were immediately used as a template for the polymerase chain reaction. Primers for the PDGF A and PDGF B chains were designed using a commercial software programme (OLIGO, National Biosciences) and screened for specificity using the EMBL database. Sequences used for PDGF A were positioned on exon 3,5' CCC CTG CCC ATT CGG AGG AAG A 3' (sense) and the exon 4-5 boundary, 5' TTG GCC, ACC TTG ACC CTG CGG TG 3' (antisense) corresponding to nucleotides of the gene. Sequences used for PDGF B were in the 3' untranslated region 5'CCG CAC CAA CGC CAA CTT CC 3' (sense) and 5'TTT GGC TCG CTG CTC CTG GG 3' (antisense) corresponding to nucleotides 1318 to 1569. The predicted sizes for PDGF A and PDGF B cDNA products were 227 and 271 bp respectively. Oligonucleotides were synthesized using an automated DNA synthesiser (Applied Biosystems), purified by butanol extraction, divided into aliquots and stored in sterile water at -20°C. For polymerase chain reaction expansion each 50µl reaction contained 10µl cDNA derived from 1 µg rNA, 7.5pmol each primer, 1.25 U T aquaticus DNA polymerase, 10 mM Tris-HCL (pH 9.0), 50mM KCl, 2.5mM $MgCl_2$ and 0.1% Triton X-100 (all from Promega,

Southampton, UK). Samples were overlaid with light mineral oil to prevent evaporation. The cycling parameters were 1 min at 95°C, 1 min at 62°C (PDGF B) or 55°C (PDGF A), and extension at 72°C for 1 min for 40 cycles with a final extension period of 6 min at 72°C. Chain reaction products were size fractionated and separated from unincorporated primers by electrophoresis through 1.5% agarose gels. Amplified DNA was visualised by EtBr staining under ultraviolet light transillumination.

Statistical Methods

Values are shown as mean ± SEM. Data were considered significant if $p < 0.05$ using the Student's t-test for unpaired data.

Results

Measurements of ATP concentration and ATP:ADP ratio were performed to examine the viability of the predominant cell type, the smooth muscle cell before and after the 24 hour culture period (Table 1). The ATP concentration for both control and angioplasty vessels remained unchanged indicating that tissue viability was maintained (296±28) and 270±19) before culture and 382±34) and 334±40) respectively after 24 hour culture). This was confirmed with the ATP:ADP ratio which again showed a similar pattern (2.15±0.05 and 2.26±0.06 before culture and 2.56±0.10 and 2.41±0.09 respectively after culture)
Total DNA was measured to assess total cell numbers and as an index of cell proliferation (Table 1). Both freshly isolated and balloon angioplasty vessels demonstrated similar DNA levels before (0.52±0.08), 0.68±0.04) respectively) and after serum free culture (0.74±0.09), 0.74±0.08) respectively) suggesting that the total cell number was maintained.

Vessel	Control	Ptca	Control	Ptca
Hours in culture	0	0	24	24
No of specimens	11	10	9	9
ATP Conc. (nmol/g wet wt)	296(28)	270(19)	382(31)	334(40)
ADP Conc. (nmol/g wet wt)	133(14)	128(15)	160(15)	125(17)
ATP/ADP ratio	2.15	2.26(0.06)	2.56(0.10)	2.41(0.09)
DNA Conc. (μg/mg wet wt)	0.52(0.08)	0.68(0.04)	0.74(0.09)	0.74(0.08)

Table 1: Variation in purine metabolites and DNA concentrations at hour 0 and hour 24 for control and angioplasty vessel in serum free organ culture (RPMI)

Morphological studies

Histological examination of freshly isolated segments of coronary artery showed an intimal layer of endothelial cells separated from the muscular media by a convoluted internal elastic lamina (Figure 1a). Histological examination of angioplasty vessels again demonstrated abundant elastic fibres and axial and longitudinally arranged smooth muscle layers in the media but now there was a clearly defined neointimal layer visible above the internal elastic lamina (Figure 1b)

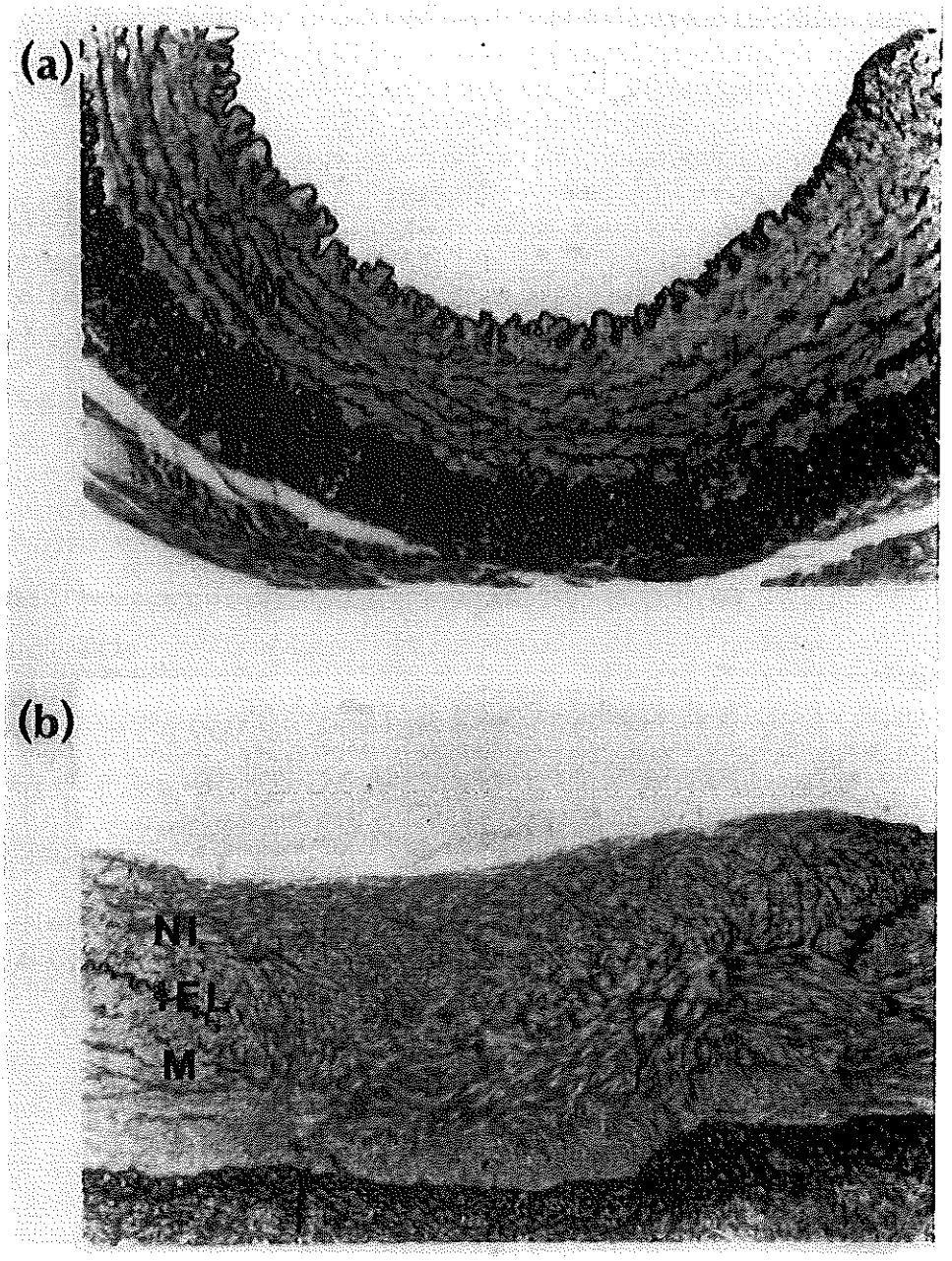

Figure 1: Transverse sections of pig coronary artery stained with Miller's elastic van Gieson stain (x37);
(a) Control vessel demonstrating abundant elastic fibres and axially and longitudinally arranged smooth muscle cell layers. Note the virtual absence of an intima.
(b) Angioplasty vessel demonstrating the development of a neointima. Note the increased degree of intimal proliferation over the ruptured internal elastic lamina.

Morphometric analyses demonstrated a markedly thicker vessel wall in angioplasty vessels (n=9) compared to control vessels (n=10) (291(26) vs 205(20), $p < 0.001$ vs control vessel). This was due to the presence of a neointima in angioplasty vessels (mean neointimal thickness, 92.8 ± 28.0 μm, n = 9) compared to the absence of a measurable intima in the control vessels. There was a marked difference in the intimal thickness over areas where the internal elastic lamina was ruptured compared to areas where the internal elastic lamina remained intact (206 ± 28 vs 39 ± 6μm, $p<0.001$). Measurement of the medial thickness demonstrated no difference between the angioplasty and control vessels (205 ± 20 vs 201 ± 16 μm) or between areas with intact or disrupted internal elastic lamina (206 ± 18 vs 202 ± 26μm respectively).
Immunocytochemistry with a monoclonal antibody to α-actin was performed to identify and localize vascular smooth muscle cells. In sections of control coronary artery most of the medial cells stained positively for α-actin. In the angioplasty vessels positive staining was observed in the media and also throughout the neointimal layer (Figure 2) suggesting that the cells in the neointimal layer were predominantly smooth muscle cells.

This was confirmed by transmission electron microscopy which showed this layer comprised of extracellular matrix and smooth muscle-like cells containing an abundance of rough endoplasmic reticulum and few actin filaments suggesting them to be in a synthetic rather than a contractile phenotype. Scanning electron microscopy showed that the endothelial monolayer in both control and angioplasty vessels was largely undamaged during preparation and these appearances were maintained after serum free culture.

The basis of the neointimal accumulation of cells was investigated by pulse labelling the vessel segments with [^{3}H]-thymidine during culture. Thymidine incorporation and autoradiography were then used to assess cell proliferation. Liquid scintillation counting reflecting overall cell proliferation (endothelial, smooth muscle and fibroblast) suggested that cell proliferation was occuring during culture in both control and angioplasty vessel (mean count of 1200dpm/mg wet wt).

Autoradiography of transverse vessel segments cultured and pulse labelled for 24 hours with [^{3}H]-thymidine was used to localise the proliferating cells. This demonstrated the presence of cells with silver grains over their nuclear region in both the neointimal layer but also the medial layers of the angioplasty vessels (Figure 3).

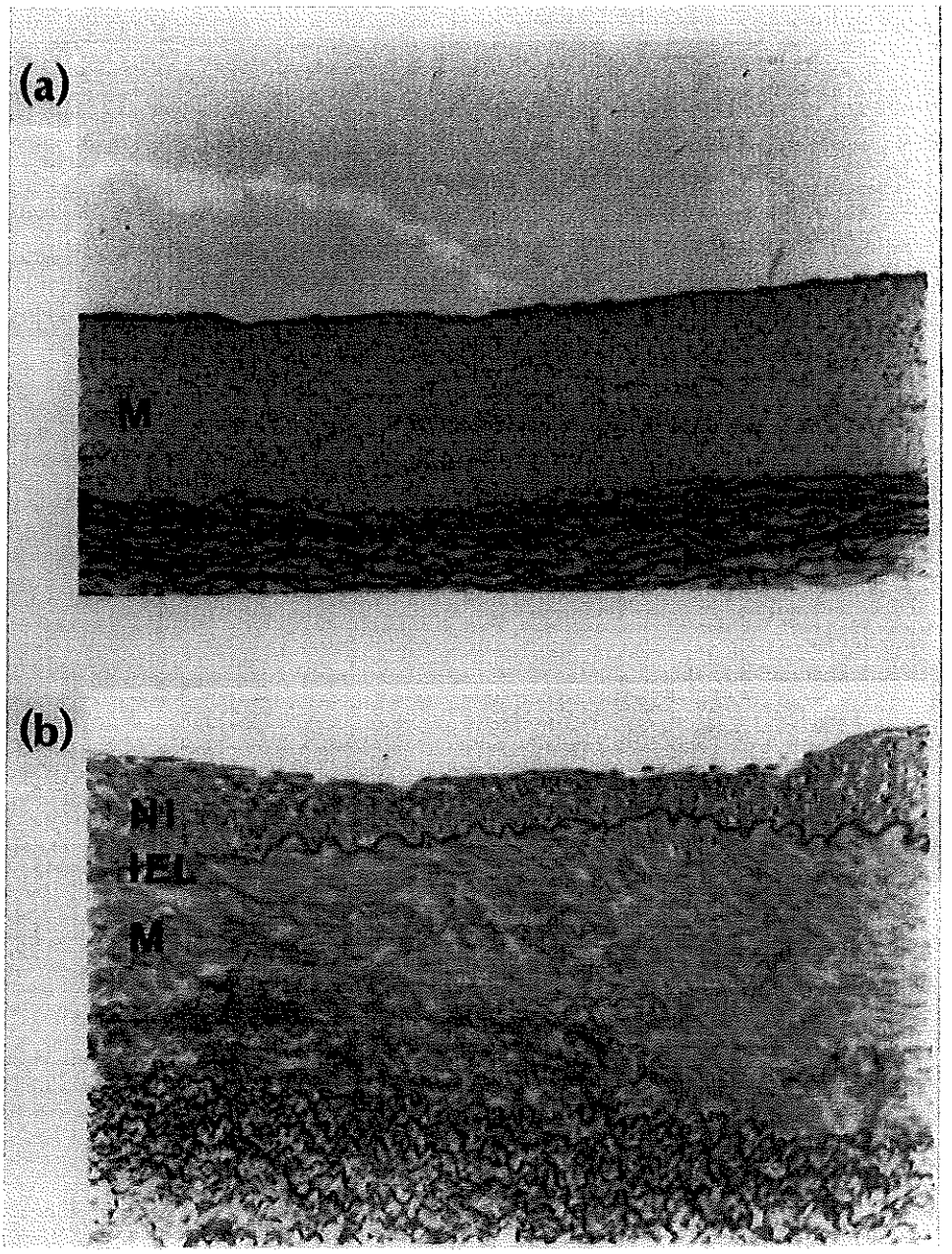

Figure 2: Transverse sections of control and angioplasty pig coronary artery stained with anti smooth muscle α-actin (x37);
(a) Control vessel demonstrating granular staining of the medial smooth muscle cells with no staining of the area of the internal elastic lamina or endothelium (Counterstained with Harris' haematoxylin)
(b) Angioplasty vessel demonstrating granular staining of the medial smooth muscle cells but also granular staining of most of the cells in the neointima (Counterstained with Harris' haematoxylin).

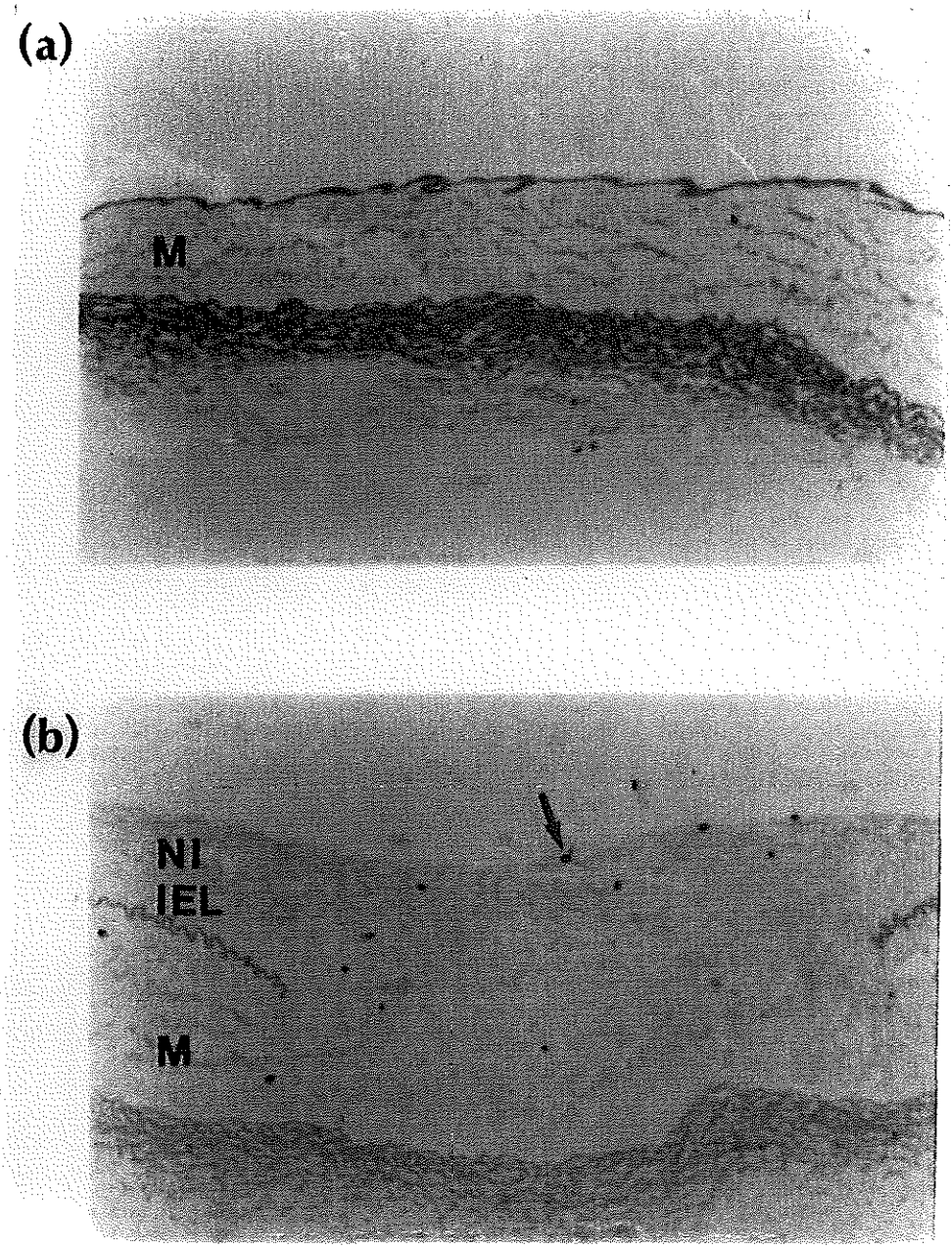

Figure 3: Transverse sections of pulse labelled control and angioplasty pig coronary artery cultured for 24 hours. Sections were autoradiographed and then post stained with haematoxylin and eosin (x37).
(a) Control vessel demonstrating few labelled nuclei
(b) Angioplasty vessel demonstrating proliferating cells both in the neointimal but also in the medial layers.

In the control vessels there were only a few proliferating cells in the medial layer. The number of [^{3}H]-thymidine labelled cells /mm^2 in both intima and media of angioplasty and control vessels are given in Table 2. There were significantly more labelled intimal and medial cells in the angioplasty vessels than control vessel suggesting that the neointimal cells were perhaps arising from both migrating cells from the media and also dividing cells in the neointima.

Vessel	Control (+IEL)	Ptca (-IEL)	Ptca
No of specimens	10	9	9
Thymidine incorporation (dpm/mg wet wt)	1289(341)	1326(226)	1326(226)
Labelled nuclei/mm			
Intimal	0	1.03(0.23)	8.05(0.84)
Medial	2.12(0.45)	3.36(0.28)*	4.53(0.41)*+
Intimal labelled nuclei /mm^2	0	26.39(8.82)*	48.74(2.57)*+
Medial labelled nuclei /mm^2	13.88(3.22)	17.65(1.17)	24.90(3.42)#

Table 2: Quantification of intimal proliferation. Vessels were exposed to [^{3}H] Thymidine for the last 24 hours. Labelled nuclei contained at least 20 silver grains. The number of nuclei is expressed per mm of intimal length. +IEL= Intact internal elastic lamina, -IEL= Ruptured internal elastic lamina. * $p<0.001$ vs control, # $p<0.05$ vs control, + $p<0.001$ vs +IEL.

Cell proliferation assay

A Swiss 3T3 fibroblast bioassay was used to assess mitogenic activity in the cultured media. Basal culture medium produced proliferation of 3T3 fibroblasts as assessed by [^{3}H]-thymidine incorporation of 10,224±1,227 dpm/well (n=12). Conditioned media from control vessel caused a 5.8±0.45 fold stimulation over basal media whilst angioplasty vessels caused a 6.4±0.44 (n=10) stimulation over basal media. By comparison 10% foetal calf serum resulted in a 5.6±0.49 stimulation over basal media.

A polyclonal neutralising antibody was used to assess the contribution of PDGF-like activity to the growth promoting activity of the conditioned media.

Compared to non immune IgG, anti PDGF did not significantly affect the mitogenic activity of the conditioned media at a concentration of either 50 or 100 µg/ml (Table 3) suggesting that either the majority of the mitogenic activity of the conditioned media must be due to other mitogenic peptides or the concentration of PDGF in the conditioned media was such as to abolish any effect from the antibody.

Media	Fold Stimuln PDGF Ab	Fold Stimuln Non immune IgG
PDGF Ab [50µg/ml)		
RPMI+10% FCS	1.6(0.27)	
Conditioned Media from		
a) Control vessel	3.2(0.45)	2.3(0.36)
Angioplasty vessel	2.5(0.34)	1.9(0.26)
PDGF Ab [100µg/ml)		
RPMI+10% FCS	0.9(0.28)	
Conditioned Media from		
a) Control vessel	3.9(0.12)	3.3(0.88)
Angioplasty vessel	3.2(0.46)	2.8(0.80)

Table 3: Mitogenic activity of conditioned media and response to PDGF neutralising antibody or non immune IgG at concentrations of 50 and 100µg/ml; The cell proliferation values are given as percent stimulation over basal culture media (mean (SEM)). FCS - Foetal Calf Serum. PDGF - Platelet Derived Growth Factor

Expression of mRNA for PDGF B

RT-PCR analysis was used to assess levels for PDGF A and B chain in angioplasty vessel and controls. Using this technique similar levels of PDGF A and B chain were observed in both types of vessel (Figure 4).

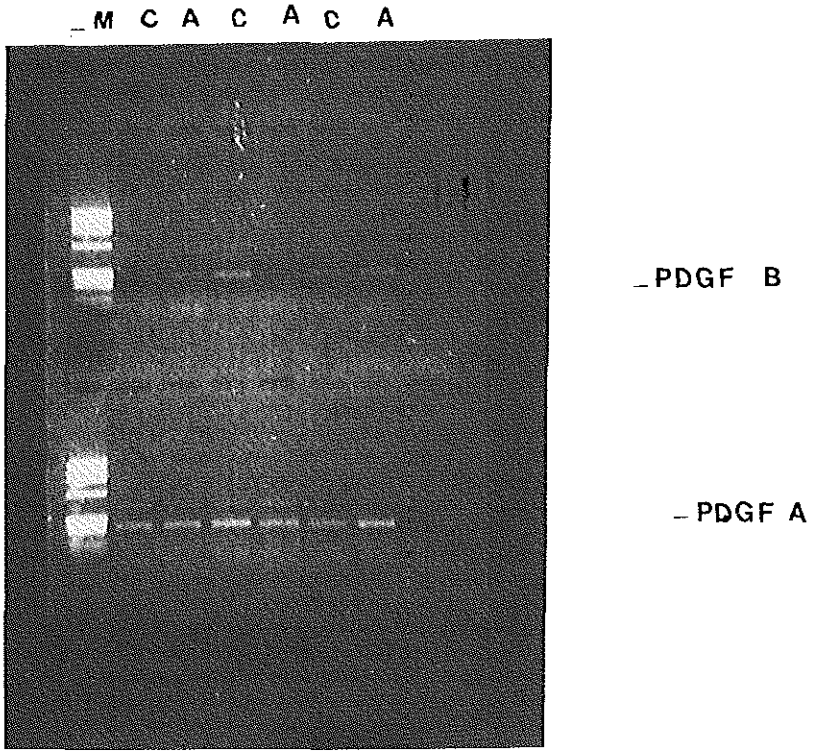

Figure 4: RT-PCR analysis of angioplasty and control vessels. Amplification of reverse transcribed DNA sequences for PDGF A and PDGF B were performed as described in the methods section. After electrophoresis of RT-PCR products from vessel segments intense bands were detected by ethidium bromide staining for the molecular weight marker(M). The upper panel illustrates amplification products for PDGF B whilst the lower panel illustrates amplification products for PDGF A. Lanes 2, 4 and 6 are control vessel and lanes 3,5 and 7 are angioplasty vessel. Similar levels of PDGF A (227 bp, lower panel) and B transcript (271bp, upper panel) are seen in both control and angioplasty vessel.

Discussion

This study has demonstrated that balloon angioplasty of normal porcine coronary arteries results in vsmc migration and proliferation with the subsequent formation of a clearly visible neointima. Furthermore, the degree of neointimal proliferation is significantly increased if the internal elastic lamina is ruptured suggesting a critical role for the internal elastic lamina in restenosis. Explanted vessels can be maintained in short term serum free organ culture with no detectable loss of tissue viability. Moreover conditioned media from these vessels induces proliferation of Swiss 3T3 fibroblasts equivalent to 10% calf serum.

The origin of this mitogenic activity is unclear but may relate to growth factor production from the vessel wall itself.

Efforts to reduce or eliminate restenosis have so far been hampered by the lack of knowledge regarding the pathophysiological mechanisms involved and the lack of an accurate animal model in which to study the phenomenon further. In the present model a reproducible neointima occurs within a reasonably short time following oversize balloon injury to a normal coronary artery. The majority of the cells in this layer were identified as smooth muscle cells by immunocytochemical staining with smooth muscle cell α-actin antibodies. Furthermore autoradiography performed on ex-vivo cultured segments of artery suggests that these cells were actively proliferating.

Why this intimally directed smooth muscle cell migration, proliferation and laying down of extracellular matrix occurs is unclear. It may relate however to the intimal and medial damage induced by balloon angioplasty. When the endothelial monolayer is denuded, it's barrier function is lost, resulting in the adherence of platelets which may then stimulate smooth muscle cell migration and proliferation by the release of different growth factors (12). Furthermore the discontinuity and dysfunction of the endothelium during endothelial regrowth may result in the loss of the physiological balance between the smooth muscle cell growth promoters and inhibitors culminating in the smooth muscle cell migration and proliferation (13, 14). Adherent platelets, injured and dead smooth muscle cells and mechanical stretching of the smooth muscle cells may also result in the release of a number of mitogens resulting in the phenotypic change of smooth muscle cells to the synthetic type (15).

The structural integrity of the internal elastic lamina appears to be crucial in minimising neointimal thickening. The mean intimal thickness increased from 39(6) μm in regions where the internal elastic lamina was intact, to 205(28)μm where it was ruptured giving an overall mean intimal thickness of 92(28)μm. Thus although rupture of the internal elastic lamina is not a prerequisite for neointimal formation it appears to amplify the phenomenon. This may be because it reflects greater vessel injury. This is unlikely however as all portions of the vessel are equally stretched when the balloon is inflated. A more likely explanation is that rupture of the internal elastic lamina exposes the medial tissues to flowing blood increasing local platelet deposition and local growth factor production. Furthermore associated medial tears result in the loss of contact inhibition, both between cells and between cells and their extracellular matrix, which mechanisms of which are known to constrain smooth muscle cell proliferation (16). Additionally, rupture of the IEL may result in the loss of any physical barrier it may represent to the intimal migration of the medial smooth muscle cells. Any or all of these mechanisms could lead to the over exuberant vessel reaction seen in these areas. Similar observations regarding the importance of the internal elastic lamina have also been seen in pig coronary arteries injured by wire stents (17) and oversize balloon injury (18). Moreover a recent report suggests that these observations may also be true in man (19). A comparable

phenomenon is also seen in atherosclerotic coronary arteries in man where intimal smooth muscle cell proliferation is often associate with gaps in the internal elastic lamina (20).

The angiographic facilities available did not allow quantitative measurements to be performed pre and post angioplasty to determine whether the degree of intimal proliferation seen resulted in a significant stenosis, but based on the histological measurements with a neointimal thickness of 92(28)μm this is unlikely. Nevertheless the underlying pathophysiological process involved in restenosis, intimally directed smooth muscle cell migration and proliferation, occurred reproducibly within a short time span.

The use of this pig model offers specific advantages over other animal models for the study of intimal hyperplasia. Pigs resemble man with respect to their coronary circulation, platelet coagulation system and lipoprotein metabolism. Furthermore pigs are known to develop atherosclerotic plaques similar to those found in man and their intima contains smooth muscle cells that participate in the response to vascular injury and in the development of spontaneous intimal thickening in older pigs (21). Additionally, the lesion produced by balloon angioplasty resemble those of human post angioplasty restenosis in being composed of smooth muscle cells.

A number of pig models have previously been described. The pig carotid artery model (22), although useful for studying the acute response of the vessel wall to angioplasty does not cause significant intimal proliferation and any significant proliferative stenoses are due to organised thrombus. Furthermore the artery used (the carotid) is an elastic vessel with proportionally more smooth muscle than the muscular coronary artery. The recently proposed coronary stent restenosis model (17, 18) although resulting in gross and histopathologic lesions virtually identical to human restenosis tissue, is limited by the excessive tissue response perhaps as a result of the vessel wall being under constant tension by the stent, as well as the high cost of the stents themselves.

The present model admittedly does not result in substantial vessel occlusion but nonetheless the underlying pathophysiological process involved occurs and can be monitored. Furthermore the use of standard catheterisation techniques means that the model may be useful for the assessment of new techniques for local drug delivery such as the microporous balloon which offers the possibility of topical delivery at concentrations which may be toxic at systemic level (23).

There are however a number of limitations. The animals used are young with normal coronaries and no underlying atherosclerotic plaque. This means that we are unable to comment on any effect the plaque/vessel wall interaction may have in the in-vivo human restenosis process. Furthermore the degree of intimal proliferation seen is unlikely to result in significant stenoses limiting our ability to comment on the influence of any local flow dynamics. These limitations can to some extent be

overcome by adaptation of the model. For example sequential balloon angioplasties at four weekly intervals may be a more realistic version of the in-vivo situation of stenosis dilatation. Furthermore the model may be adapted to produce occlusive disease by the addition of cholesterol feeding. Using cholesterol feeding and balloon denudation of the coronary arteries, for example, Lee and Lee produced significant coronary stenoses with virtual occlusion of the vessels and resulting myocardial ischaemia, myocardial infarction and sudden death within 3-6 months whilst the animals were still relatively small (40-50 Kg) (24).

We used our model to assess endogenous growth factor production using a serum free organ culture technique. Our first concern initially was to ensure that tissue viability was maintained in the absence of serum. Once we confirmed that tissue viability was maintained in short term serum-free organ culture we used the technique to, firstly, assess the presence of endogenous growth factors in the cultured media using a Swiss 3T3 fibroblast bioassay system and, secondly, to demonstrate proliferating cells in the neointimal layer.

Conditioned media from both control and angioplasty vessels resulted in a 6 fold increase in the proliferation of fibroblasts compared to a 1.5 fold increase caused by 10% serum, suggesting the presence of growth factor activity in the cultured media. The origin of this mitogenic activity is unclear. It may have originated from a number of sources including adherent platelets, dying cells, tissue injury or the cells of the vessel wall itself.

It is unlikely that the growth factor activity was from adherent platelets as the vessel segments were thoroughly irrigated with saline prior to culture and scanning electron microscopy confirmed the absence of any adherent platelets on the intimal surface of vessels prepared for culture. Similarly the maintenance of the ATP concentration and ATP/ADP ratio in culture also argue against the possibility of growth factor release from dying cells. The most likely explanation is that the growth factor activity detected was released from the vessel wall. It is unclear why the control vessel demonstrated as much growth factor activity as the angioplasty site. One possibility is that the, unavoidable, tissue damage during harvesting of the vessel may have resulted in induction of growth factor production from sublethally damaged cells.

We tried to elucidate the nature of the mitogenic activity further by the addition of a commercially available antibody to PDGF and compared the results to those of sample media incubated with non immune IgG at the same concentration. The addition of antibodies to PDGF did not result in any significant inhibition of mitogenic activity, in either the control, or the angioplasty vessel, suggesting that PDGF is not, perhaps, the major mitogen involved. An alternative explanation however is that the concentration of released PDGF in the conditioned media was such that the neutralising antibody used was swamped.

We also tried to obtain supplementary evidence that balloon angioplasty leads to the activation of growth factor gene expression in the vessel wall by performing RT-PCR analysis on segments of angioplasty and control vessel. This demonstrated the presence of PDGF B mRNA in both vessels suggesting that the message for PDGF B is present in the vessel wall. Whether this was induced during harvesting, or whether it is present in the normal vessel wall is not clear. Additionally the technique is not quantitative enough to allow us to determine whether there is increased expression of PDGF B mRNA in the angioplasty vessel.

Factors other than PDGF could also be involved. For example fibroblast growth factor is a particularly good candidate because it is synthesized by both endothelial and smooth muscle cells. Furthermore although not secreted it is released from disrupted cells (25, 26) and cell disruption during harvesting may account for the lack of difference between the control and angioplasty vessels. The nucleotide data would be against this but of course they only represent any additional cell degradation which has occurred in the 24 hour culture period and not the initial damage that the vessel may have sustained during harvesting. Whether the addition of commercially available antibodies to bFGF will inhibit this mitogenic activity is unknown.

In addition to allowing us to assess the role of growth factor production, the serum free organ culture technique also allowed us to demonstrate proliferating cells in the neointimal layer ex-vivo. This avoided the dangers involved in giving radioactive isotopes in-vivo. Little is known of the accuracy of the ex-vivo labelling of proliferating cells however. In the only study to compare in-vivo with ex-vivo labelling of proliferating cells, ex vivo labelling resulted in a patchy distribution of labelled cells which did not correspond with the [^{3}H]-thymidine labelling pattern obtained in vivo (27). This was in colonic and vaginal mucosa however, rather than the vessel wall. Furthermore the in-vivo S phase cells were labelled with [^{3}H]-thymidine injected 30 minutes before sacrifice and evaluated by autoradiography whereas the ex-vivo labelling was with Bromodeoxyuridine incubation for 60 minutes and subsequent immunohistochemistry. We were unable to confirm in our study whether the ex-vivo labelled cells corresponded to in-vivo proliferating cells. It is not however the state of proliferation that is important but the rate and certainly in our case the proliferative activity in the angioplasty vessel was much higher than in the control vessel.

Conclusion

This study demonstrates that neointimal smooth muscle cell proliferation occurs reproducibly following balloon injury to normal porcine coronary artery. Furthermore it suggests a pivotal role for the internal elastic lamina in this phenomenon. It also provides evidence for growth factor release by cells intrinsic to the vessel wall, further characterisation of which may help us understand the mechanisms involved in restenosis and guide the development of new therapies aimed at reducing its incidence.

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Chapter 6

Acute local delivery of drugs with incremental molecular size in normal porcine coronary arteries

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Submitted for publication

Abstract

Background: Local drug delivery by perfusion catheters has been proposed as a means of delivering high concentrations of active drug into the coronary arterial wall but pressure mediated trauma and duration of vessel occlusion are key limitations of these devices. We investigated a new delivery catheter which promises to overcome both these restrictions.

Methods: The Dispatch™ perfusion catheter has a triple lumen shaft with a distally located polyethylene coil balloon. The coils are connected by a membranous aerofoil which, when inflated, divides the vessel lumen into an external compartment allowing local drug delivery to the vessel wall and an internal compartment which allows perfusion of the distal coronary bed. Using an over the wire technique the device was positioned in 16 coronary arteries (luminal diameter (mm) 3.2±0.4(SD)) of 11 non-atherosclerotic pigs (25-68kg) and inflated to 9 atmospheres. Coronary flow was estimated during device inflation using a doppler flow wire. Infusion of a selection of 5 compounds with incremental molecular weight (Range 0.3-150 kDa) was then performed for 10 minutes using infusion rates of 0.2 to 1.0 ml/min. Following device deflation the animals were sacrificed and macroscopic and histological examination of the vessels performed.

Results: Inflation duration 19.9±9.7min (Range 3-60 min) did not result in any signs of ischaemia or haemodynamic compromise. Furthermore intracoronary infusion of adenosine with the catheter inflated demonstrated enhancement of coronary flow velocity similar to normal controls. Macroscopic examination demonstrated that compounds ranging from 0.3-70 kDa could be infused into the coronary wall. Microscopy showed that penetration of the compounds was limited to the subendothelium, which showed an oedematous appearance at higher flow rates. Most of the endothelial layer and the internal elastic lamina remained intact. Adventitial localization of the drugs was also observed, but this was probably due to side branches included in the target vessel segment and supplying the vasa vasorum. Compounds with sizes 150 kDa and larger did not reach the subendothelium.

Conclusion: This new drug delivery device is capable of safe prolonged infusion of a wide range of compounds up to 70 kDa in the subendothelium of normal porcine arteries. The device thus has potential for ameliorating acute occlusion and late restenosis post coronary intervention.

Introduction

Percutaneous coronary revascularisation has become increasingly complex with more advanced catheter technologies (laser, atherectomy, stents) being used to treat increasing numbers of patients with more complex coronary lesions(1). Most techniques however remain limited by acute occlusion in 3-8%, and late restenosis in 30-50% of cases with only the implantation of stents having been shown to reduce angiographic restenosis, albeit in a selected patient population and at the expense of higher haemorrhagic complications (2, 3). Although progress in solving both early and late complications has been slow, recent evidence suggests that the systemic administration of new monoclonal antibodies directed against the platelet glycoprotein IIb/IIIa can reduce both acute occlusion and the late need for coronary revascularisation procedures in high-risk angioplasty patients, again at the cost of higher bleeding complications (4, 5). Local delivery of these and other agents would, however, offer a means of ensuring high concentrations of the active drug at the intervention site whilst avoiding their systemic complications (6, 7). Moreover site specific drug therapy would also be invaluable for the catheter based treatment of intracoronary thrombus in the setting of unstable angina or acute myocardial infarction.

Most methods of local drug delivery presently rely on catheters which isolate the target vessel by balloon occlusion (7). In the coronary circulation, this approach has the serious limitation of inducing myocardial ischaemia. In addition, the first generation of porous balloon catheters was also shown to induce substantial local vessel trauma due to the high pressure jets of the drug solution (8). Although the second generation of microporous and channelled balloon catheters have minimized the degree of vessel wall injury they are still limited by the need for coronary artery occlusion during drug delivery (7). In this study we assessed a new drug infusion catheter designed to allow prolonged balloon inflation and local drug delivery with minimal vessel wall trauma. Our aims were to determine the safety of the procedure, assess coronary flow reserve during catheter inflation, assess the efficacy and depth of penetration of a range of compounds of incremental molecular weight and finally determine the amount of wall injury induced by the device.

Methods

Coronary infusion catheter.

The coronary infusion catheter studied is a non-dilation, over the wire device designed for localized delivery of solutions (Dispatch™, Scimed Life Systems, Inc., Maple Grove, MN; Figures 1 and 2). The catheter has a triple lumen shaft, the first lumen to inflate a distally located 20 mm long coil encapsulated by a thin polyurethane sheath to isolate the selected vessel wall from the lumen. A second lumen permits the use of a 0.014 inch coronary guide wire. The third larger lumen of the shaft is used to infuse solution which can enter the sheath isolated vessel wall through slits in the shaft between the coils. A radiopaque marker runs the entire length of the inflation coil to assist precise placement.

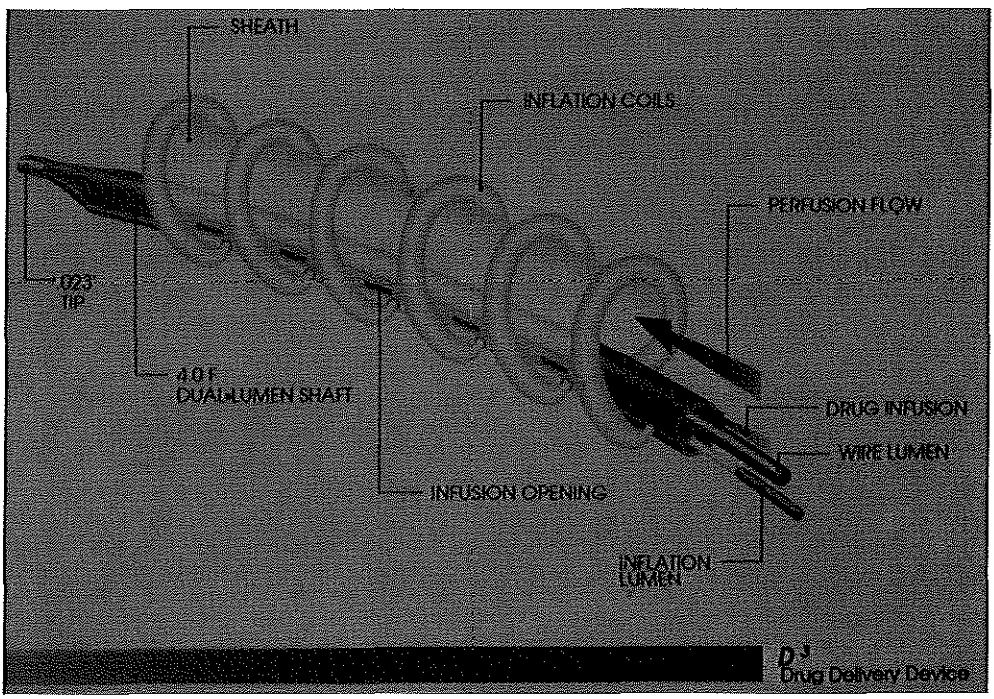

Figure 1: Schematic representation of Dispatch infusion catheter. Note distally located polyethylene coil allowing local drug delivery whilst maintaining distal perfusion.

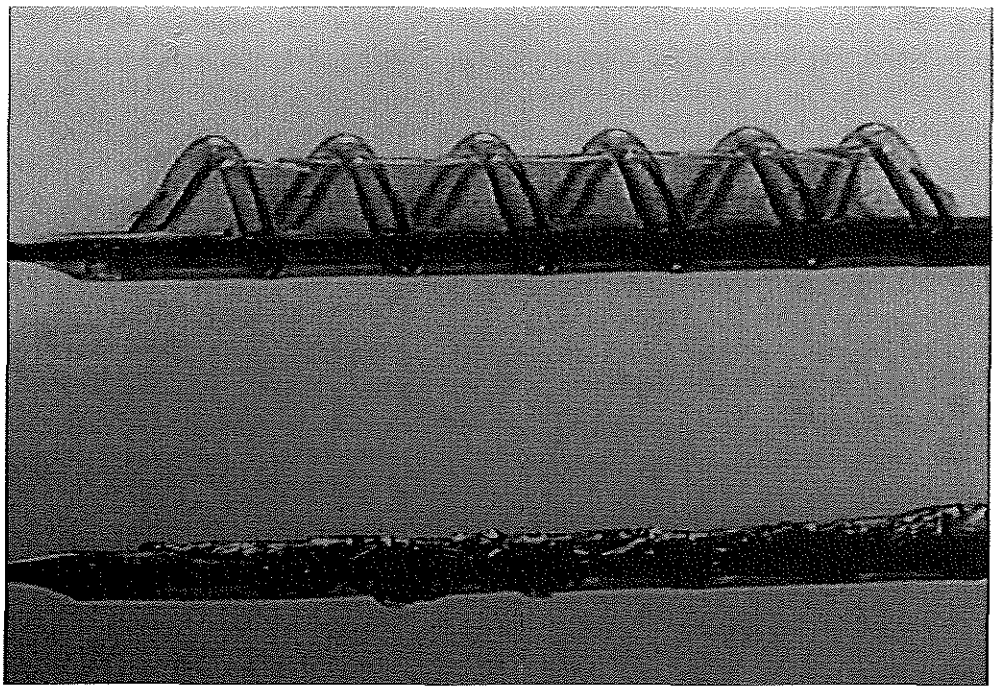

Figure 2: Lower panel shows deflated Dispatch infusion catheter whilst upper panel shows fully inflated device. Note distally located polyethylene coil allowing local drug delivery whilst maintaining distal perfusion.

Animal preparation.

Experiments were performed in 11 cross-bred Landrace Yorkshire pigs (25-68 kg in weight; HVC, Hedel, The Netherlands). The investigations were performed according to a protocol approved by the Committee on Experimental Animals of the Erasmus University. The anaesthetized animals were connected to a ventilator which administered a mixture of oxygen, nitrous oxide (1:2, v/v) and 1-4 vol % enflurane. A 9F guiding catheter was advanced to the coronary ostium through an introduction sheath placed in the left carotid artery. Coronary angiography was performed using iopamidol (Iopamiro^{R} 370) as contrast agent. Using on-line quantitative measurement of the arteriograms a segment of the left or right coronary artery was selected with a mean diameter of 2.5 - 3.8 mm for the placement of the infusion catheter (3.0, 3.5 or 4.0 mm) which was inflated to 6 Atm.

Three types of experiments were performed: 1) To determine the safety of the procedure the infusion catheter was inflated for prolonged periods of time and the occurrence of myocardial wall motion abnormalities and serious arrhythmias recorded ; 2) To determine the possibility of increasing distal coronary blood flow

during catheter inflation, coronary infusion of the vasodilator drug adenosine was performed ; 3) To assess the efficacy of acute delivery of compounds with incremental molecular size into the coronary arterial wall macroscopic and microscopic evaluation was used to determine the depth of marker penetration and the degree of wall damage.

Coronary flow velocity measurements.

In three experiments a flexible coronary doppler angioplasty guidewire with a 12 MHz piezoelectric transducer at the tip (Cardiometrics, Mountain View, CA) was positioned parallel to the infusion catheter shaft, across the inflated coil, in the distal coronary artery. After the animals had been haemodynamically stable for at least 30 min baseline recordings were made of coronary flow velocity with the infusion catheter coil inflated (9). Thereafter, a selective intracoronary dose of adenosine 20-60 µg/kg was administered and maximal flow velocity measurements repeated until the hyperaemic response subsided.

Drug infusion protocol.

After inflation of the infusion catheter coil at the selected coronary segment one of a selection of compounds or drug solutions was infused for 10 min. Target arteries, drugs and infusion rates have been summarized in Table 1. At the end of 10 min infusion period, the coil was deflated and the infusion catheter removed. In cases of non-dye administration, the site of infusion was marked with a short infusion of Evans Blue dye prior to removal of the catheter. After crossclamping the ascending aorta, the coronary arteries were perfusion fixed with 4% formaldehyde. The hearts were then excised and the coronary arteries dissected from the epicardial surface. The infused segments and adjacent non-infused artery were placed in 4% formaldehyde in phosphate buffer (pH 7.3) for 48 hours in preparation for microscopy.

Angiographic analysis.

Coronary angiograms (at baseline, after placement and inflation of the catheter coil and finally after completion of the drug infusion protocol and removal of the catheter) were performed after i.c. injection of 1 mg isosorbide dinitrate and measured on-line by quantitative coronary arteriography (QCA) using the edge-detection method (Cardiovascular Measurement System, Medis Inc, Nuenen, The Netherlands) (10).

Macroscopic examination

The delivery of Evans blue or Evans blue-albumin complex in the vessel wall was assessed macroscopically and recorded using a dissection microscope

Light- and fluorescence microscopy

The coronary vessels were fixed in 4% buffered formaldehyde, processed, sectioned and paraffin embedded. Marker penetration was then assessed qualitatively. Evans blue-albumin non-stained sections were examined with fluorescence microscopy and compared with non infused control sections. The 150 kDa anti-actin was assessed using a double antibody technique (secondary cross reaction with peroxidase labelled antibody).

Infused Compound	Arteries (LAD/LCx/RCA)	Infusion Rate (ml/min)	D3 Size (mm)	Mol weight (D)/Size
Fluorescent thiocyanate	1/1/-	0.2	2 x 3.5	300 D
Evans Blue	-/2/- 1 x 3.5	0.2	1 x 3.0	1,000 D
Evans Blue albumin complex	4/-/-	0.2	1 x 3.0 3 x 3.5	70,000 D
Anti-actin antibody	1/-/1	0.2	2 x 4.0	150,000 D
Activated Charcoal	1/1/-	0.2	1 x 3.0	10-25µm
Activated charcoal	-/3/1	1.0	1 x 3.0 2 x 3.5 1 x 4.0	10-25µm

Table 1: Summary of target artery, infused compound and infusion catheter size. FITC = fluorescent thiocyanate; EB = Evans Blue; EBA = Evans Blue-albumin complex;

Statistical analysis.

All data are expressed as mean ± SD. Haemodynamic and angiographic parameters were evaluated using paired t-test. A P-value <0.05 was considered statistically significant.

Results

In all coronary arteries, the infusion catheter could be positioned at the site selected by QCA. After inflation of the coil, the distal coronary artery received adequate baseline flow as assessed angiographically (TIMI Grade III flow in all cases, Figure 3). Continued coil inflation up to 60 min did not induce myocardial ischaemia as witnessed by direct observation of the heart after midline sternotomy, and the absence of ECG changes, while heart rate and blood pressure remained stable (Heart rate: pre 115±22, during 108±26, Mean aortic pressure: pre- 83±20, during 67±20 mm Hg (p=ns for both).

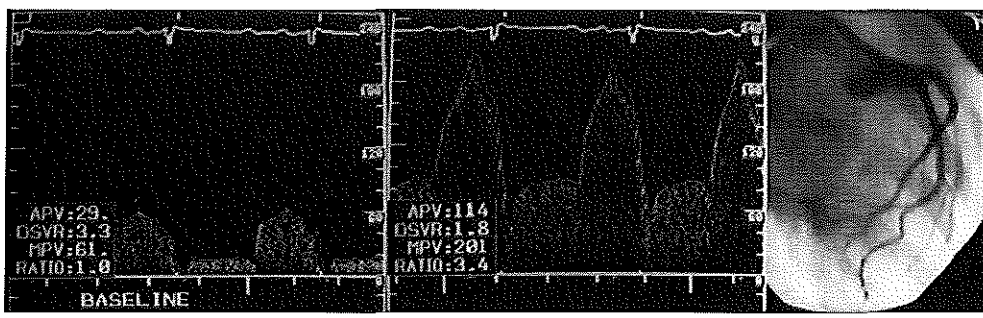

Figure 3: Intracoronary doppler flow measurements at baseline after coil inflation (left panel) and after intracoronary adenosine (middle panel). D3 Infusion catheter in position and during angiogram (right panel). Note good distal perfusion even with catheter fully inflated.

Coronary flow velocity measurements

In the three experiments where coronary flow velocity was measured at baseline, administration of adenosine 20-60 μg/kg i.c. induced reproducible increases in maximal velocity (Figure 3).

Quantitative coronary angiography

The average manufacturer specified infusion catheter coil size was 3.5 mm. Measurement of the baseline angiograms showed that the catheters were placed in coronary arteries 3.2 ± 0.4 mm in diameter. It was not possible to measure the diameter of the inflated coil during infusion because of interference by the strongly radiopaque marker. After completion of the infusion protocol and removal of the catheter the coronary diameter at the site of drug delivery had decreased to 2.7 ± 0.3 mm in the animals that had received infusion with a rate of 0.2 ml/min, and to 2.3 ± 0.8 in the experiments where a rate of 1 ml/min was used ($p<0.05$ vs baseline in both groups).

Acute drug delivery

Macroscopic examination

Macroscopic examination demonstrated that compounds ranging from 0.3-70 kDa (Evans blue and Evans blue-albumin complex respectively) could be successfully infused into the coronary wall (Figure 4).

Figure 4: Macroscopic appearances following infusion of Evans Blue. Note the 'Zebra' effect secondary to the areas where the coil was in contact with the vessel wall

Microscopic examination

Fluorescence microscopy demonstrated Evans Blue penetration into the intima and adventitia but not into the media (Figure 5).
Histological examination revealed localised endothelial damage with partial denudation (Figure 6). The subendothelial space was oedematous with separation but no rupture of the internal elastic lamina. The media was intact as was the external elastic lamina and adventitia. Charcoal particles were seen in the adventitial vasa vasorum and even in the adventitia and overlying muscle.

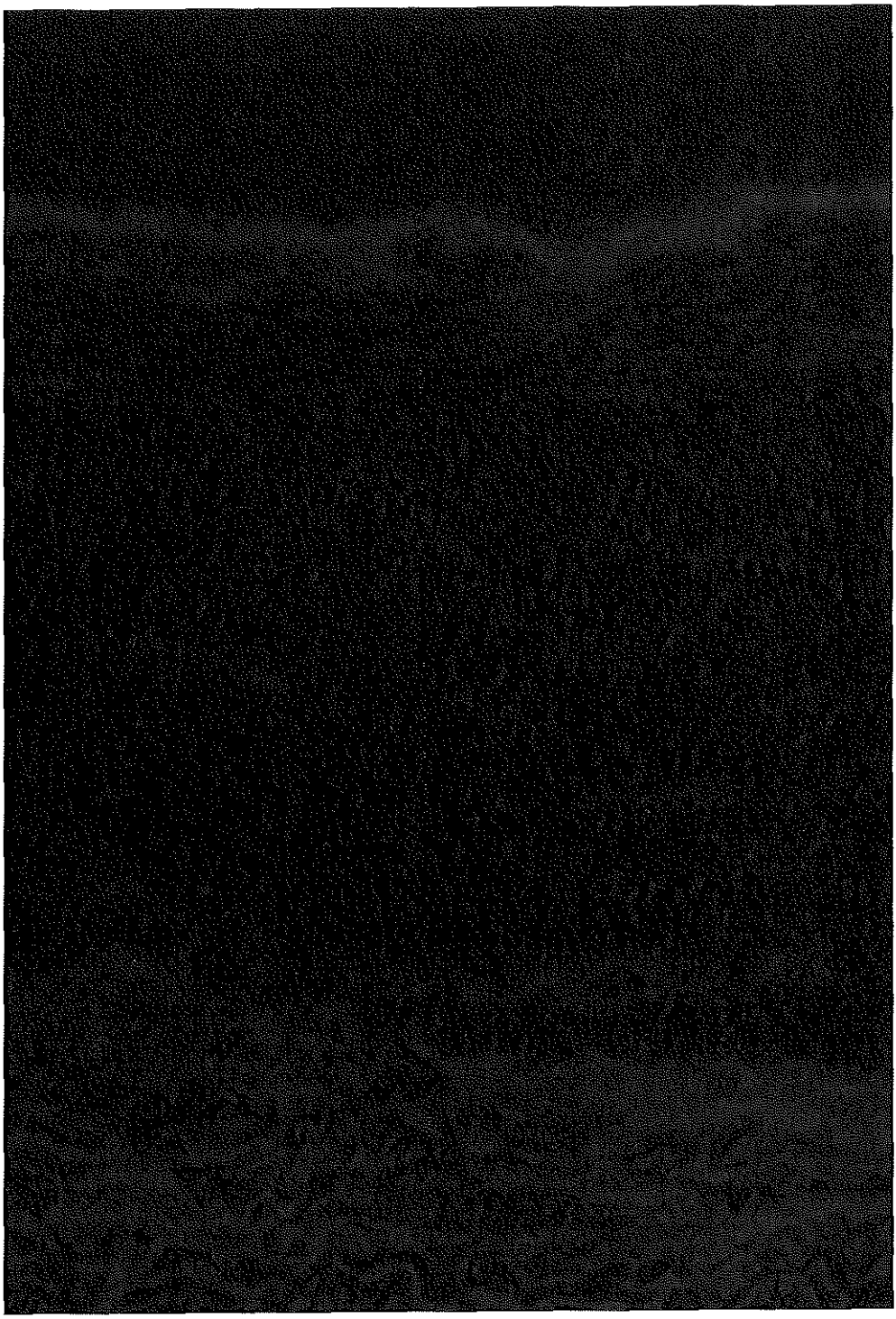

Figure 5: Histological section under fluorescence microscopy. Note the preservation of the endothelium and the presence of Evans blue in the subendothelial space and the surrounding adventitia.

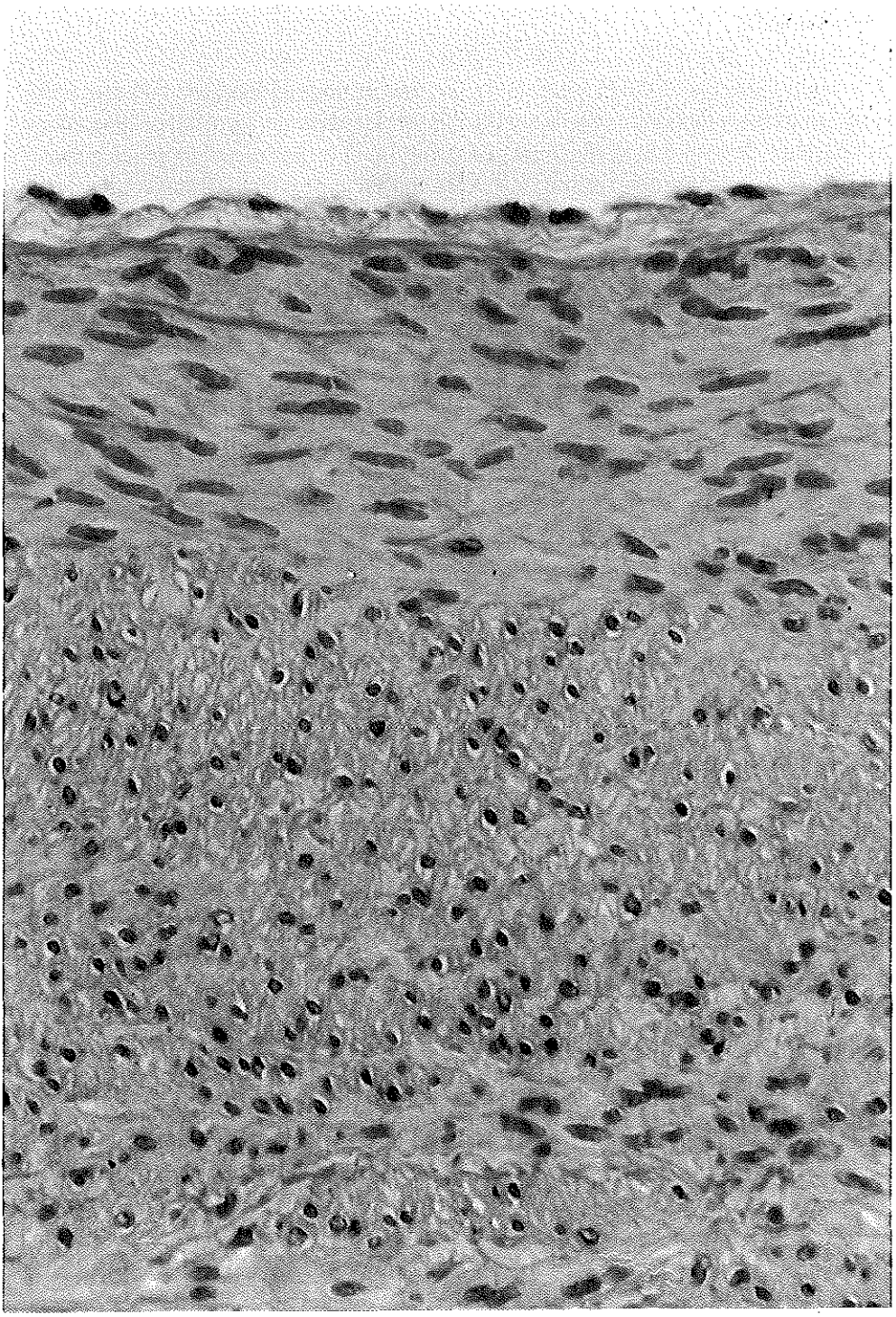

Figure 6: Histological section stained with Haematoxylin and Eosin. Note localised endothelial damage with partial endothelial denudation.

Discussion

The main findings of the present study are that prolonged infusion catheter inflation (up to 60 minutes) is feasible with this device with no significant limitations to coronary flow or haemodynamic compromise. Furthermore compounds ranging in molecular weight from 0.3-70 kDa can be successfully infused into the coronary wall using this device. This is achieved with only minimal trauma to the vessel wall where there is only localised endothelial damage with preservation of the internal elastic lamina and media.

A number of drugs have been successful in animal models of intimal proliferation but numerous large scale pharmacology trials in man have failed to demonstrate a significant reduction in clinical restenosis (11-14). These may have failed either because the drug used was ineffective in man or because the local concentration of active drug at the angioplasty site was inadequate. In animal models drugs can be given in high doses which man cannot tolerate without significant toxic side effects. Thus the systemic administration of an agent is limited by significant side effects at high doses. Delivering agents directly into the angioplasty site has the theoretical advantage that a high local dose of drug can be given without the worry of potentially toxic systemic side effects (6, 7, 15).

A number of catheters have been proposed for such local drug delivery (6, 7). These include the double balloon catheter, the porous and microporous balloons, the stented porous balloon, the weeping balloon, the inflated externally strutted porous catheter and the porous sleeve. All have limitations however. Some are limited by factors such as coupling of the perfusion pressure to the inflation pressure resulting in substantial arterial wall injury including dissection (16, 17). Others are limited by a short infusion time in the coronary vessels due to the coronary occlusive properties and expulsion of contents downstream or into side branches, either during delivery or on balloon deflation (18, 19).

One of the major advantages of the present catheter is the independence of balloon inflation and hence artery-vessel wall fit from local drug delivery conditions. This is unlike a number of catheters, including the porous and microporous balloon and weeping balloon, where both the balloon-artery fit and drug delivery conditions are determined by inflation pressure (19). This limits both the size and configuration of perforations within the balloon. Furthermore the coupling of balloon inflation and local drug delivery can also cause significant systemic administration of the catheter contents during inflation and deflation of the balloon or as a result of a poor catheter-artery fit. In contrast the present catheter allows the correct pressure for the right balloon-artery fit and an independent infusion pressure. The lack of holes which may be plugged by blood also means that the present device can be used a number of times with no limitations to its efficacy.

The other important advantage of the present device however is the ability to bathe the vessel wall in drug for a prolonged period of time (up to 60 minutes) whilst maintaining distal perfusion. The intracoronary doppler studies would suggest that even when fully inflated this catheter causes minimal obstruction to coronary flow and preserves a normal hyperaemic response. This may be a substantial advantage if duration of drug infusion is important for the local concentration of drug. In addition prolonged coil inflation also suggests that the device may be useful in acute occlusion, allowing local infusion of, for example, thrombolytic therapy whilst ensuring the physical apposition of the vessel wall layers.

The device allows prolonged local drug delivery whilst causing minimal trauma to the vessel wall. Although there is localised endothelial damage, the internal elastic lamina remains intact at low infusion rates with no damage to the media. At the higher infusion rates although the internal elastic lamina appeared oedematous there was still no microscopic evidence of deep injury and medial damage. As the degree of deep injury may relate to the subsequent neointimal thickening (20) this suggests that use of the device is unlikely to cause additional damage to the vessel wall and increase neointimal thickening.

We used 5 model compounds of incremental molecular weight to assess the range of compounds that this catheter could successfully transport into the vessel wall. The range included 0.3 kDa which is the lower limit of small synthetic drugs such as a calcium antagonist and beta blockers. Oligopeptides such as angiotensin and angiotensin II are in the range of 0.3-1 kDa whilst the range of Evans Blue Albumin complex (up to 70kDa) is that of proteins such as Cytokines, growth factors and growth factor antagonists. The range 70-150kDa includes plasminogen (90kDa) and collagen type 4 (120kDa). Our study suggests that compounds in the range of 0.3-70 kDa can be successfully infused into the coronary wall. Thus compounds such as small synthetic drugs, growth factor antagonists and oligopeptides may be successfully infused clinically using this device.

As well as knowing whether an agent can be successfully infused it is also important to know however whether the infused agent will be retained for a sufficient length of time. For example retention of heparin is less than 48hrs (21) whilst 90% of deposited colchicine and methotrexate are cleared within a few hours (22, 23). This limitation may be addressed by the incorporation of biodegradable microspheres into a matrix which can be deposited locally (24) or by using microparticles such as charcoal as carriers, allowing the controlled diffusion of impregnated drug over the course of time. A further advantage of using charcoal as the carrier particle is that the speed of release of the relevant agent from the charcoal can be substantially modulated. Although charcoal uptake into the vessel wall did not occur in our study it may do so in the clinical situation where antecedent balloon angioplasty is likely to have caused cracks, fissures and dissection planes (25) into which the charcoal may lodge. Certainly gold particles have been found in such dissection planes (26). The finding of charcoal particles in the adventitia,

carried there via the vasa vasorum also raises the possibility of local drug delivery by passive infusion inwards from the adventitia to the media.

The penetration of marker dye into the adventitia in keeping with previous reports suggesting that a large proportion of radioactive microparticles and adenoviruses delivered endoluminally are deposited in the periadventitia and overlying muscle layers (27, 28). The most likely explanation for this is leakage of the dye into the side branches, the vasa vasorum and from there into the adventitia. The effect of the delivered agent on this nontargeted tissue is unknown. Although it may detrimental if certain toxic substances are infused, if the appropriate agent is chosen this periadventitial deposition of a locally delivered agent with the resulting luminally directed diffusion, may be exploited as part of the local delivery strategy. Certainly periadventitial delivery of both heparin and dexamethasone have been shown to reduce neointimal formation (29, 30). In our experiments substantial infusion of drug into the local peri vascular environment may have occurred for the luminal diameter at the infused segment to have decreased so significantly at the time of the procedure.

In our study the internal and to a lesser extent the external elastic lamina proved a significant barrier to the penetration of the infused agent into the media. This, theoretically, may be a serious limitation if, in clinical practice, it is important that drug penetration into the media occurs for optimal treatment. It is not clear how relevant this barrier function of the IEL would be in the clinical situation however where the antecedent balloon angioplasty is likely to have already disrupted it. Even however if there is no medial drug delivery the device may still be useful for influencing surface events such as platelet adherence and thrombosis as well as for locally applied endothelial gene therapy (31, 32).

Limitations of the study

There are a number of potential limitations to the present study which must be acknowledged. As this was an acute study we only addressed the issues of feasibility, safety and delivery efficacy of local infusion. We thus did not assess the retention properties of the infused agent or the selection of an appropriately effective agent.
Secondly we only took into account the effect of molecular weight of the assessed compounds on their uptake. We do not therefore know what effect other molecular factors such as lipopholicity, ionic charge, tertiary and quartenary structure of the molecules may have on compound uptake.
Third, the device was assessed in normal coronaries with no preceding angioplasty. Our findings may thus not be completely representative of the clinical situation where the device would be used in a diseased vessel. Initial experience suggests however that the ability to maintain distal perfusion with no haemodynamic

compromise also applies to the clinical situation (33). Additionally the infusion efficacy is likely to be increased rather than decreased in the clinical situation as the presence of atherosclerosis would help the transmural flux of fluid and macromolecules (34). Furthermore the greater vasa vasorum in the diseased vessel (35), would further increase the intramural surface area for drug movement by diffusion or convection.

Conclusions

Prolonged coil inflation (up to 60 minutes) is feasible with no significant limitations to coronary flow or haemodynamic compromise. The device causes minimal trauma to the vessel wall. Although there is localised endothelial damage the internal elastic lamina remains intact at low infusion rates with no damage to the media. Compounds ranging from 0.3-70 kDa can be successfully infused into the coronary wall. The device may thus have potential for ameliorating acute occlusion and late restenosis post coronary intervention.

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Part 2- Clinical

Chapter 7

Intraluminal imaging with ultrasound.

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24. Intracoronary imaging with ultrasound

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Summary

Although intravascular ultrasound transducers were first developed and tested in the early seventies [1] it wasn't until the advent of catheter based coronary interventional techniques that a major impetus was given to their development. Because intracoronary ultrasound allows direct visualisation of the atherosclerotic plaque [2, 3], it may assist in the choice of intervention, guidance during the procedure and assessment of the results and any complications. Furthermore, direct real time visualisation of arterial wall morphology offers unique opportunities for the *in vivo* assessment of arterial pathophysiology and dynamics on a beat-to-beat basis.

Instrumentation

Two approaches are available for obtaining cross-sectional imaging of the vessel wall. Firstly by mechanical rotation, of either the ultrasound transducer itself, or an acoustic reflector in front of a fixed transducer. The advantage of these systems is high resolution imaging without the presence of a near-field artefact. Realising a driving mechanism while keeping the catheter fully flexible and steerable are, however, challenging problems. Flexibility and steerability are not a major concern with the alternative, multi-element electronic approach, where sixty-four transducer elements are mounted circumferentially in a 360° radial array perpendicular to the long axis of the catheter, but this system is limited by near-field artifact and restricted resolution and dynamic range. The choice of equipment is important as there is evidence to suggest that image quality and interpretation varies between manufacturers.

Image interpretation

Comparison with angiography

A major limitation of contrast angiography is that it provides information predominantly about the vessel lumen. Consequently, morphological changes in the vessel wall are largely inferred from their effect on the lumen, but cannot be directly visualised. Furthermore, there is high inter- and intra-

observer variability in the measurement of coronary stenosis and the commonly used percentage stenosis takes no account of the eccentricity, or otherwise of the stenosis or whether the 'normal' part of the vessel is truly normal. By contrast, ultrasound allows direct visualisation of the vessel wall and hence overcomes many of these inherent limitations of contrast angiography. Because intracoronary ultrasound almost invariably shows greater vessel involvement and/or more severe atherosclerosis, it is likely that new criteria for atherosclerotic severity, based on ultrasound findings will be required.

Nevertheless intracoronary ultrasound cannot image the complete coronary tree; therefore, contrast angiography will remain useful for road mapping, guiding the ultrasound probe to the area of interest. Ultrasound imaging will then provide more detailed information on the area of interest or target lesion. Greater integration of intracoronary ultrasound within angiographic equipment will be required to fully exploit the complimentary information provided, for improved real-time decision making during interventional procedures.

Characteristics of the normal wall

In vitro and *in vivo* studies have shown that muscular arteries can be distinguished from elastic arteries on the basis of their echographic characteristics [4]. Muscular arteries have a hypoechoic smooth muscle component in the media which results in a three-layered appearance, whereas elastic arteries and veins have a more homogeneous appearance to their vessel wall. Intimal atheroma and calcification may, however, induce diffuse attenuation or shadowing preventing the evaluation of the underlying media. Furthermore, in up to 20% of muscular arteries fibrous degeneration of the muscular component of the media results in a homogeneous appearance [5]. A homogeneous appearance is also seen in young people where the intimal layer is thin; some diffuse intimal thickening must be present before the (typical) 3–layered appearance is visualised [6].

Study of the atherosclerotic plaque

In vitro *experience*

In vitro studies have shown that two types of atherosclerotic plaque can be distinguished [4, 5]. 'Hard' plaques, composed of dense fibrous tissue, are seen as bright echoreflective lesions with acoustic shadowing and perhaps duplicate echoes in the presence of calcific deposits. 'Soft' lipid rich plaques are weakly echoreflective (Figure 24.1). Thrombi, plaque rupture and dissection after intervention are also detected with great detail.

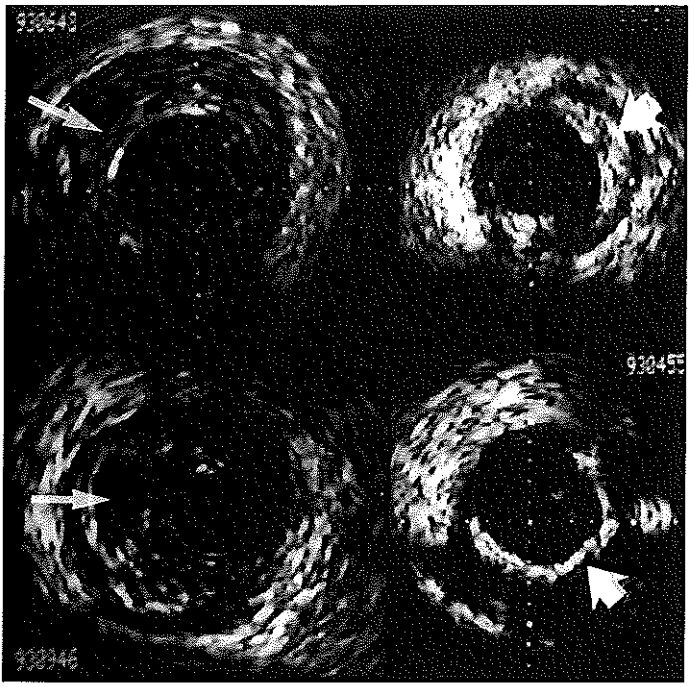

Figure 24.1. Intracoronary ultrasound images from diseased coronary arteries demonstrating: upper left panel. An eccentric, soft, weakly echo reflective plaque (arrow). Lower left panel. An eccentric, soft, weakly echo reflective, plaque with a 'lipid lake' (arrow). Upper right panel. A hard, strongly echogenic, fibrous plaque (arrow) extending from the 12 to the 5 o'clock positions. Lower right panel. A rim of superficial calcification (arrow), extending from the 1 to the 9 o'clock positions. Note the marked acoustic shadowing behind it.

In vivo *experience*

Peripheral vessels

Intravascular imaging of the aorta, iliac and femoral arteries can be obtained easily and the severity of stenosis, type and extent of atherosclerotic plaque and involvement of the medial layer assessed [7, 8]. Quantitative analysis of lumen and plaque area shows a close correlation with digital angiography [9]. Various interventions such as transluminal angioplasty, atherectomy and endovascular stent delivery are accurately monitored [10]. In comparison with angiography, intravascular ultrasound demonstrates a higher sensitivity in depicting presence and extent of dissection, length of intimal tears and characteristics of flow in the true and false lumen. Direct insertion of the ultrasound catheter during surgery can be used to replace more complex angiographic procedures in the evaluation of the efficacy of arterial bypass operation or end-arterectomy in peripheral vessels [11].

Coronary vessels

The limitations of contrast angiography for guidance of coronary interventional procedures has resulted in the development of smaller and more flexible catheters, amenable to coronary insertion. Intravascular ultrasound is able to detect and characterize atherosclerotic changes in what appear to be, angiographically, normal segments and in the presence of edge irregularities or luminal stenoses [12, 13].

Intracoronary imaging has provided direct evidence in support of previous pathology studies showing that coronary arteries undergo a progressive enlargement in relation to increases in plaque area so that a reduction of lumen area is delayed until the atherosclerotic lesion occupies more than 40% of the area circumscribed by the internal elastic lamina [14]. These findings explain why angiographically normal arterial segments show extensive atherosclerotic involvement at surgery. Hermiller and colleagues [15], have demonstrated, in 44 consecutive patients, that when coronary segments with <30% area stenosis are examined, there is an excellent correlation between internal elastic lamina (IEL) area and plaque area. In these segments the IEL area increased by 2.7 mm^2 for each 1 mm^2 increase in plaque area suggesting that arterial enlargement may overcompensate for early atherosclerotic lesions.

As well as being able to detect and characterize atherosclerotic changes in what appear to be, angiographically, normal segments, intracoronary imaging has also provided additional information in the presence of edge irregularities or luminal stenoses [12, 13]. Furthermore, directional atherectomy in conjunction with intracoronary imaging has confirmed previous *in vitro* studies suggesting that two types of atherosclerotic plaque can be distinguished [4, 5] by demonstrating a higher collagen and calcium content in echogenic 'hard' plaques and increased levels of fibrin, nuclei and lipids in 'soft' plaques [16].

As well as information on atherosclerosis, intracoronary imaging is also providing new insights into the pathogenesis of unstable coronary syndromes and accelerated atherosclerosis in transplant patients [17–23]. In the unstable coronary syndromes, ultrasound imaging has demonstrated more soft lesions and fewer mixed, calcified plaques with fewer intralesional calcium deposits than in stable angina, suggesting that ultrasound morphologic criteria are closely correlated to clinical anginal patterns [18–20]. Whether they correlate with clinical outcome, however, remains unknown.

In cardiac transplant recipients, intracoronary imaging has demonstrated that even angiographically normal vessels show a range of coronary intimal thickening, which includes occasional evidence of focal, early atheromatous lesions [22]. Furthermore, it has also demonstrated a vasoconstrictor response to acetylcholine at 1 year after transplantation suggesting endothelial dysfunction in the epicardial vessels [21] and that the vasodilatory response to nitroglycerin is attenuated during episodes of cardiac rejection, independent of the degree of intimal thickening [23].

Preliminary evidence suggests that imaging prior to interventions may be helpful for deciding which lesions may be most suitable for which specific treatment modality, and imaging post-intervention may be helpful in delineating which patients are at increased risk of acute occlusion and long-term restenosis.

Prior to intervention ultrasound allows the distinction of 'soft' plaques which are more likely to be dilated by compression, stretching and superficial intimal tears from 'hard' or clearly calcific plaques, which are at increased risk of extensive dissection after balloon angioplasty [24]. Furthermore, the presence of diffuse subendothelial calcification is associated with a lower success rate and higher risk of complications after directional atherectomy [25], indicating that alternative techniques such as rotational or laser atherectomy should be used in this situation. Although echogenic plaques are harder to resect by directional atherectomy than echolucent ones, they are also less likely to restenose [16]. These initial studies confirm previous clinical and pathological studies demonstrating that plaque morphology may affect outcome, and suggest that increased stratification of patients for specific treatment modalities, based on *de novo* plaque morphology, may help in reducing acute occlusion and long-term restenosis post-intervention.

Following intervention, intracoronary ultrasound assessment may be useful in two ways, firstly in assessing and optimising the results and secondly in assessing the risk of acute occlusion and long term restenosis. Since angiography only provides an outline of the vessel lumen, an angiographically successful angioplasty may turn out to be a 'pseudo-success', perhaps because cracks and dissection planes in the vessel wall allow contrast flow, with, in reality, very little increase in the actual luminal area. Subsequent apposition of the split wall layers may result in an angiographic restenosis (a pseudo-restenosis), a lesion being classified as restenosis whereas in fact it was never an actual success. Intracoronary ultrasound by visualising the vessel wall as well as the vessel lumen may have an important role to play in ensuring a good result, with a good luminal cross-sectional area after intervention (Figure 24.2). Furthermore, in the case of stent implantation, the clear imaging of the struts by intracoronary ultrasound ensures that the operator is aware of any incomplete stent expansion and can take appropriate action.

A further important aspect of intracoronary imaging during intervention may be in providing prognostic information regarding the subsequent risk of acute occlusion or restenosis. Angiographic studies have shown that the presence of an intimal flap increases the risk of acute occlusion six-fold. Furthermore, post-mortem studies have shown that extensive medial tears also increase the risk of abrupt closure. As intracoronary ultrasound is very sensitive in detecting the development and characteristics of intimal flaps following interventional procedures [26, 27] it may be able to predict subsequent outcome. Preliminary evidence is supportive of this showing that in-

Figure 24.2. Left panels. Quantitative angiographic assessment of an eccentric left anterior descending coronary artery stenosis prior (upper panel), and post (lower panel), successful rotablator therapy. Right panels. Angiograms prior (upper), during (middle) and post (lower) rotablator therapy with ultrasound images prior (upper) and after therapy (lower). Note the eccentric nature of the, mainly fibrous plaque, with deep seated calcification resulting in some acoustic shadowing, at the 5 o'clock position. Following rotablator therapy most of the plaque has been removed apart from the, previously, deep seated, calcification (arrow).

timal tears are twice as common and also more severe in patients who subsequently went on to develop acute occlusion [28]. Preliminary evidence also suggests that intracoronary ultrasound may also be helpful in identifying a subset of patients who have a higher incidence of restenosis [26]. Both of these possibilities now need to be confirmed by the larger ongoing multicenter studies but the preliminary data sited above are already beginning to influence therapy with preliminary data from the ongoing GUIDE (Guidance by Ultrasound Imaging for Decision Endpoints) trial suggesting that the detailed information regarding plaque morphology provided by intracoronary ultrasound leads to a change in therapy in about 50% of cases.

Pathophysiology of intervention and restenosis

Intracoronary ultrasound has made a significant contribution to our understanding of the pathophysiology of coronary interventional procedures and restenosis. Intracoronary ultrasound studies have confirmed previous studies

in peripheral vessels showing that luminal enlargement following balloon angioplasty is achieved primarily by arterial wall stretching with lesion volume remaining essentially unchanged [29]. They have also suggested that both vessel stretch and dissection are uncommon after atherectomy [30] thus confirming that plaque removal rather than a 'dotter effect' as the major mechanism for improved lumen area after this procedure.

Intracoronary ultrasound has also brought new understanding to the mechanisms involved in restenosis post-intervention. Our understanding of the mechanisms involved has previously been confined to serial angiographic assessment of the vessel lumen and post-mortem examination of limited histological tissue at specific time points in the disease process. Ultrasound has the potential to differentiate between the nature of the process (elastic recoil, mural thrombosis and intimal hyperplasia) and allow serial assessment of the *in-vivo* vessel wall, hence increasing our understanding of the pathophysiology of restenosis and allowing better targeting of therapeutic agents. These potential advantages have been recently confirmed by preliminary data from Kovach and colleagues, which suggest that chronic arterial recoil may have a far greater influence on late lumen loss and restenosis than previously thought [31].

Safety

The safety of intravascular and in particular intracoronary ultrasound should be a major concern. So far no significant adverse events have been reported when intracoronary ultrasound studies were performed prior to and following interventional procedures, including patients with acute coronary syndromes. Transient coronary spasm has been observed and the catheter can occlude flow when advanced into stenoses or small distal arteries, in which cases prompt withdrawl is clearly necessary. Serial studies in patients have not shown an increase in stenosis of the instrumented vessels when compared to those which were not instrumented making endothelial damage and accelerated atherosclerosis unlikely. These findings are of importance when one considers the use of intracoronary ultrasound in therapeutic trials for the study of regression/progression of atherosclerosis

Potential clinical and research directions

Angiography depicts only a silhouette of the vessel lumen; the extent of atherosclerotic disease and luminal narrowing, particularly in the early pre-stenotic phase, may therefore be misinterpreted. Intravascular ultrasound has the potential for detecting atherosclerotic changes in the pre-stenotic phase and allows accurate measurement of the plaque area as well as the vessel lumen. Furthermore it allows direct evaluation of plaque character-

istics. These advantages have important implications for regression studies as dietary and pharmacological interventions are likely to induce regression of the vascular changes in the 'pre-stenotic' rather than the more advanced phases of atherosclerotic disease. Intravascular ultrasound thus has the potential capability to directly visualise these vessel wall abnormalities and may differentiate lipid plaques, potentially amenable to regression from fibrocalcific plaques which are less likely to respond to an intervention. Little progress has been made to date however because of financial costs and major problems in correctly locating the identical imaging position during long-term follow-up.

When a three-layered appearance is present in a muscular artery, the middle hypoechoic layer may be used as a landmark for the detection and quantitation of intimal and medial change [32]. Intravascular ultrasound could thus be useful in assessing changes in medial thickness induced by systemic arterial hypertension and evaluating the effects of long-term antihypertensive treatment. Major limitations remain, however, as the plaque has a longitudinal architecture and is complex, requiring true 3–D reconstruction before these potential benefits may be realised.

Work is also in progress in analysis of the backscatter signals to allow a more accurate and quantitative characterisation of plaque components. This may allow better discrimination between soft plaque and thrombus, which have roughly equivalent echogenicity, as well as characterising the lipid content of specific plaques and hence their propensity to rupture and acute occlusion.

Three-dimensional reconstruction

The present imaging field is orthogonal to the catheter and is really only a two-dimensional representation of a complex three-dimensional process. Three-dimensional reconstruction of intracoronary ultrasound images is a major advance since tomographic views are now displayed longitudinally giving a more complete spatial picture of a coronary segment and any associated mural pathology (Figure 24.3). It thus offers an efficient gateway to the quantification of volumetric changes of atherosclerotic plaque, a better understanding of the complex longitudinal patho-anatomy and, when available on-line, would greatly help in guiding interventional techniques and assessing their results. Major problems remain, however, as present algorithms do not take into account catheter shift during withdrawal and lumen curvature, resulting in a straight catheter line reconstruction and hence a three-dimensional image which is not correctly reconstructed spatially.

Forward imaging

All available imaging transducers to date are side facing, requiring advancement of the transducer to the point of interest before images can be acquired.

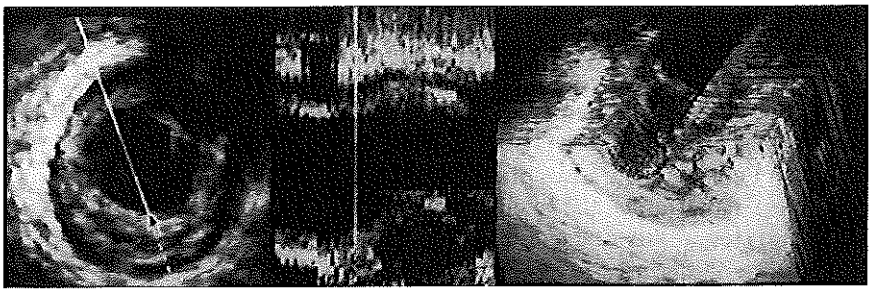

Figure 24.3. Intracoronary ultrasound cross-sectional image (left panel), taken at the level shown (middle panel), with the equivalent three-dimensional reconstruction (right panel). Note the detailed morphology visible on three-dimensional reconstruction.

Forward imaging transducers would allow a more comprehensive assessment of severe stenoses/occlusions and may make the guidance of interventions, such as laser therapy, more feasible.

Combination devices

Therapeutic

A combination device has inherent advantages over dedicated transducers by allowing real time ultrasound imaging during the procedure and obviating the need for repeat catheter exchanges. This would allow direct assessment of the immediate results and any associated elastic recoil. A number of combination devices incorporating balloons [33, 34], laser catheters [35] and atherectomy devices [36] have been developed and preliminary data is encouraging, suggesting that the on line qualitative and quantitative information regarding the vessel wall obtained during the procedure influences operator strategy in over 40% of cases.

Diagnostic

The combination of intracoronary imaging with recording of blood velocity with a Doppler transducer mounted on the same catheter or on a separate guidewire would allow measurement of basal and post-intervention absolute regional coronary flow. In this way the effects of stenoses can be adequately studied by integrating anatomic (stenosis cross-sectional area) and physiologic data (velocity increase at the site of the stenosis, regional flow reserve). The combination of intravascular imaging with simultaneous recording of high-fidelity blood pressure allows arterial compliance to be accurately calculated from the slope of the pressure-dimension relationship [37]. With this method the changes induced by disease or ageing on the arterial wall can be evaluated, and the effects of pharmacological agents monitored.

Problems and limitations

There are important limitations of the present technology which are currently being addressed. Although a marvel of miniaturising technology, imaging transducers are still relatively large precluding the visualisation of clinically significant coronary stenoses prior to intervention or the more distal coronary arteries. Furthermore, the handling characteristics such as trackability, flexibility and steerability of currently available systems are still not optimal. This has important implications, particularly for intracoronary applications, where all the elements of the catheter, including the distal end where the echo-transducer is mounted, must be fully flexible in order to allow safe and successful negotiation of tortuous vessels. Furthermore, increased steerability of the catheters is required to correct for non-coaxial or eccentric intraluminal positions, as the perpendicularity of the ultrasound beam to the vascular wall influences the intensity with which a structure is visualised and partial drop-out occurs above a critical angle [27]. In addition, the 'blooming effect' induced by off-axis positioning of the catheter, results in an overestimation of the vascular lumen and wall.

The present images are not consistently enough adequate for a complete evaluation of vascular dimensions and morphologic changes. Furthermore, relatively long acquisition times limit the study of luminal changes during the cardiac cycle. Shorter acquisition times are desirable for a more precise study of systolic-diastolic changes in luminal dimensions.

These technical problems are currently being addressed with the introduction of even smaller (2.9F), more flexible transducers and with industry talk of imaging guidewires, which would not only allow assessment of severe stenoses, but also serve as a platform for therapeutic devices allowing real time imaging, on-line during the procedure.

Our knowledge of the appearance of normal and diseased vascular walls and the effects of intervention is still in its infancy, however, and the additional diagnostic and prognostic information obtained in small studies to-date must be confirmed in large on-going trials before the expected benefit outweighs the potential costs.

Conclusions

Direct visualisation of the vessel wall by intravascular ultrasound opens up a world of opportunities to us. It may provide prognostic information on atherosclerosis, and allow the assessment of the effects of dietary and pharmacologic interventions in high risk patients. Furthermore, intracoronary ultrasound offers potentially unique information for the selection and guidance of catheter based interventional techniques.

Acknowledgwments

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Chapter 8

Comparison of coronary luminal quantification obtained from intracoronary ultrasound and both geometric and videodensitometric quantitative angiography, pre, and post, balloon angioplasty and directional atherectomy.

Ozaki Y, Violaris AG, Keane D, Camenzind E, Di Mario C, de Feyter PJ, Roelandt JTRC, Serruys PW.

Circulation 1997; 96 (2): 491-499

Comparison of Coronary Luminal Quantification Obtained From Intracoronary Ultrasound and Both Geometric and Videodensitometric Quantitative Angiography Before and After Balloon Angioplasty and Directional Atherectomy

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Background Debate exists regarding the relationship between angiographic and intracoronary ultrasound (ICUS) measurements of minimal luminal cross-sectional area after coronary intervention. We investigated this and the factors that may influence it by using ICUS and quantitative angiography.

Methods and Results Patients who underwent successful balloon angioplasty (n=100) or directional atherectomy (n=50) were examined by using ICUS and quantitative angiography (edge-detection [ED] and videodensitometry [VID]) before and after intervention. Luminal damage postintervention was qualitatively graded into three categories based on angiographic results (smooth lumen, haziness, or dissection). Correlation of minimal luminal cross-sectional area measurements by ICUS and ED was .59 before and .47 after balloon angioplasty. Correlation between ICUS and VID was .50 before and .63 after balloon angioplasty. Postintervention, the difference between ICUS and VID was less than the difference between ICUS and ED ($P<.01$). Additionally, the correlation was .74 between ICUS and ED measurements and .78 between ICUS and VID measurements in the smooth lumen group, .46 and .63, respectively, in the presence of haziness, and .26 and .46, respectively, in lesions with dissection. Similar results were obtained after directional atherectomy: the agreement between ICUS and quantitative angiography deteriorated according to the degree of vessel damage, but less so with VID than ED.

Conclusions Complex morphological changes induced by intervention may contribute to discordance between the two quantitative imaging techniques. In the absence of ICUS, VID may be a complementary technique to ED in lesions with complex morphology after balloon angioplasty and directional atherectomy. (*Circulation*. 1997;96:491-499.)

Key Words • angiography • angioplasty • imaging • coronary disease • ultrasonics

Although QCA is the gold standard in interventional cardiology, pathology studies indicate that angiography may underestimate the extent and severity of atherosclerotic disease.[1,2] While ICUS provides unique information regarding vessel wall morphology compared with angiography,[3-8] precise quantitative analysis of luminal CSA by ICUS would offer a significant advantage in the guidance of coronary intervention procedures.[9-11] A recent multicenter ICUS study in patients with coronary angioplasty indicated that postinterventional luminal dimensions obtained by ICUS but not QCA may be a significant predictor of restenosis at follow-up.[12] Additionally, another multicenter study suggests that a large residual plaque burden remains on ICUS imaging, despite optimal angiographic results.[13] Nakamura et al[9] and Colombo et al[10] also suggest that luminal measurements provided by ICUS may be helpful for optimal stent deployment. Previous studies, however, have provided conflicting evidence on the agreement between quantitative measurements derived from ICUS and those derived from QCA.[3,4,7,14-17] The aim of our study was to clarify whether ICUS measurements agree with QCA measurements and to determine which factors, if any, may play a role in any discordance between the techniques. To do this, we compared MCSA obtained from ICUS and both ED and VID computer-based QCA before and after BA and DCA.

Methods

Patients

Patients who had an ICUS examination before and after single-vessel BA (n=100) or DCA (n=50) with adequate

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Selected Abbreviations and Acronyms

BA = balloon angioplasty
CSA = cross-sectional area
DCA = directional coronary atherectomy
ED = edge detection
ED-QCA = edge-detection quantitative coronary angiography
ICUS = intracoronary ultrasound
MCSA = minimal luminal cross-sectional area
MLD = minimal luminal diameter
QCA = quantitative coronary angiography
RD = reference diameter
VID = videodensitometry
VID-QCA = videodensitometric quantitative coronary angiography

quality of ICUS and angiographic images for quantitative analysis were enrolled in this study. Preintervention, the US catheter did not cross the target lesions in 20 lesions because of proximal vessel tortuosity, the severity of the target stenosis, or transient serious arrhythmia during the ICUS examination. Additionally, the ultrasound catheter completely occluded the coronary lesion in 89 lesions. Thus, preintervention MCSA of the stenotic segment was measured in the remaining 41 lesions. Postintervention ICUS measurements were obtained in all 150 lesions in the 150 patients.

BA or DCA Procedures

All patients received full anticoagulant therapy including intravenous aspirin and heparin before ICUS examination and intervention. Coronary angiograms were recorded on cinefilm after the intracoronary administration of isosorbide dinitrate (1 to 2 mg). The size of the balloon or atherectomy device was determined to match the vessel RD obtained from the online QCA measurement. Luminal damage postintervention was qualitatively graded into three categories by angiographic assessment as none (smooth lumen), generalized haziness, or dissection. Dissection was defined according to the dissection classification types B, C, D, E, and F of the classification of the National Heart, Lung, and Blood Institute.[18]

ED-QCA

The new version of the computer-based Coronary Angiography Analysis System (CAAS II)[19,20] was used to perform the ED and VID quantitative analyses. In the CAAS analysis,[19-24] the entire 18×24-mm cineframe is digitized at a resolution of 1329×1772 pixels, and the boundaries of a selected coronary segment are detected automatically. The absolute diameters of the stenosis (MLD and RD) are determined by using the contrast-free guiding catheter as a scaling device. To standardize the method of analysis before and after intervention, all study frames selected for analysis were end-diastolic to minimize motion artifact, and arterial segments were measured between the same identifiable branch points in multiple views after the administration of isosorbide dinitrate.[20-24] MCSA was calculated as $\pi \times (MLD1) \times (MLD2) \div 4$ from measurements obtained from the ED analysis in orthogonal views (MLD1 and MLD2) before and after intervention.

VID-QCA

VID measurement is based on the relationship between the attenuating power of the lumen filled with contrast medium and the x-ray image intensity.[25] Using this relationship, a VID profile that was proportional to the CSA of the lumen was obtained. Subtraction of patient structure noise was applied after computing the linear regression line through the background pixels located on both sides of the detected luminal contours. Consecutive densitometric profiles of the analyzed segment were acquired in all scan lines perpendicular to the vessel including lesion, reference, and nondiseased areas. Conversion of the individual VID profiles to absolute values was performed after a transformation of the VID profile found in a CSA of a nondiseased segment, assuming a CSA at any point is proportional to the densitometric profiles at that point. MCSA was calculated from the average value obtained from the VID system in multiple views. The basic principles of the technique are illustrated in Fig 1.

ICUS Image Acquisition

Following angiography, an ICUS catheter (30 MHz; 2.9F, 3.2F, or 4.3F; Cardiovascular Imaging Systems) was introduced over a 0.014-in. guide wire and positioned distal to the lesion. Lesion geometry was then imaged by using a slow, continuous catheter pull-back procedure. Catheter position was documented by simultaneous fluoroscopy superimposed on the ICUS display screen. ICUS images were stored on super VHS tape for offline analysis.

Quantitative and Qualitative Assessment of ICUS

Luminal CSA was defined as the integrated area central to the intimal leading-edge echo. Images with MCSA were selected from the pull-back sequence by reviewing the position of the ICUS catheter on the angiographic image that was recorded on the same ICUS image and by reviewing the time log and audio recording of the procedure to analyze the same coronary segment as the quantitative angiogram. Total vessel CSA was defined as the area inside the interface between the plaque-media complex and adventitia (ie, the area inside the external elastic membrane). When the dissected lumen communicated constantly with the true lumen, the dissected lumen was included in the luminal area, as exemplified in Fig 2. Echo reflectivity was categorized as either low or high (plaque reflectivity lower or higher, respectively, than the bright adventitial layer).[26] Calcium deposits were defined as highly echoreflective tissue with acoustic shadowing. A lesion was considered homogeneous if the plaque consisted of >75% of one type of echo reflectivity. A lesion was defined as mixed if it contained both high and low echo-reflective areas occupying >25% of the plaque area.[26] A lesion was considered predominantly calcific if calcium occupied >180° of the vessel circumference.[26]

Luminal damage postintervention was qualitatively graded into three categories: regular lumen, irregular lumen including a small tear not extending to the media, and dissected lumen with circumferential tear behind the plaque or tear extending to the media.[5,6,27] The eccentricity ratio was calculated as the ratio between minimal and maximal wall thickness (1 indicates concentric plaque, <1 indicates increasing eccentricity).[27] To determine the interobserver variability of ICUS measurements, 30 videotapes of the complete original recording were used by two independent observers to select and measure the minimal CSA. The mean signed difference and correlation of the measurements of minimal CSA were -0.12 ± 0.79 mm^2 and 0.94, respectively.

Statistical Analysis

In the absence of the known true values, Bland and Altman[28] recommend the use of the mean and SD of the signed differences between two measurement systems as an index of agreement between the two systems. Thus, we took the mean and SD of the signed differences between ICUS and QCA measurements as an index of agreement between ICUS and QCA measurements instead of linear regression analysis. The individual measurements obtained from ICUS and QCA were compared by using the paired Student t test and correlation coefficient. A probability value of $<.05$ was considered significant.

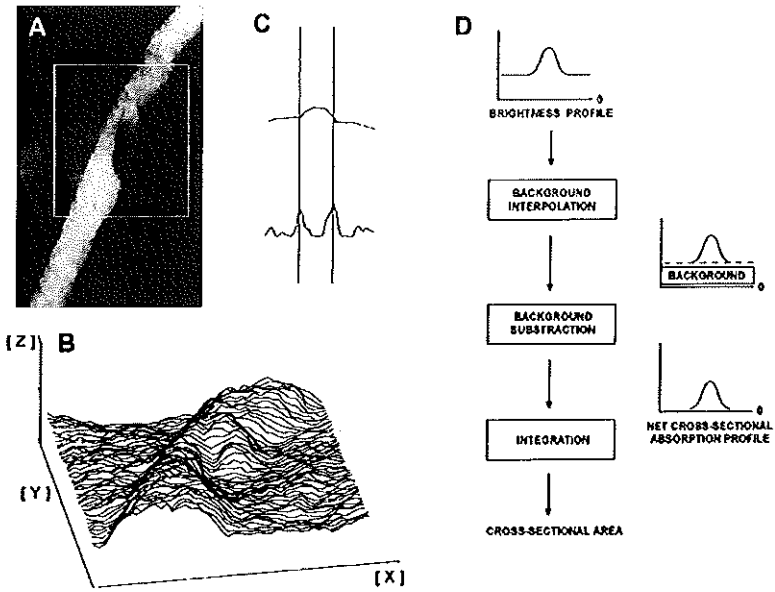

FIG 1. A, In the VID technique, a matrix is placed over the area selected for analysis from the right coronary angiogram encompassing a complex coronary obstruction. B, Pseudo-three-dimensional representation of the brightness information within the matrix and the coronary artery can be recognized as a mountain ridge with a deep pass at the site of the obstruction; the brightness profile along one particular scan line is plotted. C, Positions with maximal values of the sum of the first- and second-derivative functions left and right of the center positions of the artery correspond to the edge positions of the artery. D, Flow chart of the analysis indicates the main procedures followed for the computation of the VID area function.

Results

Baseline Clinical and Angiographic Characteristics

No difference was found in gender, age, anginal symptoms, or distribution of diseased vessels between the BA and DCA groups (Table 1). QCA measurements were obtained in 100 lesions before and after BA and in 50 lesions before and after DCA. ED-QCA indicated that the RD before and after intervention and MLD postintervention were significantly larger in the DCA than the BA patients. MCSA was measured by using ICUS in 26 lesions before BA and 15 lesions before DCA without wedge of the ICUS catheter. One hundred lesions after BA and 50 lesions after DCA were esti-

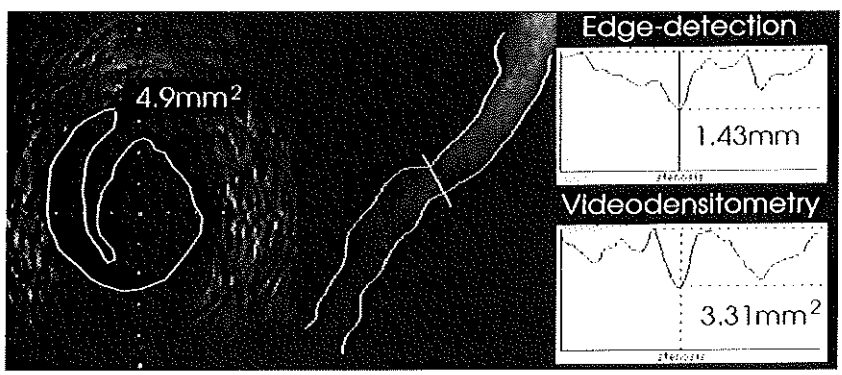

FIG 2. ED-QCA, VID-QCA, and ICUS analyses after BA in the middle segment of the left anterior descending coronary artery. Left, Dissection is clearly seen in the ICUS image. Middle, ED algorithm partially traces between the true and the dissected lumen. Right, Complex morphological changes caused by BA may explain the discordance between MCSA measurements obtained from ICUS (4.9 mm²) and ED-QCA (2.96 mm²; MLD, 1.43 mm) and VID-QCA (3.31 mm²). The VID-QCA value is nearer to the ICUS measurement than the ED-QCA value; see "Discussion."

TABLE 1. Baseline Clinical and Angiographic Characteristics of 150 Patients

	BA (n=100)	DCA (n=50)	P
No. of patients (M/F)	100 (81/19)	50 (43/7)	
Age, y	59±10	58±9	NS
Stable/unstable angina, n	53/47	24/26	NS
RCA/LAD/LCX/SVG, n	31/47/18/4	8/34/8/0	NS
Luminal diameter by ED-QCA, mm			
MLD before	1.05±0.50	1.20±0.43	NS
RD before	2.85±0.64	3.51±0.61	<.001
MLD after	2.04±0.55	2.81±0.59	<.001
RD after	2.98±0.70	3.69±0.59	<.001
Plaque composition estimated by ICUS, n			
Homogeneous plaque (echo reflectivity: poor/high with shadow/high without shadow)	64 (54/8/2)	31 (26/5/0)	NS
Mixed plaque	36	19	NS
None/<90°/≥90° calcium deposits	26/42/32	15/19/16	NS
Luminal measurement by ICUS			
Total vessel area before, mm^2	17.00±5.35	19.66±4.84	<.05
Plaque and media area before, mm^2	13.65±4.97	16.97±4.99	<.05
Plaque and media area before, %	79±7%	86±5%	<.05
Eccentricity index	0.43±0.24	0.38±0.22	NS

RCA indicates right coronary artery; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; and SVG, saphenous vein graft. All 150 lesions were analyzed by QCA before and by QCA and ICUS after intervention. Preintervention, 26 and 15 lesions that were to be treated by BA and DCA, respectively, were analyzed by ICUS; lesions with wedge of ICUS catheter (BA=74 and DCA=35) were excluded from ICUS analysis.

mated by using ICUS, which revealed that 64 lesions in BA and 31 lesions in DCA consisted of homogeneous plaque; all remaining lesions were classified as mixed. Most of the homogeneous plaque was low echo reflective. Focal calcium deposits were observed in 42 lesions in BA and 19 lesions in DCA, while moderate-to-diffuse calcification was seen in 32 lesions in BA and 16 lesions in DCA (P=NS). Total vessel CSA, plaque and medial CSA, and percent plaque and medial CSA preintervention were larger in the DCA than the BA group. Lesion eccentricity was not significantly different between the two groups.

MCSA Measured by ICUS, ED-QCA, and VID-QCA Before and After Interventions

Table 2 shows the MCSA measured by ICUS, ED-QCA, and VID-QCA before and after BA and DCA. MCSA in nonwedged lesions (BA=26 lesions and DCA=15 lesions) as obtained by ICUS was significantly larger than MCSA measured by using ED- or VID-QCA pre-BA (both P<.01) and pre-DCA (both P<.01). Post-BA MCSA in 100 lesions as obtained by ICUS was significantly larger than the MCSA measured by either ED- or VID-QCA (both P<.01). Post-DCA MCSA in 50 lesions as obtained by ICUS was significantly larger than MCSA as measured by ED-QCA (P<.01) but not VID-QCA.

Agreement Between ICUS, ED, and VID Before and After BA and DCA

Table 3 compares the agreement between the three measurement techniques, and Figs 3 and 4 display the postintervention agreement between measurements obtained from ICUS and ED-QCA (Fig 3) and from ICUS and VID-QCA (Fig 4). The correlation coefficient between the ICUS and ED measurements decreased from .59 pre-BA to .47 post-BA and from .57 pre-DCA to .44 post-DCA. The absolute difference between ICUS and ED was significantly greater post-BA and post-DCA than pre-BA and pre-DCA (both P<.05). The agreement between ICUS and ED deteriorated post-BA and DCA compared with the pre-BA and DCA agreement. The correlation coefficient between ICUS and VID measurements increased from .50 pre-BA to .63 post-BA and from .50 pre-DCA to .72 post-DCA (Table 3). The difference between ICUS and VID was not significantly different from pre-BA and DCA to post-BA and DCA. Postintervention, the difference between ICUS and VID was significantly less than the difference between ICUS and ED post-BA and post-DCA (both

TABLE 2. Comparison of MCSA Measurements Between BA and DCA

	MCSA, mm^2		
	ICUS	ED-QCA	VID-QCA
Pre-BA (nonwedged lesions)	3.36±0.99 (n=26)*†	1.93±1.36 (n=26)	2.21±1.22 (n=26)
Pre-BA (all lesions)	...	1.12±0.96 (n=100)†	1.32±1.02 (n=100)
Post-BA	5.19±1.90 (n=100)*†	3.48±1.76 (n=100)†	3.92±1.82 (n=100)
Pre-DCA (nonwedged lesions)	2.69±0.89 (n=15)*‡	1.61±1.08 (n=15)	1.64±1.02 (n=15)
Pre-DCA (all lesions)	...	1.29±0.79 (n=50)	1.26±0.77 (n=50)
Post-DCA	7.57±1.85 (n=50)*	6.58±2.43 (n=50)†	7.25±2.20 (n=50)

*P<.05 vs ED-QCA.
†P<.05 vs VID-QCA.

TABLE 3. Comparison of Agreement Between Measurements of MCSA by ICUS, ED, and VID Before and After BA and DCA

	Correlation Coefficient	Mean±SD, mm^2
ICUS vs ED		
Pre-BA (nonwedged lesions)	.59	1.43±1.12
Post-BA	.47	1.71±1.87
Pre-DCA (nonwedged lesions)	.67	1.08±1.00
Post-DCA	.44	0.95±2.30
ICUS vs VID		
Pre-BA (nonwedged lesions)	.50	1.15±1.12
Post-BA	.63	1.27±1.61
Pre-DCA (nonwedged lesions)	.50	1.05±0.96
Post-DCA	.72	0.28±1.54
VID vs ED		
Pre-BA (all lesions)	.78	0.20±0.65
Post-BA	.82	0.43±1.08
Pre-DCA (all lesions)	.74	−0.04±0.56
Post-DCA	.73	0.67±1.71

$P<.01$). The discordance between ICUS and VID was smaller than the discordance between ICUS and ED both post-BA and post-DCA. While in both pre-BA and DCA no significant difference was observed between ED and VID, in post-BA and DCA there was a significant difference between the two (BA, $P<.001$; DCA, $P<.01$).

Luminal Damage Postintervention as Assessed by Angiography and ICUS

The degree of luminal damage postintervention as assessed by using angiography and ICUS is given in Table 4. Concordance between the two qualitative imaging techniques was found in 23 (70%) of the 33 patients with angiographically detected dissected lesions and 23 (66%) of the 35 patients with dissection as detected by ICUS post-BA and in 10 (83%) of the 12 patients with angiographically detected dissected lesions and 10 (59%) of the 17 patients with dissection as detected by ICUS post-DCA.

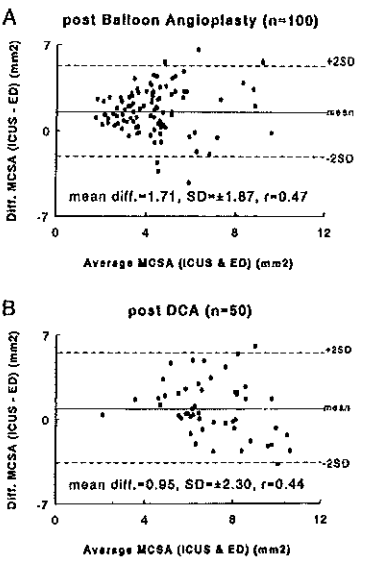

FIG 3. Plots show agreement of measurements of MCSA between ICUS and ED-QCA after (A) BA and (B) DCA according to the statistical approach proposed by Bland and Altman.[23] Diff. indicates difference.

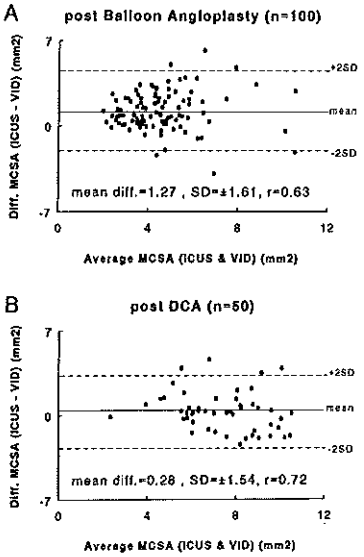

FIG 4. Plots show agreement of measurements of MCSA between ICUS and VID-QCA after (A) BA and (B) DCA according to the statistical approach proposed by Bland and Altman.[23] Diff. indicates difference.

Influence of Vessel Damage Induced by BA and DCA in the Agreement Between ICUS and QCA

The correlation coefficient of ICUS and ED quantitative measurements was .74 in lesions with an angiographically determined smooth lumen, .46 in lesions with angiographic haziness, and .26 in lesions with angiographic evidence of dissection (Table 5). The correlation of ICUS and ED quantitative measurements was .70 in lesions with a regular lumen as determined by ICUS, .52 in lesions with an irregular lumen, and .10 in

TABLE 4. Luminal Damage As Assessed by Using ICUS and Angiography in 150 Patients After Coronary Intervention

Angiographic Luminal Assessment	ICUS Luminal Assessment		
	Regular	Irregular	Dissected
BA			
Smooth (n=44)	26	13	5
Haziness (n=23)	2	14	7
Dissection (n=33)	4	6	23
DCA			
Smooth (n=24)	13	9	2
Haziness (n=14)	1	8	5
Dissection (n=12)	0	2	10

TABLE 5. Comparison of Agreement Between Measurements of MCSA by ICUS, ED, and VID According to Degree of Vessel Damage Induced by BA

	Correlation Coefficient	Mean±SD, mm²
ICUS vs ED		
Angiography		
Smooth lumen (n=44)	.74	1.21±1.27
Haziness (n=23)	.46	2.01±1.69
Dissection (n=33)	.26	2.16±2.47
ICUS		
Regular lumen (n=32)	.70	1.36±1.66
Irregular lumen (n=33)	.52	1.45±1.71
Presence of dissection (n=35)	.10	2.27±2.11
ICUS vs VID		
Angiography		
Smooth lumen (n=44)	.78	1.02±1.29
Haziness (n=23)	.63	1.46±1.34
Dissection (n=33)	.46	1.48±2.09
ICUS		
Regular lumen (n=32)	.80	1.03±1.48
Irregular lumen (n=33)	.54	1.02±1.70
Presence of dissection (n=35)	.37	1.73±1.57
VID vs ED		
Angiography		
Smooth lumen (n=44)	.93	0.19±0.75
Haziness (n=23)	.86	0.55±0.89
Dissection (n=33)	.64	0.68±1.48
ICUS		
Regular lumen (n=32)	.93	0.32±0.92
Irregular lumen (n=33)	.79	0.43±1.09
Presence of dissection (n=35)	.60	0.54±1.22

TABLE 6. Comparison of Agreement Between Measurements of MCSA by ICUS, ED, and VID According to Degree of Vessel Damage Induced by DCA

	Correlation Coefficient	Mean±SD, mm²
ICUS vs ED		
Angiography		
Smooth lumen (n=24)	.70	0.58±1.72
Haziness (n=14)	.34	1.43±2.62
Dissection (n=12)	.04	1.46±2.63
ICUS		
Regular lumen (n=14)	.72	0.30±1.58
Irregular lumen (n=19)	.49	0.77±2.39
Presence of dissection (n=17)	.28	1.68±2.61
ICUS vs VID		
Angiography		
Smooth lumen (n=24)	.79	−0.01±1.40
Haziness (n=14)	.71	0.64±1.61
Dissection (n=12)	.55	0.46±1.84
ICUS		
Regular lumen (n=14)	.83	−0.23±1.28
Irregular lumen (n=19)	.69	0.29±1.53
Presence of dissection (n=17)	.70	0.69±1.71
VID vs ED		
Angiography		
Smooth lumen (n=24)	.76	0.59±1.68
Haziness (n=14)	.74	0.80±1.68
Dissection (n=12)	.63	0.99±1.69
ICUS		
Regular lumen (n=14)	.87	0.53±1.15
Irregular lumen (n=19)	.72	0.48±1.54
Presence of dissection (n=17)	.67	1.00±2.19

lesions with ICUS evidence of dissection. Thus, the presence of vessel damage induced by BA was associated with a deterioration of agreement between ICUS and ED measurements. While agreement between ICUS and VID-QCA also deteriorated in the presence of morphological changes induced by BA, the difference of the measurements between ICUS and VID was significantly less than the difference between ICUS and ED in the presence of both angiographic ($P<.05$) and ICUS ($P<.05$) evidence of dissection. While high agreement was obtained between ED-QCA and VID-QCA, agreement between these two techniques also decreased post-BA according to the increase of vessel damage.

A similar pattern was seen when lesions treated by DCA were categorized according to their morphological characteristics (Table 6). Poor agreement was obtained in lesions with angiographic or ICUS evidence of vessel damage compared with lesions with an angiographically smooth lumen or ICUS appearance of a regular lumen. The absolute difference of the measurement between ICUS and VID was significantly less than the difference between ICUS and ED in the presence of both angiographic ($P<.01$) and ICUS ($P<.01$) evidence of dissection.

Correlation Between QCA Analyses of the Same Lesion From Multiple Views

To ensure that the better relationship between VID and ICUS was a true phenomenon and not due to a greater variation in values obtained from different views, we looked at the correlation and differences between orthogonal measurements for both VID and ED before and after intervention.[29-31] The correlation and differences between orthogonal measurements obtained by ED were 0.69 (0.21±0.62 mm²) preintervention and 0.49 (0.39±2.33 mm²) postintervention. The values obtained for VID were 0.69 (0.14±0.71 mm²) preintervention and 0.67 (−0.33±1.79 mm²) postintervention.

Discussion

The principle findings of our study were (1) that MCSA obtained by ICUS was significantly larger than MCSA as measured by either ED- or VID-QCA both before and after BA and DCA, (2) that the agreement between ICUS and ED deteriorated considerably after both BA and DCA, (3) that the complex morphological changes induced by BA and DCA contributed to the discordance of the agreement between ICUS and ED, and (4) that VID measurements were found to provide a better agreement with ICUS than ED measurements, particularly in lesions with complex morphological changes post-BA and DCA.

Agreement Between ICUS and ED-QCA in Previous Studies

Previous studies have provided conflicting evidence on whether luminal measurements obtained from ICUS agree with ED-QCA measurements in human coronary arteries. In general, studies that examined ICUS and ED measurements in normal coronary segments report a favorable correlation between the two quantitative imaging modalities,[3,7] while those that examined lesions postangioplasty report a poor correlation.[4,15,16] Naka-

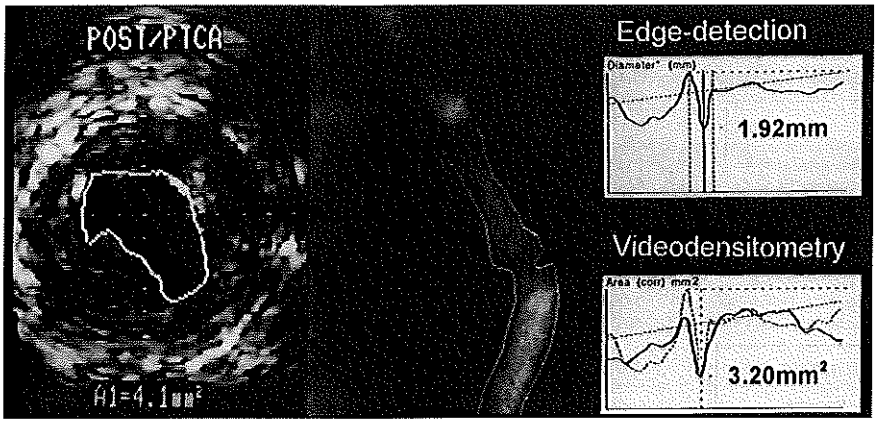

FIG 5. ED-QCA, VID-QCA, and ICUS analysis after BA in the middle segment of the left circumflex coronary artery. Left, ICUS image shows an irregular lumen with partial rupture of a soft eccentric plaque post-BA. The elliptical and irregular shape of the lumen may explain the discordance between measurements of the MCSA obtained from ICUS (4.1 mm²) and ED-QCA (2.99 mm²; MLD, 1.92 mm) and VID-QCA (3.20 mm²). The VID-QCA value is closer to the ICUS measurement than the ED-QCA value; see "Discussion."

mura and colleagues[17] report that dissection induced by BA plays a role in the discordance between ICUS and ED-QCA measurements, but there are several methodological differences between their study and ours. First, they did not examine VID measurements. Second, they showed only the correlation coefficient in groups with and without dissection, and no statistical difference was found in the difference of the individual ICUS and angiographic measurements between two groups. Finally, they did not use a computer-based QCA system but rather caliper measurements, which have a poor reproducibility and result in frequent underestimation or overestimation of stenosis severity.[32] Our study is the first to compare both ED- and VID-QCA measurements with ICUS measurements.

Factors Contributing to the Discordance of ICUS and QCA Measurements

Agreement of luminal area measurements as obtained by using ICUS and ED deteriorated considerably after BA and DCA. A progressive deterioration in the relationship between the ICUS and QCA measurements was seen in accordance with the presence of increasing vessel damage and increasing luminal complexity postintervention. Thus, the cross-sectional shape of the vessel lumen postintervention may be a significant factor in the discrepancy between ICUS and ED-QCA measurements. MLD obtained from ED-QCA depends on the angiographic projection. Although we calculated luminal area from two orthogonal views, the chances of obtaining the exact minimal and maximal diameters of the lesion CSA using ED-QCA would be small, particularly in the complex elliptical shape of the lumen postintervention (Fig 5). ICUS and VID are not projection dependent, and both would provide a measure of the "depth" as well as the "width" of the lumen cross section. ED, however, provides only a measure of one diameter (the "width") of the lumen. Consistent with this is the fact that VID-QCA provided a better agreement and less relative underestimation in relation to ICUS measurements than ED-QCA.

The underestimation by ED relative to ICUS measurements may reflect the propensity of the contour-detection algorithm to trace the change in the brightness profile in the contrast-weak channel between the true and false lumens[33] (Fig 2), while ICUS measurements and VID measurements in multiple views may include the contribution of the false lumen. Such a phenomenon, however, would not account for the relative underestimation by ED in the preintervention phase. Another possibility is that the interventional cardiologist tends to select angiographic projections that best demonstrate both the stenosis preintervention and the residual stenosis postintervention, ie, "worst view" angiography. Even using multiple orthogonal views, as we did in our study, the assumed elliptical cross section may have been based on multiple "worst views," which although they were, per protocol, >45° apart, were not necessarily a combination of truly "worst view" and "best view." Such a limitation to ED-QCA has been an inherent problem in all coronary interventional trials, and attempts to address this by three-dimensional imaging in truly orthogonal views are currently under evaluation.[34,35]

Two additional ICUS-related factors may also have contributed to the observed discordance between ICUS and quantitative angiographic measurements. Elliptical angulation of the ultrasound catheter within the longitudinal axis of the vessel may have led to overestimation of luminal dimensions by ICUS. Additionally, introduction of the ultrasound catheter may have itself resulted in tacking back of dissection flaps, with a resultant larger lumen during ICUS examinations postintervention compared with the less invasive technique of contrast angiography. Our data also suggest that quantitative angiography may yield larger luminal measurements than ICUS in a significant number of patients (Figs 3 and 4). This increase in the apparent angiographic diameter may be caused by extraluminal contrast within fissures, cracks, and dissection as seen around the true lumen.

Study Limitations

First, the coronary sites compared by ICUS and QCA may not have been exactly identical. Although we tried to ensure that this was the case by using simultaneous recording of fluoroscopy and ICUS imaging as well as landmarks such as side branches to guide us, there is no guarantee that we analyzed exactly the same point of the coronary artery in ICUS and QCA measurements. This is, however, a generic problem of any ICUS-QCA study[3,4] that would be very difficult to overcome, as the presence of the ICUS catheter at the lesion site during coronary arteriography would interfere with the QCA measurements. Second, both ED- and VID-QCA analysis were performed using only the CAAS II system. Thus, further studies would be required to confirm if our findings can be generalized to other QCA hardware or software systems.[21] It is conceivable that if an ED algorithm were inaccurate in the normal reference segment, then a systematic underestimation or overestimation of vessel diameters could be translated to subsequent VID measurements. Third, it is also possible that ultrasound image analysis fails to see the true leading intimal edge, especially if the plaque has a low fibrous component and appears relatively hypoechoic, thus overestimating luminal dimensions. Additionally, a poor dynamic range can also induce technical intimal drop-out, leading to lumen overestimation.

Clinical Implications

Although ICUS provides unique information regarding the vessel wall morphology, the clinical utility of this technique remains unproven to date. ICUS luminal measurements, however, do provide important additional information to that obtained by visual assessment for optimal stent implantation.[9,10] Whether ICUS provides more accurate information than QCA, however, has not yet been determined. Additionally, whether luminal measurements obtained from ICUS are a superior index for the short- and long-term success of interventional procedures awaits the results of recent multicenter trials.[12,13,36] While ICUS is not universally available and involves additional time and expense,[11] QCA is more widely available and less time-consuming. Our data suggest that in the absence of the known true value and assuming that ICUS gives the most accurate estimate of luminal dimensions, QCA measurements, particularly ED, may be compromised, especially in assessing the complex luminal morphology following BA or DCA. Our study suggests that VID may offer a better correlation with the true luminal dimensions, as reflected by ICUS, than ED-QCA. VID may thus be the "poor man's" ICUS, especially in lesions with complex morphology.

Conclusions

MCSA measurements obtained by ICUS are significantly larger than measurements provided by either geometric or VID-QCA both before and after intervention. Agreement between ICUS and geometric QCA measurements deteriorate considerably after intervention. Complex morphological changes induced by intervention may play a role in such a discordance between the two quantitative imaging techniques. In the absence of ICUS, VID, which is currently available in an online QCA system, may provide a better alternative than ED-QCA in lesions with complex morphology.

Acknowledgment

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Chapter 9

Intravascular ultrasound imaging combined with coronary angioplasty- Initial clinical experience.

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Lancet 1992; 339: 1571-72

Intravascular ultrasound imaging combined with coronary angioplasty

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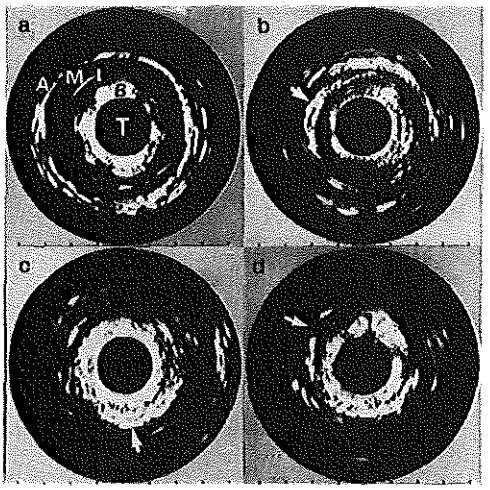
A novel catheter combining ultrasound imaging and coronary balloon angioplasty was used in the treatment of 69 coronary-artery lesions in 51 patients. The ultrasound transducer enables real-time cross-sectional imaging and qualitative and quantitative assessment of the vessel wall before and after angioplasty. The combination catheter successfully dilated 67 lesions. There was a characteristic three-layered appearance, representing intima, media, and adventitia, in 60 cases. Intravascular imaging provided information on the vessel wall unobtainable by standard contrast angiogaphy in 28 cases and influenced our management in 6 cases.

Lancet 1992; 339: 1571–72.

Technological advances have enabled miniaturisation of ultrasound transducers to such an extent that they can now be mounted in catheters and introduced into the body percutaneously.[1] Intravascular ultrasonography allows direct visualisation, in vivo, of vessel walls and assessment of the results of interventional procedures.[2] We report here our experience with a catheter that combines coronary ultrasound imaging and balloon angioplasty; the vessel wall can be viewed before and after angioplasty.

51 patients (69 lesions) of mean age 59·5 (range 41–81) years, who were undergoing clinically indicated coronary angioplasty, gave



Ultrasound images from segments of coronary arteries.

a=normal segment showing centrally located transducer (T), surrounded by air bubble artefact (B), and characteristic three-layered appearance of the normal vessel wall with a thin echogenic intima (I), sonolucent media (M), and echogenic adventitia (A); b=segment showing intimal thickening (arrow); c=stenosis that appears as one thick echogenic layer encroaching on transducer, with one highly echogenic area (arrow); d=segment of vessel wall immediately after angioplasty showing dissection (arrow).

informed consent for the use of the new catheter (Oracle; Endosonics, Pleasanton, California, USA). The device is inserted and used by standard techniques. The catheter consists of a standard polyethylene angioplasty balloon; at the proximal end is mounted a 64-element ultrasound transducer, of diameter 1·83 mm, around the catheter. Ultrasound signals are relayed from the transducer to a computer that generates real-time, cross-sectional images of the vessel wall.

67 of the 69 lesions were successfully crossed and dilated by the combination catheter. 2 lesions could not be crossed because of their severity and because the device was too wide. There were no complications from the procedure. Images were obtained for 60 lesions; in 28 cases these images provided information not available by contrast angiography.

Angiographically normal arterial wall gave a characteristic three-layered appearance (see figure, a). In 14 vessel segments apparently normal on angiography, the intima proximal to a stenosis seemed grossly thickened (figure, b). At the stenosis site the three-layered appearance was no longer present and the vessel wall appeared as one thick echogenic layer (figure, c); in 4 cases this layer included eccentrically located highly echogenic areas, with acoustic shadowing, which we thought might represent calcification. After angioplasty, imaging showed intimal disruption in almost every case. Dissections, not apparent in contrast angiography, were seen in 4 cases (figure, d).

In 6 cases, ultrasonography gave additional information that influenced our management. In 1 patient, an apparently significant stenosis on angiography was not confirmed on ultrasound imaging and dilatation was not done. In 3 patients, ultrasound imaging suggested substantial residual stenoses after dilatation despite an angiographically satisfactory result. The use of a larger balloon improved the ultrasound appearance. In the other 2 patients, ultrasonography repeatedly showed a substantial stenosis in an angiographically normal segment distal to the target lesion. After dilatation there was substantial improvement on ultrasonography, although the angiographic appearance did not change.

Ultrasound imaging gave morphological information about the coronary arteries, before and after angioplasty, that added to information available from contrast angiography in 28 cases. We were able to visualise morphological details within the vessel of interest, such as the degree of intimal thickening, calcification, and even plaque structure. This information may ultimately provide further insight into the pathogenesis of coronary artery disease and its progression. There have been attempts to characterise plaques in peripheral vessels in vitro according to their ultrasound characteristics.[3,4] Plaques could not be as readily differentiated in our study as in the in-vitro studies, perhaps because of the much smaller diameter of the coronary stenoses, but they appeared as thick echogenic layers encroaching on the centrally located transducer. Nevertheless, some seemed to have highly echogenic areas with acoustic shadowing that suggested calcification, not apparent on fluoroscopy.

We were able to assess directly the result of balloon angioplasty on the coronary stenosis. Our experience is not yet sufficient to allow us to predict events from ultrasound appearance, but published evidence on coronary angioplasty suggests that lesion morphology and wall disruption may be related to acute occlusion and long-term outcome.[5] In future, therefore, morphological features shown by intravascular ultrasonography may prove to be important predictors of outcome. Furthermore, ultrasound imaging provides a better perspective of the cross-sectional anatomy, and hence residual stenosis, than does angiography.

The catheter we used had two limitations. The imaging transducer was large in relation to coronary artery stenosis size, so many stenoses were fully visualised only after balloon dilatation. Another problem was that air bubbles could become trapped around the transducer, reducing the image quality. Modifications of the Oracle catheter have eliminated the latter difficulty and reduced the diameter to 1·2 mm.

The clinical significance of morphological information obtained with this catheter is not yet certain; long-term follow-up in larger studies is necessary to show whether morphological features visualised by intravascular ultrasonography are valuable in coronary angioplasty.

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Chapter 10

Impact of de novo vascular remodeling mode on the mechanisms of acute luminal gain and long term restenosis following coronary balloon angioplasty and directional coronary atherectomy: A serial intracoronary ultrasound study.

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Abstract :

Background: Post mortem and intravascular ultrasound studies suggest that the coronary vessels respond to atherosclerosis by either compensatory enlargement or paradoxical shrinkage. The aim of our study was to determine the pathophysiological role of this mode of de novo vascular remodeling on the subsequent mechanism of acute luminal gain and restenosis after coronary angioplasty and directional atherectomy.

Method: Serial intracoronary ultrasound examinations (pre-, post intervention and 6 month follow-up [fup]) and quantitative coronary angiography (QCA) were performed in 36 patients who underwent successful balloon angioplasty (BA, n=18) or directional atherectomy (DCA, n=18). The minimal cross sectional area (MCSA) and proximal reference segment area (cross section with the least diseased segment within 15mm proximal to the lesion) were determined during the pull-back sequence of the intracoronary ultrasound images. The vessel remodeling mode was expressed as the ratio of total vessel area (TVA) at MCSA to TVA at the reference site (TVA ratio) and classified into two categories; 1) paradoxical shrinkage (TVA ratio <1.00) (BA; 12 patients, DCA 12 patients) or 2) compensatory enlargement (TVA ratio >1.00) (BA; 6 patients, DCA; 6 patients). Plaque area (PA) was defined as=TVA-MCSA. Acute plaque reduction was defined as=PA pre-PA post, and late intimal hyperplasia as=PA fup-PA post. Acute lumen gain was defined as=MCSA post-MCSA pre, and late lumen loss as=MCSA post-MCSA fup. Acute vessel expansion was defined as=TVA post- TVA pre (at the segment of MCSA) and late vessel recoil was defined as=TVA post-TVA fup, at the segment of MCSA.

Results: Although the overall acute gain and late loss were similar between the compensatory enlargement group and paradoxical shrinkage groups after both BA and DCA, there were significant differences in the mechanism of action involved in each.

Balloon Angioplasty group (BA)- Both acute vessel stretch and late vessel recoil were significantly greater in the shrinkage group than in the compensatory enlargement group (2.4 ±1.3mm^2 versus 0.9 ±0.6mm2 and 1.8 ±0.6mm^2 versus 1.1 ±0.6mm^2, respectively, both $p<0.05$). There was also a significant positive correlation ($r=0.73$, $p=0.007$) between the degree of acute vessel stretch and chronic vessel recoil in the shrinkage group. At follow-up chronic vessel recoil accounted for 90% of late lumen loss in the shrinkage group, and for only 55% of late lumen loss in the compensatory enlargement group ($p=0.322$).

Directional Coronary Atherectomy group (DCA) - Both acute vessel stretch and chronic vessel recoil were significantly larger in the shrinkage group than the compensatory enlargement group (vessel stretch; 2.4±1.3 mm2 versus 0.3±0.6

mm², p<0.01; chronic recoil 2.4±1.3 mm² versus 0.7±0.9 mm², p<0.01). Acute plaque reduction was, however, significantly greater in the compensatory enlargement group than shrinkage group (5.8±1.0 mm² versus 3.6±1.5 mm², p<0.01). At follow-up vessel recoil accounted for 89% of late loss in the shrinkage group and 29% of late lumen loss in the compensatory enlargement group (p=0.340).

Conclusions : Our study suggests that the underlying de novo vessel remodeling mode (vessel shrinkage/compensatory enlargement) may play a key role in the acute efficacy of a coronary revascularisation device (vessel expansion or debulking) and the subsequent mechanism of restenosis (remodeling or intimal hyperplasia).

Introduction

Glagov originally proposed the hypothesis that compensatory enlargement of coronary vessels occurs as plaque volume increases until more than 40% of the vessel wall is composed of atherosclerotic plaque (compensatory enlargement) [1]. The mechanism can then no longer compensate and subsequent plaque growth results in lumen reduction [1]. The initial post mortem studies have been subsequently confirmed in vivo in man by both quantitative angiography and intravascular echocardiography [2-5]. Intravascular echocardiography has also confirmed that reverse glagovian modeling can also occur; that vessel shrinkage as well as vessel enlargement can occur in response to atherosclerosis (paradoxical shrinkage) [6,7]. We hypothesised that the mode of de novo vascular remodeling (compensatory enlargement or paradoxical shrinkage) prior to coronary intervention may be important in influencing both the mechanism of acute gain and of late restenosis after percutaneous revascularisation. The aim of our study was thus to determine the pathophysiological role of the mode of de novo vascular remodeling on the mechanism of acute luminal gain and subsequent restenosis after coronary balloon angioplasty and directional coronary atherectomy using intracoronary ultrasound and quantitative angiography pre-, post- and at 6 months after intervention.

Methods

Patient selection

Fifty-four patients who underwent successful balloon angioplasty (BA) or directional coronary atherectomy (DCA) were candidates for this study. Successful angioplasty was defined as angiographic success (<50% residual luminal diameter stenosis post intervention) with no major cardiac events (myocardial infarction, repeat intervention, coronary bypass surgery and death). For the purpose of this study patients in whom we could not obtain a complete set of high quality serial intracoronary ultrasound images either because of anatomical (total occlusion pre-intervention or at follow-up (n=4), lesions located at the origin of major side branches thus making it impossible to measure the proximal reference segment area, n=4) or technical reasons (the presence of severe calcification preventing the measurement of total vessel area, n=5) were excluded from the study. Five patients whose vessels did not show either compensatory enlargement or vessel shrinkage were also excluded. Complete sets of serial ultrasound studies with sufficient image quality were obtained in the remaining 36 patients (BA; 18 patients, DCA; 18 patients).

Balloon angioplasty (BA) or directional coronary atherectomy (DCA) procedures:

All patients received full anticoagulant therapy including aspirin and heparin before intracoronary ultrasound examination and intervention. The size of balloon or atherectomy device was determined from on-line quantitative angiographic measurement of the reference vessel diameter.

Quantitative coronary angiography (QCA)

The new version of the computer-based Coronary Angiography Analysis System (CAAS II) [8] was used to perform both the on-line quantitative analysis as well as the subsequent off-line cinefilm analysis. In the CAAS analysis which has been previously described and validated [8-10], the entire cineframe of 18 x 24mm in size is digitized at a resolution of 1329 x 1772 pixels. Correction for pincushion distortion is performed before analysis. Boundaries of a selected coronary segment are detected automatically. The absolute diameter of the stenosis (in mm) is determined using the guiding catheter as a scaling device. Using the diameter function, the luminal diameter at the site of lesion (minimal luminal diameter; MLD) and a computer-derived estimation of an interpolated reference vessel diameter (RD) at the site of the lesion were determined. To standardize the method of analysis of the initial and follow-up angiograms, measures were taken as previously described; all study frames selected for analysis were end-diastolic to minimize motion artifact and arterial segments were measured between the same identifiable branch points after the intracoronary administration of 2mg isosorbide dinitrate vasodilatory therapy [8-10]. Coronary angiograms were read and analyzed by two independent blinded observers.

Intracoronary Ultrasound (ICUS) examination

Following selective coronary angiography, a mechanical intracoronary ultrasound imaging catheter (30-MHZ, 2.9Fr or 4.3Fr, CardioVascular Imaging Systems, Sunnyvale, CA) was introduced over a 0.014 inch guidewire. After the imaging catheter was passed beyond the lesion, a slow motorized pull-back (1.0 mm/second) was performed to obtain images both proximal and distal to the target stenosis [11-13]. A simultaneous fluoroscopic image of the position of the ICUS catheter-tip was continuously displayed on the same video screen [11-13]. ICUS images were stored on super VHS videotape for off-line analysis. Cross sectional luminal area was defined as the integrated area central to the intimal leading edge echo [11-13]. Images with minimal cross sectional area (MCSA) were selected from the pull-back sequence by reviewing the position of the ICUS catheter on the angiographic image. Side branches and calcification pattern on ICUS images and

the position of the ICUS catheter on the angiographic image recorded on the same ICUS images were used as a landmark to obtain the same position, as far as possible, during serial ultrasound examinations. A reference segment was defined as the area with the least amount of plaque within 15mm proximal to the lesion but distal to a major side branch. To determine the interobserver variability of ICUS measurements, 30 lesions were independently measured by two observers. The mean signed difference and correlation of the measurements of cross sectional area were 0.02±0.37 mm^2 and 0.97, respectively.

Qualitative Intracoronary Ultrasound (ICUS) Assessment

Echo reflectivity was categorised into; 1) poorly echo reflective plaque (reflectivity is lower than bright adventitia layer; 2) highly echo reflective plaque (reflectivity is higher than bright adventitia layer) [12]. Calcium deposit was defined as highly echo-reflective tissue with acoustic shadow. A lesion was considered homogeneous if the plaque consisted of >75% of one type of echo-reflectivity. A lesion was defined as mixed if it contained both highly and poorly echo-reflective area occupying >25% of the plaque area [12]. A lesion was considered predominantly calcific in calcium occupied >1800 of vessel circumference [12].

Quantitative Intracoronary Ultrasound parameters

The vessel remodeling mode was expressed as the ratio of the total vessel area (TVA) at the minimum cross sectional area divided by the TVA at the reference site (TVA ratio). Target lesions were classified into two categories based on this ratio ; 1) compensatory enlargement (TVA Ratio >1.00) and 2) paradoxical shrinkage (TVA Ratio <1.00)).

Lumen eccentricity index was calculated as the ratio between the minimal and maximal wall thickness. Plaque area (PA) was defined as a TVA at the point of MCSA minus MCSA. Acute lumen gain was defined as =MCSA post-MCSA pre. Acute plaque reduction was defined as =PA pre-PA post. Acute vessel expansion was defined as =TVA post-TVA pre, at the segment of MCSA. Late lumen loss was defined as =MCSA post-MCSA follow-up. Late intimal hyperplasia was defined as= PA follow-up minus PA post. Late vessel recoil was defined as =TVA post-TVA follow-up, at the segment of MCSA.

Statistical analysis

All values are expressed as mean values ± standard error of the mean. Paired Student's t-test was used to compare chronological changes at the same segment in the same patients. Unpaired Student's t-test were used for intergroup comparisons. Differences between categorical variables were analyzed by chi-square with correction. A p value <0.05 was considered significant.

Results

Clinical and angiographic characteristics

The baseline clinical and angiographic characteristics of the 36 patients with balloon angioplasty (BA) and directional coronary atherectomy (DCA) are provided in Table 1. The two groups were comparable in terms of age, gender, coronary risk factors, anginal class [14] and distribution of diseased vessels. The two groups were also similar in both the type and location of lesions, but the reference diameter was significantly higher in the directional atherectomy group. Although the minimal luminal diameter (MLD) pre intervention was similar in the two groups (1.15±0.42 mm DCA versus 1.01±0.31 mm BA, p=0.288) MLD post DCA was significantly larger than post BA (2.85±0.55 mm DCA versus 2.16±0.47 mm BA, $p<0.001$). This more favourable acute result after DCA was subsequently lost however with the MLD at follow-up being similar in the two groups (1.73±0.51 mm DCA versus 1.57±0.50 mm BA, p=0.369).

	BA	DCA	p-value
Patients	18	18	
Male/female	14/4	16/2	0.658
Age (years)	58 ± 9	58 ± 8	0.846
Ischemic syndrome			
Stable angina pectoris*	12	11	0.732
Unstable angina pectoris*	6	7	0.732
Prior myocardial infarction	2	4	0.658
Prior balloon angioplasty	3	2	0.635
Prior coronary bypass surgery	0	0	-
Coronary risk factors;			
Hypercholesterolemia	2	6	0.228
Systemic hypertension	5	6	0.721
Cigarette smoking	6	4	0.710
Diabetes	0	1	1.000
Modified AHA/ACC classification			
type A	5	6	0.721
type B1	7	7	0.732
type B2	5	5	0.710
type C	1	0	1.000
Location of lesions			
RCA	6(33%)	2(11%)	0.229
LAD	8(45%)	13(72%)	0.176
LCX	4(22%)	3(17%)	0.678
Quantitative analysis			
MLD pre	1.01±0.31	1.15±0.42	0.288
RD pre	2.78 ±0.55	3.47±0.37	***<0.001***
% DS pre	63 ± 11%	67 ± 13%	0.354
MLD post	2.16 ± 0.47	2.85±0.55	***<0.001***
RD post	2.85 ± 0.47	3.66 ± 0.54	***<0.001***
% DS post	24 ± 12%	22 ± 10%	0.554
MLD follow-up	1.57 ±0.50	1.73±0.51	0.369
RD follow-up	2.73 ±0.58	3.20 ±0.46	***<0.05***
% DS follow-up	42 ±15%	46 ±15%	0.471
Balloon / vessel ratio	1.16 ± 0.34		

Table 1: Clinical and angiographic characteristics of the study population. BA- Balloon angioplasty, DCA- Directional coronary atherectomy, * angina pectoris classification [14], LAD- Left anterior descending coronary artery, RCA- Right coronary artery, MLD- Minimal luminal diameter, RD- Reference diameter, %DS- Percent diameter stenosis

Baseline ultrasonographic characteristics

Baseline intracoronary ultrasonographic characteristics are shown in Table 2. No difference was found in plaque composition (soft or mixed plaque) and the presence and location of calcium deposits between the two groups. Minimal cross sectional area (MCSA) pre and follow-up, and vessel cross sectional area (CSA) pre, post and at follow-up were also similar in the two groups. Acute luminal gain, and hence minimal cross sectional area (MCSA) post intervention were, however, significantly greater after directional atherectomy than balloon angioplasty (acute gain; 6.0±1.5 mm^2 versus 3.8 ±1.6 mm^2, p=0.0001, and MCSA post; 7.6±1.5 mm^2 versus 5.3 ±1.7 mm^2, p=0.0001, respectively, Table 2). This difference was the result of greater plaque reduction in this group (plaque reduction; 4.3 ±1.7 mm^2 in DCA versus 1.8 ±1.8 mm2 in BA, p=0.0001). Late luminal loss was higher in the DCA group however resulting in a similar MCSA at follow-up in the two groups (4.9±2.8 mm^2 (DCA) versus 3.4 ±2.0 mm^2 (BA), p=0.051).

	BA	DCA	p-value
Lesion composition			
Homogenous plaque			
poorly echo reflective	8	10	0.739
highly echo reflective	0	0	-
Mixed plaque	10	8	0.739
Calcium deposits			
Subendothelial	6	7	0.732
Basal/Center	7	6	0.732
Luminal measurement (mm^2)			
TVA ratio	0.94±0.11	0.95±0.11	0.792
Ref.TVA pre	16.6±4.1	18.5±3.9	0.162
TVA pre	15.4±3.5	17.4±3.6	0.105
MCSA pre	1.6±3.5	1.6±0.7	0.877
Plaque area pre	13.9±3.8	15.8±3.7	0.125
TVA post	17.4±3.3	19.2±3.9	0.149
MCSA post	5.3±1.7	7.6±1.5	***<0.001***
Plaque area post	12.0±3.5	11.5±3.2	0.653
TVA follow-up	15.8±3.2	17.3±3.5	0.184
MCSA follow-up	3.4±2.0	4.9±2.8	0.051
Plaque area follow-up	12.5±3.7	12.3±4.8	0.935
Eccentricity index	0.47±0.27	0.40±0.25	0.296
Acute luminal gain	3.8±1.6	6.0±1.5	***<0.001***
vessel stretch	2.0±1.3	1.7±1.5	0.677
plaque reduction	1.8±1.8	4.3±1.7	***<0.001***
Late luminal loss	2.0±1.4	2.5±2.4	0.382
vessel late recoil	1.6±0.7	1.9±1.5	0.461
intimal hyperplasia	0.4±1.7	0.6±2.5	0.618

Table 2: Baseline ultrasonographic characteristics of the study population. BA- Balloon angioplasty, DCA- Directional coronary atherectomy, TVA- Total vessel area in the segment with minimal cross sectional area. MCSA- Minimal cross sectional area. Ref TVA: Total vessel area in the reference segment. Eccentricity Index- see text

Comparison between the shrinkage and compensatory enlargement groups in Balloon Angioplasty

Whilst 12 patients showed paradoxical shrinkage (TVA ratio <1.00) (Figure 1), the remaining 6 patients had compensatory enlargement (TVA ratio >1.00).

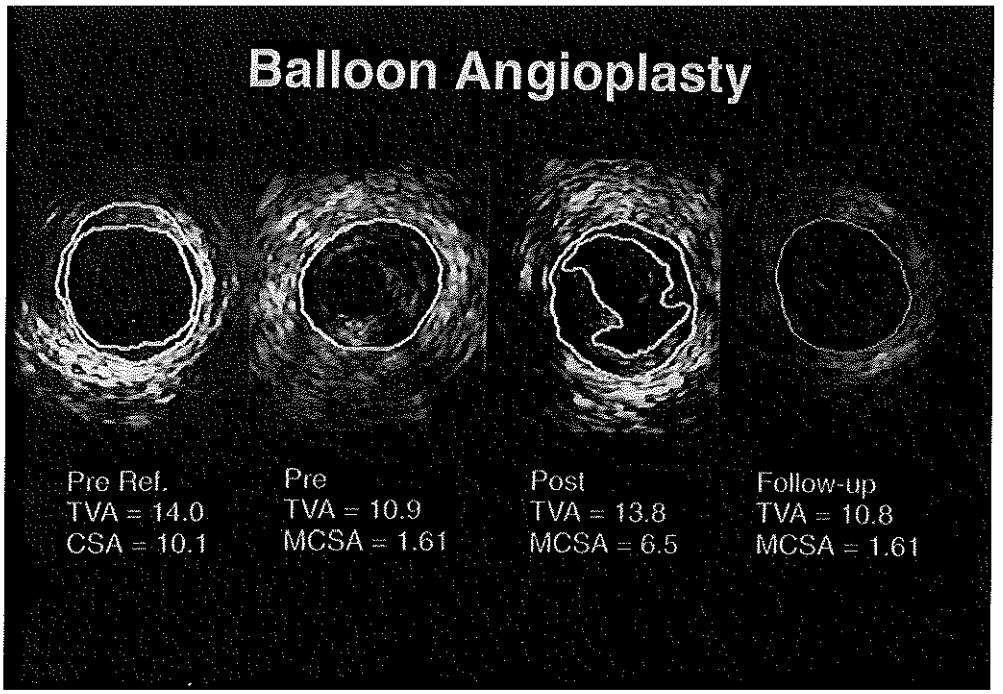

Figure 1. Intracoronary ultrasound (ICUS) images obtained from a patient with paradoxical shrinkage and treated with balloon angioplasty (BA). Reference segment showed total vessel area (TVA) was 14.0 mm2 [extreme left panel]. Pre BA TVA was 10.9 mm2 and minimal cross sectional area (MCSA) was 1.61mm2 [middle left panel]. The ratio of the total vessel area (TVA) at MCSA divided by TVA at the reference site pre intervention (TVA ratio) was 0.78, hence the de novo remodeling mode was one of paradoxical shrinkage. Post BA MCSA improved to 6.5 mm2 associated with TVA enlargement from 10.9 mm2 to 13.8 mm2 [middle right panel]. At follow-up MCSA decreased from 6.5 mm2 to 1.61 mm2 associated with TVA shrinkage from 13.8 mm2 to 10.8 mm2. In this case 39% of luminal loss was derived from intimal hyperplasia and 61% from late chronic recoil (vessel remodeling) [extreme right panel].

Minimal CSA, vessel CSA and plaque CSA pre-, post- balloon angioplasty and at follow-up as well as reference vessel CSA-pre were similar in both paradoxical shrinkage and compensatory enlargement groups (Table 3).

	paradoxical shrinkage	compensatory enlargement	p-value
Patients (n)	12	6	
Luminal measurement (mm^2)			
TVA ratio	0.87±0.07	1.07±0.02	***<0.001***
Ref.TVA pre	17.3±4.4	15.0±3.0	0.258
TVA pre	15.1±3.8	16.0±3.1	0.635
MCSA pre	1.5±0.7	1.8±0.7	0.367
Plaque area pre	13.7±4.1	14.2±3.5	0.785
TVA post	17.6±3.6	17.0±2.6	0.722
MCSA post	5.3±1.6	5.3±1.9	0.977
Plaque area post	12.2±3.7	11.6±3.1	0.746
TVA follow-up	15.7±3.5	15.9±2.9	0.897
MCSA follow-up	3.3±2.2	3.4±1.5	0.946
Plaque area follow-up	12.4±3.9	12.6±3.5	0.939
Acute luminal gain	3.9±1.4	3.5±2.1	0.670
vessel stretch	2.4±1.3	0.9±0.6	***<0.05***
plaque reduction	1.5±1.5	2.6±2.3	0.225
Late luminal loss	2.0±1.3	2.0±1.7	0.897
vessel late recoil	1.8±0.6	1.1±0.6	***<0.05***
intimal hyperplasia	0.2±1.6	0.9±1.8	0.399

Table 3: Comparison between paradoxical shrinkage and compensatory enlargement groups in Balloon Angioplasty group. TVA- Total vessel area in the segment with minimal cross sectional area. MCSA- Minimal cross sectional area. Ref TVA: Total vessel area in the reference segment.

Although overall acute gain and late loss were also similar in the two groups this masked significant differences in the mechanism of action involved in each. Acute vessel stretch was the predominant mechanism of acute luminal gain in the shrinkage group (2.4±1.3 mm^2 versus 0.9±0.6 mm^2, p=0.0163), and plaque reduction in the compensatory enlargement group (2.6±2.3 mm2 versus 1.5±1.5 mm^2) although because of the small numbers involved this did not reach statistical significance (p=0.2248). At follow-up the main mechanism for late loss was chronic vessel recoil in the shrinkage group (1.8±0.6 mm^2 versus 1.1±0.6 mm^2, p=0.0125), and intimal hyperplasia in the compensatory enlargement group (0.9±1.8 mm^2 versus 0.2±1.6 mm^2), although again, because of the small numbers involved, this did not reach statistical significance (p=0.3991). The degree of acute vessel stretch and late vessel recoil correlated well (r = 0.73, p=0.0068) in the shrinkage group, but poorly in the compensatory enlargement group (r = 0.44, p=0.1496).

Thus vessel stretch was the predominant mechanism of acute luminal gain, and chronic vessel recoil the predominant mechanism of late loss in the shrinkage group compared to the compensatory enlargement group.

Comparison between the shrinkage and compensatory enlargement groups in DCA

While 12 patients had paradoxical shrinkage, the remaining 6 patients showed compensatory enlargement (Figure 2).

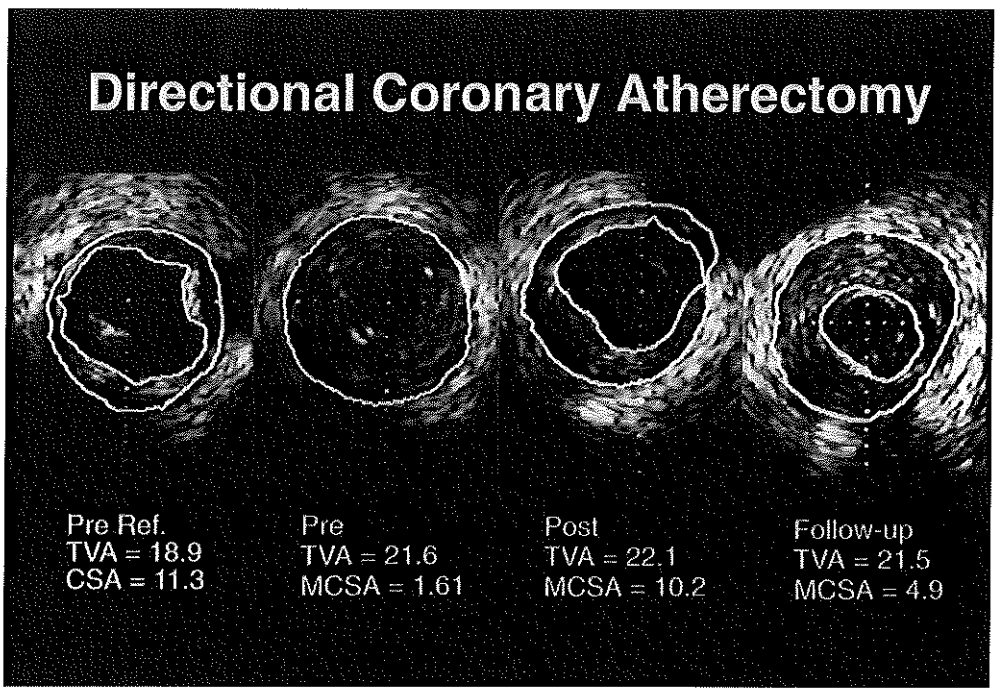

Figure 2. Intracoronary ultrasound (ICUS) images obtained from a patient with compensatory enlargement and treated with directional coronary atherectomy (DCA). Reference segment showed total vessel area (TVA) was 18.9 mm2 [extreme left panel]. Pre DCA TVA was 21.6 mm2 and minimal cross sectional area (MCSA) was 1.61 mm2 [middle left panel]. The ratio of the total vessel area (TVA) at MCSA divided by TVA at the reference site pre intervention (TVA ratio) was 1.14 hence the de novo remodeling mode was one of compensatory enlargement. Post DCA MCSA improved to 10.2 mm2 associated with TVA enlargement from 21.6 mm2 to 22.1 mm2 [middle right panel]. At follow-up MCSA decreased from 10.2 mm2 to 4.9 mm2 associated with TVA shrinkage from 22.1 mm2 to 21.5 mm2. In this case 89% of luminal loss was derived from intimal hyperplasia and 11% from late chronic recoil (vessel remodeling) [extreme right panel].

Minimal CSA, vessel CSA and plaque CSA pre-, post- directional atherectomy and at follow-up as well as reference vessel CSA-pre were similar in both paradoxical shrinkage and compensatory enlargement groups (Table 4).

	paradoxical shrinkage	compensatory enlargement	p-value
Patients (n)	12	6	
Luminal measurement (mm^2)			
TVA ratio	0.89±0.05	1.08±0.04	***<0.001***
Ref.TVA pre	19.4±3.4	16.4±4.4	0.121
TVA pre	17.2±3.1	17.8±4.9	0.756
MCSA pre	1.5±0.8	1.8±0.5	0.356
Plaque area pre	15.7±3.1	16.0±5.0	0.899
TVA post	19.7±3.3	18.1±5.3	0.461
MCSA post	7.5±1.6	7.9±1.4	0.567
Plaque area post	12.2±2.5	10.2±4.3	0.231
TVA follow-up	17.2±3.0	17.8±4.8	0.869
MCSA follow-up	4.7±2.8	5.5±2.9	0.553
Plaque area follow-up	12.5±4.2	12.0±6.3	0.825
Acute luminal gain	6.0±1.7	6.1±1.3	0.900
vessel stretch	2.4±1.3	0.3±0.6	***<0.01***
plaque reduction	3.6±1.5	5.8±1.0	***<0.01***
Late luminal loss	2.7±2.6	2.4±2.8	0.810
vessel late recoil	2.4±1.3	0.7±0.9	***<0.01***
intimal hyperplasia	0.3±2.9	1.7±2.4	0.249

Table 4: Comparison between paradoxical shrinkage and compensatory enlargement groups in directional coronary atherectomy. TVA- Total vessel area in the segment with minimal cross sectional area. MCSA- Minimal cross sectional area. Ref TVA: Total vessel area in the reference segment.

Although overall acute gain and late loss were also similar in the two groups, this masked significant differences in the mechanism of action involved in each. Acute vessel stretch was the predominant mechanism of acute luminal gain in the shrinkage group (2.4 ±1.3 mm^2 versus 0.3 ±0.6 mm^2, p=0.0014) whilst plaque reduction was the predominant mechanism of acute luminal gain in the compensatory enlargement group (5.8 ±1.0 mm^2 versus 3.6 ±1.5 mm^2, p=0.0048). At follow-up the main mechanism for late loss was chronic vessel recoil in the shrinkage group (2.4 ±1.3 mm^2 versus 0.7 ±0.9 mm^2, p=0.0073), and intimal hyperplasia in the compensatory enlargement group (1.7±2.4 mm^2 versus 0.3 ±2.9 mm^2) although because of the small numbers involved this did not reach statistical significance (p=0.2489). There was no significant correlation between acute vessel

stretch and late vessel recoil in either the shrinkage or compensatory enlargement group ($r = 0.28$, $p=0.3673$, and $r = 0.41$, $p=0.2823$, respectively).

Role of chronic vessel recoil (vessel remodeling) in the mechanism of restenosis between the shrinkage and compensatory enlargement groups in BA and DCA

The mode of de novo vessel remodeling was found to play a significant role in the mechanisms involved in late restenosis. While in the shrinkage group of balloon angioplasty chronic vessel recoil (remodeling) accounted for 90% ($1.8mm^2$ / $2.0mm^2$) of late lumen loss, in the compensatory enlargement group chronic vessel recoil accounted for only 55% of late lumen loss ($1.1mm^2$ / $2.0mm^2$). While in the shrinkage group of directional atherectomy chronic vessel recoil accounted for 89% ($2.4mm^2$ / $2.7mm^2$) of late lumen loss, in the compensatory enlargement group chronic vessel recoil accounted for only 29% ($0.7mm^2$ / $2.4\ mm^2$) of late lumen loss. Thus the de novo mode of vessel remodeling, vessel shrinkage versus compensatory enlargement, was seen to substantially influence the subsequent mechanism of restenosis following both balloon angioplasty and directional atherectomy.

Discussion

The principle findings of our study were: 1) Overall initial luminal gain and late loss were similar in the two different de novo vessel remodeling states for both BA and DCA; 2) at the time of intervention significantly larger vessel stretch was obtained in the shrinkage group for both BA and DCA, whilst significantly greater plaque removal was obtained by DCA in the compensatory enlargement group; 3) at follow-up late vessel recoil (vessel remodeling) was significantly greater in the shrinkage group after either BA or DCA; 4) a high correlation ($r = 0.73$) was observed between the degree of acute stretch and late vessel recoil in the shrinkage group in BA; 5) while in the shrinkage group chronic vessel recoil (remodeling) accounted for 89% to 90% of late lumen loss after BA and DCA respectively, in the compensatory enlargement group remodeling accounted for only 55% to 30% of late lumen loss after BA and DCA respectively.

Role of de novo vessel remodeling mode in acute luminal gain after coronary intervention

Recent intracoronary ultrasound studies indicate that plaque reduction accounts for 52% to 67% and vessel stretch for the remaining 33% to 48% of acute luminal

gain after BA and DCA [13,15] This is in keeping with our study where plaque reduction accounted for 47% of overall acute luminal gain after BA and 72% of overall acute gain after DCA in the total study population. However, our tomographical quantification based on the underlying de novo vessel remodeling mode indicates that in the shrinkage group of BA vessel stretch accounted for 62% of acute gain, while in the compensatory enlargement group of BA vessel stretch accounted for only 26% of acute gain. In the shrinkage group of DCA plaque reduction accounted for 60% of acute gain, while in the compensatory enlargement group of DCA plaque reduction accounted for 95% of acute gain. These findings indicate that the de novo vessel remodeling mode may have an important role in determining the mechanism of acute luminal gain (vessel stretch or plaque reduction) after coronary intervention. Pasterkamp and colleagues also recently examined the influence of the de novo vessel remodeling mode on the mechanism of balloon angioplasty in femoral arteries and again found that vessel stretch was significantly greater in the shrinkage group than in the compensatory enlargement group [7]. Our findings expand on their initial observations and suggest that the same mechanisms may also be operative in the coronary vessels. Furthermore vessel stretch also appears to be a major mechanism of action in the paradoxical shrinkage group treated by directional atherectomy. The reasons for this are not clear. One possible explanation is that paradoxical shrinkage may be the result of structural changes in the vessel wall, such as alterations in the relative proportions of elastin or collagen, resulting in alterations in the elastic properties of the vessel. Our findings also indicate that vessels which have undergone compensatory enlargement are much more susceptible to plaque removal by directional atherectomy than vessels which have undergone paradoxical shrinkage, perhaps again reflecting underlying differences in vessel wall characteristics. Against this however is the fact that we did not find any significant differences in ultrasound plaque characteristics between the paradoxical shrinkage and compensatory enlargement groups. ICUS visual plaque characteristic assessment however is still a fairly rough method of assessing plaque composition. Whether newer techniques such as angular dependent backscatter analysis by ultrasound may result in increased precision in cell wall characterisation and allow us to differentiate plaque composition further remains to be determined [16].

Role of de novo vessel remodeling mode in restenosis

The classical paradigm regarding the mechanisms of restenosis after coronary angioplasty has been one of neointimal thickening from the migration, proliferation and extracellular matrix synthesis by vascular smooth muscle cells [17]. More recently this paradigm has been challenged by both experimental [18,19] and human intravascular ultrasound studies which suggest that restenosis relates more to unfavourable late vascular remodeling than neointimal hyperplasia [20-23]. The mechanisms involved in this however are unclear but are thought to

involve a combination of vessel wall fibrosis (particularly of the adventitia) in response to deep wall injury, apoptosis (programmed cell death), qualitative and quantitative changes in the extracellular matrix and changes in local flow dynamics [22-26]. What factors may influence this process remains unknown. Our serial intracoronary ultrasound study clearly indicates a major role for the underlying de novo vascular remodeling mode on late restenosis after both balloon angioplasty and directional coronary atherectomy. Previous intracoronary ultrasound studies have reported that various degree of chronic vessel recoil ("vascular remodeling" ; from 42% to 84%) account for late loss after BA and DCA [13,15]. Our study was consistent with these studies suggesting that chronic recoil accounts for 71% of late lumen loss after BA and for 69% of late lumen loss after DCA in the overall patient population. However, our study also suggests that within the overall picture, the relative contributions of chronic vessel recoil and intimal hyperplasia to restenosis can vary widely, depending on the de novo vessel remodeling mode. For example, in the shrinkage group subjected to balloon angioplasty chronic vessel recoil (remodeling) accounted for 90% of late lumen loss, whilst in the compensatory enlargement group of directional atherectomy remodeling accounted for only 29% of late lumen loss whilst intimal hyperplasia accounted for the remaining 71% of late lumen loss. The reasons for these differences are not clear but again are likely to be secondary to structural changes in the vessel wall as a result of whatever process underlies the initial de novo vascular remodeling mode.

Relation of acute stretch and late recoil

Glagov and colleagues suggested that in a coronary artery compensatory enlargement without luminal narrowing was frequently observed until plaque area increased up to 40% [1]. A recent intravascular ultrasound study indicated that in a femoral artery compensatory enlargement was mainly observed in plaque area less than 25% and paradoxical shrinkage was frequently seen in plaque area more than 25%, although no strong relation existed between the decrease of luminal area and the increase of the plaque area [6]. In our patients with significant fixed stenoses 33% of the patients showed compensatory enlargement of the vessel and the remaining 67% of the patients had paradoxical shrinkage. Compensatory enlargement and paradoxical shrinkage may be natural stages in the atherosclerotic process itself and may be associated with structural changes in the media and adventitia [1, 27]. In the paradoxical shrinkage stage the elastin content of the vascular wall may be decreased and the rigidity of the wall hence increased [27], whilst in the compensatory enlargement stage vessel wall extensibility may be preserved. Our study would suggest that following balloon angioplasty of vessels which have undergone paradoxical shrinkage the dilated vessel wall may constrict gradually during the process of restenosis and that the degree of this constriction relates directly to the original degree of vessel stretch at the time of the procedure.

This relationship is however altered by directional atherectomy. Atherectomy specimen has been frequently reported to involve the adventitia [28], which may substantially weaken the vessel wall, and thus reduce and alter the degree of late vessel recoil (remodeling) at follow-up. This phenomenon may be responsible for the poor correlation between the degree of vessel stretch and remodeling after directional atherectomy. An alternative mechanism may be that factors responsible for the initial paradoxical shrinkage vessel remodeling mode are still present and may therefore influence the subsequent vessel response to balloon angioplasty, whereas atherectomy, by removing vessel wall constituents, alters the local plaque burden, composition and characteristics thus altering the local vessel milieu and hence the vessels response to the procedure.

Study limitations

Our study has a number of limitations. Firstly, the reference segment site used is critically important for our definition of vascular remodeling and hence our results. For our reference we used the least diseased segment within 15mm proximal to the narrowing but distal to a major side branch, as this is likely to be the site of least vessel wall remodeling and hence the most likely site to approximate the original vascular dimensions. We cannot discount the fact however, that a different, perhaps even more proximal, reference segment may provide a different value of TVA and hence affect the estimated remodeling mode. Secondly, performing serial intracoronary ultrasound measurements of the same arterial segment at an interval of 6 months poses significant technical and logistic problems. We tried to overcome these however and ensure accurate localisation of the ICUS catheter by using permanent landmarks, such as side branches and areas of calcification, for guidance and by carefully observing the position of the ICUS catheter-tip on the fluoroscopic image which was recorded simultaneously on a split screen. Thirdly, pre intervention several patients showed wedging of the ICUS catheter and in these patients the site of minimal luminal cross sectional area may have already been slightly dilated by the ICUS catheter itself. However, the minimal cross sectional area pre intervention calculated from QCA was $0.92mm^2$ whilst the cross sectional area of the most of ICUS catheter used is $0.73mm^2$ so any "Dotter effect" would have been minimal. Finally, our patients were, to an extent, selected on the basis of a successful interventional procedure with subsequent high quality serial ICUS images. We are unable to comment therefore on the role of de novo vascular remodeling on the acute success rate of the procedure. As the number of patients reported in this early experience is also small our findings will need to be confirmed in larger multicenter studies.

Clinical implications

Our study suggests that the underlying de novo vessel remodeling mode may significantly contribute to the efficacy of a device (vessel expansion or debulking) and the subsequent mechanism of restenosis (remodeling or intimal hyperplasia). This has important clinical implications with regard to the optimal use of new devices. Our study suggests that in a vessel with paradoxical shrinkage stent implantation, to act as a scaffold for the vessel wall and prevent late vessel recoil, may be the optimal device to prevent restenosis. In vessel with compensatory enlargement plaque reduction (debulking) during the initial interventional procedure and concomitant medical treatment to prevent the growth of intimal hyperplasia using local drug delivery [29] or endovascular radiation [30] may be the most useful way of reducing restenosis. Thus observation of de novo vessel remodeling mode provides novel information with which to determine the most suitable interventional device for each specific lesion. Our study also suggests that the de novo vessel remodeling mode may be substantially more important to both the mechanism of acute luminal gain and late restenosis than plaque characteristics. It may thus provide some insight into why intravascular ultrasound studies, to date, have not been wholly supportive of the role of plaque characteristics on the subsequent acute gain and late loss and may provide important lessons for the ongoing OARS and BOAT studies [15,31]. Finally our study once again highlights the limitations of quantitative coronary angiography which, although the gold standard for interventional work, is unable to differentiate between the different pathophysiological mechanisms involved in acute luminal gain and late restenosis and hence guide optimal use of revascularisation devices.

Conclusions

Our study suggests that the underlying de novo vessel remodeling mode (vessel shrinkage/compensatory enlargement) may play a key role in the acute efficacy of a coronary revascularisation device (vessel expansion or debulking) and the subsequent mechanism of restenosis (remodeling or intimal hyperplasia).

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Part 3- Epidemiology

Chapter 11

Influence of serum cholesterol and cholesterol subfractions on long term luminal renarrowing following coronary angioplasty. A quantitative angiographic analysis.

Violaris AG, Melkert R, Serruys PW.

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Influence of Serum Cholesterol and Cholesterol Subfractions on Restenosis After Successful Coronary Angioplasty

A Quantitative Angiographic Analysis of 3336 Lesions

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Background Previous reports have suggested that hyperlipidemia may be associated with increased restenosis after successful coronary angioplasty. These studies have been compromised, however, by their retrospective nature, the small numbers involved, differences in the definition of restenosis, and inadequate quantitative angiographic follow-up at a prespecified time interval. The objective of the study was to examine the relation between serum cholesterol and long-term restenosis after coronary angioplasty, using quantitative angiography, at a predetermined time interval.

Methods and Results The study population comprised 2753 patients (3336 lesions) prospectively enrolled and successfully completing four major restenosis trials. Cineangiographic films were processed and analyzed at a central angiographic core laboratory with the use of an automated interpolated edge-detection technique. Serum total cholesterol was measured at trial entry and at 6 months. Hypercholesterolemia was defined as total cholesterol >7.8 mmol · L^{-1} at trial entry. Two approaches were used to assess restenosis: first, a categorical approach using the cutoff point of >50% diameter stenosis at follow-up and second, a continuous approach examining changes in minimal luminal dimensions, the absolute loss (change in minimum luminal diameter after PTCA to follow-up, in mm) and relative loss (absolute loss corrected for vessel size), which may give a better understanding of the underlying pathological process involved. One hundred sixty patients with 191 lesions (5.73%) had hypercholesterolemia (total cholesterol, >7.8 mmol · L^{-1}; mean±SD, 8.46±0.75 mmol · L^{-1}) and 2593 patients with 3145 lesions (94.27%) normal cholesterol (5.67±1.06 mmol · L^{-1}). The restenosis rate was similar in patients with and without hypercholesterolemia (31.9% versus 33.7%, respectively; relative risk, 0.975; 95% CI, 0.882 to 1.077; P=.68). Similarly, there was no difference in either the absolute or relative loss between patients with and without hypercholesterolemia (0.31±0.53 versus 0.32±0.53 mm and 0.12±0.20 versus 0.13±0.21, respectively, P=NS for both). Conversely, the total serum cholesterol in patients with restenosis (using the categorical definition) was similar to those without restenosis (5.84±1.24 versus 5.81±1.22 mmol/L, respectively, P=NS). Dividing the population into deciles according to total cholesterol and examining the categorical restenosis rate (by χ^2) as well as the absolute and relative loss by ANOVA again revealed no significant differences between deciles. Subgroup analysis of 579 patients (667 lesions) with HDL and LDL cholesterol levels available again revealed no differences in the categorical restenosis rate (by χ^2) or the absolute or relative loss between deciles according to LDL, HDL, or LDL:HDL ratio, suggesting no influence of these cholesterol subfractions on restenosis.

Conclusions Our results indicate that there is no association between cholesterol and restenosis by either a categorical or continuous approach, suggesting that measures aimed at reducing total cholesterol are unlikely to significantly influence postangioplasty restenosis. (*Circulation*. 1994;90:2267-2279.)

Key Words • angioplasty • cholesterol • stenosis • angiography

Restenosis after successful coronary angioplasty remains a major limitation of the technique.[1] Postmortem examination suggests that this is the result of intimal hyperplasia secondary to the intimally directed migration and proliferation of vascular smooth muscle cells.[2] Experimental in vitro cell studies have shown that vascular smooth muscle cell migration and proliferation may both be influenced by cholesterol and cholesterol subfractions.[3] Furthermore, in vivo animal work also suggests that serum cholesterol may be an important determinant of intimal hyperplasia after balloon angioplasty.[4] Because of this body of experimental evidence and also clinical evidence linking elevated serum lipid fractions to atherosclerosis,[5,6] a number of studies have sought to investigate the relation between serum lipids and restenosis. The results from these studies have been equivocal (Table 1), with some suggesting a positive correlation between the two[7-10] and others suggesting no association.[11,12] These differences arise for a number of reasons. First, most studies have been retrospective analyses using small numbers of patients. Second, angiographic follow-up was mostly incomplete and performed for the recurrence of symptoms rather than at a predetermined time interval, thus introducing selection bias. Finally, studies have almost invariably used visual assessment to estimate angiographic severity. This is subject to wide interobserver and intraobserver variability, which further limits the reliability of the results.[13] This study attempted to overcome these limitations by using a validated automated edge-detection technique to evaluate the effect of serum cholesterol on restenosis prospectively in a

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TABLE 1. Summary of Studies in Which the Relation Between Lipid Levels and Subsequent Restenosis Was Examined

Study	No. of Patients	Angio Follow-up, %	Definition	Restenosis Rate, %	Association
Austin et al[7]	443	100	Loss >50% AG	41	TC, female Aspirin Rx gp
Austin et al[11]	103	74	?	36	None
Arora et al[25]	723	100	Loss >50% AG	41	Inverse relation to TC at fup
Johansson et al[8]	157	100	>50% DS fup	33	Low HDL (M) High HDL (F)
Reis et al[9]	186	37	>70% DS fup	32	TC:HDL cholesterol
Foley et al[12]	100	82	Loss >50% AG Incr %DS >30%	45	None
Shah and Amin[10]	68	37	>70% DS fup	41	Low HDL
Present study	2753	80	>50% DS fup A/Rloss ANG/NGI	33.6	None

Angio indicates angiographic; AG, absolute gain; DS, diameter stenosis; fup, at follow-up; A/Rloss, absolute/relative loss; ANG, absolute net gain; NGI, net gain index; TC, total cholesterol; and Rx gp, treatment group.

large series of patients undergoing successful balloon angioplasty and routine follow-up angiographic assessment at a predetermined time interval.

Methods

Patients

The study population comprised 3582 patients with significant primary stenoses in native coronary arteries who were prospectively enrolled into four major restenosis trials.[14-17] These demonstrated that active therapy had no effect on restenosis or clinical outcome in the first 6 months after balloon angioplasty, so for the purposes of this study, the data for the active and placebo groups were pooled. Patients were eligible for study entry if they were symptomatic or asymptomatic men, or women without child-bearing potential, with stable or unstable angina pectoris and proven angiographically significant narrowing in one or more major coronary arteries. Informed consent was obtained in all cases before the coronary angioplasty procedure. Patients with developing myocardial infarction and significant left main coronary artery disease were excluded from the study.

Serum Cholesterol Measurements

Serum cholesterol measurements were taken before percutaneous transluminal coronary angioplasty (PTCA) and at the time of 6-month follow-up angiography by each individual center. Sample analysis was carried out locally in all participating centers. Hypercholesterolemia was defined as a serum cholesterol level >7.8 mmol · L^{-1} at trial entry.[18] A cholesterol level of <7.8 mmol · L^{-1} was, for the purposes of this study, defined as "normal" cholesterol.

Angioplasty Procedure and Follow-up Angiography

Coronary angioplasty was performed with a steerable, movable guide wire system by the femoral route. Standard balloon catheters were used. The choice of balloon type and brand as well as inflation pressure and duration were left to the discretion of the operator. Patients were followed up for 6 months, at which time a follow-up study was performed. If symptoms recurred within 6 months, coronary angiography was carried out earlier. If no definite restenosis was present and the follow-up time was <4 months, the patient was asked to undergo further coronary arteriography at 6 months.

Quantitative Angiography

Three coronary angiograms, in total, were obtained for each patient: before PTCA, after PTCA, and at angiographic follow-up. The angiograms were recorded in such a manner that they were suitable for quantitative analysis by the computer-assisted Coronary Angiography Analysis System (CAAS). To standardize the method of data acquisition and to ensure exact reproducibility of the angiographic studies, measures were taken as previously described, and all angiograms were processed in a central angiographic core laboratory.[14-17] All cineangiograms were analyzed with the computer-assisted CAAS technique, which was described and validated earlier.[13,19] Because the computer algorithm is unable to measure total occlusions, a value of 0 mm was substituted for the minimal lumen diameter and a value of 100% for the percent diameter stenosis before PTCA. In these cases, the postangioplasty reference diameter was substituted for vessel size.

Angiographic Definitions Used

Vessel size refers to the reference diameter of the relevant coronary segment and is represented by the interpolated reference diameter before PTCA, since this is the closest and most objective approximation of the disease-free vessel wall. Minimum luminal diameter (MLD) is the point of maximal luminal narrowing in the analyzed segment.

Many criteria have been proposed for the assessment of restenosis.[20] For the purposes of this study, two approaches were used: first, the categorical approach widely used by clinicians who favor the traditional cutoff point of >50% diameter stenosis at follow-up; and second, a continuous approach using absolute and relative losses, which reflect the behavior of the lesion during and after angioplasty and may be better representations of the pathological process involved during follow-up.[21,22]

Absolute gain and absolute loss represent the improvement in minimal luminal diameter achieved at intervention and the absolute change during follow-up, respectively, measured in mm. Absolute gain is defined as MLD after PTCA minus MLD before PTCA. Absolute loss is MLD after PTCA minus MLD at follow-up.

Relative gain and relative loss depict the improvement in minimal luminal diameter achieved at intervention and the change during follow-up, respectively, normalized for vessel size. Relative gain is (MLD after PTCA minus MLD before

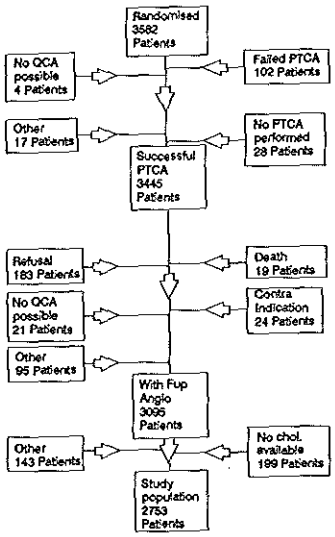


FIG 1. Flow chart of 3582 randomized patients. In 102 patients, angioplasty (angio) procedure was unsuccessful; in 28, percutaneous transluminal coronary angioplasty (PTCA) was not performed; in 25, quantitative analysis was not possible; and in 199, no cholesterol level was available. The study population comprised 2753 patients with an angiographic follow-up rate of 80% of successful procedures. QCA indicates quantitative coronary arteriography; Fup, follow-up; and chol., cholesterol.

PTCA) divided by vessel size. Relative loss is (MLD after PTCA minus MLD at follow-up) divided by vessel size.

The absolute net gain is the MLD at follow-up minus MLD before PTCA. The net gain index is the net gain normalized for the vessel size. Net gain index is (MLD at follow-up minus MLD before PTCA) divided by vessel size.

Statistical Analysis

Data were analyzed by the SAS statistical software package. All values are expressed as mean±SD. A χ^2 test was used to assess differences in categorical variables. Student's t test was used to assess differences in continuous variables between two groups or one-way ANOVA for more than two groups. Serum total cholesterol, HDL and LDL cholesterol, and LDL:HDL ratio were evaluated by linear regression analysis for their relation with absolute and relative loss during follow-up as well as absolute net gain and the net gain index. A lesion-based analysis was used, but since multiple lesions within a given patient are not independent with respect to their cholesterol level, repeat patient-based analysis using a single randomly selected lesion in patients with multivessel angioplasty was performed if the previous lesion-based analysis suggested statistical significance. Values of $P<.05$ were considered significant.

Results

Patient Characteristics and Procedural Results

The study population comprised 2753 patients (3336 lesions, 1.21 lesions per patient) who successfully completed the study (Fig 1). Of the 3582 patients initially randomized, the angioplasty procedure was unsuccessful in 102 patients, PTCA was not performed in 28, quantitative analysis was not possible in 25, no cholesterol level was available in 199, and 475 were excluded for other reasons, such as patient refusal for follow-up angiography, no quantitative coronary arteriography possible, or left main disease. Thus, the overall angiographic restudy rate was 77% of all patients initially randomized and 80% of all patients undergoing successful PTCA and no other interventional procedure. Within the study population, 160 patients with 191 lesions (5.73%) had hypercholesterolemia (mean total cholesterol, 8.46±0.75 mmol·L^{-1}) and 2593 patients with 3145 lesions (94.27%), normal cholesterol (5.67±1.06 mmol·L^{-1}) at trial entry. The clinical and angiographic characteristics of the patient population according to the presence or absence of hypercholesterolemia are summarized in Tables 2 and 3.

TABLE 2. Clinical Variables in Patients With and Without Hypercholesterolemia*

Clinical Variable	Cholesterol <7.8 mmol/L	Cholesterol >7.8 mmol/L
No. of patients	2593	160
Men, %	82.0	73.8
Age, y	57±9.3	55.3±9.3
Ever smoked, %	45.3	57.5
Current smoker, %	10.2	11.2
History of hyperlipidemia, %	31.4	48.4†
History of diabetes, %	10.2	9.4
History of previous MI, %	41.7	36.9
Previous PTCA, %	2.7	0
Previous CABG, %	2.9	3.2
CCS class, %		
0	2.5	. . .
I	13.9	14.9
II	35.4	38.0
III	33.2	36.4
IV	15.0	10.7
Drug therapy at trial entry, %		
Nitrates	18.9	17.9
β-Blockers	26.3	25.2
Calcium antagonists	35.9	40.4
Lipid-lowering agents	9.7	17.2
Drug therapy at 6 months, %		
Nitrates	21.5	18.7
β-Blockers	35.2	30.7
Calcium antagonists	40.8	30.7†
Lipid-lowering agents	13.9	26.7†

MI indicates myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass graft; and CCS, Canadian Cardiovascular Society anginal classification.
*Serum total cholesterol >7.8 mmol·L^{-1}.
†$P<.05$.

The incidence of known hyperlipidemia, as would be expected, was significantly higher in the high-cholesterol group; otherwise, the two groups were comparable in terms of known risk factors for coronary artery disease. In particular, smoking, either past or current,

TABLE 3. Lesion Location, Angiographic Characteristics, and Quantitative Angiographic Analysis of 160 Patients (191 Lesions) With and 2593 Patients (3145 Lesions) Without Hypercholesterolemia*

Angiographic Parameter	Cholesterol <7.8 mmol/L (n=3145)	Cholesterol >7.8 mmol/L (n=191)	P
Lesion location, %			
LAD	42.9	42.9	NS
Circumflex	25.0	24.6	NS
RCA	32.1	32.5	NS
Lesion characteristics, %			
Concentric	47.1	47.2	NS
Tandem lesion	4.2	4.2	NS
Branch point lesion	59.2	58.4	NS
Calcification in lesion	12.33	9.0	NS
Presence of thrombus	4.4	3.9	NS
Total occlusion pre-PTCA	7.4	7.9	NS
Dissection post-PTCA	34.6	27.5	NS
Vessel size, mm			
Minimal luminal diameter, mm	2.60±0.53	2.59±0.52	NS
Before angioplasty	1.00±0.39	0.98±0.39	NS
After angioplasty	1.75±0.36	1.75±0.34	NS
At follow-up	1.44±0.58	1.44±0.59	NS
Differences in MLD			
Absolute gain, mm	0.75±0.41	0.76±0.43	NS
Relative gain	0.30±0.16	0.30±0.16	NS
Absolute loss, mm	0.32±0.53	0.31±0.53	NS
Relative loss	0.13±0.21	0.12±0.20	NS
Absolute net gain, mm	0.17±0.23	0.18±0.24	NS
Net gain index	0.17±0.23	0.18±0.24	NS
Percent stenosis, %			
Before angioplasty	60.92±14.47	61.36±14.64	NS
After angioplasty	33.39±8.40	33.64±7.88	NS
At follow-up	45.86±19.16	45.47±19.46	NS
DS at follow-up >50%, %	33.7	31.9	NS

LAD indicates left anterior descending coronary artery; RCA, right coronary artery; PTCA, percutaneous transluminal coronary angioplasty; MLD, minimal luminal diameter; and DS, diameter stenosis.

*Serum total cholesterol >7.8 mmol · L^{-1}.

was present in about two thirds of the population and was similar in the two study groups. The anginal class of the two study groups also did not differ to any significant extent, most being in Canadian Cardiovascular Society anginal class II to III. Finally, there was no significant difference in drug therapy, although, perhaps not surprisingly, more patients with high cholesterol were taking a lipid-lowering agent (Table 2). By the time of the follow-up angiography, this difference persisted, but in addition, a significantly lower percentage of patients with hyperlipidemia were taking calcium antagonists. The disease was predominantly mild to moderate, with 63% of the total population having one-vessel disease, 31% two-vessel disease, and only 6% three-vessel disease. Lesion characteristics were comparable in the two groups, with a similar incidence of concentric, tandem, and branch point lesions, thrombus present before- or after- PTCA, total occlusions before PTCA, and dissections after PTCA (Table 3).

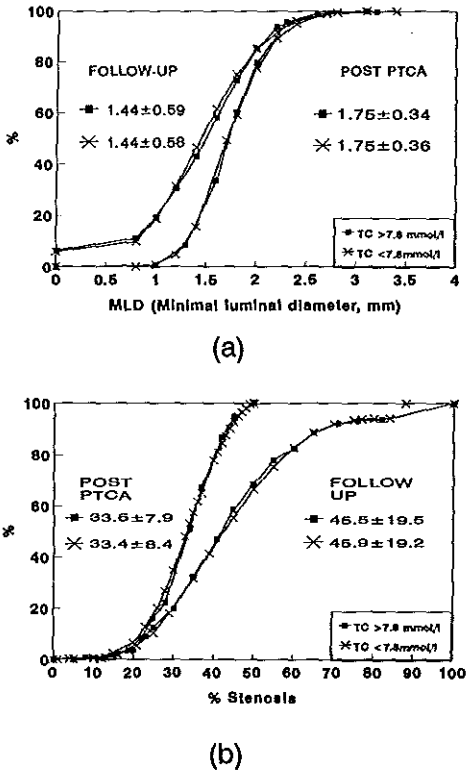
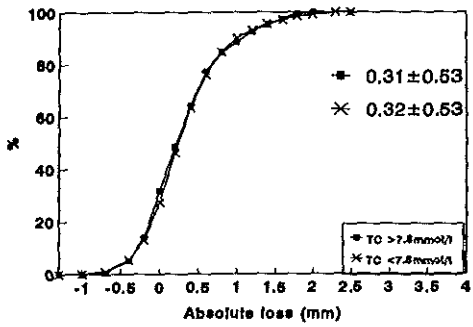


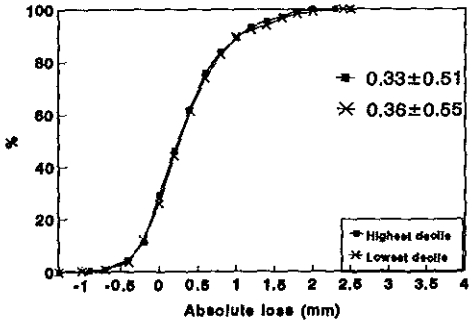
FIG 2. (a), Cumulative distribution curve of minimal luminal diameter (MLD) after percutaneous transluminal coronary angioplasty (PTCA) and at follow-up for patients with and without hypercholesterolemia. (b), Cumulative distribution curve of percentage stenosis after PTCA and at follow-up for patients with and without hypercholesterolemia. TC indicates total cholesterol.

Quantitative Angiographic Analysis at Baseline and 6 Months

A mean of 2.12 matched angiographic projections per lesion had satisfactory quantitative analysis performed at the central angiographic core laboratory before PTCA, after PTCA, and at follow-up (Table 3). The distribution of lesions was similar in the two groups, with around 43% in the left anterior descending coronary artery, 32% in the right coronary artery, and 25% in the circumflex artery. There were no significant differences in the MLD and percent stenosis before PTCA between patients with and patients without hypercholesterolemia, suggesting that the two groups were well matched angiographically. After PTCA, the MLD, percent residual stenosis, and absolute and relative gains were similar for the two groups (Table 3, Fig 2),



(a)



(b)

FIG 3. (a), Cumulative distribution curve of absolute loss for patients with and without hypercholesterolemia. (b), Cumulative distribution curve of absolute loss for patients in the top and bottom deciles of total cholesterol (TC).

again confirming the similarity in acute angiographic outcome between the two groups. Similar results were also obtained with patient rather than lesion analysis.

Restenosis According to the Presence of Hypercholesterolemia at Trial Entry

At the 6-month angiographic follow-up, the overal restenosis rate for the study population was 33.6% by the categorical (>50% stenosis at follow-up) approach. The MLD at follow-up was 1.44±0.59 mm for patients with and 1.44±0.58 mm for patients without hypercholesterolemia (Fig 2). The percent stenosis at follow-up was again similar, 45.47±19.46% for patients with and 45.86±19.16% for patients without hypercholesterolemia (Fig 2). The absolute loss was also similar for the two groups (Fig 3a), as were the relative loss, net gain, and net gain index (Table 3). By the criterion of >50% diameter stenosis at follow-up, there were no differences in restenosis rates between patients with and without hypercholesterolemia (31.9% versus 33.7%, respectively; relative risk, 0.975; 95% CI, 0.882 to 1.077; P=.68). Additionally, the total serum cholesterol in patients with restenosis (according to the criterion of >50% diameter stenosis at follow-up) was similar to those without restenosis (5.84±1.24 versus 5.81±1.22 mmol/L, respectively, P=NS).

Deciles According to Total Cholesterol at Trial Entry

Since definitions for hypercholesterolemia vary[18,23] and previous population studies suggest that even within the total cholesterol population there is a gradation of atherosclerosis risk,[5,6] the population was divided into deciles according to total cholesterol at trial entry to further examine the role of serum cholesterol on restenosis and whether there is any evidence for a nonlinear relation between the two. There were no significant differences between groups in either the categorical restenosis rate or the absolute or relative loss (Table 4). The net gain and net gain index were also similar in all groups. Additionally, cumulative distribution curves of the top and bottom deciles for cholesterol showed no differences in either MLD at follow-up, percent stenosis at follow-up (Fig 4), or absolute loss (Fig 3b).

Linear Regression Analysis of Total Cholesterol

Linear regression analysis was used to further evaluate the relation between total cholesterol at trial entry

TABLE 4. Restenosis Rates, Absolute Loss, Relative Loss, Net Gain, and Net Gain Index Per Decile According to Total Cholesterol Level

Decile Rank	n	TC, mmol/L	RR, %	Absolute Loss, mm	Relative Loss	Net Gain, mm	Net Gain Index
1	337	3.73±0.68	35.9	0.36±0.55	0.14±0.22	0.15±0.23	0.16±0.29
2	329	4.62±0.13	35.0	0.34±0.52	0.13±0.20	0.18±0.22	0.16±0.19
3	330	5.02±0.11	33.3	0.34±0.51	0.14±0.21	0.17±0.23	0.13±0.23
4	338	5.37±0.09	36.7	0.32±0.52	0.13±0.21	0.17±0.23	0.16±0.23
5	332	5.67±0.09	30.1	0.31±0.50	0.13±0.20	0.17±0.23	0.18±0.19
6	341	5.95±0.09	32.0	0.31±0.53	0.12±0.22	0.17±0.23	0.17±0.24
7	327	6.23±0.09	36.1	0.33±0.54	0.14±0.22	0.16±0.24	0.20±0.23
8	335	6.57±0.12	31.0	0.27±0.55	0.11±0.23	0.18±0.24	0.15±0.27
9	334	7.06±0.16	32.3	0.26±0.54	0.11±0.21	0.18±0.22	0.16±0.21
10	333	8.08±0.72	32.3	0.33±0.51	0.13±0.21	0.17±0.23	0.20±0.19

TC indicates total cholesterol; RR, restenosis rate.

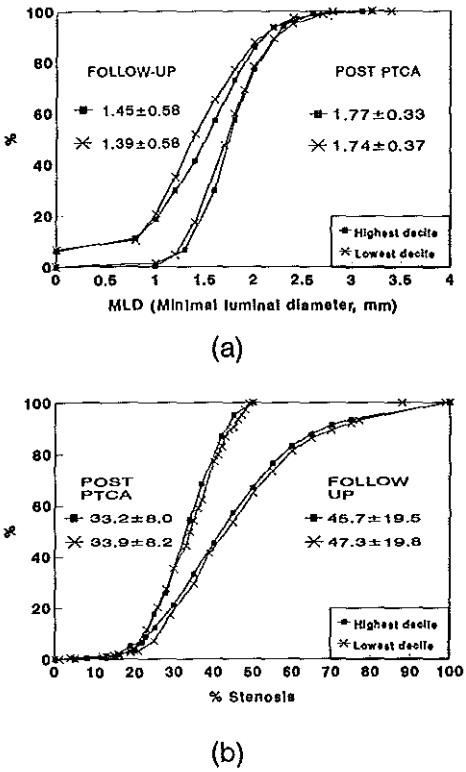


Fig 4. (a), Cumulative distribution curve of minimal luminal diameter (MLD) after percutaneous transluminal coronary angioplasty (PTCA) and at follow-up for patients in the top and bottom deciles of total cholesterol. (b), Cumulative distribution curve of percentage stenosis after PTCA and at follow-up for patients in the top and bottom deciles of total cholesterol.

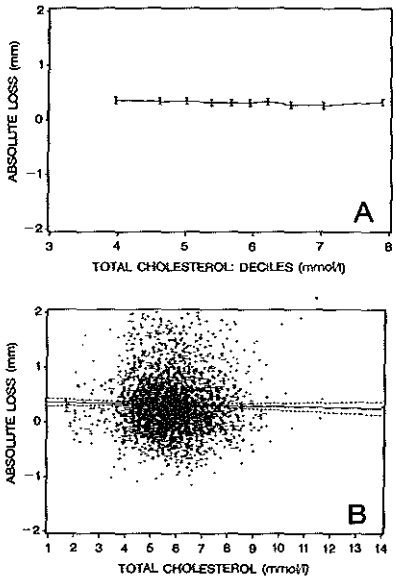


Fig 5. Plots showing relation between absolute loss (y axis) and total cholesterol (x axis). A, Deciles; mean with 95% confidence intervals. B, Scattergram with regression line and 95% confidence intervals.

and absolute loss (Fig 5), as well as relative loss, net gain, and net gain index (Table 5). There were no significant relations between any of the measured variables and total cholesterol (Table 5).

The change in cholesterol level during follow-up was normally distributed around the zero mark. There was a high correlation between cholesterol level at trial entry and at the 6-month angiographic follow-up (r=.61, P<.00001). Linear regression analysis of the cholesterol level at 6 months, the mean cholesterol level, and the change in cholesterol level over this period again failed to demonstrate any relation between these and any angiographic parameters of restenosis (Table 5).

Exclusion of Covert Influence of Trial Medication

To exclude any covert influence by the trial medication on lipid levels, subgroup analysis was performed, examining the change in cholesterol levels (mmol · L^{-1}) from trial entry to the 6-month follow-up in the separate studies. There were no significant differences between the active drug groups (A) and the placebo groups (P) in any study [CARPORT, 0.13±1.19 (A) versus 0.06±1.05 (P); MARCATOR, 0.10±1.02 versus 0.11±1.05; MERCATOR, 0.13±1.07 versus 0.03±1.05; PARK, 0.05±0.97 versus 0.07±1.07].

LDL and HDL Subgroup Analysis

Subgroup analysis was performed on 667 lesions in 579 patients in whom cholesterol subfractions were available to examine the influence of LDL cholesterol, HDL cholesterol, and LDL:HDL cholesterol ratio on restenosis during follow-up. This patient population was divided into deciles according to these cholesterol parameters, and we examined for differences between deciles for restenosis rates, absolute loss, relative loss, net gain, and net gain index (Table 6). There were no significant differences in any of the examined parameters between deciles or between patients in the top and bottom deciles for HDL and LDL cholesterol or LDL:HDL ratio.

Linear regression analysis was used to further evaluate the relation between LDL (Fig 6), HDL (Fig 7), and LDL:HDL ratio (Fig 8) and absolute loss as well as relative loss, net gain, and net gain index (Tables 7, 8, and 9). There was a negative relation between increasing LDL levels and absolute loss (Fig 6, Table 7). This negative relation between increasing LDL levels and absolute as well as relative loss (Fig 6, Table 7) persisted even when the data were analyzed on a population basis (P=.03 and P=.02, respectively). The negative relation between increasing LDL levels and absolute loss was due solely to 29 lesions that had occluded at follow-up angiography. When these were removed from the analysis, there was no longer any significant relation between increasing LDL levels and absolute loss (P=.264). Interestingly, the mean LDL cholesterol level was signif-

TABLE 5. Linear Regression Analysis to Evaluate the Respective Contribution of Total Cholesterol Before PTCA, at 6 Months, Mean Total Cholesterol, and Change in Total Cholesterol to Absolute Loss, Relative Loss, Net Gain, and Net Gain Index

Variable	Intercept	Regression Coefficient	Standard Error of Regression Coefficient	P	R^2
Total cholesterol before PTCA					
Absolute loss	0.38	−0.010	0.008	.20	.0005
Relative loss	0.15	−0.003	0.003	.30	.0003
Net gain	0.40	+0.007	0.008	.43	.0002
Net gain index	0.15	+0.003	0.003	.38	.0002
Total cholesterol at 6 months					
Absolute loss	0.35	−0.007	0.008	.37	.0003
Relative loss	0.14	−0.003	0.003	.34	.0003
Net gain	0.42	+0.005	0.009	.59	.0001
Net gain index	0.16	+0.002	0.004	.53	.0001
Mean total cholesterol					
Absolute loss	0.39	−0.012	0.008	.14	.0007
Relative loss	0.15	−0.005	0.003	.18	.0006
Net gain	0.38	+0.010	0.009	.29	.0003
Net gain index	0.15	+0.004	0.004	.27	.0004
Change in total cholesterol					
Absolute loss	0.31	−0.000	0.009	.99	.0000
Relative loss	0.12	−0.001	0.004	.72	.0000
Net gain	0.45	+0.003	0.010	.74	.0000
Net gain index	0.17	+0.001	0.004	.82	.0000

PTCA indicates percutaneous transluminal coronary angioplasty.

icantly lower in lesions that occluded at follow-up (3.61±1.59 versus 4.13±1.26 mmol · L^{-1}, P=.03). Logistic regression analysis was also suggestive of a relation between lower LDL levels and probability of occlusion at follow-up (P=.033). Although there was a significant relation between HDL cholesterol at 6 months and absolute as well as relative loss and net gain index (Table 8), this relation became insignificant once the data were analyzed on a patient rather than a lesion basis (P=.07, .06, and .09, respectively). There was no relation between LDL:HDL cholesterol ratio and any of the angiographic parameters of restenosis (Table 9).

Discussion

Our study has demonstrated that there is no significant association between restenosis, by either a categorical or continuous approach, and serum cholesterol levels. This is in a large patient population with 80% quantitative angiographic follow-up at a predetermined time interval. Although the study has a number of limitations, it does suggest that measures aimed at reducing total cholesterol are unlikely to significantly influence postangioplasty restenosis.

Epidemiological, pathological, and experimental studies have shown that serum total cholesterol is a causal factor in atherosclerosis and ischemic heart disease.[23] Furthermore, evidence from the MR-FIT study[5] suggests that there is a continuous curvilinear relation between serum total cholesterol and mortality from ischemic heart disease. It is not surprising, therefore, that if restenosis after angioplasty is viewed as an accelerated form of atherosclerosis, risk factors for coronary artery disease should also be risk factors for this process.

A number of studies have previously attempted to examine the relation between restenosis and serum lipid levels (Table 1). Shah and Amin[10] found a strong correlation between a low HDL cholesterol level and the subsequent risk of restenosis. The study was limited, however, by the categorical definition of restenosis used (>70% diameter stenosis at follow-up) and the poor angiographic follow-up rate of 37%. Reis and colleagues[9] found that in a group of 186 patients enrolled in a trial of fish oil for restenosis after angioplasty, serum lipid levels, in particular high triglyceride and a higher ratio of total cholesterol to HDL cholesterol were associated with an increased risk of clinical restenosis after angioplasty. Contrary to this, Johansson and coworkers,[8] in a group of 157 patients, did not find any significant relation between serum lipid levels and restenosis except in subgroup analysis, in which they found that low HDL cholesterol in men and high HDL cholesterol in women were associated with increased risk of restenosis. A number of other studies have been negative.[11,24]

Discrepancies between studies have arisen for a number of reasons. First, most have been retrospective analyses using small numbers of patients[11] and, as such, subject to a type B error. Second, they have almost invariably used visual assessment of the angiogram, which previous studies

TABLE 6. Restenosis Rates, Absolute Loss, Relative Loss, Net Gain, and Net Gain Index Per Decile According to (A) LDL Level, (B) HDL Level, and (C) LDL:HDL Ratio

Decile Rank	n	LDL-C, mmol/L	RR, %	Absolute Loss	Relative Loss	Net Gain	Net Gain Index
A							
1	50	1.70±0.70	30	0.35±0.68	0.14±0.29	0.45±0.64	0.17±0.25
2	49	2.82±0.18	45	0.45±0.61	0.19±0.24	0.29±0.62	0.12±0.26
3	50	3.32±0.12	30	0.32±0.44	0.13±0.19	0.41±0.54	0.15±0.20
4	49	3.78±0.12	29	0.30±0.51	0.12±0.20	0.42±0.51	0.16±0.19
5	53	4.09±0.07	40	0.34±0.48	0.13±0.20	0.40±0.63	0.15±0.26
6	48	4.32±0.05	38	0.28±0.57	0.11±0.21	0.43±0.52	0.17±0.20
7	46	4.51±0.09	24	0.24±0.58	0.09±0.24	0.48±0.61	0.20±0.24
8	50	4.87±0.11	36	0.22±0.46	0.09±0.18	0.46±0.58	0.17±0.22
9	49	5.31±0.13	29	0.24±0.51	0.10±0.23	0.53±0.55	0.20±0.26
10	51	6.31±0.68	27	0.27±0.48	0.10±0.19	0.44±0.53	0.16±0.20
B		HDL-C					
1	57	0.65±0.07	47	0.43±0.54	0.18±0.22	0.39±0.49	0.16±0.20
2	57	0.79±0.02	25	0.26±0.52	0.09±0.19	0.54±0.55	0.20±0.21
3	61	0.87±0.03	33	0.29±0.60	0.11±0.23	0.39±0.64	0.15±0.24
4	55	0.95±0.02	33	0.28±0.61	0.12±0.27	0.44±0.55	0.17±0.23
5	56	1.02±0.02	36	0.30±0.52	0.13±0.24	0.41±0.56	0.14±0.22
6	61	1.10±0.02	34	0.28±0.47	0.11±0.20	0.39±0.61	0.15±0.25
7	61	1.20±0.02	30	0.21±0.48	0.09±0.19	0.54±0.44	0.21±0.16
8	54	1.28±0.03	26	0.23±0.52	0.09±0.20	0.56±0.69	0.21±0.26
9	64	1.41±0.05	38	0.33±0.50	0.13±0.21	0.37±0.48	0.14±0.21
10	53	2.11±1.09	32	0.40±0.65	0.15±0.26	0.36±0.64	0.14±0.26
C		LDL:HDL Ratio					
1	49	1.08±0.61	31	0.45±0.76	0.18±0.31	0.41±0.74	0.16±0.29
2	49	2.36±0.23	24	0.28±0.58	0.11±0.24	0.42±0.51	0.16±0.19
3	49	2.95±0.11	39	0.35±0.52	0.14±0.21	0.35±0.60	0.13±0.23
4	51	3.35±0.11	35	0.31±0.50	0.12±0.21	0.41±0.56	0.16±0.23
5	48	3.68±0.08	40	0.31±0.45	0.12±0.17	0.45±0.47	0.18±0.19
6	50	4.03±0.12	20	0.21±0.51	0.08±0.20	0.47±0.61	0.17±0.24
7	47	4.43±0.12	34	0.25±0.43	0.10±0.18	0.49±0.52	0.20±0.23
8	52	4.95±0.15	40	0.27±0.57	0.12±0.25	0.40±0.64	0.15±0.27
9	49	5.81±0.31	29	0.37±0.56	0.14±0.21	0.41±0.54	0.16±0.21
10	48	7.58±1.18	33	0.23±0.42	0.09±0.16	0.53±0.52	0.20±0.19

have shown to have wide interobserver and intraobserver variability.[13] Furthermore, the angiographic follow-up rate has been generally poor.[10,13] Finally, and most importantly, they have used varying but always categorical definitions of restenosis, such as >50% diameter stenosis at follow-up,[8] >70% diameter stenosis at follow-up,[9,10] or loss >50% acute gain.[7,25] Although the >50% diameter stenosis at follow-up criterion may give an indication of the functional significance of a stenosis at the time of follow-up angiography, it provides little information about the biological process involved, which hyperlipidemia would be expected to influence. Furthermore, the use of percent measurements is inherently flawed, since it relies on the normal vessel wall, which may itself undergo restenosis.[26] Finally, arbitrary categorical definitions may be misleading. For example, if we have two lesions of identical reference diameter (3 mm), successfully dilated to a 40% (1.8-mm) and 25% (2.25-mm) residual stenosis, respectively, if both undergo the same reduction in luminal diameter of 0.6 mm during follow-up, the first lesions would be classified as restenosis (1.2 mm, 60% stenosis) and the second as no restenosis (1.65 mm, 45% stenosis) even though the absolute luminal loss is the same in both lesions.

We thus believe that although the clinician is best served by the "present/not present" assessment of restenosis, the biological process itself may be best analyzed by measurement of absolute angiographic dimensions or changes in absolute angiographic dimensions on a continuous scale.[21,22] This is for two main reasons. First, as

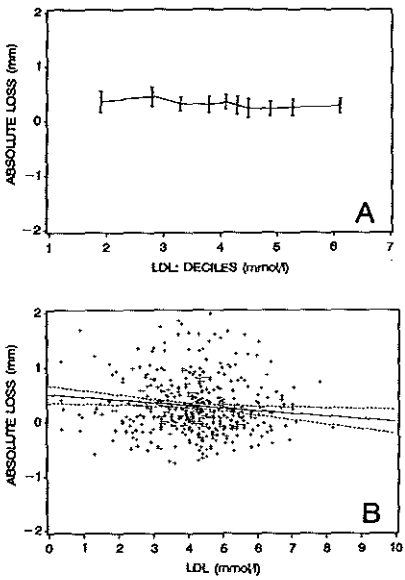


FIG 6. Plots showing relation between absolute loss (*y* axis) and LDL cholesterol (*x* axis). A, Deciles; mean with 95% confidence intervals. B, Scattergram with regression line and 95% confidence intervals.

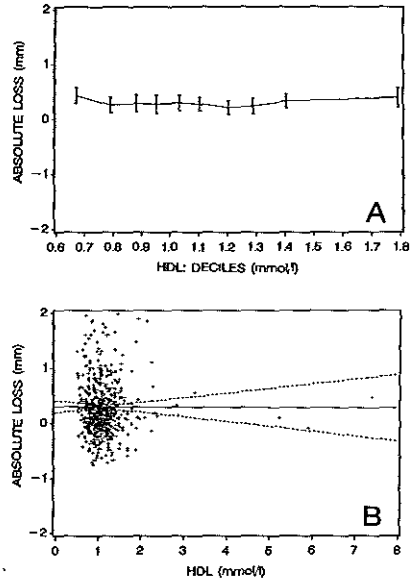


FIG 7. Plots showing relation between absolute loss (*y* axis) and HDL cholesterol (*x* axis). A, Deciles; mean with 95% confidence intervals. B, Scattergram with regression line and 95% confidence intervals.

we[27] and others[28] have previously demonstrated, all lesions undergo restenosis to some extent during follow-up, in a gaussian distribution. Second, if we treat restenosis as a continuous variable, more information can be gleaned from the available data regarding the underlying process itself.[22] Furthermore, we have further expanded on experimental[29] and pathological evidence[30] suggesting that a greater acute gain after intervention is associated with a greater loss during follow-up by introducing the concept of relative gain to represent vessel injury and relative loss to represent "intimal hyperplasia."[31] Thus, the application of quantitative angiography and the principles of absolute and relative loss provides a useful tool for the objective measurement of the degree of biological renarrowing during the weeks and months after intervention.[21,22]

The application of this to our data allows us to comment on the biological processes occurring after intervention and examine the hypothesis that hyperlipidemia influences restenosis in detail. We have shown that hypercholesterolemia is not a significant risk factor for restenosis. Furthermore, we have shown that within the angioplasty population, there is no significant relation between serum cholesterol before PTCA and at 6-month follow-up, mean cholesterol level or change in cholesterol level during follow-up, and restenosis. Subgroup analysis also demonstrated that HDL and LDL:HDL ratios have no significant influence on subsequent restenosis, although there was a negative relation between LDL cholesterol level and absolute loss. Finally, we have shown that even if we take the two extreme deciles, again there is no difference in any angiographic measure of restenosis. Thus, the assumption that total cholesterol, a risk factor for atherosclerosis, may also be a risk factor for restenosis after angioplasty has not been confirmed by this study.

The negative relation between increasing LDL levels and absolute loss is interesting and was due solely to 29 lesions that had occluded at follow-up angiography. When these were removed from the analysis, there was no longer any significant relation between increasing LDL levels and absolute loss. Intriguingly, the mean LDL cholesterol level was significantly lower in lesions with than without occlusion at follow-up. This is a paradoxical finding, the reasons for which are unclear. Although LDL cholesterol is known to promote platelet aggregation[32] and increases in plasma viscosity have been reported in hyperlipidemic patients,[33] it is unclear why a low LDL cholesterol should increase the likelihood of occlusion at follow-up angiography. Furthermore, this goes against recent hypotheses that LDL cholesterol reduction, by depleting lipid from fatty lesions prone to rupture, stabilizes the atherosclerotic plaque.[34] We are unable to explain this finding, particularly since the occlusions at follow-up are likely to have been thrombotic events. There is evidence in the literature, however, to suggest that there may be a J-shaped LDL coronary heart disease risk function that puts a sizable subset of these patients with low LDL cholesterol at increased risk of cardiac death associated with lifestyle characteristics.[35] Similar mechanisms may have been operating in our cohort of patients.

There are a number of possible reasons why cholesterol, a risk factor for atherosclerosis, is not a risk factor for restenosis after angioplasty. First, although cholesterol has been shown to be a risk factor for atherosclerosis, this is over the course of years,[5,6] whereas careful

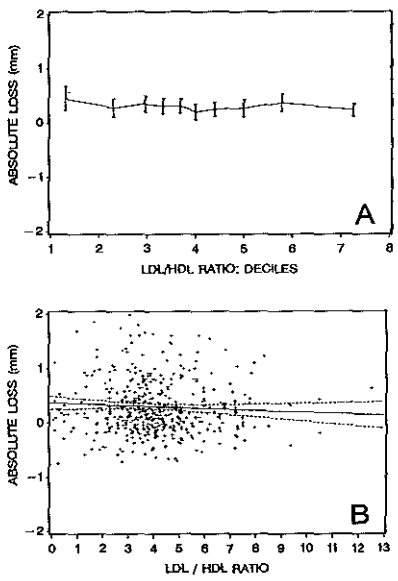


FIG 8. Plots showing relation between absolute loss (*y* axis) and LDL:HDL cholesterol ratio (*x* axis). A, Deciles; mean with 95% confidence intervals. B, Scattergram with regression line and 95% confidence intervals.

serial quantitative angiographic studies have shown restenosis to occur in the first 3 to 6 months after intervention.[1,36] Thus, total cholesterol may have little influence on the process over this short time frame. Second, the mechanisms of restenosis are still incompletely understood and are likely to involve slow elastic recoil,[37] thrombus incorporation,[38,39] vessel remodeling,[40] and late myointimal hyperplasia.[2,30] Since the possible contribution of each of these factors may vary within individual patients as well as within the general population, it is perhaps not surprising that total cholesterol, which is likely to influence only late myointimal hyperplasia, is not significantly related to restenosis. Furthermore, although quantitative coronary angiography is the most objective and reproducible method currently available to describe the changes in stenosis geometry after intervention, it measures the process only indirectly without defining its actual nature (recoil, organized mural thrombus, neointimal hyperplasia). Finally, restenosis is likely to be a multifactorial process with different contributions from different factors, some of which are ill understood. For example, most multivariate models can explain only a low percentage of the available variance in the measurements, even when all available variables are incorporated.[41,42] The most accurate way to quantify restenosis would be with the direct measurement of neointimal thickening after coronary intervention. This, however, is not currently possible in humans, although the advent of new imaging modalities such as intravascular ultrasound,[43,44] particularly with

TABLE 7. Linear Regression Analysis to Evaluate the Respective Contribution of LDL Cholesterol Before PTCA, at 6 Months, Mean LDL Cholesterol, and Change in LDL Cholesterol to Absolute Loss, Relative Loss, Net Gain, and Net Gain Index

Variable	Intercept	Regression Coefficient	Standard Error of Regression Coefficient	*P*	R^2
LDL cholesterol before PTCA					
Absolute loss	0.50	−0.046	0.019	.02	.0120
Relative loss	0.21	−0.020	0.008	.01	.0135
Net gain	0.35	+0.022	0.021	.29	.0024
Net gain index	0.13	+0.009	0.008	.30	.0023
LDL cholesterol at 6 months					
Absolute loss	0.34	−0.010	0.022	.64	.0005
Relative loss	0.14	−0.006	0.009	.52	.0009
Net gain	0.38	+0.017	0.023	.47	.0012
Net gain index	0.14	+0.008	0.009	.39	.0017
Mean LDL cholesterol					
Absolute loss	0.45	−0.034	0.021	.09	.0053
Relative loss	0.18	−0.015	0.008	.07	.0063
Net gain	0.41	+0.011	0.022	.63	.0004
Net gain index	0.15	+0.004	0.009	.62	.0005
Change in LDL cholesterol					
Absolute loss	0.30	+0.030	0.024	.21	.0041
Relative loss	0.12	+0.012	0.010	.22	.0039
Net gain	0.42	−0.030	0.025	.25	.0035
Net gain index	0.16	−0.013	0.010	.19	.0045

PTCA indicates percutaneous transluminal coronary angioplasty.

TABLE 8. Linear Regression Analysis to Evaluate the Respective Contribution of HDL Cholesterol Before PTCA, at 6 Months, Mean HDL Cholesterol, and Change in HDL Cholesterol to Absolute Loss, Relative Loss, Net Gain, and Net Gain Index

Variable	Intercept	Regression Coefficient	Standard Error of Regression Coefficient	P	R^2
HDL cholesterol before PTCA					
Absolute loss	0.32	−0.006	0.046	.90	.0000
Relative loss	0.13	−0.002	0.019	.92	.0000
Net gain	0.46	−0.018	0.048	.71	.0003
Net gain index	0.18	−0.007	0.019	.71	.0003
HDL cholesterol at 6 months					
Absolute loss	0.15	+0.151	0.072	.04	.0082
Relative loss	0.06	+0.059	0.029	.04	.0081
Net gain	0.59	−0.132	0.076	.08	.0057
Net gain index	0.24	−0.062	0.029	.04	.0083
Mean HDL cholesterol					
Absolute loss	0.26	+0.048	0.059	.42	.0011
Relative loss	0.10	+0.020	0.024	.41	.0011
Net gain	0.53	−0.066	0.063	.30	.0018
Net gain index	0.21	−0.030	0.025	.24	.0023
Change in HDL cholesterol					
Absolute loss	0.31	+0.040	0.047	.39	.0016
Relative loss	0.12	+0.016	0.019	.39	.0016
Net gain	0.43	−0.020	0.049	.68	.0004
Net gain index	0.17	−0.012	0.019	.55	.0008

PTCA indicates percutaneous transluminal coronary angioplasty.

three-dimensional reconstruction and quantitative analysis of plaque volume,[45] may one day make this possible.

Our finding that cholesterol level has no significant bearing on long-term restenosis has several important clinical implications. First, it explains why trials designed to reduce overall cholesterol level have failed to influence restenosis[46] and why studies still pending are negative.[47] Although one study has been positive,[48] this was in a small patient population with a poor follow-up rate; thus, the results were biased. Second, it suggests that although lipid lowering is generally desirable, lowering cholesterol and its subfractions per se will not reduce restenosis. Whether newer compounds such as lovastatin, which also act on the vessel wall, reducing smooth muscle cell migration and proliferation as well as affecting lipid subfractions, may be more effective remains to be answered. Finally, it suggests that, ideally perhaps, future studies should include intravascular ultrasound assessment of the acute results of intervention and the mechanism of subsequent restenosis, differentiating between slow recoil, thrombus formation/incorporation, and intimal hyperplasia, to have a a better likelihood of demonstrating benefit from lipid lowering.

Limitations of the Study

A number of limitations of the present study are to be acknowledged. First, the study was a retrospective analysis of prospectively gathered data and is hence subject to the limitations inherent in any retrospective analysis. Additionally, because of the nature of the data, we are also unable to comment on the importance or otherwise of any lipid subfractions, such as Lp(a), on long-term restenosis.[49] Second, all cholesterol measurements were performed locally at the participating center and not by a central laboratory, so we are unable to comment on the variability of these measurements. Since all participating centers were large, internationally recognized institutions previously vetoed for trial participation, we have assumed that the variability in cholesterol measurements was small. Furthermore, in view of the large numbers involved, this is unlikely to have been a significant limitation. Third, certain patients with normal cholesterol levels at study entry were on treatment for hypercholesterolemia either with diet or with cholesterol-lowering drug therapy. It is not clear what effect this may have had on our results, but the absence of any trend even when cholesterol levels were analyzed in terms of deciles makes it unlikely that this would have a significant influence on our results. Finally, data were amalgamated from the four previously described studies[14-17] to form our patient study population. We believe that this amalgamation of data is justified because the number of patients in each individual study was limited, the data amalgamated were those common to all studies, and the angiographic criteria were standardized, with one central angiographic core laboratory performing the quantitative angiographic analysis in all studies. Furthermore, it provides a unique opportunity to obtain accurate quantitative angiographic data at a predetermined time interval in a field where few such data exist to date.

TABLE 9. Linear Regression Analysis to Evaluate the Respective Contribution of LDL:HDL Cholesterol Ratio Before PTCA, at 6 Months, Mean LDL:HDL Cholesterol Ratio, and Change in LDL:HDL Cholesterol Ratio to Absolute Loss, Relative Loss, Net Gain, and Net Gain Index

Variable	Intercept	Regression Coefficient	Standard Error of Regression Coefficient	P	R^2
LDL:HDL cholesterol ratio before PTCA					
Absolute loss	0.37	−0.015	0.014	.28	.0025
Relative loss	0.15	−0.007	0.006	.23	.0031
Net gain	0.40	+0.010	0.015	.53	.0009
Net gain index	0.15	+0.004	0.006	.46	.0012
LDL:HDL cholesterol ratio at 6 months					
Absolute loss	0.41	−0.029	0.017	.09	.0065
Relative loss	0.16	−0.012	0.007	.07	.0075
Net gain	0.36	+0.021	0.018	.26	.0029
Net gain index	0.13	+0.009	0.007	.19	.0038
Mean LDL:HDL cholesterol ratio					
Absolute loss	0.41	−0.027	0.016	.09	.0054
Relative loss	0.17	−0.011	0.006	.07	.0064
Net gain	0.40	+0.013	0.017	.44	.0012
Net gain index	0.15	+0.006	0.007	.36	.0016
Change in LDL:HDL cholesterol ratio					
Absolute loss	0.30	−0.010	0.017	.54	.0010
Relative loss	0.12	−0.005	0.007	.49	.0013
Net gain	0.43	−0.003	0.018	.88	.0001
Net gain index	0.16	−0.002	0.007	.80	.0002

Conclusions

Our results indicate that there is no association, by either a categorical or continuous approach, between restenosis and serum cholesterol levels, suggesting that measures aimed at reducing total cholesterol are unlikely to significantly influence postangioplasty restenosis.

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Chapter 12

Influence of past and present smoking habits on six month clinical and angiographic outcome after successful coronary angioplasty.

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J Am Coll Cardiol (Re-submitted after peer review)

Abstract

Background: Although smoking is a well known risk factor for atherosclerosis it's influence on post angioplasty restenosis, is unclear.

Methods: We investigated the incidence of smoking on restenosis in 2,948 patients prospectively enroled and successfully completing 4 major restenosis trials utilising quantitative angiography prior, immediately post successful angioplasty and at six months

Results: Within the study population there were 530 current smokers, 1690 ex smokers and 728 patients non smokers. Smokers were more likely to be male (85.9% vs 87.5% vs 65.3%, current vs ex vs never, $p<0.001$), to be younger (54.0±9.0 vs 57.0±9.1 vs 59.9±9.4 years, $p<0.001$), to have peripheral vascular disease (7.2% vs 5.5% vs 2.3%, $p<0.001$), have sustained a previous myocardial infarction (42.9% vs 43.9% vs 37.9%, $p=0.022$) and be in a worse anginal class ($p=0.017$), but less likely to be diabetic (9.1% vs 9.5% vs 12.6%, $p=0.043$) or hypertensive (24.9% vs 29.3% vs 37.2, $p<0.001$). They were more likely to have the dilated lesion in the RCA (36.2% vs 34.1% vs 25.9%, $p=0.002$) and their lesions required a longer duration of inflation (344±288secs vs 323±264 vs 301±267, $p=0.021$) at higher pressures (8.7±2.5 vs 8.6±2.5 vs 8.2±2.5 atm, $p<0.001$). There was no significant difference in the categorical (>50%DS) restenosis rate at 6 months (35.28% vs 35.33% vs 37.09%, current vs ex vs never, or the absolute/relative loss (Absolute loss 0.29±0.54 vs 0.33±0.52 vs 0.35±0.55mm, $p=0.172$, relative loss 0.12±0.22 vs 0.13±0.21 vs 0.14±0.22 respectively, $p=0.085$).

Conclusion: Thus smokers, despite having a lower incidence of known predisposing risk factors for atherosclerosis, require coronary intervention almost 6 years earlier than non smokers and 3 years earlier than ex smokers. Once they undergo successful coronary angioplasty however their short term (six month) outcome is similar to non smokers.

Introduction

Restenosis after successful coronary angioplasty remains a major limitation of the technique (1,2). Post mortem and recent intracoronary ultrasound studies suggest that it involves a combination of slow elastic recoil, vessel remodelling, thrombus incorporation and late myointimal hyperplasia (3-7). Cigarette smoking can theoretically be involved in any of these mechanisms. Studies have shown that cigarette smoke can inhibit prostacyclin production by the vascular endothelial cells (8), impair endothelial function (9,10), activate platelets (11) and lower the baseline fibrinolytic activity in blood (12) thus enhancing platelet aggregation and thrombosis (9, 13), all of which may be involved in the restenosis process (14,15). Cigarette smoke and its constituents can also cause acute coronary vasoconstriction (16), substantially altering local flow dynamics at the angioplasty site resulting in increased platelet deposition and local thrombus formation (17), further increasing the possibilities of acute occlusion and long term restenosis (18). Because of these theoretical consideration and also the large body of experimental and clinical evidence linking smoking habits with atherosclerosis, resulting in an increased risk of myocardial infarction, unstable angina and sudden death (19-21), a number of studies have investigated the role of smoking on restenosis after successful coronary angioplasty (22-26). The results have been conflicting however with two studies suggesting a positive relationship (22,23) and others suggesting no relationship (24-26) between the two. Discrepancies between studies have arisen for a number of reasons. Firstly most have been retrospective analyses using small patient numbers and as such subject to a type B error (23). Second they have almost invariably used visual assessment of the angiogram which previous studies have shown to have wide inter- and intra- observer variability (27). Furthermore the angiographic follow up rate has been generally poor and performed for recurrence of symptoms (22, 24), thus introducing important selection bias. We attempted to overcome these limitations by using a validated automated edge detection technique to evaluate the effect of smoking habits on restenosis in a large series of patients undergoing successful balloon angioplasty and routine follow up angiographic assessment at a pre determined time interval.

Methods

Patients

The study population comprised 3,582 patients with significant primary stenoses in native coronary arteries who were prospectively enrolled into four major restenosis trials (28-31). These demonstrated that active therapy had no effect on restenosis or clinical outcome in the first 6 months after balloon angioplasty so for the purposes of this study the data for the active and placebo groups were pooled. Patients were eligible for study entry if they were symptomatic or asymptomatic

men, or women without child bearing potential, with stable or unstable angina pectoris and proved angiographically significant narrowing in one or more major coronary arteries. Informed consent was obtained in all cases before the coronary angioplasty procedure. Patients with developing myocardial infarction and significant left main disease were excluded from the study.

Smoking history

A history of smoking was requested as part of the routine work up. Patients were asked if they had ever smoked and whether they were continuing to smoke and their answers recorded on the data sheet. In a subgroup of 1048 patients (those taking part in the Park and Carport studies) smoking status was also ascertained at both the 1 and 6 month follow up visits.

Angioplasty procedure and follow up angiography

Coronary angioplasty was performed with a steerable, moveable guide wire system by the femoral route. Standard balloon catheters were used. The choice of balloon type and brand as well as inflation pressure and duration were left to the discretion of the operator. Patients were followed up for 6 months at which time a follow up study was performed. If symptoms recurred within 6 months coronary angiography was carried out earlier. If no definite restenosis was present and the follow-up time was below 4 months, the patient was asked to undergo further coronary arteriography at 6 months.

Quantitative angiography

Three coronary angiograms, in total, were obtained for each patient, pre-, post-PTCA and at angiographic follow up. The angiograms were recorded in such a manner that they were suitable for quantitative analysis by the computer assisted Coronary Angiography Analysis System (CAAS) which has been described and validated earlier (32). To standardise the method of data acquisition and to ensure exact reproducibility of the angiographic studies, measures were taken as previously described and all angiograms were processed in a central angiographic core laboratory (28-31). Because the computer algorithm is unable to measure total occlusions, a value of 0mm was substituted for the minimal lumen diameter and a value of 100% for the percent diameter stenosis pre PTCA. In these cases the post angioplasty reference diameter was substituted for vessel size.

Angiographic Definitions used

Vessel size refers to the reference diameter of the relevant coronary segment and is represented by the interpolated reference diameter pre-PTCA.

Minimum luminal diameter (MLD) is the point of maximal luminal narrowing in the analysed segment.

Restenosis was assessed using both a categorical and a continuous approach (33, 34). For the categorical approach we used a cut-off point of >50% diameter stenosis at follow up. For the continuous approach we examined the absolute and relative loss which may reflect the behaviour of the lesion during and after angioplasty better, and may therefore be better representations of the pathological process involved during follow up (33).

Absolute Gain and Absolute loss represent the improvement in minimal luminal diameter achieved at intervention and the absolute change during follow up respectively, measured in mm. Absolute Gain = MLD post PTCA - MLD pre PTCA. Absolute loss = MLD post PTCA - MLD at follow up.

Relative gain and relative loss depict the improvement in minimal luminal diameter achieved at intervention and the change during follow up respectively, normalized for vessel size. Relative gain = [MLD post PTCA - MLD pre PTCA]/Vessel size. Relative loss = [MLD post PTCA - MLD at follow up]/Vessel size.

The absolute net gain is the MLD at follow up - MLD pre-PTCA.

The net gain index is the net gain normalised for the vesel size. Net gain index= [MLD at follow up-MLD pre-PTCA]/Vessel size.
The loss index is the late loss expressed as a fraction of the acute gain (loss/gain).

Statistical analysis

Data was analysed using the SAS statistical software package. All values are expressed as mean value ± 1 standard deviation. Differences in categorical variables were assessed using the Chi-square test. Analysis of variance was used to assess differences in continuous variables between the 3 groups. Whenever the difference between 2 of the 3 subgroups were tested, Bonferroni correction was applied. Comparisons of ranked variables (clinical end points) were tested using the Kruskal-Wallis test. The difference in event free survival time between the three groups was evaluated using the Kaplan-Meier method with the log rank and Wilcoxon test. As multiple lesions within a given patient are not independent with respect to smoking, a patient based analysis, using a single, randomly selec-

ted lesion in patients with multivessel angioplasty, was performed. To study the relationship between a binary outcome parameter (the occurence of a clinical event) and multiple categorical and continuous determinants multiple logistic regression analysis was used. To study the relationship between continuous outcome parameters and multiple categorical and continuous determinants multiple linear regression analysis was used. p values <0.05 were considered significant.

Results

Baseline patient clinical characteristics

The study population comprised 2,948 patients (3,581 lesions, 1.22 lesions per patient) who successfully completed the study and had follow up quantitative angiography. The overall angiographic restudy rate was 86% of all patients undergoing successful PTCA. Within the study population there were 728 patients (889 lesions) who had never smoked 1690 patients who were ex smokers (2,057 lesions) and 530 patients who were still smoking (635 lesions) at the time of the index procedure. Within the subgroup of 1048 patients (those taking part in the Park and Carport studies) where smoking status was also ascertained at the 1 and 6 month follow up visits, 2 (0.72%) of 279 patients who never smoked previously, took up smoking by 1 month and continued to six months. Fifty two (8.79%) of 603 ex smokers restarted smoking by six months (26 (4.31%) by 1 month) whilst 64 (38.55%) of 166 current smokers had given up by the six month follow up visit (43 (25.90%) by 1 month).

The clinical and angiographic characteristics of the three groups are summarised in Tables 1 and 2.

Lesion type	Never smoked	Ex Smoker	Current smoker	Significance Level
Number of patients	728	1690	530	
Men	65.3%	87.5%	85.9%	**0.000**
Age (years).	59.9±9.4	57.0±9.1	54.0±9.0	**0.000**
Previous Myocardial Infarction	37.9%	43.9%	42.9%	**0.022**
Previous CABG	3.6%	4.6%	4.0%	0.470
Previous PTCA	4.1%	5.1%	5.5%	0.486
Diabetes mellitus	12.6%	9.5%	9.1%	**0.043**
Insulin dependent diabetes mellitus	1.8%	0.7%	0.4%	**0.009**
History of hypertension	37.2%	29.3%	24.9%	**0.000**
History of hypercholesterolaemia	32.0%	31.5%	31.9%	0.965
History of Peripheral vascular disease	2.3%	5.5%	7.2%	**0.000**
Anginal class				**0.032**
None	6.3%	5.8%	4.3	
CCS Class I	9.8%	11.7%	11.3%	
CCS Class II	34.6%	32.6%	29.4%	
CCS Class III	31.5%	29.9%	29.1%	
CCS Class IV	17.9%	20.2%	25.9%	
Duration of angina (weeks)	120±208	111±209	94±202	0.205
Days since deterioration of angina	80±198	79±181	65±127	0.524
Medication at screening				
Beta blockers	52.3%	49.2%	51.5%	0.321
Calcium antagonists	65.4%	70.4%	72.3%	**0.016**
Nitrates	62.1%	66.7%	66.2%	0.659
Anticoagulants	0.8%	1.5%	2.5%	0.067
Thrombocyte aggregation inhibitor	64.3%	63.5%	67.7%	0.203
Aspirin	77.9%	83.4%	83.1%	**0.032**
Persantin	10.6%	11.6%	15.2%	0.121
Laboratory Investigations				
Haemoglobin	8.72±0.86	8.89±0.80	8.99±0.86	**0.000**
Haematocrit	0.41±0.04	0.42±0.04	0.43±0.04	**0.000**
White cell count	7.05±2.94	7.34±2.09	8.26±2.24	**0.000**
Platelet count	260±80	255±66	258±70	0.276
Total cholesterol (mmol/l)	5.88±1.31	5.80±1.21	5.88±1.20	0.249
HDL cholesterol (mmol/l)	1.20±0.34	1.13±0.56	1.11±0.69	0.477
LDL cholesterol (mmol/l)	4.15±1.18	4.16±1.33	3.94±1.20	0.519

Table 1. Baseline clinical characteristics of current smokers, ex-smokers and non smokers. CCS- Canadian classification system. CABG- Coronary artery bypass graft. PTCA- Percutaneous transluminal coronary angioplasty. * $p < 0.05$ vs never, + $p < 0.05$ vs ex smoker, ^ $p < 0.05$ vs current

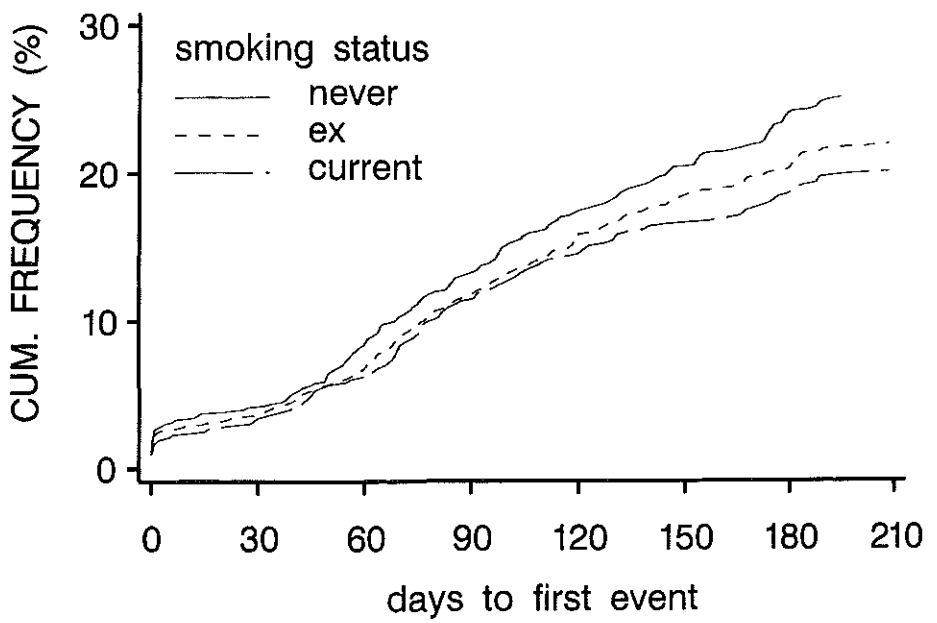
Lesion type	Never smoked	Ex Smoker	Current smoker	Significance Level
No of diseased vessels				0.347
1-VD	65.0%	62.9%	66.0%	
2-VD	26.4%	29.5%	25.7%	
3-VD	7.8%	7.2%	7.6%	
Lesion Location				**0.002**
LAD	50.6%	42.0%	41.1%	
LCx	22.8%	24.7%	23.8%	
RCA	26.5%	33.3%	34.9%	
Lesion characteristics				
Total occlusion pre PTCA	7.8%	7.5%	7.0%	0.852
Concentric	46.2%	43.8%	44.6	0.606
Tandem lesion	3.8%	4.2%	3.9%	0.871
Multiple Irregularities	8.3%	7.5%	8.4%	0.742
Branch point in stenosis	37.3%	32.2%	26.8%	0.054
Branch point in dilatation site	65.4%	65.5%	61.8%	0.635
Coronary artery bend	19.9%	19.7%	18.3%	0.772
Calcified lesion	12.5%	12.8%	10.2%	0.275
Thrombus visible (pre or post PTCA)	5.4%	4.5%	6.0%	0.316
Degree of collateral supply				**0.005**
No collaterals	78.5%	85.1%	87.7%	
Slight (minimal perfusion)	5.9%	4.2%	3.1%	
Medium (partial perfusion)	6.8%	4.4%	4.6%	
Major (complete perfusion)	4.0%	3.7%	1.6%	
Not assessed	4.8%	2.7%	2.9%	
PTCA procedure				
Nominal size of largest balloon (mm)	2.87±0.42	2.89±0.43	2.87±0.42	0.342
Balloon to artery ratio	1.13±0.18	1.12±0.18	1.12±0.19	0.402
Total number of inflations	3.6±2.6	3.6±2.2	3.8±2.4	0.412
Total duration of inflation (secs)	301±267	323±264	344±288*	**0.021**
Maximum Inflation pressure (atm)	8.2±2.5	8.6±2.5*	8.7±2.5*	**0.001**
Post PTCA result				
Dissection at the dilated site	37.5%	34.2%	31.3%	0.069
Dissection Type				0.255
Type A	16.0%	14.9%	15.7%	
Type B	16.7%	15.1%	11.1%	
Type C	4.1%	3.7%	4.0%	
Type D	0.2%	0.0%	0.0%	
Type E	0.2%	0.2%	0.0%	
Type F	0.2%	0.0%	0.0%	
Days to follow up	161±45	162±44	163±44	0.553

Table 2. Baseline angiographic and procedural data of current smokers, ex-smokers and non smokers. 2-VD Two vessel disease, 3-VD, three vessel disease. LAD, left anterior descending, LCx, left circumflex artery, PTCA, percutaneous transluminal coronary angioplasty, RCA, right coronary artery, SVD, Single vessel disease. *=p<0.05 vs never, + p <0.05 vs ex smoker, ^ p<0.05 vs current

There were marked differences in the baseline demographic characteristics of the three groups. There was a significantly higher proportion of men in the two smoking categories and they were also younger. In addition they were more likely to have had a previous myocardial infarction and a history of peripheral vascular disease. Smokers were also less likely to be diabetic or have a history of hypertension. There were also marked differences in anginal class with smokers more likely to have angina in CCS class III and IV and in medication with smokers and ex smokers more likely to be taking calcium antagonists and aspirin. Haematologically smokers were distinguished by having higher haemoglobin levels, haematocrit and white cell counts.

Clinical follow up.

One hundred and eight (20.3%) of the current smokers, three hundred and seventy five (22.2%) of the previous smokers and one hundred and eighty five (25.41%) of the non smokers had a clinical end points (Redo PTCA, CABG, Acute Myocardial Infarction or death) during follow up (p=0.085) When we compared the event free survival by way of the Log rank test the p value was 0.088 whilst the Wilcoxon test, which places more emphasis on early events rendered a p value of 0.095 which is comparable. The individual components for current, ex and non smokers were: death, 0% vs 0.12% vs 0.55%, myocardial infarction, 2.64% vs 2.66% vs 3.57%, Coronary artery bypass grafting, 1.89% vs 2.66% vs 2.75% and and re-PTCA, 15.85% vs 16.75% vs 18.54% respectively (p=0.057). The time course of clinical end points are summarised in Figure 1.



The mean time to clinical follow up was similar in the three groups (Current smokers 163±44, ex smokers 162±44, non smokers 161±45).
Figure 1. Cumulative distribution curve of clinical end points over time for current smokers, ex-smokers and non smokers.

In order to exclude the possibility of selection bias influencing our results we also examined the clinical end points in the 14% of the population in whom no QCA measurements were available, either pre, post or at follow up, and who were therefore excluded from the study population. Of these patients 22.5% of current smokers, 22.6% of previous smokers and 23.2% of the non smokers had a clinical end points (Redo PTCA, CABG, Acute Myocardial Infarction or death) during follow up. The difference was not statistically significant (p=0.99). The individual components of worse clinical end point such as death, myocardial infarction, Coronary artery bypass grafting and re-PTCA were 7.14%. 2.04%, 6.12% and 7.14% respectively for current smokers 3.17%, 4.37%, 4.76% and 10.32% for ex smokers and 0.89%. 3.57%, 7.14% and 11.61% for non smokers. The differences in the individual clinical end points between the three groups were not significant (p=0.340).

In order to exclude the possibility of cross over from the current smokers to ex smokers influencing our results we also examined the clinical end points in the sub group of the study population who stopped smoking after the index procedure and theoretically would have crossed over from the current smokers to the ex smokers group. Of these 27.7%, had a clinical end point compared to 23.8% of patients who continued to smoke (p=0.60)

Quantitative angiographic analysis and coronary angioplasty procedure.

A mean of 2.12 matched angiographic projections per lesion had satisfactory quantitative analysis performed, at the central angiographic core laboratory, pre-, post- PTCA, and at follow up (Table 2 & Table 3). The distribution of lesions was significantly different in the three groups with non smokers having more lesions in the LAD and less in the right coronary than smokers and ex smokers.
Non smokers were also more likely to have visible collaterals on baseline angiography. (Table 2)

There were no significant differences in the baseline quantitative angiographic measurements between the three groups apart from a borderline significant higher mld pre PTCA in current smokers (Table 3).

	Never smoked	Ex Smoker	Current smoker	Significance Level
Reference Diameter (mm)				
Before angioplasty	2.60±0.54	2.65±0.53	2.64±0.54	0.116
After angioplasty	2.65±0.52	2.69±0.50	2.67±0.51	0.129
At follow up	2.67±0.56	2.71±0.56	2.71±0.54	0.200
Minimal luminal diameter (mm)				
Before angioplasty	0.96±0.40	1.00±0.40	1.02±0.38	**0.046**
After angioplasty	1.75±0.37	1.78±0.36	1.76±0.34	0.144
At follow up	1.40±0.62	1.44±0.57	1.46±0.59*	0.096
Differences in MLD				
Absolute Gain	0.78±0.43	0.78±0.41	0.74±0.40	0.128
Relative Gain	0.31±0.16	0.30±0.16	0.29±0.15	0.074
Absolute Loss	0.35±0.55	0.33±0.52	0.29±0.54	0.172
Relative Loss	0.14±0.22	0.13±0.21	0.12±0.22	0.085
Absolute Net Gain	0.43±0.61	0.45±0.56	0.45±0.61	0.870
Net Gain Index	0.16±0.25	0.17±0.22	0.16±0.24	0.282
Loss Index	0.41±2.37	0.60±5.01	0.49±1.34	0.545
Percentage stenosis				
Before angioplasty	62.35±14.38	61.75±14.32	60.74±14.02	0.142
After angioplasty	33.57±8.43	33.55±8.33	33.70±7.81	0.929
At follow up	47.32±20.34	46.19±18.91	45.69±19.11	0.278
DS at follow up >50%	37.09%	35.33%	35.28%	0.687

Table 3. Quantitative angiographic analyses of current smokers, ex-smokers and non smokers. DS, diameter stenosis. MLD, Minimal luminal diameter. *=p<0.05 vs never.

Smokers however required a higher duration of inflation (current) and inflation pressure (both current and ex) for a successful angioplasty procedure. Post-PTCA all quantitative angiographic measurements and derived parameters were similar for the three groups (Table 3, Figure 2) again confirming the similarity in acute angiographic outcome.

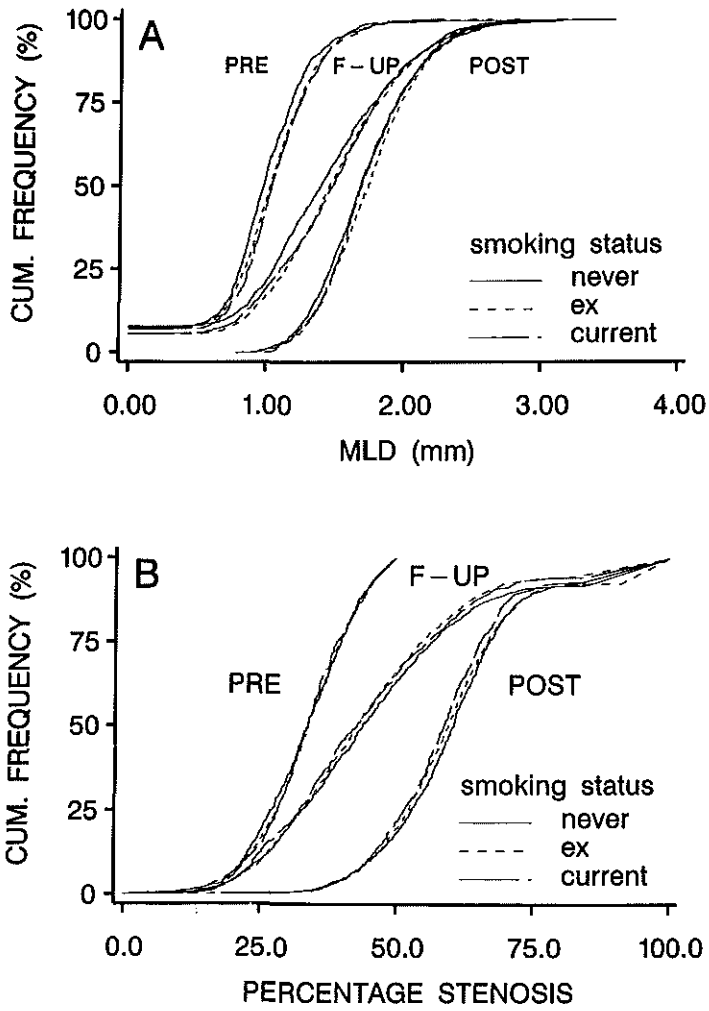


Figure 2. Upper panel: Cumulative distribution curve of MLD post PTCA and at follow up for current smokers, ex-smokers and non smokers. Lower panel: Cumulative distribution curve of percentage stenosis post PTCA and at follow up for current smokers, ex-smokers and non smokers.

At the 6 month angiographic follow up there was no significant difference in angiographic outcome between the three groups (Table 3, Figure 2). The overall restenosis rate for the study population was 35.8% using the categorical (>50% stenosis at follow up) approach (Current smokers 35.28%, Ex smokers 35.33%, non smokers 37.09%, p=0.687). Additionally the absolute and relative loss were also similar between current, ex smokers and non smokers (Absolute loss 0.29±0.54 vs 0.33±0.52 vs 0.35±0.55mm, p=0.172, relative loss 0.12±0.22 vs 0.13±0.21 vs 0.14±0.22 respectively, p=0.085) (Table 3, Figure 3).

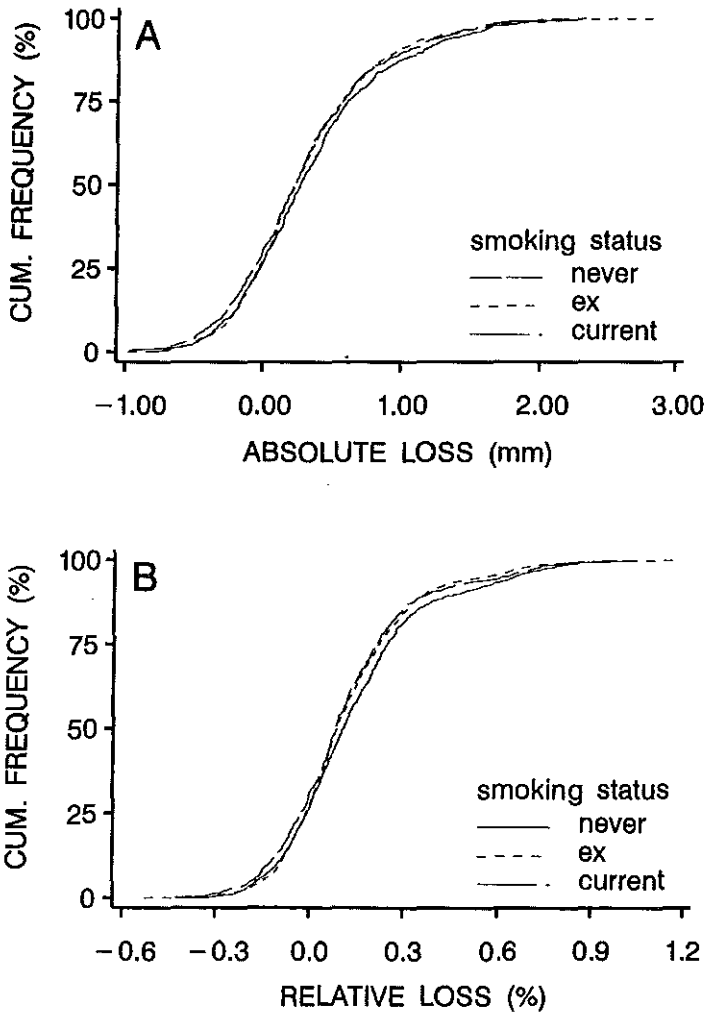


Figure 3. Upper panel: Cumulative distribution curve of absolute loss for current smokers, ex-smokers and non smokers. Lower panel: Cumulative distribution curve of relative loss for current smokers, ex-smokers and non smokers.

Multiple linear regression analysis

We have previously demonstrated that Vessel size, mld pre-PTCA, absolute gain and Lad location make a significant contribution to late angiographic outcome (35). Adding smoking to this model did not significantly improve it's predictive value. Least Squares means for absolute loss were 0.348, 0.311, 0.307 for non smokers, ex smokers and current smokers respectively. The p value of adding the variable smoking to the model was 0.15, which is not significant.

In order to ascertain whether the tendency towards improved clinical outcome in patients who smoke was related to differences in the underlying baseline characteristics we corrected for these variables to see whether smoking had any significant, independent, predictive value. We performed logistic regression with the above mentioned baseline characteristics as covariates resulting in a p value for the variable smoking of 0.122 suggesting that differences in clinical outome were related to differences in baseline characteristics.

Discussion

Our results indicate that there are marked differences in baseline clinical, angiographic and procedural characteristics between smokers and non smokers undergoing coronary angioplasty. Smokers, despite having a lower incidence of known predisposing risk factors for atherosclerosis, require coronary intervention much earlier than non smokers but once they undergo successful coronary angioplasty their short term (six month) outcome is similar to non smokers.

Two previous studies have suggested that smoking may be associated with an increased risk of restenosis (22, 23). Both studies however were in small patient populations, with a low angiographic follow up rate, performed for recurrence of symptoms, and without quantitative angiographic analysis. Using a large patient population with a high quantitative angiographic follow up rate at a predetermined, six month, time interval our study has shown that smoking, a risk factor for atherosclerosis in general, is not a significant risk factor for restenosis.

There are a number of possible reasons for this. First, although cigarette smoking has been shown to be a risk factor for atherosclerosis this is over the course of years (19-21), whereas careful serial quantitative angiographic studies have shown restenosis to occur in the first 3 to 6 months after intervention (1,2). Thus cigarette smoking may have little influence on the process over this short time frame. Second, the mechanism(s) of restenosis are still incompletely understood and likely to involve differing contributions of slow elastic recoil (4), thrombus incorporation (5, 36), vessel remodelling (6,7) and myointimal hyperplasia (3,4) in each individual patient. Cigarette smoking is unlikely to influence all of these mechanisms. Third, it is possible that the sudden withdrawl of cigarettes during and immediately after the procedure in the smokers may have had some favorable effect on vascular or haematological systems (37, 38) that discouraged local platelet deposition, mural thrombus formation and consequent restenosis. Fourth, there may be substantive differences in plaque characteristics between smokers and non smokers which may ameliorate any thrombogenicity associated with smoking. In support of this are the higher inflation pressures and duration of inflation required for successful dilatation of the atherosclerotic plaque in the smoking group and evidence in the literature suggesting that lesions in smokers have a higher content

of collagen (39) and that the type of lesion which precipitates myocardial infarction in smokers is less severe and possibly generated by a different mechanism in smokers (38).

Differences in baseline characteristics

There were significant differences in the baseline clinical, angiographic and procedural characteristics between the smoking classes which could have been responsible for, or associated with, the outcome of the procedure. Smokers were younger, more likely to be male, to have peripheral vascular disease and to have sustained a previous myocardial infarction. Conversely, they were were less likely to have diabetes mellitus or a history of hypertension. These differences in baseline characteristic may be related to age. For example as smokers tend to develop atherosclerosis at a younger age they would be less likely to develop diseases of the older age group such as hypertension and diabetes. Many epidemiological studies have however also reported that smokers have significantly lower blood pressures than ex smokers or non smokers (40), but the mechanism for this negative relationship is unknown. These differences in baseline clinical characteristics may have influenced clinical and angiographic outcome in a number of ways. For example, diabetics are more likely to have restenosis than non diabetics (24), whilst dilatation of a vessel supplying previously infarcted territory is more likely to result in occlusion at the time of follow up angiography.

There were also significant differences in baseline haematological characteristics with smokers having a significantly higher haemoglobin, haematocrit and white cell count than non smokers, with ex smokers somewhere in between. Evidence in the literature suggests that smokers are also more likely to have a higher fibrinogen level and blood viscocity (41). What influence these variables may have on clinical and angiographic outcome after intervention is unclear. Although elevated fibrinogen levels have not been associated with a higher restenosis rate in a small series (42) elevated white cell counts (43) are known to be strong predictors of myocardial infarction (44, 45) and to be a marker of important cellular injury.

There were also differences in the distribution of lesions with smokers having more lesions in the RCA and less in the LAD than non smokers, and ex smokers somewhere in between. This is in keeping with other evidence in the literature suggesting a more proximal location of coronary lesions in hypercholesterolaemic non smokers with more distally situated lesions in normocholesterolaemic smokers (46). The lower incidence of LAD lesions with their known higher incidence of restenosis (35) may be responsible for the trend towards less absolute and relative loss in smokers with concordance.from current smokers, through ex smokers to non smokers.
There were also significant differences in procedural characteristics with lesions

in smokers requiring a longer inflation time at a higher pressure. This would suggest that there may be differences in plaque characteristics between the two groups which may have affected clinical and angiographic outcome in both a positive and a negative way. This is supported by evidence in the literature suggesing that smokers have a significantly higher content of collagen in coronary endarterectomy specimens (39), whilst smoking has also been shown to increase arterial wall stiffness- a change which may be associated with reduction of medial porosity and reduces flow pulsatility (47).

Clinical implications

Smokers should be strongly discouraged from smoking. In our study population, smokers, despite having a lower incidence of known predisposing risk factors for atherosclerosis, such as hypertension, diabetes and hypercholesterolaemia, required coronary intervention almost 6 years earlier than non smokers and 3 years earlier than ex smokers. This was on a background of increased risk of previous myocardial infarction and peripheral vascular disease. If the patient is completely unable to stop smoking however, continued smoking per se should not be a contraindication for coronary angioplasty.

Limitations of the study

Our study has a number of limitations. Firstly, it was a retrospective analysis of prospectively gathered data and is hence subject to the limitations inherent in any retrospective analysis. For example it is possible that unknown variables not examined in our logistic regression model may account for the lack of a worse clinical and angiographic outcome in smokers. Second, our data only apply to successful angioplasty procedures and to a 6 month follow up period. Thus we do not know if smoking reduces the chances of acute success or increases the immediate acute complications of the procedure. Additionally, although restenosis is usually manifest in the first 6 months, we do not know whether a longer follow up period may in fact demonstrate progression of the angioplasty lesion in smokers. Third, we do not have objective verification of the patient's self reported smoking status as we did not specifically assess, perhaps by periodic measurement of urinary products, whether supposed ex-smokers had truly stopped or whether they were continuing to smoke. This is especially pertinent as patients may give misleading answers in order to ensure the doctor's approval. It is also possible that, given the large effects of secondhand smoke on arterial function, passive smoking could have affected the data in the ex smokers and to a lesser extent the non smokers and thus lead to an underestimate of the effects of smoking. Conversely we also do not have information on how many current smokers stopped smoking after angioplasty, and on the contribution of this to their improved outcome. In the

small subgroup in which this information is available however, a large proportion (38%) of smokers, stopped smoking by the time of the six month follow up visit. A further possibility is that our study may have been underpowered to detect a small enough difference between the groups. Given the number of patients and the restenosis rate (both categorical and continuous (absolute loss)) in the never smoked group, our study has a power of 90% to detect a difference of 20% in the smoking group for the categorical restenosis rate and 0.10 mm for the absolute loss. We would consider both of these to be clinically significant but the fact remains that if the effect of smoking is below these levels we would have been unable to detect it. Ideally, future studies should include intravascular ultrasound assessment of the acute results of intervention (48) and the mechanism of subsequent restenosis, differentiating between slow recoil, thrombus formation/incorporation and intimal hyperplasia (6, 49), to have a better likelihood of demonstrating benefit from cessation of smoking.

Conclusions

Our results indicate that smokers, despite having a lower incidence of known predisposing risk factors for atherosclerosis, require coronary intervention almost 6 years earlier than non smokers and 3 years earlier than ex smokers. This is on a background of increased risk from previous myocardial infarction and peripheral vascular disease. Once they undergo successful coronary angioplasty however their short term (six month) outcome is similar to non smokers. Our findings thus warrant continuing strong efforts to discourage smoking in our patients.

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Chapter 13

Role of angiographically identifiable thrombus on long term luminal renarrowing following coronary angioplasty: A quantitative angiographic analysis.

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Role of Angiographically Identifiable Thrombus on Long-term Luminal Renarrowing After Coronary Angioplasty

A Quantitative Angiographic Analysis

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Background Experimental studies suggest that mural thrombus may be involved in postangioplasty restenosis. The aim of our study was to examine the role of angiographically identifiable thrombus in the clinical situation.

Methods and Results The study population comprised 2950 patients (3583 lesions). The presence of angiographically identifiable thrombus either before or after the procedure was defined as the presence of a generalized haziness or filling defect within the arterial lumen. Restenosis was assessed by both a categorical (>50% diameter stenosis at follow-up) and a continuous approach (absolute and relative losses). The study population included 160 lesions with and 3423 lesions without angiographically identifiable thrombus. The categorical restenosis rate was significantly higher in lesions containing angiographically identifiable thrombus: 43.1% versus 34.4%, $P<.01$; relative risk, 1.449; CI, 1.051 to 1.997. The absolute and relative losses were also higher in lesions containing angiographically identifiable thrombus (absolute loss, 0.43±0.66 versus 0.32±0.52; relative loss, 0.16±0.26 versus 0.13±0.21; both $P<.05$). The higher restenosis in these lesions was due primarily to an increased incidence of occlusion at follow-up angiography in this group: 13.8% versus 5.7%, $P<.001$. When lesions that went on to occlude by the time of follow-up angiography were excluded from the analysis, the restenosis rate between the two groups was similar by both the categorical (34.1% versus 30.4%, P=NS; relative risk, 1.183; CI, 0.824 to 1.696) and continuous (absolute loss, 0.23±0.46 versus 0.24±0.42, P=NS; relative loss, 0.09±0.17 versus 0.09±0.16, P=NS) approaches.

Conclusions Our results indicate that the presence of angiographically identifiable thrombus at the time of the angioplasty procedure is associated with higher restenosis. The mechanism by which this occurs is through vessel occlusion at follow-up angiography. Measures aimed at improving outcome in this group of patients should be focused in this direction. (*Circulation*. 1996;93:889-897.)

Key Words • angioplasty • thrombus • angiography • trials • meta-analysis

Since the introduction of coronary angioplasty by Gruentzig et al[1] and the subsequent refinements in equipment, the indications for the technique have been expanded to include patients with unstable angina[2-4] and acute myocardial infarction.[5] In these situations, however, angioplasty carries increased risks thought to relate, in part, to the presence of thrombus. A number of studies have demonstrated that the presence of angiographically identifiable thrombus either before or after dilatation of a coronary stenosis carries an increased risk of acute occlusion.[6,7] The influence of thrombus on long-term restenosis, however, is less clear. Experimental work suggests that local platelet deposition with the subsequent release of a number of chemotactic and mitogenic factors, such as platelet-derived growth factor and thrombin,[8] may mediate the fibroproliferative response. Recurrent platelet aggregation at the site of injury with associated vasoconstriction and the consequent increased frequency and severity of cyclic coronary blood flow variations may also play an important role in the subsequent development of neointimal proliferation.[9] Although one study suggested that thrombus formation and incorporation into the vessel wall may play a pivotal role in restenosis,[10] this has not been confirmed by other investigators.[11] Few clinical studies have actually assessed the role of angiographically identifiable thrombus on subsequent restenosis. The aim of this study was to examine the role of angiographically identifiable thrombus on long-term restenosis in a large series of patients undergoing successful balloon angioplasty and routine follow-up QCA assessment.

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Methods

Patients

The study population was taken from the 3582 patients with significant primary stenoses in native coronary arteries who were prospectively enrolled into four major restenosis trials.[12-15] These demonstrated that active therapy had no effect on restenosis or clinical outcome in the first 6 months after balloon angioplasty, so for the purposes of this study, the data for the active and placebo groups were pooled. Patients, men or women, were eligible for study entry if they were symptomatic or asymptomatic, had stable or unstable angina pectoris, and showed angiographically significant narrowing in one or more major coronary arteries. Patients with recent (<1 week)

Selected Abbreviations and Acronyms

LAD = left anterior descending coronary artery
MLD = minimum luminal diameter
PTCA = percutaneous transluminal coronary angioplasty
QCA = quantitative coronary angiography
RCA = right coronary artery

or evolving myocardial infarction and those with significant left main coronary artery disease were excluded from the study.

Angioplasty Procedure and Follow-up Angiography

Coronary angioplasty was performed with a steerable, movable guide-wire system by the femoral route. Standard balloon catheters were used. The choice of balloon type and brand as well as inflation duration and inflation pressure were left to the discretion of the operator. Patients were followed up for 6 months, at which time a follow-up study was performed. If symptoms recurred within 6 months, coronary angiography was carried out earlier. If no definite restenosis was present and the follow-up time was <4 months, the patient was asked to undergo further coronary arteriography at 6 months.

Quantitative Coronary Angiography

Three coronary angiograms, in total, were obtained for each patient: before and after PTCA and at angiographic follow-up. To standardize the method of data acquisition and to ensure exact reproducibility of the angiographic studies, measures were taken as previously described and all angiograms were processed in a central angiographic core laboratory.[12-15] The angiograms were recorded in such a manner that they were suitable for QCA by the computer-assisted Coronary Angiography Analysis System (CAAS), which was described and validated earlier.[16] Because the computer algorithm is unable to measure total occlusions, a value of 0 mm was substituted for the MLD and a value of 100% for the percent diameter stenosis before PTCA. In these cases, the postangioplasty reference diameter was substituted for vessel size.

Definitions

Angiographically identifiable thrombus was defined as the presence of a filling defect within the coronary lumen, surrounded by contrast material, seen in multiple projections and in the absence of calcium within the filling defect.[17,18] Alternatively, the persistence of contrast material within the lumen or visible embolization of intraluminal material downstream was also taken to represent intracoronary thrombus.

Total occlusion was present if no anterograde filling beyond the lesion was visible or if faint, late anterograde opacification of the distal segment was present in the absence of a discernible luminal continuity.[19] Occlusion at follow-up angiography was similarly defined.

Vessel size refers to the reference diameter of the relevant coronary segment and is represented by the interpolated reference diameter before PTCA. MLD is the point of maximal luminal narrowing in the analyzed segment.

Restenosis: Many criteria have been proposed for the assessment of restenosis.[20,21] For the purposes of this study, we used, first, the categorical approach with the traditional cutoff point of >50% diameter stenosis at follow-up and second, a continuous approach using absolute and relative losses.[21]

Absolute gain and absolute loss represent the improvement in MLD achieved at intervention and the absolute change during follow-up, respectively, measured in millimeters. Absolute gain is the MLD after PTCA minus MLD before PTCA. Absolute loss is the MLD after PTCA minus MLD at follow-up.

Relative gain and relative loss depict the improvement in MLD achieved at intervention and the change during follow-up, respectively, normalized for vessel size. Relative gain is [MLD after PTCA minus MLD before PTCA] divided by vessel size. Relative loss is [MLD after PTCA minus MLD at follow-up] divided by vessel size.

Absolute net gain is the MLD at follow-up minus MLD before PTCA.

Net gain index is the net gain normalized for the vessel size. Net gain index is [MLD at follow-up minus MLD before PTCA] divided by vessel size.

Statistical Analysis

Data were analyzed with the SAS statistical software package. A χ^2 test was used to assess differences in categorical variables. Student's t test was used to assess differences in continuous variables. To test the assumption that the variances were equal, the folded-form F statistic was used. Whenever this assumption was violated, the Cochran and Cox approximation of the t test was used. Differences in variables with an ordinal scale (severity of clinical outcome) were assessed with the Wilcoxon rank-sum test. The difference in event-free survival time between the two groups was evaluated by the Kaplan-Meier method with the log rank and Wilcoxon tests. To study the relation between a binary outcome parameter (occlusion at follow-up, the occurrence of a clinical event) and multiple categorical and continuous determinants, multiple logistic regression analysis was used. To study the relation between continuous outcome parameters and multiple categorical and continuous determinants, multiple linear regression analysis was used. Lesion characteristics were investigated with a lesion-based analysis and patient characteristics with a per-patient analysis in which a single lesion was randomly selected in patients with multivessel angioplasty. Values of $P<.05$ were considered significant.

Results

Baseline Patient Characteristics, Procedural Results, and Clinical Follow-up

The study population comprised the 2950 patients (3583 lesions, 1.21 lesions per patient) who successfully completed the study and had follow-up QCA. The overall QCA restudy rate was 86% of all patients undergoing successful PTCA with a residual QCA stenosis of <50%. Of 3583 lesions in 2950 patients, 160 lesions in 158 patients complied with the angiographic definition of thrombus present either before or after PTCA.

The two groups were comparable in terms of age and sex, but patients with angiographically identifiable thrombus at PTCA were more likely to have sustained a previous myocardial infarction and less likely to have had a previous PTCA (Table 1). There were, however, substantial differences in lesion and procedural characteristics between the two groups (Table 2). Thrombotic lesions were more likely to be located in the RCA than in the LAD and had a much higher proportion of total occlusions and multiple irregularities. They were also more likely to require a larger balloon and a greater number and duration of inflations. After the procedure, this group of lesions was also more likely to have a dissection (Table 2).

Forty-four (28%) of the patients with angiographically identifiable thrombus present and 625 (22.4%) of the patients without thrombus had a clinical end point during follow-up ($P=.116$). The individual components of death, myocardial infarction, coronary artery bypass graft surgery, and repeat PTCA were 0%, 8.3%, 2.6%, and 17.2%, respectively, for lesions containing angiographically identifiable thrombus and 0.2%, 2.6%, 2.5%, and 17.0% for lesions without thrombus ($P=.053$). The mean time to clinical end point was significantly less in

TABLE 1. Demographic Data of Patients With and Without Thrombus Preangioplasty or Postangioplasty Included in Analysis

Clinical Variable	Thrombus Present	Thrombus Absent
Patients, n	158	2792
Lesions, n	160	3423
Men, %	83	82
Age, y	57±9.3	55.3±9.3
Ever smoked, %	74	75
Current smoker, %	22	18
Hypertension, %	36	31
Diabetes, %	8	11
Hyperlipidemia, %	35	32
History of previous PTCA, %	1	4*
History of previous MI, %	61	42†
Previous CABG, %	3.5	4.5
Pain at rest, %	28	34
No. of vessels diseased, %		
1 VD	61	59
2 VD	33	32
3 VD	6	9

MI indicates myocardial infarction; CABG, coronary artery bypass graft surgery; and VD, vessels diseased.
*P<.05, †P<.001.

the angiographically identifiable thrombus group (63±63 versus 92±56 days, P<.05, Fig 1a), and when we compared the pattern of occurrence of clinical end points by way of the log rank test, the probability value was .051, whereas the Wilcoxon test, which places more emphasis on early survival times, rendered a value of P=.026, indicating the diverging survival curves in the beginning. When lesions that went on to occlude at the time of follow-up angiography were excluded from the analysis, there was no significant difference in the mean time to clinical end point (Fig 1b), and the log rank test gave a value of P=.209, whereas the Wilcoxon test rendered a value of P=.159, suggesting that the excess early events were related to the occlusions at follow-up angiography.

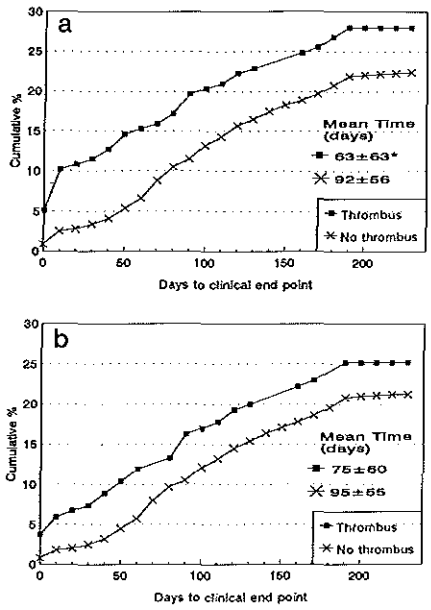

FIG 1. a, Cumulative distribution curve of clinical end points over time for patients with and without the presence of thrombus at the time of angioplasty. b, Cumulative distribution curve of clinical end points over time for patients with and without the presence of thrombus at the time of angioplasty (excluding lesions that went on to occlude at the time of follow-up angiography). Numbers given are mean±SD. *P<.05.

To exclude the possibility of a selection bias influencing our results, we also examined the incidence of thrombus-laden lesions and clinical end points in the 14% of the population in whom full QCA measurements

TABLE 2. Baseline Angiographic and Procedural Data of Lesions

Lesion Type	Thrombus	No Thrombus	Significance Level
Number of lesions	160	3423	
Lesion location, %			.0040
LAD	33.8	43.2	
LCx	20.6	25.0	
RCA	45.6	31.8	
Type of lesion, %			
Multiple irregularities	13.0	7.6	.0400
Total occlusion	20.0	6.8	.0000
Tandem lesion	1.3	4.2	.1120
Branch point in lesion	28.0	32.8	.4660
Lesion calcification	16.9	12.1	.0990
PTCA procedure			
Nominal size of largest balloon, mm	3.00±0.43	2.85±0.43	.0001
Balloon-to-artery ratio	1.12±0.18	1.13±0.19	.4345
Total number of inflations	4.2±2.9	3.53±2.3	.0013
Total duration of inflation, s	380±325	308±266	.0016
Maximum inflation pressure, atm	8.35±2.53	8.48±2.50	.5423
Post-PTCA result			
Dissection at the dilated site, %	54.4	33.1	.0000

LCx indicates left circumflex artery.
*P<.05.

TABLE 3. Quantitative Analysis of 160 Lesions With and 3423 Lesions Without Thrombus Before or After Angioplasty Included in Analysis

Thrombus Present	Thrombus	No Thrombus	Significance Level
No.	160	3423	
Reference diameter, mm			
Before angioplasty	2.77±0.54	2.60±0.54	.0006
After angioplasty	2.80±0.53	2.65±0.51	.0007
At follow-up	2.87±0.64	2.67±0.56	.0000
MLD, mm			
Before angioplasty	0.88±0.52	1.01±0.39	.0001
After angioplasty	1.80±0.35	1.75±0.36	.0868
At follow-up	1.37±0.71	1.44±0.58	.1552
Differences in MLD			
Absolute gain, mm	0.92±0.53	0.75±0.41	.0000
Relative gain	0.34±0.19	0.29±0.16	.0002
Absolute loss, mm	0.43±0.66	0.32±0.52	.0070
Relative loss	0.16±0.26	0.13±0.21	.0339
Absolute net gain, mm	0.49±0.72	0.43±0.57	.1970
Net gain index	0.18±0.27	0.17±0.23	.5050
Loss index	1.57±13.59	0.46±2.44	.0002
Percentage stenosis			
Before angioplasty	67.78±18.27	60.63±14.30	.0000
After angioplasty	35.15±7.45	33.38±8.37	.0039
At follow-up	51.71±23.70	45.71±19.00	.0001
DS at follow-up >50%, %	43.13	34.36	.0280

DS indicates diameter stenosis.
*P<.05, + P<.01, ^P<.001.

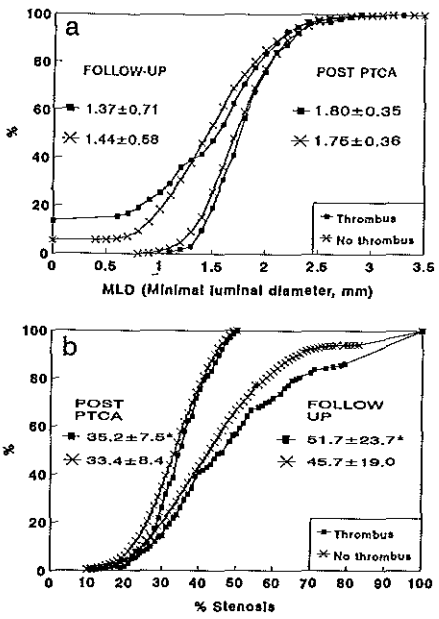

FIG 2. a, Cumulative distribution curve of MLD after PTCA and at follow-up for patients with and without the presence of thrombus at the time of angioplasty. b, Cumulative distribution curve of percent stenosis after PTCA and at follow-up for patients with and without the presence of thrombus at the time of angioplasty. Numbers given are mean±SD. *P<.05.

were not available and who were therefore excluded from the study population. The incidence of thrombus in these patients (6.7%) was comparable to that in our study population (5.4%, P=NS). Of these patients with thrombus, 22.6% and of patients without thrombus, 22.6% had a clinical end point during follow-up (P=1.000). The individual worst clinical end-point components of death, myocardial infarction, coronary artery bypass graft surgery, and repeat PTCA were 3.2%, 9.7%, 3.2%, and 6.5%, respectively, for lesions with and 3.5%, 3.2%, 5.8%, and 10.2% for lesions without thrombus (P=.403).

QCA Analysis

Satisfactory QCA was performed in a mean of 2.12 matched angiographic projections per lesion (Table 3). The reference diameter did not change from before to after the procedure but was significantly larger in lesions containing angiographically identifiable thrombus, and this difference remained at follow-up (Table 3). Although the MLD before angioplasty was significantly smaller in lesions containing angiographically identifiable thrombus, the MLD after angioplasty was similar. The residual percent stenosis after PTCA was higher in the angiographically identifiable thrombus group, as were the absolute and relative gains (Table 3, Fig 2). At follow-up, although the MLD was similar in both groups, the percent stenosis was significantly higher in lesions containing thrombus (Fig 2, Table 3), as were the categorical restenosis rate (43.1% versus 34.4%, P<.01; relative risk, 1.449; CI, 1.051 to 1.997) and the absolute and relative losses (Table 3, Fig 3).

The higher restenosis rate in the angiographically identifiable thrombus group was predominantly due to an increased number of occlusions at follow-up angiography (13.8% versus 5.7%, P<.001; relative risk, 2.639; 95% CI, 1.645 to 4.233). When lesions that went on to occlude at follow-up angiography were excluded from the analysis, there remained a tendency for a higher categorical restenosis rate in the thrombus group (34.1% versus 30.4%; relative risk, 1.183; CI, 0.824 to 1.696), but this was no longer statistically significant (P=.411). The absolute and relative losses were now also similar (0.23±0.46 versus 0.24±0.42 and 0.09±0.17 versus 0.09±0.16, respectively, both P=NS).

Multiple Linear Regression Analysis

We have previously demonstrated that vessel size, MLD before PTCA, absolute gain, and LAD location make a significant contribution to late angiographic outcome.[22] Adding thrombus to this model significantly improved its predictive value. Least-squares means for absolute loss were 0.404 for lesions with thrombus and 0.318 for lesions without thrombus. The probability value of adding the variable thrombus to the model was .037. Adding thrombus to the model when lesions that went on to occlude at follow-up angiography were excluded did not improve its predictive value. Least-squares means for absolute loss were 0.222 for lesions with thrombus and 0.243 for lesions without thrombus. The probability value of adding the variable thrombus to the model was .549.

To ascertain whether the trend toward a worse clinical outcome in patients with thrombus was related to differences in the underlying baseline characteristics, we corrected for these variables to see whether thrombus had an independent predictive value. We performed

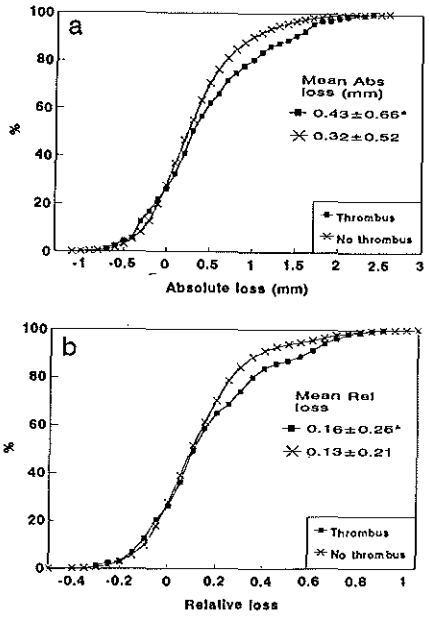

FIG 3. a, Cumulative distribution curve of absolute (Abs) loss (change in MLD from before PTCA to follow-up) for lesions with and without thrombus. b, Cumulative distribution curve of relative (Rel) loss (change in MLD from before PTCA to follow-up corrected for vessel size) for lesions with and without thrombus. Numbers given are mean±SD. *P<.001.

logistic regression with the above-mentioned baseline characteristics as covariates resulting in a value for the variable thrombus of P=.038, implying that thrombus has a positive relation with the probability of a clinical end point. Performing the analysis when lesions that went on to occlude at follow-up angiography were excluded gave a value of P=.183, suggesting that the positive relation with the probability of a clinical end point was related to occlusion at follow-up angiography.

Univariate and Multivariate Analyses of Occlusions at Follow-up Angiography

The finding that the higher restenosis in lesions containing angiographically identifiable thrombus was predominantly due to an increased number of occlusions at follow-up angiography prompted us to examine the time to clinical and angiographic follow-up and a number of variables predictive of late occlusion in this group (Table 4). The time to clinical and angiographic follow-up was significantly shorter in lesions that occluded at the time of angiographic follow-up. These lesions had a higher incidence of total occlusion, a tighter stenosis before PTCA, a longer duration of inflation with the use of a smaller balloon in a smaller vessel with a tighter residual MLD, and a greater likelihood of a dissection after PTCA. Logistic regression analysis confirmed the presence of a total occlusion before PTCA and total inflation time (seconds) to be positively related and the reference diameter after PTCA (millimeters) to be negatively related to occlusion at follow-up angiography (Table 5).

Discussion

Our study has specifically addressed the role of angiographically identifiable thrombus in long-term restenosis in a large patient population with a control group, a high angiographic follow-up rate, and QCA at a predetermined time interval. We have demonstrated, using both a categorical and a continuous approach, that restenosis is significantly increased by the presence of angiographically identifiable thrombus during coronary angioplasty. Furthermore, we have also shown that the mechanism for this is an increased rate of occlusion at follow-up angiography and that this is positively related to the presence of a total occlusion before PTCA and the total duration of balloon inflation and negatively related to the residual stenosis after intervention. If lesions that subsequently occlude at follow-up angiography are excluded from the analysis, then restenosis is similar in both groups. These findings support and expand our current understanding regarding the role of thrombus in long-term luminal renarrowing and occlusion after successful PTCA.

Our findings support a role for thrombus in restenosis after successful PTCA in terms of both clinical and angiographic outcomes. They suggest that the contribution thrombus makes to restenosis relates to vessel occlusion by the time of follow-up angiography. The timing of this occlusion is unclear. If it occurred early, it is likely to have been the end result of an acute thrombotic process, whereas if it occurred late, it would be the final end result of the process of restenosis itself. We do not know when the occlusion at follow-up angiography occurred in our patients, so our results must be speculative. We suspect, however, that it occurred early. In support of this is the higher incidence of previous myocardial infarction in the thrombus group (successful dilatation of the infarct-related vessel is associated with a higher rate of early silent occlusion[23]) and the Wilcoxon test indicating early divergence in the survival curves when occlusions at follow-up angiography are included. Additional evidence comes from the much earlier occurrence of clinical and angiographic end points in the thrombus-laden lesions that had occluded by the time of follow-up angiography and the fact that the excess in clinical end points is driven by a much higher incidence of acute myocardial infarction. Our hypothesis that the occlusions occurred early is also supported by evidence in the literature suggesting that 2% to 8% of elective PTCA lesions[24] occlude during the first 24 hours, silently in many cases. Thus, although our data support a role for thrombus in vessel occlusion by the time of follow-up angiography and hence restenosis, they do not provide any strong evidence to support a role for angiographically identifiable thrombus in late myointimal hyperplasia. Further prospective studies are thus required to evaluate this important matter further.

Univariate regression analysis was suggestive of a number of procedural and angiographic variables related to occlusion at follow-up angiography. These included the presence of a total occlusion and a tighter stenosis before PTCA, a longer duration of inflation with the use of a smaller balloon diameter in a smaller vessel with a tighter residual MLD, and a greater likelihood of a dissection after PTCA at the dilated site. Thus, the more difficult dilatation of a more complex

Table 4. Univariate Analysis of Patient-, Lesion-, and Procedure-Related Characteristics Relevant to Occlusion at Follow-up Angiography in 160 Lesions Containing Thrombus

Lesion Type	Occlusion at Follow-up (n=22)	No Occlusion at Follow-up (n=138)	Significance Level
Anginal class, %			.365
None	4.6	11.6	
CCS class I	9.1	13.8	
CCS class II	40.9	26.8	
CCS class III	31.8	22.5	
CCS class IV	13.6	25.4	
Duration of angina, wk	106±219	63±132	.326
Medication at screening, %			
Anticoagulants	0	0.7	1.000
Thrombocyte aggregation inhibitor	68.2	65.2	.976
Aspirin	80.0	74.2	.872
Dipyridamole	20.0	7.8	.317
Laboratory investigations			
Hemoglobin	8.70±1.00	8.81±0.86	.593
Hematocrit	0.42±0.04	0.42±0.04	.863
Platelet count	274±55	267±80	.691
Lesion location, %			.085
LAD	13.7	37.0	
LCx	22.7	20.3	
RCA	63.7	42.8	
Lesion characteristics, %			
Concentric	26.3	36.6	.542
Multiple irregularities	10.5	13.4	1.000
Branch point in stenosis	16.7	16.4	1.000
Coronary artery bend	15.6	24.1	.615
Calcified lesion	31.8	14.5	.088
Total occlusion	45.5	15.6	.003*
Degree of collateral supply, %			.138
No collaterals	66.7	81.3	
Slight (minimal perfusion)	0	7.3	
Medium (partial perfusion)	26.7	7.3	
Major (complete perfusion)	0	1.0	
Not assessed	6.7	3.1	
PTCA procedure			
Nominal size of largest balloon, mm	2.81±0.40	3.03±0.43	.0264
Balloon to artery ratio	1.10±0.19	1.12±0.18	.7172
Total number of inflations	4.5±3.2	4.2±2.9	.6687
Total duration of inflation, s	530±484	356±289	.0248*
Maximum inflation pressure, atm	9.10±2.93	8.24±2.45	.2159
Post-PTCA result			
Dissection at the dilated site, %	77.3	50.7	.036
Quantitative angiographic measurements			
Reference diameter, mm			
Before angioplasty	2.62±0.53	2.79±0.54	.3101
After angioplasty	2.54±0.39	2.84±0.54	.0036*
MLD, mm			
Before angioplasty	0.55±0.56	0.93±0.49	.0052
After angioplasty	1.66±0.30	1.82±0.35	.0277
Differences in MLD, mm			
Absolute gain	1.11±0.60	0.89±0.51	.1161
Relative gain	0.44±0.23	0.33±0.18	.0360
Percentage stenosis			
Before angioplasty	79.00±20.40	65.99±17.33	.0088
After angioplasty	34.35±8.38	35.28±7.35	.6276
Lesion length post-PTCA, mm	6.14±1.99	6.26±2.35	.8032
Days to follow-up	127±79	160±46	.007

CCS indicates Canadian Cardiovascular Society angina classification; LCx, left circumflex artery. Values are mean±SD.
*Retained in multivariate model.

lesion in a smaller vessel with a less satisfactory result would be more likely to occlude by the time of follow-up angiography. Multivariate regression analysis confirmed the presence of total occlusion before PTCA and a longer total inflation time to be positively related to the risk of subsequent occlusion and the reference diameter

TABLE 5. Result of Multiple Logistic Regression Analysis to Evaluate the Respective Contributions of Clinical, Angiographic, and Procedural Variables to Occlusion at Follow-up Angiography in Lesions Containing Thrombus

Variable	Regression Coefficient	Standard Error of Regression Coefficient	P
Presence of total occlusion pre-PTCA	.379	.163	.021
Total inflation time, s	.002	.0006	.002
Reference diameter post-PTCA, mm	−1.634	.630	.009

after PTCA to be negatively related. The relation between total occlusion and subsequent risk of occlusion may be secondary to the highly thrombogenic surface generated by the successful dilatation of a total occlusion, without a preexisting endothelial lining.[25] Successful dilatation of a total occlusion may also expose flowing blood to activated thrombin bound to fibrin in the internal layers of a previously formed thrombus. The prothrombotic processes stimulated by the activated thrombin would be even more severe than those associated with the deeply injured artery and would further accelerate thrombosis after PTCA in these lesions,[26,27] thus contributing to both enhanced local thrombus formation after successful dilatation of these lesions and an increased likelihood of thrombotic occlusion. The total inflation time may represent the more complex dilatation of a total occlusion, multiple irregularities, or a more complicated angioplasty. This is further supported by the higher incidence of dissections requiring prolonged inflation in lesions that occlude by the time of follow-up angiography. The negative relation between increasing vessel size and subsequent occlusion is probably representative of the local flow dynamics.[28]

Our study has a number of limitations. First, it was a retrospective analysis of prospectively gathered data and is hence subject to the limitations inherent in any retrospective analysis. For example, there are significant differences in the baseline clinical, angiographic, and procedural characteristics between the two groups that could have been responsible for, or associated with, the outcome of the procedure, including the presence of thrombus. Patients with angiographic evidence of thrombus before or after angioplasty had a significantly greater history of previous myocardial infarction and a significantly lower proportion of previous coronary angioplasty, both of which may have had an impact on the clinical and angiographic outcomes. There is evidence for a silent early occlusion after successful acute dilatation of infarct-related vessels[5,23] and evidence that after stent implantation in coronary vessels supplying an infarcted segment, the low flow makes the vessel more prone to thrombotic occlusion.[29] Similar mechanisms may be operating in our study, but we do not know whether the vessel dilated was the infarct-related vessel, and we do not know the length of time since myocardial infarction, except that it was longer than 1 week. Similar arguments also apply to the history of previous PTCA. Again, we do not know whether the present procedure was performed at the same site, and it is not possible to draw conclusions about what effect it may have had on subsequent clinical and angiographic outcomes.

There were also significant differences in lesion location and lesion characteristics. There was a higher proportion of lesions containing thrombus in the RCA and less in the LAD. This may have had an impact on angiographic outcome in two ways. First, the RCA is significantly larger than the LAD, and this may explain why the reference diameter in lesions containing thrombus was significantly larger. Second, there are significant differences between the two vessels in terms of local flow dynamics, vessel geometry, and external compressive forces[30] that may have a substantial influence on the subsequent risk of occlusion.[31] Although lesion location was not a major risk factor in our multivariate analysis of occlusions at follow-up angiography, it is nonetheless interesting to note that the trend was for lesions that occluded to be in the RCA (P=.085). Thus, similar mechanisms may be operating in our study.

The type of lesion was also significantly different, with a greater proportion of total occlusions in the thrombus group. Successful dilatation of these may have enhanced local thrombus formation and may have contributed to the increased incidence of occlusion at follow-up angiography.[23,32] It may also partly explain the smaller MLD before PTCA and greater absolute and relative gains in this group of lesions. Differences in lesion location and characteristics could also have been responsible for the significant differences in the PTCA procedure. For example, the prevalence of RCA lesions could explain the larger nominal size of the largest balloon, whereas the greater number of inflations and total duration of inflation may reflect the more complex dilatation of a total occlusion, multiple irregularities, or a more complicated angioplasty.

Although we tried to compensate for these differences in baseline characteristics by using multivariate analysis and demonstrated that thrombus has a predictive value on restenosis and clinical outcome independent of the underlying clinical and angiographic characteristics, nonetheless, we cannot exclude the possibility of covert factors not available in the study influencing outcome. For example, we do not know what proportion of the angiographically identifiable thrombus group had a successfully treated occlusive dissection, a recognized risk factor for restenosis,[33] and total occlusion as a late outcome.[34]

Second, although the angiographic definition of thrombus we used is the standard definition found in the literature,[17,18] the individual sensitivity and specificity of the three criteria have, to the best of our knowledge, never been addressed. In addition, contrast angiography, although the gold standard for randomized studies, has a poor sensitivity for intracoronary thrombus.[18] When we used the above angiographic definition and coronary angioscopy as the gold standard, we found the specificity of contrast angiography to be good (100%) but the sensitivity to be poor (19.4%). This is in keeping with other evidence in the literature. Coronary angioscopy,

for example, suggests a very high incidence of macroscopic mural thrombus, not identifiable by contrast angiography, after balloon angioplasty,[35,36] whereas directional atherectomy suggests that thrombus may contribute to arterial narrowing in 8% to 25% of restenosis cases.[37] Thus, although our results apply to angiographically identifiable thrombus, they may not apply to patients with mural thrombus not visualized by contrast angiography.

Finally, the study relies on data pooled from four separate restenosis trials.[12-15] We believe that the pooling of data was justified, however, since the number of patients with angiographically identifiable thrombus present in each individual study was limited. Furthermore, the entry criteria for the studies were broadly similar, the data pooled were those common to all studies, and the angiographic criteria were standardized, with one central angiographic core laboratory performing the QCA analysis in all studies. In addition, the resulting large study population provides a unique opportunity to obtain accurate QCA data at a predetermined time interval in a field in which few such data exist to date.

Clinical Implications

Our data support previous work suggesting that local thrombus formation may result in acute occlusion[7,38,39] and expand it to include late subacute occlusion and hence restenosis. This may have important clinical implications with regard to recent studies using monoclonal antibodies and synthetic peptides directed against the platelet glycoprotein IIb/IIIa receptor.[40-42] Although preliminary data suggest that they reduce the need for coronary revascularization procedures in high-risk angioplasty patients,[42] most of the reduction occurred in the first 30 days after intervention, and the effects were not verified at the angiographic level. Our data would suggest that perhaps some of their improved clinical outcome may relate to eliminating subacute occlusion in a subset of the population without necessarily affecting the restenosis process.

Conclusions

Our results indicate that the presence of angiographically identifiable thrombus at the time of the angioplasty procedure is associated with a higher rate of angiographic restenosis. The mechanism by which this occurs is through increased vessel occlusion at follow-up angiography. Measures aimed at improving outcome in this group of lesions should be focused in this direction.

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Chapter 14

Long term luminal renarrowing after successful elective coronary angioplasty of total occlusions: a quantitative angiographic analysis.

Violaris AG, Melkert R, Serruys PW.

Circulation 1995; 91: 2140-2150

Long-term Luminal Renarrowing After Successful Elective Coronary Angioplasty of Total Occlusions

A Quantitative Angiographic Analysis

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Background The long-term angiographic outcome after successful dilatation of coronary occlusions remains unclear. The objective of this study was to examine long-term restenosis after successful balloon dilatation of coronary occlusions at a predetermined time interval with quantitative angiography and compare this with a control population of stenoses.

Methods and Results The study population comprised 2950 patients (3583 lesions) prospectively enrolled in and successfully completing four major restenosis trials (86% quantitative angiographic follow-up). Cineangiographic films were processed and analyzed at a central core laboratory with the use of an automated interpolated edge detection technique. The study population comprised 266 occlusions (7%) defined as total when there was absent anterograde filling beyond the lesion (109 lesions) and functional (157 lesions) when faint, late anterograde opacification of the distal segment was seen in the absence of a discernible luminal continuity; 3317 lesions were defined as stenoses (93%). Restenosis was significantly higher after successful dilatation of occlusions than of stenoses. With the categorical (>50% diameter stenosis at follow-up) approach, the restenosis rate was 44.7% in occlusions compared with 34.0% in stenoses ($P<.001$; relative risk, 1.575; CI, 1.224 to 2.027). Similarly, the absolute loss (defined as the change in minimal lumen diameter between post coronary angioplasty and follow-up; in millimeters, mean±SD) (0.43±0.68) in occlusions was significantly higher than in stenoses (0.31±0.51, $P<.001$), as was the relative loss, defined as the change in minimal lumen diameter between postangioplasty and follow-up, adjusted for the vessel size (0.17±0.28 versus 0.12±0.20, $P<.001$). The higher restenosis rate in the occlusions group was due predominantly to an increased number of occlusions at follow-up angiography in this group (19.2% compared with 5.0% for stenoses, $P<.001$). Within the occlusions group, there were no significant differences in long-term outcome between total and functional occlusions (restenosis rate, 45.0% versus 44.6%; reocclusion rate, 23.9% versus 15.9%; absolute loss, 0.53±0.69 versus 0.36±0.67; relative loss, 0.21±0.28 versus 0.15±0.28; P=NS).

Conclusions These results indicate that successfully dilated coronary occlusions, both total and functional, have a higher rate of angiographic restenosis at 6 months than stenoses. This is due chiefly to a higher rate of occlusion at follow-up angiography in this group of lesions. Measures aimed at reducing restenosis after successful dilatation of coronary occlusion should be focused in this direction. **(*Circulation*. 1995;91:2140-2150.)**

Key Words • angioplasty • occlusions • angiography

After the introduction of coronary angioplasty by Grüntzig et al in 1979,[1] the indication for its use were at first tentatively and subsequently more strikingly expanded to include patients with total occlusions.[2-6] Although a number of factors are known to influence the acute success rate,[4,7-9] relatively little is known regarding long-term luminal renarrowing after successful dilatation of total occlusions. A number of studies have investigated this, but uncertainty continues with restenosis rates in the literature varying from 20% to 65%.[2,3,5,6,10-17] This variation has three primary sources. First, most studies have been retrospective analyses that use small patient numbers and no control group.[2,3,11,12,15,16] Second, the angiographic follow-up rates have been low and performed for the recurrence of symptoms rather than at a predetermined time interval, thus introducing important selection bias.[5,6,17] Finally, some have used visual assessment to estimate angiographic severity, which is subject to wide interobserver and intraobserver variability.[18] This study attempted to overcome these limitations by using a validated automated edge detection technique to evaluate restenosis prospectively in a large series of patients undergoing successful balloon angioplasty and routine follow-up angiographic assessment at a predetermined time interval. Furthermore, a control cohort of patients with stenoses was used to directly compare the long-term results.

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Methods

Patients

The study population comprised 3582 patients with significant primary stenoses in native coronary arteries who were prospectively enrolled in four major restenosis trials[19-22] and who underwent successful coronary angioplasty (postprocedural stenosis <50%). These trials demonstrated that active therapy had no effect on restenosis or clinical outcome in the

first 6 months after balloon angioplasty, so for the purposes of this study, the data for the active and placebo groups were pooled. Men or women were eligible for study entry if they were symptomatic or asymptomatic with stable or unstable angina pectoris and proven angiographically significant narrowing in one or more major coronary arteries. Informed consent was obtained in all cases before the coronary angioplasty procedure. Patients with evolving myocardial infarction and significant left main disease were excluded from the study.

Angioplasty Procedure and Follow-up Angiography

Coronary angioplasty was performed with a steerable, moveable guide wire system by the femoral route. Standard balloon catheters were used. The choice of balloon type and brand, inflation duration, and inflation pressure was left to the discretion of the operator. Patients were followed up for 6 months; then a follow-up study was performed. If symptoms recurred within 6 months, coronary angiography was performed earlier. If no definite restenosis was present and the follow-up time was less than 4 months, the patient was asked to undergo further coronary arteriography at 6 months.

Quantitative Angiography

In total, three coronary angiograms were obtained for each patient—before and after percutaneous transluminal coronary angioplasty (PTCA) and at angiographic follow-up. To standardize the method of data acquisition and to ensure exact reproducibility of the angiographic studies, measures were taken as previously described, and all angiograms were processed in a central angiographic core laboratory.[19,20,22] The angiograms were recorded in such a manner that they were suitable for quantitative analysis by the computer-assisted CORONARY ANGIOGRAPHY ANALYSIS SYSTEM, which was described and validated earlier.[23,24] Because the computer algorithm cannot measure total occlusions, a value of 0 mm was substituted for the minimal lumen diameter and a value of 100% for the pre-PTCA percent diameter stenosis. In these cases, the post-PTCA reference diameter was substituted for vessel size.

Definitions

Total Occlusion

As in previous studies, total occlusions were divided into absolute occlusions (Thrombolysis in Myocardial Infarction [TIMI] flow grade 0) where no anterograde filling beyond the lesion was visible and functional occlusions (TIMI grade 1) where faint, late anterograde opacification of the distal segment was present in the absence of a discernible luminal continuity.[17,25]

Occlusion at Follow-up Angiography

Occlusion at follow-up angiography was defined as the presence of an absolute or functional occlusion at the previously dilated angioplasty site on angiographic follow-up.

Angiographic Parameters Assessed

Vessel size refers to the reference diameter of the relevant coronary segment and is represented by the interpolated reference diameter pre-PTCA because this is the closest and most objective approximation of the disease-free vessel wall.

Minimum luminal diameter (MLD) is the point of maximal luminal narrowing in the analyzed segment.

Many criteria have been proposed for the assessment of restenosis.[26-28] For the purposes of this study, two approaches were used: the categorical approach with the traditional cutoff point of >50% diameter stenosis at follow-up and a continuous approach that used absolute and relative loss, which reflect the behavior of the lesion during and after angioplasty and may be more representative of the pathological process involved during follow-up.[28,29]

Absolute gain and absolute loss represent the improvement in MLD achieved at intervention and the absolute change during follow-up respectively, measured in millimeters. Absolute gain is post-PTCA MLD minus pre-PTCA MLD. Absolute loss is post-PTCA MLD minus MLD at follow-up.

Relative gain and relative loss depict the improvement in MLD achieved at intervention and the change during follow-up respectively, normalized for vessel size. Relative gain is (post-PTCA MLD minus pre-PTCA MLD) divided by vessel size. Relative loss is (post-PTCA MLD minus MLD at follow-up) divided by vessel size.

The absolute net gain is the MLD at follow-up minus pre-PTCA MLD. The net gain index is the net gain normalized for the vessel size. The net gain index is (MLD at follow-up minus pre-PTCA MLD) divided by vessel size.

The loss index is the relation of late loss to acute gain: loss index is (MLD at follow-up minus post-PTCA MLD) divided by (post-PTCA MLD minus pre-PTCA MLD).

Statistical Analysis

Data were analyzed with the SAS statistical software package. Categorical variables are presented as absolute numbers (%). Continuous variables are expressed as mean value±SD. Differences between groups were evaluated with adjusted χ^2 tests for categorical variables and Student's t tests for continuous variables. The contributions of clinical, angiographic, and procedural variables to the categorical outcome parameters were evaluated with logistic regression analysis. For the continuous outcome parameter (absolute loss), multiple linear regression analysis was used. Categorical variables were dichotomized and entered into the analysis as indicator variables with values of 0 and 1. Selection of variables was achieved in a stepwise fashion. The adjusted R^2 was used as the criterion for model selection. Probability values $<.05$ were considered significant.

Results

Patient Characteristics and Clinical Follow-up

The study population comprised 2950 patients (3583 lesions, 1.21 lesions per patient) who successfully completed the study and had follow-up quantitative angiography. The overall angiographic restudy rate was 86% of all patients undergoing successful PTCA. We found that 266 lesions in 249 patients out of 3583 lesions in 2950 patients complied with the angiographic definition of total occlusion. Of these, 109 were absolute and 157 were functional total occlusions.

Tables 1 and 2 summarize the clinical and angiographic characteristics of the 249 patients with occlusions compared with the 2701 with stenoses. Patients with total occlusions were younger and had a significantly higher rate of previous myocardial infarction than patients with stenoses. Furthermore, the duration of angina in this group was significantly shorter than in stenoses. Additionally, the presence of thrombus either before or after PTCA was significantly higher in occlusions. Of the procedural characteristics, the nominal size of the largest balloon was significantly higher in stenoses, while the total number of balloon inflations required, total duration, and maximum inflation pressure were higher in occlusions. Within the occlusions group, there were no significant differences between lesions with total and functional occlusions.

Seventy-one (28.5%) of the patients with successfully dilated total occlusions and 598 (22.1%) of the patients with stenoses had clinical end points (additional PTCA, coronary artery bypass graft [CABG] surgery, acute

TABLE 1. Baseline Clinical Characteristics of Patients With Occlusions and Stenoses Before Coronary Angioplasty

	Lesion Type	
	Occlusion	Stenoses
Patients, n	249	2701
Men, %	85.1	81.4
Age, y	54.4±9.6	57.4±9.3*
Smoking status, %		
Ever smoked	73.1	75.5
Current smoker	17.2	18.3
Previous myocardial infarction, %	54.2	41.1*
Previous CABG, %	1.6	4.5
Previous PTCA, %	3.6	5.0
Diabetes mellitus, %	10.8	10.2
History of hypertension, %	30.1	30.5
History of hypercholesterolemia, %	33.3	31.5
History of peripheral vascular disease, %	3.6	5.1
CCS anginal class, %		
None	6.0	5.6
I	10.0	11.2
II	33.7	32.4
III	28.9	30.2
IV	21.3	20.6
Duration of angina, wk	69±126	114±207*
Medication at screening, %		
Nitrates	57.6	66.2
Calcium antagonists	69.1	69.5
β-blockers	57.4	49.8
Anticoagulants	1.6	1.5
Aspirin	80.2	82.0
Laboratory investigations		
Total cholesterol	5.58±1.30	5.85±1.23
Hemoglobin	8.89±0.80	8.86±0.83
Hematocrit	0.42±0.04	0.42±0.04
Platelet count	259±58	257±71

CABG indicates coronary artery bypass grafts; PTCA, percutaneous transluminal coronary angioplasty; and CCS, Canadian Cardiovascular Society.
*$P<.05$.

TABLE 2. Baseline Angiographic and Procedural Data of Patients With Occlusions and Stenoses Before Coronary Angioplasty

	Lesion Type	
	Occlusions	Stenoses
Lesions, n	266	3317
Lesions per patient, n	1.07	1.23
Number of diseased vessels, %		
SVD	61.0	64.2
2-VD	34.5	27.5
3-VD	3.6	7.8
Lesion location, %		
LAD	39.4	44.2
LCx	26.9	24.0
RCA	33.7	31.7
Type of lesion, %		
Multiple irregularities	6.9	8.0
Side branch in lesion	21.1	20.2
Lesion calcification	12.0	12.4
PTCA procedure		
Nominal size of largest balloon, mm	2.74±0.43	2.89±0.43*
Balloon to artery ratio	1.14±0.18	1.13±0.19
Total number of inflations	4.6±3.2	3.47±2.2*
Total duration of inflation, s	404±341	303±261*
Maximum inflation pressure, atm	8.80±2.59	8.45±2.50*
Post-PTCA result, %		
Dissection at the dilated site	39.8	34.5
Thrombus visible (before or after PTCA)	12.9	4.2*

SVD indicates single-vessel disease; 2-VD, two-vessel disease; 3-VD, three-vessel disease; LAD, left anterior descending; LCx, left circumflex artery; RCA, right coronary artery; and PTCA, percutaneous transluminal coronary angioplasty.
*$P<.05$.

myocardial infarction, or death) during follow-up. The difference was statistically significant ($P=.026$). The individual components of death, myocardial infarction, CABG, and re-PTCA were 0%, 3.6%, 3.6%, and 21.3%, respectively, for occlusions and 0.2%, 2.8%, 2.4%, and 16.7%, respectively, for stenoses. The differences in the individual clinical end points between the two groups were also statistically significant ($P=.022$). Fig 1 summarizes the time course of clinical end points. Although the mean time to clinical follow-up was similar in the two groups, when we compared the pattern of occurrence of clinical end points by way of the log-rank test, clinical end points in the occlusions group were found to occur earlier than in stenoses ($P=.022$).

Interestingly, when lesions that went on to occlude at follow-up angiography were excluded from the analysis, 50 patients (25.1%) with occlusions and 541 patients (21.1%) with stenoses reached a clinical end point, and the difference was no longer statistically significant ($P=.217$). The individual clinical end points and the time to a clinical end point were also no longer significantly different ($P=.238$ and .214, respectively).

Quantitative Angiographic Analysis

A mean of 2.12 matched angiographic projections per lesion had satisfactory quantitative analysis performed at the central Angiographic Core Laboratory before and after PTCA and at follow-up (Table 3). The reference diameter was significantly lower in occlusions than in stenoses, and this difference remained at follow-up. As expected, the MLD increased substantially more after dilatation of occlusions than of stenoses, which was reflected in the substantially greater relative gain. Nevertheless, the post-PTCA MLD was significantly lower in occlusions, perhaps reflecting the smaller vessel diameter in this group (Fig 2a). When this was taken into account, the percent stenosis after PTCA was similar in both groups (Fig 2b). At follow-up, previously occluded

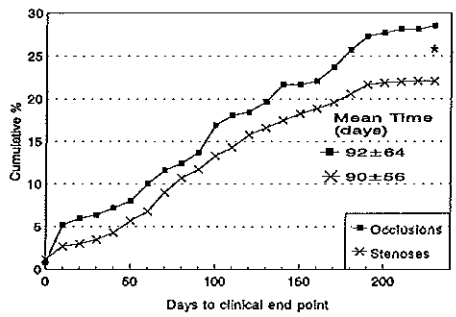

FIG 1. Graph showing cumulative distribution curves of clinical end points over time for total occlusions and stenoses. Numbers given are mean±SD (days). *$P<.05$.

TABLE 3. Quantitative Analyses of 266 Lesions in 249 Patients With Occlusions and 3317 Stenoses in 2701 Patients Included in the Analysis

	Lesion Type		
	Occlusions, n=266	Stenoses, n=3317	Significance Level
Reference diameter, mm			
Before angioplasty	...	2.61±0.54	...
After angioplasty	2.46±0.49	2.67±0.51	<0.001
At follow-up	2.60±0.55	2.69±0.56	0.026
MLD, mm			
Before angioplasty	0	1.08±0.29	...
After angioplasty	1.61±0.34	1.77±0.36	<0.001
At follow-up	1.18±0.69	1.46±0.57	<0.001
Differences in MLD, mm			
Absolute gain	1.61±0.34	0.69±0.34	<0.001
Relative gain	0.66±0.09	0.27±0.13	<0.001
Absolute loss	0.43±0.68	0.31±0.51	<0.001
Relative loss	0.17±0.28	0.12±0.20	<0.001
Absolute net gain	1.18±0.69	0.38±0.53	<0.001
Net gain index	0.48±0.28	0.14±0.21	<0.001
Loss index	0.26±0.43	0.53±3.88	0.255
Percentage stenosis			
Before angioplasty	100	57.83±9.86	...
After angioplasty	34.16±8.46	33.40±8.33	0.156
At follow-up	54.08±25.67	45.33±18.52	<0.001
DS at follow-up >50%, %	44.74	33.95	<0.001

MLD indicates minimal luminal diameter; DS, diameter stenosis.

lesions deteriorated significantly more in terms of both absolute and relative loss (Fig 3, Table 3), resulting in a smaller follow-up MLD and higher percent stenosis (Fig 2a and 2b, Table 3). Thus, in addition to restenosis being higher when the continuous, absolute, and relative loss approach was used, the restenosis rate when the categorical approach was used was also significantly higher (44.74% in occlusions compared with 33.95% in stenoses; $P<.001$; relative risk, 1.575; CI, 1.224 to 2.027).

Although angiographic restenosis was higher in occlusions than stenoses, there were no significant differences in the presentation of restenosis between the two groups. Of recanalized total occlusions with angiographic restenosis, 40% were symptom-free, while 18.5% complained of angina and 41.5% had a clinical end point. This compares with 39.9%, 18.7%, and 41.4%, respectively, for stenoses with angiographic restenosis.

The higher restenosis rate in the occlusions was due predominantly to an increased number of occlusions at follow-up angiography in this group (19.2% versus 5.0% for stenoses, $P<.001$). Thus, recanalized occlusions accounted for 23.5% of all occlusions at follow-up angiography even though they formed only 7% of the total study population. The relative risk for occlusion at follow-up angiography was 4.503 (95% CI, 3.196 to 6.344).

Subgroup Analysis of Occlusions Group

Subgroup analysis of the occlusions group revealed 109 absolute and 157 functional occlusions (Table 4). The reference diameter after angioplasty was significantly higher in absolute than in functional occlusions, but this difference did not persist to follow-up angiography. There were no significant differences in the MLD or

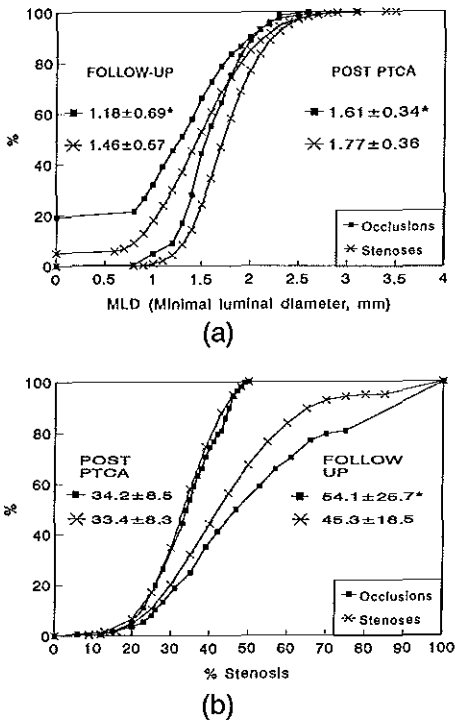

FIG 2. Graphs showing (a) cumulative distribution curves of mean luminal diameter (MLD) after percutaneous transluminal coronary angioplasty (PTCA) and at follow-up for total occlusions and stenoses and (b) cumulative distribution curves of percentage stenosis after PTCA and at follow-up for total occlusions and stenoses. Numbers given are mean±SD. *$P<.001$.

percent stenosis after PTCA or at follow-up (Table 4) between the two groups. The categorical restenosis rate (>50% diameter stenosis at follow-up) was almost identical (44.95% versus 44.59%) in the two groups. Although the reocclusion rate in absolute occlusions tended to be higher than in functional occlusions (23.9% versus 15.9%), this did not reach statistical significance ($P=.06$). By use of the continuous measurements of restenosis, the absolute and relative loss tended to be lower in functional occlusions (Table 4), resulting in a significantly higher net gain index and a significantly lower loss index.

Univariate and Multivariate Analyses of Restenosis in Total Occlusions

Univariate analysis of the available clinical-, procedural-, and lesion-related characteristics was performed to assess whether any of these variables were associated with an increased categorical restenosis rate (Table 5). The only significant associations were with a shorter duration of angina, a longer balloon total inflation time, higher residual stenosis after PTCA, and a lower relative gain. Stepwise logistic regression analysis was used to further evaluate the

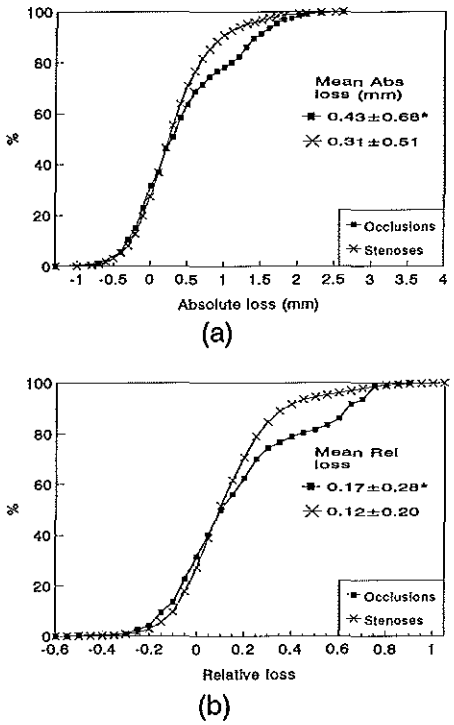

FIG 3. Graphs showing (a) cumulative distribution curves of absolute loss (Abs) (change in mean luminal diameter [MLD] from before percutaneous angioplasty to follow-up) for total occlusions and stenoses and (b) cumulative distribution curves of relative (rel) loss (change in MLD from before percutaneous angioplasty to follow-up corrected for vessel size) for total occlusions and stenoses. Numbers given are mean±SD. *$P<.001$.

relation between this dichotomous definition of restenosis and the above variables, as well as all other clinical, lesion, and procedural characteristics. The number of diseased vessels and the percent stenosis after PTCA were positively related; duration of angina (in days) was negatively related to the probability of restenosis at follow-up (Table 6).

Univariate analysis of clinical, procedural, and lesion characteristics related to absolute loss showed the only significant associations to be with total inflation time ($P=.0004$), presence of thrombus ($P=.0039$), post-PTCA MLD ($P=.0144$), duration of angina ($P=.0262$), left anterior descending coronary artery location ($P=.0418$), and lesion calcification ($P=.0443$). Multiple linear regression analysis was used to further evaluate absolute loss. Of the variables assessed, total inflation time (in seconds) and the presence of thrombus were both positively related to absolute loss (Table 6).

Univariate and Multivariate Analysis of Occlusions at Follow-up Angiography

The finding that the higher restenosis rate after dilatation of occlusions was due predominantly to an increased number of reocclusions at follow-up angiography in this group prompted us to examine a number of variables predictive of reocclusion (Table 7). The clinical characteristics of the two groups were similar, although lesions that went on to reocclude had a shorter duration of angina (27±52 versus 74±132 weeks). Procedural characteristics were also similar, although lesions that went on to reocclude required a longer balloon inflation (537±478 versus 373±294 seconds). Logistic regression analysis was performed for the above and for all other clinical, angiographic, and procedural parameters thought to be associated with the occlusion at follow-up. Of these variables, the total inflation time (in seconds) was positively related and the post-PTCA reference diameter (in millimeters) was negatively related to reocclusion at follow-up angiography (Table 6).

TABLE 4. Quantitative Angiographic Analysis of 109 Total and 157 Functional Occlusions Included in the Analysis

	Lesion Type		
	TIMI 0, n=109	TIMI 1, n=157	Significance Level
Reference diameter, mm			
Before angioplasty	...	...	...
After angioplasty	2.55±0.52	2.40±0.56	0.015
At follow-up	2.66±0.55	2.56±0.54	0.200
MLD, mm			
Before angioplasty	...	...	...
After angioplasty	1.65±0.35	1.58±0.34	0.096
At follow-up	1.12±0.74	1.21±0.65	0.285
Differences in MLD, mm			
Absolute gain	1.65±0.35	1.58±0.34	0.096
Relative gain	0.65±0.09	0.66±0.08	0.440
Absolute loss	0.53±0.69	0.36±0.67	0.051
Relative loss	0.21±0.28	0.15±0.28	0.063
Absolute net gain	1.12±0.74	1.21±0.65	0.285
Net gain index	0.44±0.29	0.51±0.28	0.037
Loss index	0.32±0.44	0.22±0.42	0.042
Percentage stenosis			
Before angioplasty	...	...	0.285
After angioplasty	34.61±8.69	33.86±8.32	0.481
At follow-up	57.52±26.38	51.70±24.96	0.072
DS at follow-up >50%, %	44.95	44.59	1.000

TIMI indicates Thrombosis in Myocardial Infarction classification; MLD, minimal luminal diameter.

Discussion

Our study specifically addressed the problem of long-term luminal renarrowing after dilatation of total coronary occlusions in a large patient population with a control group, a high angiographic follow-up rate, and quantitative angiography at a predetermined time interval. We demonstrated using both a categorical and a continuous approach that restenosis is significantly greater after balloon dilatation of occlusions than of stenoses. Furthermore, we also showed that this is mainly the result of an increased rate of occlusion at follow-up angiography, which is specifically related to total inflation time and reference diameter after intervention. We also demonstrated that restenosis and reocclusion occur to an equal extent in both absolute and functional occlusions. These findings support and expand our current understanding of the long-term angio-

TABLE 5. Univariate Analysis of Patient-, Lesion-, and Procedure-Related Characteristics Relevant to Long-term Restenosis* in 266 Occlusions

	Restenosis at Follow-up, n=119	No Restenosis at Follow-up, n=147	Significance Level
CCS anginal class, %			0.790
None	6.7	4.8	
I	8.4	11.6	
II	31.1	34.7	
III	31.1	29.3	
IV	22.7	19.7	
Duration of angina, wk	40±76	85±146	0.018†
Medication at screening, n			
Anticoagulants	1.7	1.4	1.000
Thrombocyte aggregation inhibitor	70.6	70.8	1.000
Aspirin	79.8	81.6	0.896
Persantin	14.3	9.2	0.400
Laboratory investigations			
Hemoglobin	8.83±0.77	8.95±0.80	0.235
Hematocrit	0.42±0.04	0.42±0.04	0.398
Platelet count	261±63	257±56	0.564
Lesion location, %			0.554
LAD	37.0	43.5	
LCx	27.7	24.5	
RCA	35.3	32.0	
Lesion characteristics, %			
Concentric	19.3	15.6	0.645
Multiple irregularities	5.7	8.0	0.661
Branch point in stenosis	30.2	20.8	0.430
Coronary artery bend	9.4	7.2	0.707
Calcified lesion	14.3	10.2	0.408
Degree of collateral supply, %			0.704
No collaterals	52.3	47.1	
Slight (minimal perfusion)	7.0	10.1	
Medium (partial perfusion)	25.6	21.9	
Major (complete perfusion)	12.8	16.8	
Not assessed	2.3	4.2	
PTCA procedure			
Nominal size of largest balloon, mm	2.74±0.43	2.75±0.43	0.846
Balloon to artery ratio	1.13±0.18	1.15±0.19	0.293
Total number of inflations	4.6±3.4	4.6±3.0	0.925
Total duration of inflation, s	452±390	365±293	0.043
Maximum inflation pressure, atm	8.92±2.63	8.69±2.44	0.464
Post-PTCA result, %			
Dissection at the dilated site	42.0	38.1	0.601
Thrombus visible (before or after PTCA)	15.1	9.5	0.227
Quantitative angiographic measurements			
Reference diameter after PTCA	2.48±0.48	2.44±0.50	0.590
Minimal luminal diameter after PTCA	1.59±0.35	1.62±0.34	0.474
Stenosis after PTCA, %	35.50±7.99	33.08±8.70	0.019†
Absolute gain	1.59±0.35	1.62±0.34	0.474
Relative gain	0.64±0.08	0.67±0.09	0.024
Lesion length after PTCA, mm	6.29±2.35	6.44±2.71	0.631
Time to follow-up, d	147±55	167±41	<0.001

CCS indicates Canadian Cardiovascular Society angina classification; LAD, left anterior descending; LCx, left circumflex; RCA, right coronary artery; and PTCA, percutaneous transluminal coronary angioplasty. Values are mean±SD.

*>50% diameter stenosis at follow-up angiography.

†Retained in multivariate model.

graphic outcome after successful angioplasty of coronary occlusions.

Our overall restenosis rate of 44.7% with the categorical (>50% diameter stenosis) approach compares with the previously reported recurrence rate of 65% from our center[3] and is within the 20% to 65% range described previously.[2,3,5,6,10-16] Our restenosis rate, however, is significantly lower than those of the two largest studies published to date,[5,6] perhaps as a result of our high angiographic follow-up rate. With univariate analysis, a number of risk factors have been postulated for the higher restenosis rate after dilatation of chronic occlu-

TABLE 6. Regression Analyses to Evaluate the Respective Contributions of Clinical, Angiographic, and Procedural Variables on the Categorical Restenosis Rate,* Absolute Loss, and Reocclusion During Follow-up

Variable	Regression Coefficient	Standard Error of Regression Coefficient	P
Categorical restenosis rate			
Duration of angina, d	−0.0007	0.0003	.032
Diseased vessels, n	0.649	0.307	.034
Stenosis after PTCA, %	0.043	0.023	.049
Absolute loss			
Total inflation time, s	0.0005	0.0001	.0004
Presence of thrombus	0.379	0.163	.021
Reocclusion at follow-up angiography			
Total inflation time, s	0.002	0.0006	.002
Reference diameter after PTCA, mm	−1.634	0.630	.009

PTCA indicates percutaneous transluminal coronary angioplasty. Logistic regression analysis was used for the categorical restenosis rate and reocclusion at follow-up. Multiple linear regression analysis was used for absolute loss.
*>50% diameter stenosis at follow-up.

sions. These include anatomic factors such as lesion location (left anterior descending and circumflex)[6,14] and absence of functional occlusion,[6] procedural factors such as multivessel dilatations[6] and increased balloon inflations at higher pressures,[10] and residual stenosis after intervention.[6,12,14,15] A final risk factor is thought to be the presence of collateral vessels, which may exert competitive pressure even after they are no longer visibly functional[30] and thus lead to an increased restenosis rate.

Univariate analysis in our study confirmed significant associations between the categorical definition of restenosis (>50% diameter stenosis at follow-up) and a shorter duration of angina, a longer balloon total inflation time, higher residual stenosis after PTCA, and a greater relative gain. Multivariate analysis, however, suggested that the only significant positive relations were with the number of diseased vessels and the percent stenosis after PTCA, while duration of angina was negatively related to the probability of restenosis at follow-up. The positive relation between the number of diseased vessels and the percent stenosis after PTCA with a categorical definition of restenosis is in keeping with previous studies in both occlusions[6] and stenoses.[31] The negative relation with duration of angina is also in keeping with previous studies suggesting a positive relation between recent onset of symptoms and a higher risk of restenosis.[32-34]

Interestingly, when we looked at the absolute loss as a marker of restenosis, an outcome measure that may better indicate the underlying pathological process involved, the above variables were no longer significant. The only significant relations were found to be with total inflation time and the presence of thrombus. Both of these were positively related to the subsequent absolute loss. The duration of balloon inflation as a positive risk factor may represent the sum total of the lesion characteristics and a more difficult, more complex procedure with a consequently higher risk of occlusion, thereby markedly influencing the absolute loss. In keeping with this is the fact that we also demonstrated total inflation time to be positively related to the subsequent risk of occlusion at follow-up angiography. The positive relation between the presence of thrombus and the subsequent absolute loss may also reflect a greater likelihood of subsequent occlusion, although we were unable to demonstrate this in our study. Previous studies, however, showed thrombus to be a risk factor for subsequent occlusion,[35,36] perhaps acting as a nidus for further platelet deposition.

The main reason for the significantly higher rate of angiographic restenosis in recanalized occlusions in our study was the high rate of occlusion at follow-up angiography in this group (19.2% versus 5.0% after dilatation of stenoses). A number of reasons may account for this. First, this group may represent a subset of the total population that has an intrinsic hematological propensity to thrombosis. Although our laboratory data did not confirm this, there are other hematologic factors such as fibrinogen levels that we did not measure that may influence reocclusion covertly.[35]

Second, there are substantial morphological differences between lesions,[37] which may have considerable influence on the subsequent risk of occlusion. Angiography provides little information on this or on the pathway of subsequent recanalization, which may vary from subintimal to periatheromatous and transatheromatous.[38] What effect these variables may have on the subsequent reocclusion and indeed restenosis rates is unknown. Differences in these lesion characteristics may be reflected in the total inflation time, which was significantly higher in lesions more likely to reocclude.

Third, the coronary vasomotor responses after balloon angioplasty of chronic total occlusions may be abnormal. Distal vasoconstriction occurs frequently after angioplasty and correlates well with coronary perfusion pressure, suggesting that chronic hypoperfusion resets epicardial coronary autoregulation and that restoration of normal perfusion pressure after PTCA may provoke reflex vasoconstriction,[39] thus precipitating early reocclusion.

Finally, the increased reocclusion rate may relate to local flow dynamics. The reference diameters before and after PTCA and at follow-up were significantly lower in occlusions than in stenoses. Furthermore, within the occlusions group multivariate analysis suggested that

TABLE 7. Univariate Analysis of Patient, Lesion, and Procedural Characteristics Relevant to Reocclusion During Follow-up in 266 Occlusions

	Reocclusion at Follow-up, n=51	No Occlusion at Follow-up, n=215	Significance Level, Univariate Analysis
CCS anginal class, %			0.125
None	9.8	4.7	
I	3.9	11.6	
II	25.5	34.9	
III	39.2	27.9	
IV	21.6	20.9	
Duration of angina, wk	27±52	74±132	0.044
Medication at screening, %			
Anticoagulants	0	1.8	0.733
Thrombocyte aggregation inhibitor	64.7	72.1	0.384
Aspirin	73.5	82.4	0.344
Persantin	11.8	11.5	1.000
Laboratory investigations			
Hemoglobin	8.82±0.81	8.91±0.78	0.505
Hematocrit	0.42±0.03	0.42±0.04	0.960
Platelet count	256±55	259±60	0.706
Lesion location, %			0.400
LAD	37.3	41.4	
LCx	23.5	26.5	
RCA	39.2	32.1	
Lesion characteristics, %			
Concentric	16.3	20.7	0.653
Multiple irregularities	2.3	8.0	0.325
Branch point in stenosis	13.3	22.8	0.365
Coronary artery bend	9.3	8.0	1.000
Calcified lesion	15.7	11.2	0.514
Degree of collateral supply, %			0.779
No collaterals	44.7	50.3	
Slight (minimal perfusion)	7.9	9.0	
Medium (partial perfusion)	31.6	21.6	
Major (complete perfusion)	13.2	15.6	
Not assessed	2.6	3.6	
PTCA procedure			
Nominal size of largest balloon, mm	2.67±0.47	2.76±0.42	0.214
Balloon to artery ratio	1.15±0.18	1.14±0.18	0.665
Total number of inflations	4.5±3.1	4.6±3.2	0.777
Total duration of inflation, s	537±478	373±294	0.003*
Maximum inflation pressure, atm	9.10±2.80	8.72±2.46	0.370
Post-PTCA result, %			
Dissection at the dilated site	43.1	39.1	0.708
Thrombus visible (before or after PTCA)	19.6	10.2	0.107
Quantitative angiographic measurements			
Reference diameter after PTCA	2.36±0.47	2.48±0.49	0.105*
Minimal luminal diameter after PTCA	1.54±0.36	1.62±0.34	0.123
Stenosis after PTCA, %	34.52±8.33	34.08±8.51	0.735
Absolute gain	1.54±0.36	1.62±0.34	0.123
Relative gain	0.66±0.08	0.66±0.09	0.693
Lesion length after PTCA, mm	6.17±2.47	6.42±2.58	0.519

CCS indicates Canadian Cardiovascular Society; LAD, left anterior descending; LCx, left circumflex; RCA, right coronary artery; and PTCA, percutaneous transluminal coronary artery. Values are mean±SD.
*Retained in multivariate analysis.

there is a negative relation between vessel size and subsequent risk of reocclusion. Thus, successfully dilated occlusions in larger vessels were less likely to reocclude than those in smaller vessels. This is in keeping with experimental work that suggested that smaller vessel diameters and hence higher shear rates favor local platelet activation and deposition,[40,41] resulting in a greater likelihood of occlusion.

In addition to being responsible for the higher rate of angiographic restenosis, the high reocclusion rate in recanalized occlusions also seems to have been responsible for the higher rate of clinical events in this group of patients. Patients with recanalized occlusions had a significantly higher proportion of clinical events, mainly in terms of myocardial infarction, CABG, and repeated PTCA. Furthermore, these occurred earlier in occlu-

sions than in stenoses. When patients with occlusions that went on to occlude at the time of follow-up angiography were removed from the analysis, the differences between the two groups were no longer significant, suggesting that the excess risk relates to the higher rate of occlusion at follow-up angiography. Interestingly, despite the higher rate of reocclusion in recanalized occlusions, there were no significant differences in the presentation of restenosis between the two groups, with approximately 40% of patients with angiographic restenosis in both groups being symptom-free.

Although studies of total occlusions suggest that there are important differences in the acute success rates between total and functional occlusions,[7,8] our data suggest that in terms of restenosis and reocclusion, there is little difference between the two groups. Although reocclusion after successful dilatation of total occlusions was higher than in functional occlusions (23.9% versus 15.9%), this did not reach statistical significance. However, there was a tendency for functional occlusions to mount less of a fibroproliferative response, which was reflected in the significantly greater net gain index and lower loss index. The cause for this is unclear but is likely to reflect differences in the underlying pathological substrate.

Clinical Implications

Up to 20% of patients undergoing diagnostic catheterization have one or more total occlusions; thus, they make up 10% to 20% of the total angioplasty population.[42] Furthermore, in multivessel disease, they may make the difference in referring the patient for angioplasty or for CABG surgery. Although great attention has been paid to increasing the acute success rate with the use of sophisticated new devices such as the low-speed rotational angioplasty[43] and the Excimer laser,[44] relatively little attention has been paid to long-term restenosis and its amelioration. Our results suggest that the higher restenosis in these lesions is likely to relate in part to the very high rate of occlusion at follow-up angiography in this group. Thus, it suggests that if we can identify these lesions and stop reocclusion, then the angiographic and clinical courses of recanalized total occlusions are likely to be similar to those of stenoses. The question of how we do this, however, is a vexing one. The clinical course in our patient population and previous studies would suggest that reocclusions tend to occur early.[45] A number of pharmacological and mechanical approaches have been tried. Ellis and colleagues[17] demonstrated that drug therapy with aspirin, dipyridamole, or warfarin did not influence the overall restenosis rate. In a prospective study, Di Sciascio and colleagues[46] demonstrated that restenosis is not modified by a longer balloon inflation time. A more recent study assessed the value of coronary stenting for dissection with threatened closure following recanalization of total occlusions.[47] The restenosis rate at follow-up was high at 57%, as was the recurrent occlusion rate (20%). When stenting was used routinely after recanalization of chronic total occlusions and hence when vigorous anticoagulation was used, the restenosis rate was low at 24% in a small patient population (65 patients) with an 80% angiographic follow-up rate.[48] Thus, whether the reocclusion rate can be ameliorated by additional (eg, stenting) or alternative percutaneous revascularization techniques (high-speed rotational atherectomy or laser angioplasty) with or without concomitant pharmacological therapy remains to be established.

Study Limitations

A number of limitations of the present study are to be acknowledged. First, the study was a retrospective analysis of prospectively gathered data and hence is subject to the limitations inherent in any retrospective study.

Second, there were minor variations in the entry criteria of the studies. In CARPORT and PARK, for example, patients were randomized before PTCA, whereas in MERCATOR and MARCATOR, patients were entered into the trial only after a successful angioplasty procedure.[19-22] Thus, we are unable to comment on clinical, angiographic, or procedural factors influencing the acute success rate of the procedure. Furthermore, patients taking part in large multicenter studies like these are selected in certain ways; therefore, care needs to be taken in extrapolating our results to the broader angioplasty population.

Third, for obvious reasons, the reference diameter in the occlusions group before PTCA could not be reliably measured, so we took the reference diameter after dilatation as the reference diameter before dilatation. The statistical analyses remain valid, however, because even if we substitute the post-PTCA reference diameter for the pre-PTCA reference diameter in the stenoses group, the significant differences between the two groups remain.

Fourth, because of the nature of the data, we unfortunately are unable to comment on whether certain previously documented risk factors for restenosis such as the degree of collateral supply[11] or the measured coronary wedge pressure[49,50] may relate to reocclusion rather than to long-term restenosis.

Finally, data from the active therapy group and the placebo group were amalgamated. Although the drugs did not seem to influence the overall restenosis rate, the Ketanserin in the PARK study and the thromboxane A_2-receptor blocker in the CARPORT studies may have had a covert influence on the reocclusion rate after dilatation of occlusions that we were unable to detect.

Nonetheless, we believe that the merging of the data is justified because the data amalgamated were common to all studies and the angiographic criteria were standardized, with one central laboratory performing the quantitative angiographic analysis in all studies. Furthermore, the resulting large study population provides a unique opportunity to obtain accurate quantitative angiographic data at a predetermined time interval in a field where such few data currently exist.

Conclusions

These results indicate that successfully dilated coronary occlusions, both total and functional, have a higher rate of angiographic restenosis at 6 months than stenoses. This is due chiefly to a higher rate of occlusion at follow-up angiography in this group of lesions. Measures aimed at reducing restenosis after successful dilatation of coronary occlusions should therefore be focused in this direction.

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Chapter 15

Luminal enlargement after coronary angioplasty: A quantitative angiographic analysis.

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Circulation (Resubmitted after peer review)

Abstract

Background. Experimental and human intravascular ultrasound studies suggest that unfavourable vascular remodelling may contribute to restenosis after coronary angioplasty. We evaluated whether favourable remodeling and hence luminal enlargement, defined as a negative absolute loss, may also occur following successful balloon angioplasty and which factors may influence this.

Methods and Results. Our study population consisted of 3,583 lesions in 2,950 patients taking part and successfully completing four major restenosis studies. Within the study population there were 980 lesions (27.3%) which underwent luminal enlargement during follow up and 2,603 lesions (72.7%) which did not. As expected the categorical restenosis rate was significantly lower in lesions which underwent favourable remodeling (1.53% vs 47.25%, $p<0.0001$) as was the rate of clinical events during follow up (8.23% vs 26.91%, $p<0.0001$). Multivariate analysis suggested that diuretic therapy ($p=0.024$), MLD pre PTCA ($p=0.0003$), diameter stenosis pre PTCA ($p=4.E^{-18}$), balloon artery ratio ($p=6.E^{-12}$) and dissection post PTCA ($p=0.0084$) increased the probability of luminal enlargement whilst age ($p=0.036$), heparin therapy ($p=1.E^{-07}$), number of vessels diseased ($p=0.037$), lesion length ($p=0.0001$), LAD location ($p=0.001$), total inflation time ($p=0.0139$) and relative gain ($p=0.E^{+00}$) decreased the probability of luminal enlargement during follow up.

Conclusions. Luminal enlargement may occur after successful coronary angioplasty and can be substantially influenced by clinical, angiographic and procedural variables.

Introduction

The classical paradigm regarding the mechanism of restenosis after successful coronary angioplasty has been one of neointimal thickening from the migration, proliferation and extracellular matrix production by smooth muscle cells(1-3). More recently this paradigm has been challenged by both experimental (4,5) and human (6-8) intravascular ultrasound studies which suggest that restenosis relates more to unfavourable vascular remodeling than neointimal hyperplasia (9-11). The data regarding this however are still controversial with some studies confirming this whilst others refute it (12). We hypothesized that favourable arterial remodeling and hence luminal enlargement may also occur, increasing luminal dimensions during follow up. The aim of our study was to assess whether such luminal enlargement occurs in man and define which, if any, factors may influence it.

Methods

Patients

Our study population was taken from the 3,582 patients with significant primary stenoses in native coronary arteries who were prospectively enroled into four major restenosis trials (13-16) and consisted of the 2,950 patients with coronary angioplasty and quantitative angiographic follow up. Patients were eligible for study entry if they were symptomatic or asymptomatic men, or women, with stable or unstable angina pectoris and proved angiographically significant narrowing in one or more major coronary arteries. Informed consent was obtained in all cases before the coronary angioplasty procedure. Patients with evolving myocardial infarction and significant left main disease were excluded from the study.

Angioplasty procedure and follow up angiography

Coronary angioplasty was performed with a steerable, moveable guide wire system by the femoral route. Standard balloon catheters were used. The choice of balloon type and brand as well as inflation duration and inflation pressure were left to the discretion of the operator. Patients were followed up for 6 months at which time a follow up study was performed. If symptoms recurred within 6 months coronary angiography was carried out earlier. If no definite restenosis was present and the follow-up time was below 4 months, the patient was asked to undergo further coronary arteriography at 6 months.

Quantitative angiography

Three coronary angiograms, in total, were obtained for each patient, pre-, post-PTCA and at angiographic follow up. The angiograms were recorded in such a manner that they were suitable for quantitative analysis by the computer assisted Coronary Angiography Analysis System (CAAS) which has been described and validated earlier (17,18). To standardise the method of data acquisition and to ensure exact reproducibility of the angiographic studies, measures were taken as previously described and all angiograms were processed in a central angiographic core laboratory (13-16). Because the computer algorithm is unable to measure total occlusions, a value of 0mm was substituted for the minimal lumen diameter and a value of 100% for the percent diameter stenosis pre PTCA. In these cases the post angioplasty reference diameter was substituted for vessel size.

Definitions

Luminal enlargement

Luminal enlargement was defined as an increase in minimal luminal diameter during angiographic follow up (ie a negative absolute loss).

Clinical end point

A clinical end point was defined as the occurrence of death, myocardial infarction, Coronary artery bypass grafting and repeat PTCA during follow up (13-16).

Angiographic definitions

Reference diameter refers to the reference diameter of the relevant coronary segment and is represented by the interpolated reference diameter pre-PTCA.

Minimum luminal diameter (MLD) is the point of maximal luminal narrowing in the analysed segment.

Many criteria have been proposed for the assessment of restenosis (20,21). For the purposes of this study a categorical approach with the traditional cut-off point of >50% diameter stenosis at follow up was used.

Absolute Gain and Absolute loss represent the improvement in minimal luminal diameter achieved at intervention and the absolute change during follow up

respectively, measured in mm. Absolute Gain = MLD post PTCA - MLD pre PTCA. Absolute loss = MLD post PTCA - MLD at follow up.

Relative gain and relative loss depict the improvement in minimal luminal diameter achieved at intervention and the change during follow up respectively, normalized for vessel size. Relative gain = [MLD post PTCA - MLD pre PTCA]/Vessel size. Relative loss = [MLD post PTCA - MLD at follow up]/Vessel size.

The absolute net gain is the MLD at follow up - MLD pre-PTCA. The net gain index is the net gain normalised for the vessel size. Net gain index= [MLD at follow up-MLD pre-PTCA]/Vessel size.

Statistical analysis

Data was analysed using the SAS statistical software package. A chi square test was used to assess the difference in categorical variables. A Student t-test was used to assess differences in continuous variables. To test the assumption that the variances were equal, the Folded form F statistic was used. Whenever this assumption was violated the Cochran and Cox approximation of the t test was used. Differences in variables with an ordinal scale (severity of clinical outcome) were assessed with the Wilcoxon rank-sum test. The difference in event free survival time between the two groups was evaluated using the Kaplan-Meier method with the log rank and Wilcoxon test. To study the relationship between a binary outcome parameter (the occurrence of a clinical event) and multiple categorical and continuous determinants multiple logistic regression analysis was used. To study the relationship between continuous outcome parameters and multiple categorical and continuous determinants multiple linear regression analysis was used. P values <0.05 were considered significant.

Results

Baseline patient characteristics, procedural results and clinical follow up

The study population comprised 2,950 patients who underwent successful coronary angioplasty and quantitative angiographic follow up. 980 lesions (27%) in 668 patients out of 3583 lesions in 2,950 patients underwent luminal enlargement uring angiographic follow up (Figure 1).

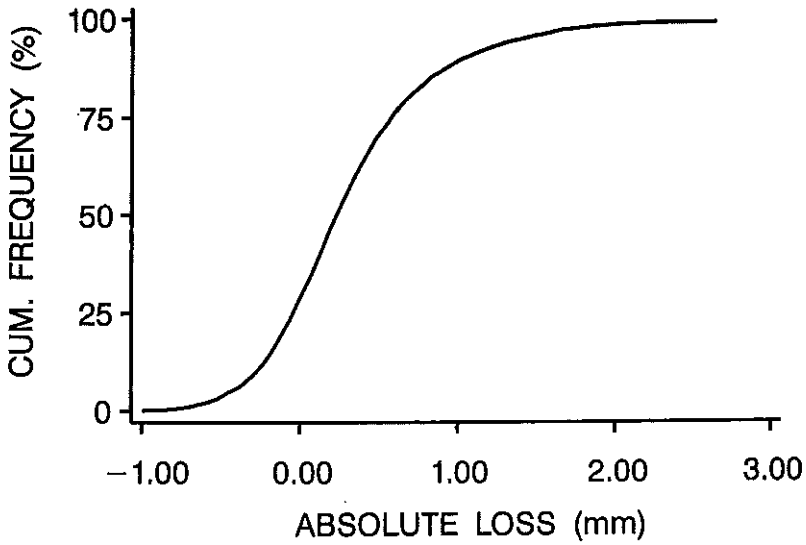

Figure 1. Cumulative distribution curve of absolute loss (change in MLD from before percutaneous angioplasty to follow up) for all lesions in study. Note that 28% have luminal enlargement (a negative absolute loss).

The clinical and angiographic characteristics of the 980 lesions which underwent luminal enlargement compared with the 2,603 which did not are summarised in Tables 1 and 2. The two groups were broadly comparable in terms of baseline demographic characteristics although patients who underwent luminal enlargement were less likely to have grade 3-4 angina. They were also less likely to be on heparin and a thrombocyte aggregation inhibitor and more likely to be on oral diuretics (Table 1). The lesion characteristics were also broadly similar although lesions which underwent luminal enlargement were less likely to have a branch point in the middle of the stenosis (Table 2).

Remodeling	Yes	No	Significance Level
Patient No	668	2282	
Lesion No	980	2,603	
Men			
	82%	81%	0.672
Age (years).	57±9.4	57.4±9.3	0.247
Ever smoked	75%	75%	0.913
Current smoker	20%	18%	0.173
Hypertension	30%	31%	0.679
Diabetes	8.6%	10.7%	0.101
Hyperlipidaemia	32%	32%	0.906
History of previous PTCA	5.1%	4.9%	0.806
History of previous MI	42%	43%	0.493
Previous CABG	3.8%	4.4	0.472
Pain at rest	40%	42%	0.461
Vessels diseased			0.058
1VD	68%	63%	
2VD	24%	29%	
3VD	7%	8%	
Anginal class			**0.047**
None	5.3%	5.8%	
CCS Class I	11.5%	10.9%	
CCS Class II	35.8%	31.5%	
CCS Class III	30.6%	30.0%	
CCS Class IV	16.8%	21.8%	
Duration of angina (weeks)	114±189	110±205	0.627
Medication at screening			
Anticoagulants	1.8%	1.5%	0.516
Thrombocyte aggregation inhibitor	59.7%	65.8%	**0.004**
Aspirin	79.1%	82.6%	0.125
Persantin	12.5%	11.9%	0.763
Heparin	36.5%	48.3%	**<0.0001**
Beta blocker	47.9%	51.2%	0.136
Calcium antagonist	67.4%	70.0%	0.189
Nitrates	65.1%	65.8%	0.866
Diuretic	9.28%	6.53%	**0.015**
Cholesterol lowering agent	9.9%	9.8%	0.934
Laboratory Investigations			
Haemoglobin	8.87±0.82	8.86±0.83	0.8779
Haematocrit	0.42±0.04	0.42±0.04	0.8729
White cell count	7.36±2.08	7.46±2.47	0.2971
Platelet count	255±69	257±71	0.4866
Total cholesterol (mmol/l)	5.91±1.23	5.81±1.23	0.0641
HDL cholesterol (mmol/l)	1.12±0.51	1.15±0.55	0.5561
LDL cholesterol (mmol/l)	4.32±1.18	4.06±1.31	0.0753

Table 1. Demographic data of patients with and without luminal enlargement included in analysis. CABG indicates coronary artery bypass grafts, PTCA, percutaneous transluminal coronary angioplasty and CCS, Canadian Cardiovascular Society angina classification.

Remodeling No.	Yes 980	No 2,603	Significance Level
Lesion location			0.120
LAD	39.6%	43.9%	
LCx	25.5%	24.5%	
RCA	34.8%	31.5%	
Type of lesion			
Concentric	44.4%	44.8%	0.884
Multiple Irregularities	8.4%	7.7%	0.599
Tandem lesion	3.6%	4.3%	0.400
Branch point in stenosis	26.9%	35.0%	**0.005**
Branch point in dilatation site	61.4%	65.2%	0.209
Coronary artery bend	21.7%	19.6%	0.222
Lesion calcification	12.2%	12.4%	0.940
Thrombus (pre/post PTCA)	4.2%	4.6%	0.681
Total occlusion pre PTCA	8.5%	7.0%	0.164
Lesion length pre	6.136±2.165	6.337±2.331	**0.020**
Degree of collateral supply			0.224
No collaterals	85.7%	84.1%	
Slight (minimal perfusion)	4.0%	4.2%	
Medium (partial perfusion)	4.4%	5.0%	
Major (complete perfusion)	3.9%	3.1%	
Not assessed	2.1%	3.7%	
PTCA procedure			
Nominal size of largest balloon (mm)	2.88±0.45	2.85±0.43	0.1915
Balloon to artery ratio	1.13±0.19	1.13±0.19	0.4183
Total number of inflations	3.3±2.2	3.7±2.4	**0.0002**
Total duration of inflation (secs)	275±262	325±271	**<0.0001**
Maximum Inflation pressure (atm)	8.32±2.63	8.53±2.45	0.0258
Post PTCA result			
Dissection at the dilated site	35.3%	33.5%	0.339
Table 2 (continued)			
Dissection Type			0.055
Type A	13.7%	15.3%	
Type B	18.2%	13.5%	
Type C	3.9%	3.5%	
Type D	0.0%	0.1%	
Type E	0.1%	0.1%	
Type F	0.0%	0.1%	

Table 2. Angiographic baseline data of patients. SVD, Single vessel disease, 2-VD Two vessel disease, 3-VD, three vessel disease. LAD, left anterior descending, LCx, left circumflex artery, RCA, right coronary artery. PTCA, percutaneous transluminal coronary angioplasty. CCS, Canadian Cardiovascular Society angina classification. Mean values ± SD.

As our data set was composed of four negative restenosis trials we also assessed whether active drug therapy had any effect on luminal enlargement in each individual data set. There was no difference in the Carport Mercator or Marcator studies (31.89 vs 28.16, 31.23 vs 26.58, 22.65 vs 26.95, respectively, active therapy vs placebo, p=ns) but a tendency in the Park study for less patients on the active drug therapy (Ketanserin) having luminal enlargement during follow up (26.35% vs 36.09%, p=0.08).

Fifty five (8.2%) of the patients with lesions which underwent luminal enlargement compared to 614 (26.6%) of the patients without luminal enlargement had a clinical end points (Redo PTCA, CABG, acute myocardial infarction or death) during follow up (p<0.0001). The individual components of death, myocardial infarction, Coronary artery bypass grafting and re-PTCA were 0.15% vs 0.22%, 2.40% vs 3.02%, 1.05% vs 2.98% and 4.64% vs 20.68% respectively for lesions which underwent luminal enlargement vs lesions which did not. The differences in the individual clinical end points between the two groups were also statistically significant (p<0.0001). The time course of clinical end points are summarised in 2, which also demonstrate that the two curves only begin to diverge after the first 40 days of follow up. The mean time to clinical end point was significantly longer in the luminal enlargement group (172±38 vs 159±46 days, p=0.0001).

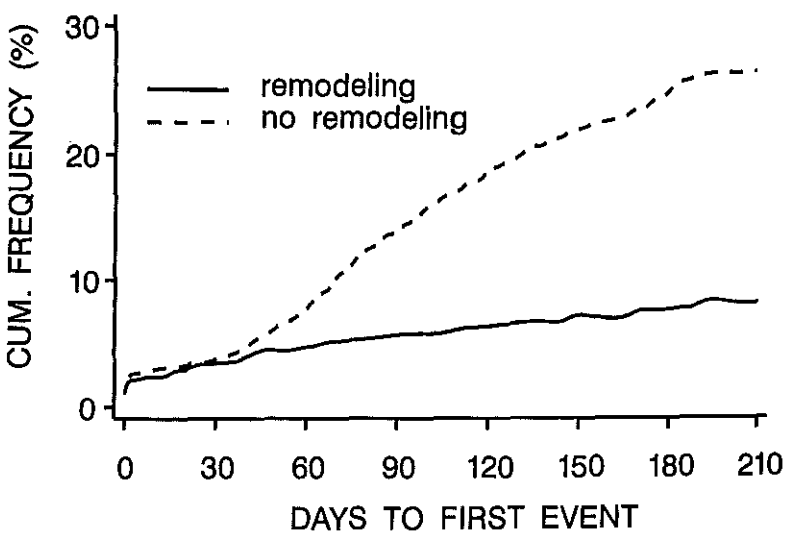

Figure 2. Cumulative distribution curve of clinical end points over time for patients with and without luminal enlargement during angiographic follow up.

Quantitative angiographic analysis and angioplasty procedure

A mean of 2.12 matched angiographic projections per lesion had satisfactory quantitative analysis performed, pre-, post- PTCA, and at follow up. Lesions which subsequently underwent luminal enlargement had a shorter length and needed fewer inflations for a shorter duration at a lower pressure (Table 2). Both the absolute and relative gain were significantly lower in lesions which underwent remodeling. Post-PTCA the MLD was significantly lower and the % residual stenosis significantly higher in the group which underwent luminal enlargement.

Remodeling No.	Yes 980	No 2,603	Significance Level
Reference Diameter (mm)			
Before angioplasty	2.62±0.56	2.60±0.53	0.2626
After angioplasty	2.62±0.53	2.67±0.51	0.0243
At follow up	2.80±0.56	2.63±0.56	<0.0001
Minimal luminal diameter (mm)			
Before angioplasty	1.02±0.43	0.99±0.38	0.1602
After angioplasty	1.68±0.35	1.78±0.36	<0.0001
At follow up	1.92±0.40	1.25±0.53	0.0001
Differences in MLD			
Absolute Gain (mm)	0.67±0.41	0.79±0.41	<0.0001
Relative Gain	0.26±0.16	0.31±0.16	<0.0001
Absolute Loss (mm)	-0.24±0.20	0.53±0.45	0.0001
Relative Loss	-0.10±0.08	0.21±0.19	0.0001
Absolute Net Gain (mm)	0.91±0.44	0.26±0.53	0.0001
Net Gain Index	0.36±0.18	0.10±0.21	0.0001
Loss Index	-0.61±2.22	0.93±4.09	0.0001
Percentage stenosis			
Before angioplasty	60.71±15.23	61.05±14.30	0.5573
After angioplasty	35.39±8.09	32.73±8.32	<0.0001
At follow up	30.76±8.75	51.71±19.04	0.0001
DS at follow up >50%	1.53%	47.25%	<0.0001

Table 3. Quantitative angiographic analysis of 980 lesions which underwent luminal enlargement compared with 2,603 which did not. MLD, Minimal luminal diameter. DS, diameter stenosis.

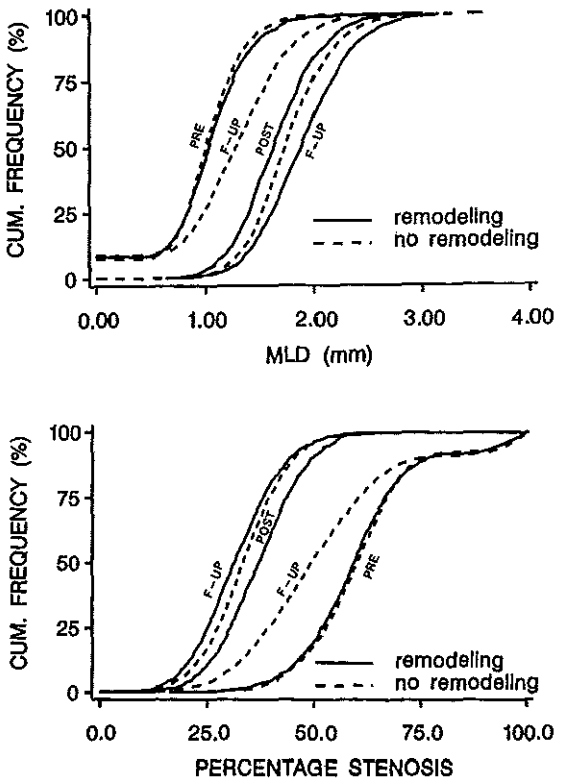

Figure 3. Upper panel: Cumulative distribution curve of MLD pre, post PTCA and at follow up for patients with and without luminal enlargement during follow up. Lower panel: Cumulative distribution curve of percentage stenosis pre, post PTCA and at follow up for patients with and without luminal enlargement during follow up.

At follow up the reference diameter was significantly higher in the luminal enlargement group suggesting that the whole vessel size had been reset (Table 3). As would be expected the categorical restenosis rate was significantly lower at 1.53% in lesions with luminal enlargement compared to 47.25 in lesions without.

Multiple linear regression analysis

Univariate analysis suggested that anginal class as well as drug therapy and procedural characteristics such as balloon pressure, number and duration of inflation may influence subsequent luminal enlargement (Table 1 and 2) so logistic regression analysis was performed for the above as well as all other clinical, angiographic and procedural parameters listed in tables 1-3. Of these variables diuretic therapy (regression co-efficient 0.41), MLD and diameter stenosis pre PTCA (1.11, 0.08 respectively), balloon artery ratio (2.70) and dissection post PTCA (0.27)

Variable	Regression Co-efficient	Standard Error of Regression co-efficient	p value	Odds ratio
Remodeling at follow up angiography				
Intercept	-0.704	1.182	$3.E^{-09}$	0.001
Diuretic therapy	0.407	0.180	0.0240	1.502
MLD pre PTCA	1.109	0.303	0.0003	3.032
Diameter stenosis pre PTCA	0.084	0.010	$4.E^{-18}$	1.088
Balloon artery ratio	2.700	0.392	$6.E^{-12}$	14.88
Dissection post PTCA	0.265	0.101	0.0084	1.303
Age	-0.011	0.005	0.036	0.989
Heparin therapy	-0.549	0.103	$1.E^{-07}$	0.578
No of vessels diseased	-0.168	0.081	0.037	0.845
Lesion length	-0.078	0.024	0.0001	0.925
LAD location	-0.336	0.102	0.001	0.715
Total inflation time	-0.001	0.000	0.0139	0.999
Relative gain	-6.001	0.590	$0.E^{+00}$	0.002

Table 4. Regression analyses to evaluate the respective contributions of clinical, angiographic and procedural variables to remodeling at follow up angiography in these lesions. Logistic regression analysis was used for this. Incl, Including. Excl, Excluding. Fup, follow up. PTCA, Percutaneous Transluminal Coronary Angioplasty. MLD. Minimal Luminal Diameter.

increased the probability of luminal enlargement whilst age (regression co-efficient -0.01), heparin therapy (-0.55), number of vessels diseased (-0.17), lesion length (-0.08), LAD location (-0.34), total inflation time (-0.001) and relative gain (-6.00) decreased the probability of luminal enlargement (Table 4).

Discussion:

Our results indicate that luminal enlargement may occur after successful coronary angioplasty, resulting in improved clinical outcome, and that a number of factors can substantially influence this. Future prospective studies are required to investigate this phenomenon further.

The reasons for the luminal enlargement in the angiographic follow up period are unclear. It may however reflect improvements in local flow dynamics and resetting of the vessel wall during follow up. The increase in blood flow velocity following successful dilatation of a stenosis results in an increase in wall shear stress which may stimulate endothelial dependent vasodilatation and resetting of the vessel wall (22-25). The higher residual stenosis and lower MLD post intervention in the luminal enlargement group however auger against this. It may still be a plau-

sible explanation however if there were covert differences in endothelial vasodilator function between the two groups (24). An alternative explanation is that the process reflects differences in wall and plaque composition and hence susceptibility to regression or favourable "Glagovian" arterial remodeling. In support of this are the differences in lesion length and procedural characteristics between the two groups, with fewer inflations at a lower pressure for a shorter time in the group which underwent luminal enlargement, perhaps reflecting a softer lesion, more likely to undergo regression and remodeling during follow up.

Multivariate analysis was suggestive of a number of clinical procedural and angiographic variables related to luminal enlargement. Those which increased the probability of luminal enlargement included diuretic therapy, mld and % stenosis pre PTCA, balloon artery ratio and dissection post PTCA, whilst those which decreased the probability of luminal enlargement included age, heparin therapy, no of vessels diseased, LAD location, lesion length, total inflation time and relative gain.

Both sets of variables revolve around lesion characteristics. The increased likelihood of luminal enlargement with balloon artery ratio and dissection may reflect enough damage to the vessel wall to allow freeing of the atherosclerotic plaque. The characteristics which decrease the probability of luminal enlargement again revolve around lesion characteristics and may reflect less likelihood of luminal enlargement in more advanced, more calcified, atherosclerotic disease. This is reflected in increased age, increased involvement of other vessels, increased lesion length and the requirement for increased total inflation time.

The relationship with drug therapy was also interesting and warrants further investigation. The positive relationship with diuretic therapy may reflect the influence of a third factor, such as hypertension, on vascular remodeling. Additionally both hypomagnasaemia and spironolactone can inhibit growth of vascular cells and decrease collagen synthesis, both of which may weaken the reparative processes in the vessel wall and allow luminal enlargement. The negative relationship with heparin therapy may provide one possible explanation as to why heparin which has been shown to suppress smooth muscle proliferation in vitro (26) and reduce neointimal hyperplasia in animal models of vascular injury (27,28) has failed to influence restenosis in the clinical situation (29). If restenosis is a result of both neointimal hyperplasia and vascular remodeling the negative effect of heparin on luminal enlargement may obviate any differential effect on neointimal hyperplasia. It is not clear why heparin should exert a negative influence on luminal enlargement. One possible explanation may be through strengthening of the vessel wall. Heparin is known to stimulate endothelial cell migration and angiogenesis which may result in faster healing, increased strengthening of the vessel wall and hence minimisation of any luminal enlargement. In addition heparin is also known to activate Transforming growth factor beta, an enhancer of matrix secretion and matrix contraction, which again may minimise any luminal enlargement.

Clinical implications: Our finding that luminal enlargement may occur after coronary angioplasty has important clinical implications for coronary intervention. If we can differentiate lesions which undergo favourable luminal enlargement we may be able to target newer percutaneous revascularisation techniques such as coronary stenting more effectively. Furthermore, by identifying clinical and procedural factors favourably influencing luminal enlargement and applying them to the other group we may be able to reduce restenosis. Additionally our data also suggest that luminal enlargement can be influenced by drug therapy in both a positive and a negative manner. This may open a second therapeutic window in tackling the problem of restenosis after coronary angioplasty and may also explain why agents such as heparin, which has been shown to suppress smooth muscle proliferation neointimal hyperplasia in animal models of vascular injury have failed to influence restenosis in the clinical situation. Future prospective studies, ideally utilising intracoronary ultrasound imaging to directly visualise the atherosclerotic plaque, are required to investigate this phenomenon further.

Limitations of the study: Our study could have been confounded by a number of methodological problems including measurement variability. We feel this is unlikely however for 2 reasons. Firstly previous work from our group (30) has shown that the re-measurement of the same lesion renders a difference between the measurements of 0.007mm with a standard deviation of 0.2mm. So we assume that 0.2mm is the standard deviation of the measurement error. In a worst case scenario where we assume all lesions to have a loss of 0mm we would expect 95% of the observations to be within 1.96 times the standard deviation, so even in this hypothetical situation, we would expect only 2.5% of the lesions to have a negative loss of 0.4 mm or more. In our database, we find, in fact 6.0% of lesions in this category. Testing for the difference between these proportions, we find a p-value of <0.00001. More importantly however as the mean loss in fact is much grater than zero at 0.30mm, measurement variability is extremely unlikely to be responsible for the negative loss which we see. Thus our results are unlikely to be due to measurement variability but are more likely to reflect real luminal increases over time. Our results may also relate to local dissection and inappropriate contour detection by the CAAS system. Again, this is unlikely as there was no significant difference in the overall incidence or type of dissection between the two groups. Another possible explanation is variability in vasomotor tone at the different time points. We controlled for this however with intracoronary nitrates administered immediately before each angiogram, again making this an unlikely explanation. Furthermore, as can be seen from Figure 2 the two curves do not begin to diverge until after 40 days, again suggesting that the cause of this is unlikely to be coronary spasm at the time of the procedure. We thus believe that we are seeing a true phenomenon, which is reflected in the low rate of clinical events in the luminal enlargement group. The fact that the process also affects the reference vessel segment, which has, in the past been shown to undergo the same restenosis process as the lesion itself (31) adds additional strength to this argument.

Our study was also a retrospective analysis of prospectively gathered data and is hence subject to the limitations inherent in any retrospective analysis. Additionally, contrast angiography can only provide an image of the vessel lumen and not the vessel wall. It is thus unclear whether the mechanism for luminal enlargement is plaque regression or vessel expansion over time or any combination of these factors.

Conclusion: Our results indicate that luminal enlargement may occur after successful coronary angioplasty, resulting in improved clinical outcome. They also suggest that lesion related characteristics, reflected by procedural variables, and drug therapy may substantially influence this process.

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Chapter 16

Randomised trials, registries or experience-based common sense?

Violaris AG, Serruys PW.

Ellis SG, Holmes DR (Editors) Strategies for coronary intervention: a practical approach. 1995 (Williams and Wilkins Publishers), pp190-204

The Thoraxcenter Perspective

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Percutaneous transluminal coronary angioplasty (PTCA), introduced by Grüntzig in the late 1970s, has been a major breakthrough in the treatment of obstructive coronary artery disease. Increasing operator experience and improvements in balloon catheter technology have resulted in a high immediate success rate and a low incidence of complications, which in turn has resulted in an increasing number of patients with more advanced coronary artery disease now being considered candidates for balloon angioplasty. Substantial limitations still remain, however, which include technical factors relating to the coronary anatomy that limit the intervention to particular subgroups of patients with coronary artery disease, acute occlusion resulting in increased morbidity and mortality, and longer-term restenosis in 30 to 40% of patients. Because of these limitations inherent in balloon angioplasty, pharmacologic agents to reduce restenosis and a plethora of new devices designed to improve the short- and long-term results of catheter-based revascularization have been introduced.

Considerable difficulties exist, however, in assessing long-term restenosis and making valid comparisons between the different treatment modalities available. We therefore need a means of rapidly and effectively assessing not only the acute efficacy and safety of new devices but also of comparing their long-term results with those of balloon angioplasty. Randomized trials, registries, and personal experience all go some way toward answering these questions. In this monograph we review the use of these three methods and propose a compromise approach, based on matching patient and lesion characteristics, that may result in the more rapid and efficient assessment of new devices and pharmacologic agents.

RANDOMIZED STUDIES

The best way to compare the short- and long-term clinical and angiographic results of different coronary interventions or pharmacologic agents is with a randomized study. The proper design of a randomized study to compare different interventional techniques requires the selection of the appropriate angiographic variables reflecting the restenosis process. In a recent study we examined which angiographic parameter best described functional status 6 months after successful single-vessel coronary angioplasty (1). Sensitivity and specificity curves for the prediction of anginal status and exercise electrocardiography of four quantitative angiographic variables were constructed, and the point of highest diagnostic accuracy for the variables was determined at the intersection of the sensitivity and specificity curves. The points of highest diagnostic accuracy using minimal luminal diameter were similar for both anginal status and exercise electrocardiography at 1.45 and 1.46 mm, respectively. This suggests that the minimal luminal diameter at follow-up may be useful for predicting clinical events such as recurrence of angina. In addition, the use of a central angiographic core lab, using a well-validated system of quantitative coronary angiography, with the expertise required to ensure the accuracy of all collated data and to minimize the possibility of introducing methodologic errors and bias is required. Also required are a sufficient number of patients undergoing repeat angiography at a predetermined time. This number depends on the variability in outcome among the patients, the magnitude of the difference in outcome, and the alpha and beta errors. Changing the power of the study in one way or another will affect the number of patients needed. Accepting a larger chance of missing

an effective treatment (large beta error and thus a reduction in power) means substantially less patients are needed.

A randomized study has several theoretical advantages. It guarantees population homogeneity in terms of clinical and angiographic variables and thus allows the direct comparison of different therapeutic treatment modalities with no bias regarding selection of treatment. Furthermore a well-designed randomized study may ensure that if a device is used, the device has a fair trial by requiring adequate expertise of all participating investigators. For example, with most devices there is often a steep learning curve, and therefore to ensure the fair evaluation of a device a minimum requirement would be that participating personnel have performed a set number of procedures using the device, with acceptable success and complication rates.

Problems Related to All Studies

Despite the above advantages randomized studies also have some inherent, logistic limitations. These include patient exclusions by virtue of the inclusion/exclusion criteria and inconsistencies in the timing of randomization, follow-up duration, and definitions of clinical and angiographic primary endpoints. We would like to expand on these using as an example four, recently completed, multicenter, restenosis trials: MERCATOR (randomized, double-blind, placebo-controlled, restenosis prevention trial of cilazapril 5 mg) (2), MARCATOR (randomized, double-blind, placebo-controlled, restenosis prevention trial of cilazapril 1, 5, or 10 mg) (3), CARPORT (randomized, double-blind, placebo-controlled, restenosis prevention trial of a thromboxane A_2 receptor antagonist) (4), and PARK (randomized, double-blind, placebo-controlled trial of ketanserin 40 mg) (5).

Patient Selection Bias—Inclusion/Exclusion Criteria

The inclusion/exclusion criteria for a study may have a substantial influence on both the power of a study and the applicability of the results to the general angioplasty population. The main inclusion/exclusion criteria in most studies relate to the coronary anatomy (single-vessel versus multivessel disease)/lesion morphology, the pattern of angina (stable versus unstable), a previous history of myocardial infarction, and concomitant drug therapy or device use.

SINGLE-VESSEL VERSUS MULTIVESSEL DISEASE

Large centers likely to participate in randomized studies often report that a large proportion of their patients have multivessel disease requiring multilesion angioplasty. If you therefore confine your study to single-vessel disease, you will limit the number of eligible patients. If you decide to include multivessel angioplasty, however, the problem then arises of how to handle restenosis. Do you perform a lesion- or a patient-related analysis. If you choose a patient-related analysis, how do you handle three dilated lesions in the same patient? It is much easier in statistics to work with single numbers than a collection of numbers. Because restenosis is likely to be a lesion-related rather than a patient-related problem (even the so-called patient-related factors such as unstable angina or recent angina actually reflect lesion instability) (6), we tend to reconcile lesion with patient analysis by using the average MLD (minimal luminal diameter) of multiple lesions obtained from multiple views so that statistically you deal with one MLD per patient. It is interesting, however, that despite the onus in the planning stage to include multivessel dilation, over 80% of patients randomized in the large multicentre trials have single-vessel disease with 16% two-vessel disease and 4% three-vessel disease.

STABLE VERSUS UNSTABLE ANGINA PECTORIS

One reason for including unstable patients is that in multivariate analysis the greatest loss in MLD occurs in the unstable patients.

For example, in the CARPORT study the change in MLD at follow-up was -0.37 ± 0.59 mm in patients with duration of angina <2.3 months (n = 210), while it was only -0.26 ± 0.53 mm in patients with duration of angina >8.5 months (n = 229). A similar pattern was seen in the MERCATOR study where the change in MLD at follow-up was -0.33 ± 0.55 mm in patients with duration of angina <86 days (n = 252), while it was only -0.19 ± 0.48 mm in patients with duration of angina >305 days (n = 256). The importance of this is in the power calculation. If unstable patients are excluded, then the calculation of the power of the trial becomes extremely difficult and large numbers of patients are required. In the ongoing FLARE study looking at fluvastatin for restenosis, the drug has to be started 3 weeks prior to PTCA which means unstable patients are excluded from the study, increasing the number of patients required.

If you do decide to include patients with unstable angina, the problem then arises that there is no accepted definition of this term. A practical definition, used in the CARPORT study, was pain at rest controlled by intravenous nitrates. Even within this group, however, there is a marked heterogeneity of patients, and a better definition and subdivision of unstable angina is needed. In the ongoing HELVETICA study (randomized, double-blind, placebo-controlled, restenosis prevention trial of recombinant hirudin) patients are entered using the Braunwald classification, which will hopefully provide better information on the long-term restenosis rate in the various subgroups of unstable angina.

PREVIOUS MYOCARDIAL INFARCTION

Most studies have systematically excluded patients with recent myocardial infarction from trial entry. In the MERCATOR study, for example, patients with a myocardial infarct within 3 weeks were excluded (Table 12C.1). In the MARCATOR study an amendment was passed 3 months into the trial allowing patients within 5 days of myocardial infarction to be entered into the study. This high rate of recent myocardial infarction may be responsible for the increased loss in MLD in the MARCATOR compared with the MERCATOR study (0.37 versus 0.28 mm).

Table 12C.1.
Screening Results of 17 Log-Keeping Clinics Participating in the MERCATOR Multicenter Randomized Study of Cilazapril[a]

Reason for Exclusion	Number[b]	%
History of sustained essential hypertension	271	21.2
Previous and/or failed PTCA at the same site	268	21.0
Q-wave MI less than 4 weeks prior to study entry	174	13.6
Follow-up coronary angiography unlikely	109	8.5
Logistic reasons	67	5.2
Significant concomitant disease	50	3.9
Older than 75 years	43	3.4
Dilatation of bypass graft	40	3.1
Primary perfusion therapy	39	3.1
No informed consent given	39	3.1
Current evidence of prior history of heart failure	28	2.2
Participation in other trial	14	1.1
Atherectomy/stent	13	1.0
Other reasons[c] (less than 1% each)	122	9.6

[a]From MERCATOR study group. Circulation 1992; 86–100–101 with permission.

[b]Screening results of 17 clinics which recruited 65% of the patients.

[c]Left main disease, history of type II hypercholesterolemia, previous cerebro-vascular accident, previous participation in MERCATOR, hypotension, contraindication to ACE inhibition/aspirin, women of childbearing potential, insulin-dependent diabetes, miscellaneous.

EXCLUSION CRITERIA BASED ON TRIAL MEDICATION

The trial medication may also play a significant role in selection bias by excluding patients with contraindications to the medication or those on concomitant therapy that may interact with the trial medication. For example, in the MERCATOR and MARCATOR studies hypertension was a major exclusion criterion because of the associated hypotensive effect of cilazapril (Table 12C.1). In the MERCATOR study 15% of all patients screened were excluded because of sustained essential hypertension. In the CARPORT and PARK studies the use of platelet-inhibiting or nonsteroidal antiinflammatory drugs within 7 days preceding the study was responsible for the exclusion of 28% of patients. The use of oral anticoagulants at the time of the procedure excluded a further 9% of patients.

EXCLUSION CRITERIA BASED ON TECHNOLOGY

Most randomized pharmacologic studies on restenosis have specifically excluded the use of new devices. This means that when the results are published, a possible criticism will be that they do not reflect and therefore do not apply to the real world. Statistically, as long as the use of new devices occurs in less than 10% of randomized patients, dealing with them is not a problem. Once they reach 50%, however, stratification of patients according to treatment modality would be required. Future trials are thus likely to include all new technologies, apart from stents. It is of interest, however, that in the ongoing HELVETICA study, where the use of new technology is allowed, these only occur in 4% of the patients entered, so there is no need for posthoc stratification.

Stringent entry criteria designed to minimize the randomization of high-risk patients mean that only a minority of eligible patients may be recruited for a particular study. In the MERCATOR study, for example, 1755 patients were screened but only 478 (27.2%) were actually recruited into the study. The reasons for exclusion included a history of sustained essential hypertension (271 patients, 15.4%) and previous and/or failed PTCA at the same site (268 patients, 15.3%), as well as a variety of other less frequent factors (Table 12C.1). In the PARK study even fewer screened patients were enrolled into the study (704/5636, 12.5%).

The exclusion of high-risk patients may have a significant influence, however, on the power of a trial. Historically, the loss in MLD during follow-up has been around 0.4 mm (7). For the trial power calculations, for a 30% reduction in the treatment group the expected loss would be 0.25 mm. For all the recent trials, however, the MLD loss has been much lower (CARPORT 0.31 mm, MERCATOR 0.30 mm, PARK 0.26 mm), resulting in a reduction in the power of the trials and making it more difficult to demonstrate significant benefit. This is because they have taken the minimalist approach toward patient recruitment—only enrolling patients with single-vessel disease and stable angina pectoris and specifically excluding high-risk patients such as those with recent myocardial infarction and unstable angina.

Inclusion of such patients should increase the loss in MLD during follow-up and hence increase the power of a trial. Thus future trials are likely to take a "maximalist" approach by including patients with multivessel disease, unstable angina, recent myocardial infarction, and previous PTCA and restenosis. In the ongoing HELVETICA trial, for example, only difficult unstable patients are included, which will hopefully increase the power of the study.

In addition, often little indication is given of the proportion of eligible patients entered into the study and the likely outcome of patients excluded from the study. Therefore the results of a study may not apply to the large proportion of patients seen in clinical practice.

Timing of Randomization

The optimal timing of randomization is also unclear. Do you randomize the patients before the procedure as in the PARK, HELVETICA, and CARPORT studies, or do you only randomize them once the procedure has been successfully performed as in the MERCATOR and MARCATOR studies? In the PARK and CARPORT studies, since the mechanism of drug action implied that it would prevent both acute occlusion caused by platelet aggregation–induced thrombus formation and late restenosis caused by platelet aggregation–induced hyperplasia, trial medication was started before the procedure, that is, before wall injury occurred. This had major consequences for the definition of the clinical endpoint. On the one hand, all failure between first balloon inflation and the end of the procedure could have been influenced by the trial medication and were therefore counted as clinical endpoints. On the other hand, as the aim of this trial was to study the effect of ketanserin on the inhibition of neointimal hyperplasia following balloon wall injury, it seemed reasonable to exclude from the analysis of the main clinical endpoints those patients in whom no balloon inflation had occurred.

In the MERCATOR and MARCATOR studies, concern was voiced over the possible hypotensive effect of the medication if it was given prior to the procedure. The debate centered around whether the medication should be given prior to the procedure and any perioperative hypotension treated with volume expansion or whether it would be safer to administer the study medication after the procedure. Since preliminary animal experiments had shown no major difference in inhibition of neointimal proliferation whether the drug was given 1 hour before or within 2 days after wall injury, we were able to screen patients before the procedure but only enroll those who had undergone a successful coronary angioplasty into the study. This approach eliminated problems relating to the influence of the drug on the success of the procedure but may also have introduced selection bias. For example, if the investigator noted the presence of thrombus postangioplasty, this may have influenced the likelihood of a patient being subsequently entered into the study. This may explain why in a multivariate analysis of the CARPORT study the presence of thrombus was associated with the relative loss, whereas this was not the case in the MERCATOR study.

Timing of Follow-Up Duration

One of the consequences of having systematic, follow-up angiography at 6 months is that it distorts the natural occurrence of clinical endpoints (reinterventions). As a consequence, all indications for a revascularization procedure that have been triggered by the 6-month repeat angiogram are counted as endpoints, provided that the indication is also substantiated by anginal symptoms or positive findings at exercise testing. In the CARPORT study clinical events were counted up to the 6-month angiogram minus 2 weeks. This meant that the therapeutic implications of the diagnostic angiogram were circumvented, resulting in a lower rate of events compared with similar studies. This led to two problems. Firstly, it reduced the power of the study to detect a difference in restenosis, and secondly, it may have tempted investigators to await recatheterisation before further therapy. To overcome these problems, in the MERCATOR study we increased the cutoff point to 7 months. This, however, tended to overestimate the clinical event rate by including cosmetic dilatations following the 6-month angiograms and thus introduced a different problem into the analysis. For the PARK study we again extended the study to 7 months, but excluded cosmetic dilatation by insisting that for a repeat PTCA to be a clinical event the clinician had to justify his decision on the basis of objective evidence of ischemia. Furthermore, the decision to redo the PTCA was counted as a clinical event

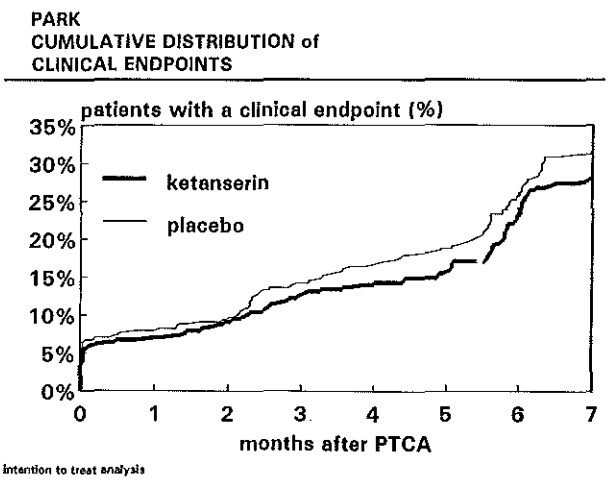

Figure 12C.1. Cumulative distribution curve of clinical endpoints for the Post Angioplasty Restenosis Ketanserin (PARK) trial. Note the distribution of endpoints and how the timing of recatheterization may influence the results. (Reprinted by permission from Serruys PW et al. Circulation 1993;88:1588–1601.)

even if it did not physically occur during the follow-up period. This did not completely solve the problem, however, as certain patients were marked down for repeat interventions that never occurred for a variety of reasons. Thus the optimal time for restudy may play a role in the clinical endpoints as shown graphically in Figure 12C.1.

It may be best in the future therefore to have a primary endpoint at 7 months and a secondary endpoint at 12 months.

Definition of Primary Endpoints

The primary goal in restenosis trials is an improvement in the short- and long-term clinical outcome in patients undergoing PTCA. It is assumed that the improvement in clinical outcome relates directly to the prevention of the stenosis recurrence. This may not be completely true, however.

CLINICAL ENDPOINTS

For the MERCATOR study as our primary clinical endpoints we chose a combination of the major untoward clinical events, namely, death, myocardial infarction, referral for coronary artery bypass surgery, or an indication for repeat angioplasty. The advantages are obvious in that if the endpoint is based on these so-called "hard" criteria, the endpoint is evaluable in all randomized patients and leads to simple effect estimates with corresponding 95% confidence intervals. Because hard endpoints only occur, however, in approximately 20% of cases, our ability to detect even a 50% difference in restenosis rate between intervention and control requires a very large sample size with all the associated logistic problems. Surrogate, soft endpoints such as recurrence of angina or noninvasive evidence of myocardial ischemia may also be introduced to increase the event rate to 50% and hence to reduce the sample size required (Table 12C.2). Because of the subjective nature of these, however, bias is introduced into the study.

For example, in trials testing pharmacologic compounds with possible antiischemic or antianginal effects unrelated to postinjury hyperplasia, subjective, soft endpoints may be misleading and obscure the reasons for observed improvement. In the MERCATOR study fewer patients in the cilazapril-treated group experienced anginal pain during exercise testing compared with the control group, despite similar workloads, double product, and ST-segment changes (Table 12C.3). This was regardless of angiographically similar restenosis rates suggesting that cilazapril had no effect on intimal hyperplasia but perhaps

Table 12C.2.
Total Number of Events and Ranking Scale in the CARPORT Study[a]

	Total No. of Events During 6-Month Follow-Up				Ranking of Clinical Status 6 Months After PTCA			
	Control N = 346		GR32191B N = 351		Control N = 346		GR32191B N = 351	
Death								
-Late	6		4					
-All	6	(2%)	4	(1%)	6	(2%)	4	(1%)
Myocardial infarction								
-Procedural	5		5					
-Early	11		7					
-Late	6		6					
-All	22	(6%)	18	(5%)	22	(6%)	18	(5%)
CABG								
-Procedural	3		7					
-Early	5		2					
-Late	18		18					
-All	26	(8%)	27	(8%)	19	(6%)	22	(6%)
Repeat angioplasty								
-Early	9		6					
-Late	59		54					
-All	68	(20%)	60	(17%)	52	(15%)	49	(14%)
*@CCS IV	5	(2%)	1	(0.3%)	5	(2%)	1	(0.3%)
*@CCS III	19	(6%)	18	(5%)	11	(3%)	11	(3%)
*@CCS II	36	(11%)	47	(14%)	23	(7%)	30	(9%)
*@CCS I	26	(8%)	32	(9%)	14	(4%)	19	(5%)
None	254	(75%)	249	(72%)	194	(56%)	197	(56%)

[a]From Serruys PW et al. Circulation 1991; 84:1568–1580 with permission.

*, For 687 patients alive at 6-month follow-up; @, secondary endpoint; *CCS*, Canadian Cardiovascular Society angina classification.

Table 12C.3.
Exercise Test Results of 564 Patients Randomized in the MERCATOR Study[a]

	Control N = 291 Pts	Cilazapril N = 273 Pts	*P* Value
Maximum workload (Watts)	146 ± 39	151 ± 44	NS
Exercise time (sec)	446 ± 124	454 ± 127	NS
Systolic blood pressure at peak exercise (mm Hg)	196 ± 27	192 ± 28	NS
Heart rate at peak exercise (bpm)	142 ± 22	142 ± 21	NS
Double product (mm Hg 100/bpm)	279 ± 65	275 ± 66	NS
ST deviation >1 mm	102 (36%)	99 (37%)	NS
Anginal symptoms during test	74 (25%)	42 (15%)	0.03
Combination of ST >1 mm and symptoms	39 (13%)	25 (9%)	NS

[a]From MERCATOR study group. Circulation 1992; 86:100–101 with permission.

Pts, Patients.

may have affected endothelial function. A similar effect was seen in the PARK study where patients receiving ketanserin demonstrated a trend toward a lower incidence of revascularization procedures (77 versus 95 in the control group). This may have been secondary to the arteriolar dilating effect of ketanserin, resulting in left ventricular unloading, or perhaps to its effect on rheologic parameters, resulting in increased coronary blood flow.

A further confounding variable is the fact that not all variables are of equal importance. For example, death is of a different order of magnitude from myocardial infarction, which in turn is of a different order of magnitude from repeat angioplasty. Furthermore, the total count of events only gives a general impression of complications as events are not mutually exclusive and may overlap. A patient may, for example, sustain an acute occlusion resulting in myocardial infarction and subsequent death despite emergency coronary artery bypass grafting; in terms of total count of events this patient might be counted four times.

Ranking endpoints in terms of relative descending importance (Table 12C.2) may overcome some of these problems, but the problem of quantifying each complication and its relative importance then arises. For example, how many myocardial infarctions are equivalent to death? Such methods are standard in the field of decision analysis, however, and a similar composite endpoint has been recently proposed for late follow-up after coronary angioplasty (8, 9). Although such an approach requires prospective valuation, it clearly provides the ability to combine both clinical and angiographic endpoints and may thus be useful in early, phase 2 clinical trials to improve statistical power.

In attempting to address these questions other models have also been proposed. Friedrich and colleagues showed that the constant-hazard model cannot be applied to coronary interventional techniques as the risk is not constant but varies over time (10). Furthermore, this rate combines two underlying hazards: an early time-dependent hazard caused by restenosis of the target vessel and a more constant hazard caused by the progression of coronary atherosclerosis or comorbid disease. This temporal sequence of events is highest at the time of the initial procedure and at the follow-up catheterization, where there is an artificial increase in the rate of events (Fig. 12C.1).

An alternative means of overcoming some of these problems is also with the use of event-free survival curves. These provide in a graphic form information on the time course, as well as on the incidence of endpoints.

NONINVASIVE EVALUATION OF RESTENOSIS

As far as detection of restenosis by noninvasive diagnostic tests is concerned, it can generally be said that an abnormal exercise ECG response or myocardial perfusion defect on a thallium-201 scintigram is usually associated with either an angiographically demonstrable restenosis of the dilated segment, a functionally inadequate original dilation, or the presence of additional disease. The sensitivity and specificity of the test may therefore be increased by performing the test prior to the procedure to document a baseline result, immediately after angioplasty to filter out the presence of additional disease or inadequate functional dilation, and subsequently at 6 months to document restenosis.

Logistic problems arise, however, in trying to perform the exercise test. Firstly, most of the centers participating in large multicenter studies are regional referral centers draining large areas, whose first contact with the referred patient is at the time of the procedure. It is logistically difficult for them therefore to ensure that all patients have an exercise test prior to the procedure. Secondly, problems arise in performing the exercise test immediately after the procedure with doubts raised as to the safety of this policy. Thus

insurmountable difficulties arise in trying to arrange the exercise test prior to and immediately after angioplasty. The concept is advanced, however, that perhaps we do not need to do this and, since all we are doing is comparing two groups—an active and a placebo group, perhaps we should just exercise test all patients at 6 months. For the reasons outlined above, however, the positive predictive value of late treadmill testing varies from 39 to 64% (Table 12C.4), making it difficult to make valid intergroup observations. The only useful role we have found for the exercise test is to substantiate the need for a repeat therapeutic PTCA at follow-up.

Similar arguments can also be used against the role radioisotopes. The need for pre-, immediate, post-, and follow-up scans also raises two additional issues. Firstly, objections are raised regarding the repeated use of radioisotopes and the long-term result of doing so. Secondly, problems arise in the objective assessment of the results as there is still no agreed standardized procedure for image acquisition and interpretation.

For all the above reasons exercise testing or thallium scintigraphy are of little value in the assessment of long-term restenosis. Their only role may be to substantiate the therapeutic procedure if repeat angiography is suggestive of a significant restenosis.

ANGIOGRAPHIC ENDPOINTS

The most accurate way to quantify the proliferative response as part of restenosis would be with the direct measurement of neointimal thickening following coronary intervention. This is not, however, currently possible in man, although the advent of new imaging modalities such as angioscopy and, more especially, intravascular ultrasound may one day make it possible. For debulking devices that may alter both acute dimensional gain and the later proliferative response, currently quantitative coronary angiography is the most objective and reproducible method available to describe the changes in stenosis geometry following intervention. Although it only measures the process indirectly without defining its nature (recoil, organized mural thrombus, neointimal hyperplasia), it has nonetheless become the accepted gold standard in documenting restenosis.

There is still, however, no agreed on angiographic definition of restenosis (11, 12). To date there have been a total of 13 different definitions used in the literature, but none have become widely accepted. Although the clinician is best served by the "present/not present" assessment of restenosis, the biologic process of restenosis itself may be best analyzed by measuring the ab-

Table 12C.4.
Detection of Restenosis by Exercise Treadmill Testing[a]

Author	Angiographic Follow-Up %	Restenosis %	PPV %	NPV %	Timing of Test
O'Keefe	100	13	29	73	<1 month
Scholl	83	12	40	27	1 month
Wijns[b]	74	35	50	65	3–7 weeks
Wijns[b]	89	40	60	52	3–8 weeks
Bengston	96	51	39	84	6 months
Rosing	100	34	47	76	8 months
Ernst	100	4	50	95	4–8 months
Honan	88	58	57	64	6 months
Scholl	83	12	64	50	6 months

[a]Modified from Califf et al. Restenosis: the clinical issues. In: Topol EJ, ed. Textbook of interventional cardiology. Philadelphia: WB Saunders, 1990.

[b]Thoraxcenter

PPV, Positive predictive value; *NPV*, negative predictive value.

solute angiographic dimensions on a continuous scale. This is for two main reasons. Firstly, as we have previously demonstrated, all lesions undergo restenosis to some extent during follow-up in a Gaussian distribution. Secondly, if we treat restenosis as a continuous variable, more information can be gleaned from the available data regarding the underlying process. Furthermore, the use of a continuous variable in statistical analysis allows us to reduce the number of patients required to demonstrate benefit in a specific study by two-thirds. For example, if treatment reduces the loss of luminal diameter from 0.40 mm under placebo to 0.25 mm under active medication (SD = 0.5 mm), 233 patients per group would be required to have a power of 90%. The above reduction corresponds to restenosis rates (defined as a loss of minimal luminal diameter of 0.72 mm) of 25% and 17.5%, respectively. This difference, however, can be statistically detected with a power of 90% with 620 patients per treatment group. Thus the quantitative outcome determined from direct measurements of continuous variables can be evaluated statistically with only one-third the number of patients required for the categorical outcome.

The minimal luminal diameter provides more reliable and meaningful information than percentage diameter stenosis with regard to the hemodynamic significance of a coronary artery lesion and has been shown to correlate at follow-up with the recurrence of angina or exercise-induced myocardial ischemia (1). The minimal luminal diameter at follow-up thus best describes the lesion severity at this point in time, while the process of restenosis can best be addressed by measuring the changes in luminal diameter from postintervention to follow-up in pharmacologic studies looking at restenosis. In studies examining devices, however, because of differences in the initial gain/loss, it is impossible to use the change in luminal diameter at follow-up and is best to look at a static parameter such as the MLD at follow-up to assess restenosis.

Are Quantitative Angiographic Parameters a Good Surrogate for Clinical Events?

One of our convictions is that the difference in minimal luminal diameter at follow-up between two populations will be translated into clinical terms. This has been generally true, as illustrated recently by the results of the BENESTENT and STRESS trials. A discordance, however, was noted in the CAVEAT I trial wherein a modest improvement in luminal dimensions at follow-up after directional atherectomy compared with PTCA did not "translate" into clinical benefit.

A more detailed analysis is available for the CARPORT study. In that study the mean difference in coronary diameter between postangioplasty and follow-up angiogram was similar in the control and GR32191B groups (-0.31 ± 0.54 mm and -0.31 ± 0.55 mm, respectively), and this was reflected in similar rates of clinical events. If you separate the population into two, however, on the basis of symptoms or positive ergometry, the cumulative distribution curves for change in minimal luminal diameter clearly separate (Fig. 12C.2), suggesting that the change in MLD may indeed reflect clinical events. Furthermore, in the MERCATOR study when the patient population was stratified according to the minimal lumen diameter at follow-up (Table 12C.5), the percentage of patients reaching one of the predefined clinical endpoints was as high as 65% in the worst category (MLD at follow-up <1.15), whereas the percentage of event-free patients ranged from 63 to 78% in the other categories. Additionally, 41% of the patients in the worst versus only 6% in the best anatomic category had reintervention irrespective of the initial dilatation site. Besides the prognostic value, the anatomic results also had a clear functional impact because only 2% of the patients in the best anatomic category had a positive exercise test versus 26% in the worst.

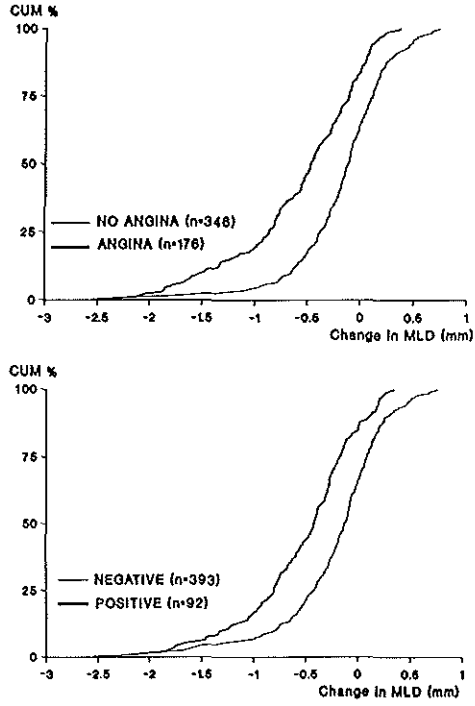

Figure 12C.2. Top panel. Cumulative distribution (CUM %) curve for change in minimal luminal diameter (MLD) for symptomatic and asymptomatic patients at follow-up angiography. **Bottom panel.** Cumulative distribution (CUM %) curve for change in minimal luminal diameter (MLD) for patients with positive and negative ergometry at follow-up angiography. (Reprinted by permission from Serruys PW et al. Circulation 1991; 84:1568–1580.)

Limitations of Differences in MLD Between Various Trials

Over the last few years it has become apparent that the loss in MLD in different trials could vary considerably, and in the placebo groups the loss in MLD ranged from 0.24 to 0.36 mm. The reasons for this are unclear, although one possibility is that these differences in mean luminal diameter at follow-up reflect differences in baseline demographic data. Factors such as the percentage of recruited patients in angina class 4, patients with recent onset of angina, unstable patients, and diabetic patients and the frequency of total occlusion have a major impact on the loss in MLD at follow-up. Popma and colleagues suggest that in future trials high-risk patients not be excluded to therefore avoid a misrepresentation of the population of typical patients undergoing angioplasty (9). Furthermore, they suggest that by selecting elective patients the expected late loss in MLD may be reduced, resulting in a requirement for a larger study population.

Limitations of Quantitative Angiography

Quantitative coronary angiography (QCA) provides an outline of the vessel lumen and not the vessel wall, which is where the un-

Table 12C.5.
Prognostic Value of Minimal Luminal Diameter at Follow-Up in the Preprotocol MERCATOR Population Divided Into Five Equal Segments*

MLD Follow-Up (mm)	Exercise Test		Clinical Outcome			
	<1 mm STT Changes and No Chest Pain	≥1 mm STT Changes and Chest Pain	MI	Reintervention	Angina	None
<1.10	70 (75%)	24 (26%)	5 (4%)	49 (41%)	24 (20%)	41 (35%)
1.10 to 1.39	88 (88%)	12 (12%)	1 (1%)	18 (15%)	25 (21%)	74 (63%)
1.39 to 1.63	103 (90%)	11 (10%)	2 (2%)	10 (8%)	31 (26%)	77 (64%)
1.63 to 1.91	99 (93%)	8 (7%)	1 (1%)	7 (6%)	21 (15%)	89 (75%)
1.91 or more	111 (98%)	2 (2%)	1 (1%)	7 (6%)	18 (15%)	94 (78%)
	471 Pts	57 Pts	10 Pts	91 Pts	119 Pts	375 Pts

*From MERCATOR study group. Circulation 1992; 86:100–101 with permission.

MLD, Minimal luminal diameter; *mm*, millimeter; *MI*, myocardial infarction; *Pts*, patients.

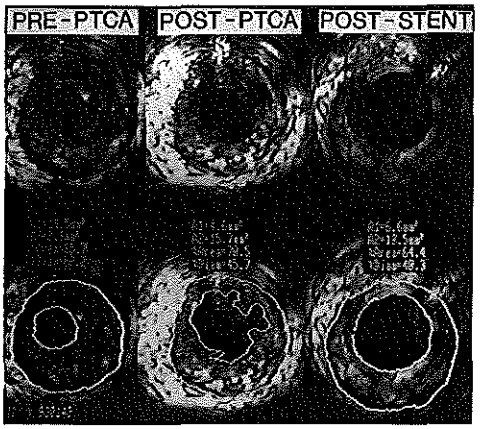

Figure 12C.3. Ultrasound imaging prior to balloon angioplasty and following angiographically successful balloon dilatation. It can be clearly seen post-PTCA that the angiographic success is as a result of eccentric tears in the vessel wall with only a small increase in the residual luminal area (pseudosuccess). Following successful stent implantation there is a good residual luminal area. (Images courtesy of Dr. C. diMario, Thoraxcenter, Erasmus University, Rotterdam, The Netherlands.)

derlying pathologic process is occurring. Thus much of what may be an angiographic success may turn out to be a pseudosuccess, perhaps because of cracks and dissection planes in the vessel wall giving the impression of a successful result whereas in reality there may be little increase in the actual luminal area (Fig. 12C.3). Thus a split in the vessel wall may provide an angiographically successful result (a pseudosuccess), but subsequent apposition of the split-wall layers may result in an angiographic restenosis (a pseudorestenosis), a lesion being classified as restenosis whereas in fact it was never an actual success.

Thus we may have to separate the clinical outcome, which may be measured by QCA, from the pathologic process occurring in the vessel wall. Whether new concepts in the visualisation of the vessel wall such as angioscopy or, perhaps more importantly, intravascular ultrasound can refine our understanding of the underlying pathologic process is still unclear but holds a great deal of promise.

Ideal Trial

As outlined above, although randomized studies are the best means we have of comparing the acute and long-term results of different interventional devices and the impact of pharmacologic therapies, they remain limited by inherent logistic problems. Our ideal randomized trial would be based purely on clinical follow-up of hard endpoints at 1 year. This would assess the effect of the intervention, whether it is drug therapy or a device compared with stand-alone angioplasty without the interference from the recatheterization procedure.

MATCHING STUDIES

In view of the large number of pharmacologic and mechanical interventions available to us and the time, energy, and financial burdens involved in conducting a large randomized trial, we have developed the concept of matching to use as a surrogate for a randomized study. Matching may offer insight into the effects of different interventional techniques, screen devices, and yield statistically helpful information such as a required sample size for the proper design of a randomized trial.

The principles of matching by quantitative angiography are threefold. Firstly, the angiographic dimensions of matched lesions are assumed to be identical. Secondly, the observed differences between the two identical lesions must be within the range of system-analysis reproducibility (0.1 mm; 1 SD). Finally, the reference diameter of the lesions to be matched must be selected to be within a range of ± 0.3 mm (3 SD; confidence limits 99%). Matching can also be extended to include lesion location and clinical parameters such as male sex, stable/unstable angina, diabetes, total occlusions, and other risk factors that may confound the incidence of restenosis.

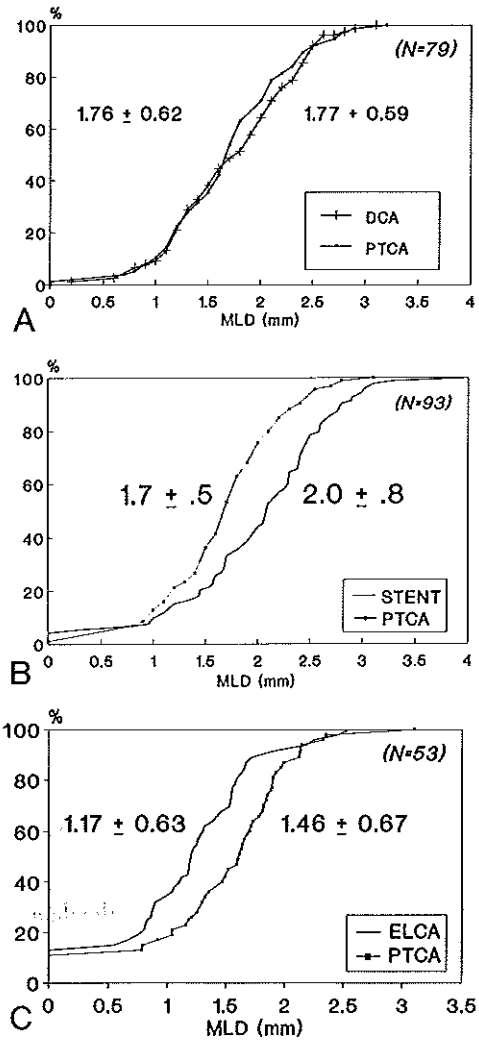

Figure 12C.4. Cumulative distribution curves of MLD at follow-up for matched populations. **A.** Atherectomy versus PTCA. **B.** Stent implantation versus PTCA. **C.** Excimer laser versus PTCA.

Matching in Action

We have used the principles outlined above to assess late luminal renarrowing in atherectomy, stent implantation, and excimer laser versus balloon angioplasty (Fig. 12C.4).

In atherectomy versus balloon angioplasty we demonstrated that matching for clinical and angiographic variables resulted in two comparable groups with identical baseline stenosis characteristics (13). Atherectomy resulted in a more pronounced increase in minimal luminal diameter than did balloon angioplasty (1.17 ± 0.29 to 2.44 ± 0.42 mm versus 1.21 ± 0.38 to 2.00 ± 0.36 mm), but this favorable immediate result was subsequently lost during late angiographic follow-up, as the minimal luminal diameter at follow-up did not differ between the two groups (1.76 ± 0.62 versus 1.77 ± 0.59 mm, atherectomy versus balloon angioplasty) (Fig. 12C.4*A*). This suggests that in matched populations, atherectomy induces a greater initial gain in minimal luminal diameter than balloon angioplasty but that the greater vascular wall injury induced by this device is associated with a more pronounced late loss. The results of this study were subsequently reflected in the CCAT and CAVEAT studies.

Using coronary stenting we demonstrated that Wallstent implantation results in a superior immediate increase in minimal luminal diameter (from 1.22 ± 0.34 to 2.49 ± 0.40 mm) compared with balloon angioplasty (from 1.21 ± 0.29 to 1.92 ± 0.35 mm) (14). Despite a greater decrease in minimal luminal diameter after Wallstent implantation compared with balloon angioplasty (0.48 ± 0.74 versus 0.20 ± 0.46 mm), the minimal luminal diameter at follow-up was significantly greater after stent implantation (2.01 ± 0.75 versus 1.72 ± 0.54 mm) (Fig. 12C.4*B*). Although different stents were evaluated, our results paralleled those of the recently reported BENESTENT and STRESS trials.

Using excimer laser we demonstrated that despite similar acute increases in minimal luminal diameter (from 0.73 ± 0.47 to 1.77 ± 0.41 mm, excimer laser, versus 0.74 ± 0.46 to 1.78 ± 0.34 mm, coronary angioplasty), the minimal luminal diameter after excimer laser was significantly lower at follow-up (1.17 ± 0.63 versus 1.46 ± 0.63 mm) (Fig. 12C.4*C*), suggesting that in successfully treated matched coronary lesions

there is reduced long-term efficacy after excimer laser compared with balloon angioplasty. Whether these preliminary results will be reflected in clinical outcome will be answered in the ongoing AMRO randomized study of excimer laser versus balloon angioplasty.

Limitations of Matching

The major limitation of a matching study is its retrospective design and the inevitable presence of selection bias. It controls for bias only in those variables taken into account. It is not possible to match for all variables because of practical difficulties in finding patients who meet all the matching criteria. Moreover, if categories of matching are relatively crude, there may be room for substantial differences between matched groups. For example, if clinical variables such as unstable angina or perhaps diabetes, which are known to predispose to a higher restenosis rate, are not included in the matching, substantial differences will occur between the two groups that may influence the validity of statistical comparisons.

Role in Clinical Decision Making

The use of matching studies as a surrogate for randomized studies offers the potential to rapidly screen a number of devices and to assess which are most likely to represent advances in balloon angioplasty. These can then be examined in larger randomized studies. Furthermore, matching yields statistically helpful information regarding the proper design of subsequent randomized trials such as required sample size.

REGISTRIES

The advantage of a registry is that data are collated in a coordinating center that allows information to be gleaned regarding the success and acute complications in a self-selected group of patients from a variety of centers with varying expertise. It therefore gives an impression of the acute results at the ground level and may, if adequate follow-up is encouraged, give an indication of the long-term restenosis rate. Information is obtained at little expense in terms of time or money as it is normally freely available at each institution.

There are a number of major limitations to extrapolating the results from registries to the general population, however. Firstly, the patients are entered on an ad hoc basis by the operator and may not be representative of the totality of patients on whom the device was used. This may explain, for example, why the *reported complication rates from registries are consistently lower than the complication rates observed in clinical practice.* Secondly, there are usually no central angiographic core lab and no independent verification or audit of the data supplied by the investigator to ensure accuracy and reproducibility. Furthermore, the angiographic analysis is normally performed by the operator with visual assessment of the coronary angiogram, a technique introducing bias and inaccuracies. Finally, the analysis is usually performed on a post hoc basis with multiple statistical comparisons being made on a small number of selected patient subgroups.

Because of the inherent limitations outlined above it is difficult to interpret the data from the various registries and to draw meaningful conclusions (15). The only useful information that may be gleaned from registries may be some idea about trends in various patient subgroups; for example, that calcification may not be good for atherectomy.

EXPERIENCE-BASED COMMON SENSE

Whatever the results of large randomized studies, it is ultimately the skill and judgment of the individual doctor in assessing and treating a particular lesion in an individual patient that will determine the safety and efficacy of the procedure. The main advantage of experience-based common sense is that it will limit cardiologists to the use of specific devices that are safe in their hands on specific

lesions in their particular circumstances. Experience-based common sense, however, will tell them little regarding long-term restenosis.

Furthermore, there are a large number of other potential limitations to experience-based common sense. Virtually all new devices have a steep learning curve so that an individual doctor may never do enough cases to get past this learning curve. Thus an operator who has never attained a sufficient level of skill in using the device will be prejudiced against it and perhaps only use it in specific patient subgroups. This combination of individual expertise and selection bias means that the safety and efficacy of particular devices can never be adequately quantified and that any differences between devices would be extremely difficult to prove.

SUMMARY

Adverse coronary anatomy, acute occlusion, and long-term restenosis remain major limitations of coronary angioplasty. Over the last 5 years atherectomy, stenting, and laser techniques have been introduced as potentially safer alternatives to balloon angioplasty with improved short- and long-term results. Considerable difficulty exists, however, in making valid comparisons between the different modalities with regard to the above outcome measures. Device registries may be useful for the initial assessment of the initial safety and success of the device. Matching studies may help to screen devices for subsequent randomized studies to delineate the precise role of each new device. We must never forget, however, that irrespective of the results of registries and matching and randomized studies, the final arbiter of the success or failure of the procedure will be the cardiologist and the team treating the individual patient.

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Part 4- Future directions

Chapter 17

Endovascular Stents: a 'break through' technology. Future challenges.

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Endovascular stents: a 'break through technology', future challenges *

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Abstract

Coronary stents were developed to overcome the two main limitations of balloon angioplasty, acute occlusion and long term restenosis. Coronary stents can tack back intimal flaps and seal the dissected vessel wall and thereby treat acute or threatened vessel closure after unsuccessful balloon angioplasty. Following successful balloon angioplasty stents can prevent late vessel remodeling (chronic vessel recoil) by mechanically enforcing the vessel wall and resetting the vessel size resulting in a low incidence of restenosis. All currently available stents are composed of metal and the long-term effects of their implantation in the coronary arteries are still not clear. Because of the metallic surface they are also thrombogenic, therefore rigorous antiplatelet or anticoagulant therapy is theoretically required. Furthermore, they have an imperfect compromise between scaffolding properties and flexibility, resulting in an unfavourable interaction between stents and unstable or thrombus laded plaque. Finally, they still induce substantial intimal hyperplasia which may result in restenosis. Future stent can be made less thrombogenic by modifying the metallic surface, or coating it with an antithrombotic agent or a membrane eluting an antithrombotic drug. The unfavourable interaction with the unstable plaque and the thrombus burden can be overcome by covering the stent with a biological conduit such as a vein, or a biodegradable material which can be endogenous such as fibrin or exogenous such as a polymer. Finally the problem of persisting induction of intimal hyperplasia may be overcome with the use of either a radioactive stent or a stent eluting an antiproliferative drug.

Introduction

Coronary stents were developed to overcome the two main limitations of balloon angioplasty, acute occlusion and long term restenosis. Coronary stents can tack back intimal flaps and seal the dissected vessel wall and thereby treat acute or threatened vessel closure after unsuccessful balloon angioplasty. Following successful balloon angioplasty stent can prevent late vessel remodeling (chronic vessel recoil) by mechanically enforcing the vessel wall and resetting the vessel size resulting in a low incidence of restenosis. These theoretical advantages of coronary stenting have been tested in two major randomised trials [1, 2]. Both BENESTENT and STRESS confirmed the theoretical advantages of coronary stenting by demonstrating a reduction in angiographic restenosis and clinical events during follow-up [1, 2]. This reduction in restenosis was achieved by a greater luminal gain despite the accommodation of a greater absolute loss in lumen diameter in the stent group, suggesting greater neointimal hyperplasia in this group. The reduction in long-term restenosis was counterbalanced by bleeding complications related to the anticoagulant therapy. Therefore, a number of limitations have to be overcome before coronary stenting achieve its full potential.

Currently available stents

The currently available stents, a description of their design, and the year of their clinical introduction are listed in Table 1 and illustrated in Figure 1. In the absence of prospective randomized interstent comparative trials, it is difficult to draw conclusions on the relative merits and demerits of each stent design. Individual experience and registry data from each stent,

* Andonis G. Violaris was the recipient of a Wellcome Trust International Research fellowship 1992–1995.

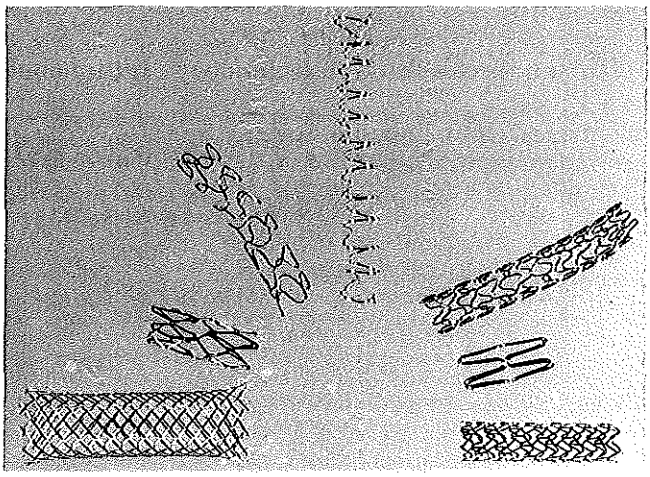

Figure 1. Coronary stents which have undergone clinical evaluation. These are going clockwise from the left. Wallstent (Schneider), Palmaz-Schatz (Johnson & Johnson), Wiktor (Medtronic), Gianturco-Roubin (Cook), Cordis, Micro (A.V.E.), Multilink (A.C.S.)

Table 1. Currently availabele stents undergoing clinical evaluation

Coronary Stent	Design	Deployment	Premounted	Delivery	Diameter (mm)	Length (mm)	First Clinical Implantation
Wallstent	Wire mesh	Self-expanding	balloon not required	over-the-wire	3.5–6.0	12–42	1986 1991 (Less shortening)
Palmaz-Schatz	Slotted tube	Balloon-expandable	pre-mounted & unmounted	over-the-wire & both	3.0–4.0	8–18	1988 1994 (Heparin coated)
Gianturco-Roubin	Incomplete coil clam shell loop	Balloon-expandable	premounted	over-the-wire	2.5–4.0	20–40	1989 (GR-I) 1995 (GR-II)
Wiktor	Sinusoidal helical coil	Balloon-expandable	premounted	over-the-wire or monorail	3.0–4.5	16	1991 1995 (Short wave)
Multilink	Multiple rings with multiple links	Balloon-expandable	premounted	over-the-wire	3.0–3.5	15	1993
Cordis	Sinusoidal helical coil	Balloon-expandable	premounted	over-the-wire	3.0–4.0	15	1994
AVE Micro	Zig-Zag axial struts	Balloon-expandable	premounted	monorail	2.5–4.0	6–36	1994 (Micro-I) 1995 (Micro-II)
NIR	Expandable uniform cellular mesh	Balloon-expandable	unmounted	both	2.0–5.0	9–32	1995

Wallstent – Schneider Bulach, Switzerland; Palmaz-Schatz – Johnson & Johnson, Warren, New Jersey, USA; Gianturco-Roubin – Cook, Bloomington, Indiana, USA; Wiktor – Medtronic, Minneapolis, Minnesota, USA; ACS – Advanced Cardiovascular Systems, London, U.K.; Cordis, Cordis, Miami, Florida, USA; AVE Micro, Advanced Vascular Engineering, Santa Rosa, California, USA; NIR, Medino, Tel Aviv, Israel.

however, allow preliminary impressions to be made on the advantages and limitations of each stent.

Wallstent

The Wallstent was the pioneer of stents [3, 4] through which we learnt the risk profile and indications for

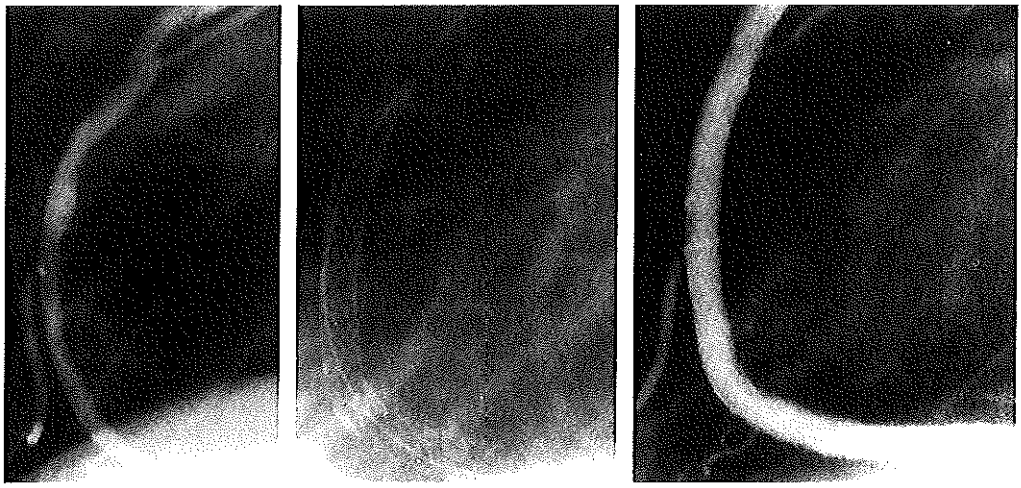

Figure 2. Coronary angiography of the right coronary artery in a patient with total occlusion pre-balloon angioplasty. Post balloon angioplasty diffuse narrowing associated with a proximal dissection was observed [left panel]. The radiopaque stent struts can be seen post Wallstent deployment [middle panel]. An excellent angiographic result [right panel].

coronary stenting and the necessity and adverse effects of antithrombotic measures. The new Less Shortening Wallstent has been developed recently with a change in the braiding angle and results of the first clinical implantation of this second generation stent in coronary vein grafts have been promising [5, 6]. The unique advantages of the Wallstent include the extensive range of diameters and lengths available, thereby allowing the Wallstent to be used for the management of long spiral dissections [7], and for vessel reconstruction [8]. The sheathed 'balloon-less' delivery system in combination with the free unconnected wire mesh design, render the Wallstent one of the most trackable, pushable and flexible stents for negotiating tortuous vessel and passing through proximally deployed stents (Figure 2). Furthermore recent modification of the delivery system allows recapture of the stent prior to final deployment and also improved positioning, raising the possibility of shorter Wallstents in clinical practise. Its fine cross-hatched mesh design provides excellent scaffolding properties; particularly well suited to entrap friable material in diffusely diseased vein grafts. Additionally enforced mechanical remodeling produced by oversizing Wallstent implantation conveys a favourable 6 month clinical and angiographic outcome in both stenosis and total occlusions in native coronary arteries both [7, 8]. Its primary limitations are the longitudinal shortening of the stent upon radial expansion and motion of the stent during retraction of the rolling membrane rendering the stent less suitable for ostial lesions.

Palmaz-Schatz stent

The Palmaz-Schatz stent has been extensively investigated in a broad range of coronary lesions [9–18]. It is the only stent to date to have completed prospective randomized trials comparing the clinical and angiographic outcome with that of balloon angioplasty [1, 2]. The angiographic results following Palmaz-Schatz stent implantation are predictable and the slotted tube design allows the performance of high pressure intrastent balloon inflations without risk of structural deformation. The low radiopacity of the Palmaz-Schatz stent, however, can render the positioning of a non- compliant balloon for post deployment high pressure intrastent inflations difficult. Additionally, a higher incidence of restenosis has been reported at the site of the central articulation [19]. To overcome this limitation a recent model has been developed without the central articulation (spiral articulation design). This model has a higher radiopacity compared to the standard single articulation design. The availability of a free unmounted Palmaz-Schatz stent which can be crimped by the operator on any balloon provides more

procedural versatility and results in a lower profile during stent delivery. It does, however, increase the risk of losing the stent during deployment.

Gianturco-Roubin stent

Prior to obtaining FDA approval for non-investigational clinical use, most of the clinical data on the Gianturco-Roubin stent was gathered in single and multicenter registries in the U.S.A. [20–25]. The indication for the Gianturco-Roubin stent for which most data has been gathered is for the bailout of subocclusive and occlusive dissections following balloon angioplasty in native coronary arteries. The data gathered compares well with historical controls treated by repeated and prolonged balloon angioplasty alone. The results of the first randomized trial of the Gianturco-Roubin in bailout therapy (GRACE) are awaited with interest [26]. The principle advantages of the Gianturco-Roubin stent include its range of lengths and longitudinal flexibility. While the relatively large interstrut intervals of 1mm raise questions over the suitability of this stent for the management of friable vein graft lesions, the advantages of the Gianturco-Roubin stent design include minimal risk of 'jailing' side branches and the potential to perform coronary interventional procedures in side branches through the interstrut spaces. While this stent excels in long dissections in curved coronary segments, its more generalised use was hindered by the high profile of the first generation. The new generation flex II stent overcome these problems with a lower profile and a higher visibility.

Wiktor stent

The available clinical data on the Wiktor stent arises from observational studies and registry data [27–30], the principle registry of which has been in the management of restenotic lesions. Like the Gianturco-Roubin stent, the Wiktor stent is a coil stent which offers marked flexibility and thus conformability with the vessel curvature. However, also like the Gianturco-Roubin stent, the Wiktor stent is unlikely to excel in the treatment of friable vein grafts on account of the large interstrut interval of the Wiktor stent and subsequently reduced scaffolding properties. The radiopacity of the Wiktor stent allows exquisite positioning of the stent in ostial and focal lesions, while the flexibility of the stent makes it suitable for short dissections on curved coronary segments. The recent introduction of the option of a monorail delivery system improves the user-friendliness of the device. The limitations of the initial prototype of the Wiktor stent included the availability of only one length and the limited scaffolding properties with the potential for the protrusion of intimal flaps through the interstrut intervals. These limitations have been overcome by the new generation short wave form Wiktor stent, although the radiopacity of this tantalum stent can still interfere with on-line quantitative angiographic analysis of the stented segment [31].

Multilink stent

The ACS stent currently has the least clinical experience of the currently available stents having just completed its first 100 patient registry conducted at five European centers [32]. The advantages of this new stent include the flexibility and low profile of the sheathed stent and delivery system. Although the Multilink stent design manages to provide remarkable scaffolding properties, the metallic burden to the stented vessel remains very low by virtue of the small diameter of the corrugated struts. The limitations of the stent include its radiolucency rendering the positioning of non-compliant balloons for post-delivery high pressure intrastent inflations very difficult. The current availability of only one length of 15mm, means that the 1st prototype can only be used for very focal lesions. Following some minor modifications this stent may offer some significant advantages over the earlier generation of stent designs. The attachment of radiopaque tips on the stent would present a significant enhancement.

AVE Micro stent

The AVE Micro stent is now undergoing clinical evaluation in a large number of countries [33]. The high degree of radiopacity and balloon expandable deployment should render this stent ideal for exquisite positioning in highly focal and ostial lesions (Figure 3). However, by virtue of the longitudinal (axial) orientation of its eight struts, the 4mm AVE Micro stent units may be prone to proximal migration and protrusion and therefore preferably longer welded AVE Micro stents should be deployed in ostial locations. The customized range of short lengths make the AVE Micro stent ideal as an adjunctive complementary device for multiple stenting, filling in the gaps between longer stents and improving the inflow or outflow of longer stents. Additionally the high flexibility and low thrombogenesity of the AVE micro stent make it an ideal stent for bailout management after failed balloon angioplasty

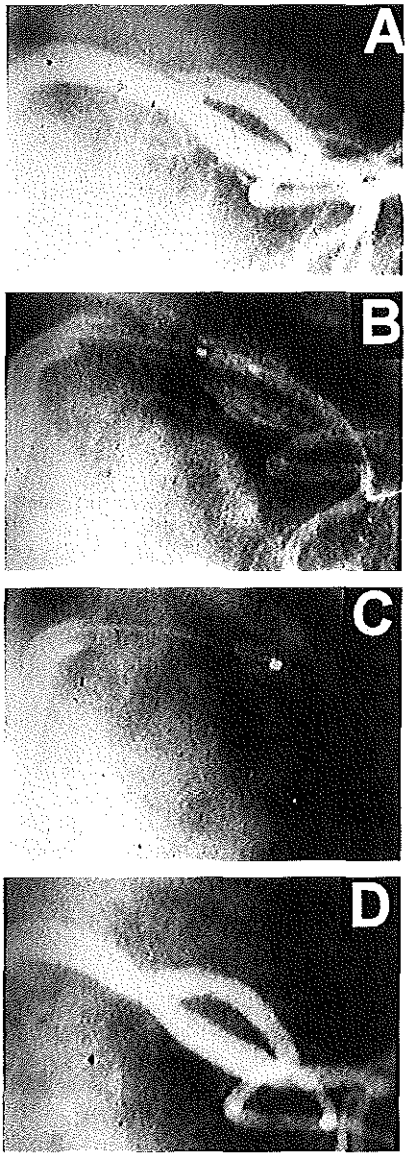

Figure 3. (*A*) Short dissection in the origin of the left circumflex coronary artery following balloon angioplasty. (*B*) An AVE Micro stent is delivered to the target vessel through an 8F Amplatz guiding catheter. The two radiopaque markers at the extremities of the stent are clearly seen. (*C*) Following delivery of the stent, the thick radiopaque stainless steel struts facilitate the precise positioning of a short non-compliant balloon (single marker) within the stent for subsequent high pressure inflations and optimization of stent deployment (*D*).

[33]. Despite the absence of a protective sheath, the low profile and longitudinal strut orientation, make the AVE micro stent one of the easiest stent to pass through other proximally deployed stents. The monorail delivery system increases the user-friendliness of the device which has a short learning curve. The strong radial support proffered by the thick struts should make the stent suitable for the prevention of recoil and restenosis. Care should be taken, however, to avoid the positioning of junctions between the unconnected 4mm units at the site of the minimal lumen diameter of lesions in order to prevent intimal protrusion. Recent developments include the helicoildal welding of multiple 3mm length units (Micro stent - II) to provide multiple lengths of up to 36mm.

Cordis stent

The Cordis stent continues to undergo early evaluation in the clinical arena [31]. Like the A.C.S. stent, the Cordis stent offers some advantages over the first generation stents by virtue of its low profile, flexibility, and comprehensive scaffolding properties. The absence of a protective sheath on the delivery system, however, increases the possibility of stent dislodgement or disruption during delivery. The operator should be aware of the protrusion of the delivery balloon beyond the limits of the Cordis stent if inflations of higher pressure are considered. While the strongly radiopaque tantalum struts allow exquisite positioning (Figure 4) of the stent in short dissections in curved coronary segments, the radiopacity may pose problems for online quantitative angiographic assessment, particularly during assessment of luminal renarrowing at 6 month angiographic follow-up [31]. The relative merits of this stent are currently being evaluated in a European multicenter registry and by Dr. Nobuyoshi and colleagues in Japan [34].

NIR stent

The NIR stent is a recently developed balloon expandable stent currently undergoing clinical evaluation in some centers in Israel and Europe. Although this stent has a low radiopacity, the stent has a high flexibility and comes in a wide range of customized sizes (diameter 2 to 5mm) and lengths (9 to 32mm). Balloon unmounted model allows to choose various types of balloon for the stent delivery and may spare the usage of an additional balloon catheter only for stent delivery. A preliminary report indicates a high deployment success rate despite most of the lesions were difficult to

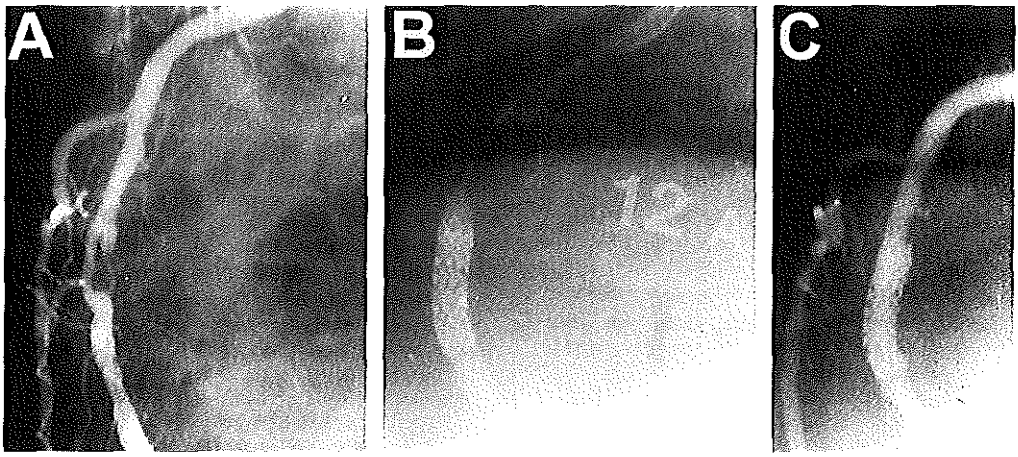

Figure 4. (*A*) Complex lesion of the mid-segment of the right coronary artery. (*B*) Positioning of the Cordis stent was facilitated by the radiopacity of the tantalum struts which are clearly seen during inflation of the contrast-filled intrastent balloon to 12 atmospheres. (*C*) Angiography post stent deployment reveals the stent struts to be embedded in the vessel wall external to the contour of the contrast-filled lumen.

treat because of vessel tortuosity, distal location and long lesion [35]. The restenosis rate has not yet been determined.

Future stents

All currently available stents are composed of metal and the long-term effects of their implantation in the coronary arteries are still not clear. Because of the metallic surface they are thrombogenic, therefore rigorous antiplatelet or anticoagulant therapy is theoretically required. Furthermore, they have an imperfect compromise between scaffolding properties and flexibility, resulting in an unfavourable interaction between stent and unstable plaque or thrombus burden. Finally, they still induce substantial intimal hyperplasia which may result in restenosis. Future stent can be made less thrombogenic by modifying the metallic surface, or coating it with an antithrombotic agent or a membrane eluting an antithrombotic drug. The unfavourable interaction with the unstable plaque and the thrombus burden can be overcome by covering the stent with a biological conduit such as a vein, or a biodegradable material which can be endogenous such as fibrin or exogenous such as a polymer. Finally the problem of persisting induction of intimal hyperplasia may be overcome with the use of either a radioactive stent or a stent eluting an antiproliferative drug.

Coated stents

Metal coated

In vitro work suggests that surface potential may exert a substantial effect on both the thrombogenicity and antiproliferative effect of metals. High surface potential is associated with pronounced attraction of negatively changed particles such as platelets and plasma proteins thus resulting in high thrombogenicity. On the positive side, however, metals with high surface potential also have a substantial antiproliferative effect on fibroblasts suggesting that by varying surface charge we may be able to influence the thrombogenicity and neointimal hyperplasia after stent implantation. One way of doing this would be by modifying the base metal [36].

Metals can be modified either by Galvanization or by Ion bombardment. Galvanisation involves the electrochemical deposition of metal 3.3 μm in thickness on the stent and results in 100% of the stent surface being covered prior to stent expansion. Ion bombardment consists of spluttering a thin metal film onto the stent followed by a bombardment with argon ions. The resulting implantation of metal onto the stent surface is 20nm in thickness and 75% stent surface coverage is required prior to expansion.

Preliminary experience suggests that coating steel stents with platinum, gold or copper results in higher in vitro surface potentials but that the incidence of

thrombosis in vivo is increased particularly in stents coated using galvanization [36]. Thus in contrast to the in vitro suggestion metal charge does not seem to play a major role in stent thrombogenicity in vivo. Furthermore a low stent charge appeared to correlate with an increased neointima formation. Thus modifying stainless steel stents by covering them with gold, platinum or copper is unlikely to be the solution to either increased thrombogenicity of neointimal hyperplasia.

Cell seeding of stents

Coating of metallic stents with endothelial cells, particularly genetically engineered cells with increased cell surface fibrinolytic activity may improve their thrombogenic nature. Preliminary work has demonstrated the feasibility of this approach [37, 38]. More recently in vitro work suggests that genetically engineered endothelial cells woould allow increased fibrinolysis to be promoted by the surface localisation of urokinase [39]. Questions remain however over the number of cells that will remain attached under flow conditions as well as the legal responsibility in case of failure of endothelial cell function.

Immobilised drug coatings

Coating of the stent surface with an antithrombotic agent such as heparin [40–42] provides a novel solution to the problem of increased thrombogenicity of metallic stents and the subsequent need for intensive anticoagulation resulting in increased morbidity and costs. Following encouraging preliminary experience with a heparin coated Palmaz Schatz stent in pig coronary arteries the Benestent 2 study evaluated the safety of reducing and eliminating anticoagulant therapy in patients receiving a heparin coated stent. Initial results from the pilot study suggests that subacute stent thrombosis does not occur using the heparin coated stent which has virtually eliminated the bleeding complications following stent implantation and reduced the in hospital stay to 3 days [43]. The 6 month angiographic follow up also indicates that these coated stents do nod induce an excess of intimal hypeplasia [44]. Follow up of phase 4 (where coumadin and heparin are replaced by ticlodipine and aspirin) are awaited.

Polymer coated stents

The stent metal surface can be rendered less thrombogenic by coating it with a thin layer of a synthetic polymer. Initial results suggested that although this may protect against acute thrombotic events it does not reduce the extent of subsequent neointimal hyperplasia [45]. The advantage of a polymer coated stent however is that it can be loaded with antithrombotic or antiproliferative agents directed against the neointimal reparative process [46] (Figure 5).

Fibrin stent

The fibrin film stent has several theoretical advantages. It is a membrane stent and can thus cover the balloon angioplasty injury site providing a natural healing matrix and reducing local thrombus formation. It may also be useful in vein grafts where the membrane may prevent distal embolisation of friable material. Preliminary work suggests that the fibrin film stent is both bioabsorbable and biocompatible [47]. It also appears to be safe in pigs with the use of an antiplatelet agent. It had little effect on neointimal proliferation however.

Vein coated stents

An autologous vein graft coated stent consisting of a conventional stent covered by a vein graft may be the ideal conduit for percutaneous revascularisation minimising stent thrombogenicity and local tissue reaction stent. Preliminary experience in 13 patients suggests that the technique is feasible and safe resulting in an excellent immmediate angiographic result [48] but further studies are warranted to investigate the effect of the procedure on subacute thrombosis and long term restenosis.

Nitinol stent

Nitinol has a number of properties which make it ideal for stent composition. It is known to be highly biocompatible and highly malleable allowing 0.006 inch struts without sacrificing flexibility. Furthermore it has unique thermoelastic properties which allow for stent collapse and removal as well as self expansion [49–52]. Finally it is amenable to surface coating for local drug delivery and is transmutable into a radioactive emitter for local radiation therapy. Preliminary work in a pig coronary subacute thrombosis model has confirmed some of these theoretical advantages of a nitinol stent demonstrating that nitinol stents, particularly polished nitinol stents, develop significantly less thrombus (as measured by thrombus weight and thrombus grade) in comparison to stainless steel stents of similar design [49]. The results on neointimal hyperplasia are still awaited although preliminary results from a separate

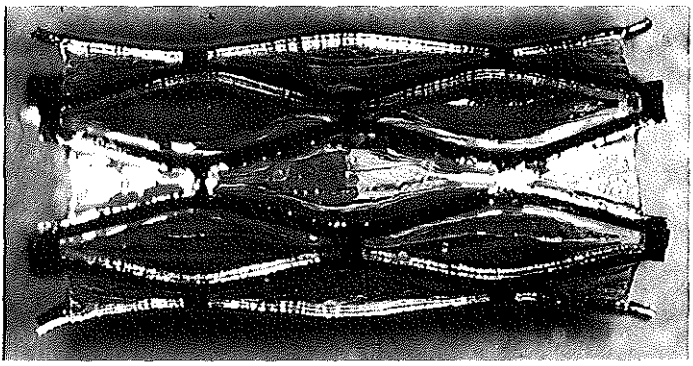

Figure 5. Eluting stent consisting of a stent surrounded by an elastic sleeve made of biodegradable polymer loaded with 40% antithrombotic agent.

group suggest that a self expanding nitinol stent exerts a more favourable effect on vascular remodeling and neointimal formation than a balloon expandable tubular slotted stent [50]. Initial experience in 20 patients suggests that a nitinol stent is safe and effective in the treatment of suboptimal results [52].

Polymer stent

Polymer stents have several potential advantages. They can be loaded with antithrombotic and/or antiproliferative pharmaceutical agents in high concentration for sustained local delivery. They may have less of a mechanical mismatch with the vessel wall than metal stents and may avoid the potential for late stage complications. They thus have a synergistic mechanical and local pharmacological therapy providing sustained structural support throughout the healing phase to avoid early and late elastic recoil, while local high dose drug delivery will avoid thrombosis, neointimal proliferation and systemic side effects. There is however only limited strength if a large% of drug is loaded onto the stent. Initial animal experiments, however, have demonstrated a marked inflammatory response resulting in substantial luminal encroachment with polymer stents in porcine coronary arteries [53, 54]. There is no vascular tissue reaction with HMW PLLA, however, and this remains a promising avenue of investigation.

Composite (metal augmented) polymer stents

Composite polymer stents guarantee a minimal mechanical mismatch between the stent and the vessel wall, leaving a delicate metal skeleton after biodegradation. They provides protection of tissues from deep strut laceration and allow large amounts of drug (up to 40%) for slow local release without affecting hoop strength. Such a composite polymeric stent, capable of excellent mechanical strength as well high dose local drug delivery, has been developed and evaluated in porcine coronary arteries. All stent designs could be placed in the selected coronary arteries. Preliminary histological analysis showed that neointimal hyperplasia and some degree of inflammatory response was present in all groups. Unfortunately implantation of bicomponent stents caused a reduction in lumen diameter for all designs. Further research will be directed to the assessment of the relative contribution of stent geometry, polymer type and incorporated drug to the overall response.

Radioactive stents

Radiation selectively kills proliferating cells independent of any stimulus for cell growth. As a major component of restenosis is neointimal hyperplasia secondary to vascular smooth muscle cell proliferation it seems reasonable to assume that radiation therapy may reduce restenosis. Multiple animal studies have now confirmed this ability of radiation therapy to inhibit neointimal hyperplasia and reduce restenosis [55,56]. Radioactive stents have the potential to deliv-

er an appropriate dose of radiotherapy to the area of interest thus reducing restenosis, while minimising the total dose given to the patient.

Two methods are currently in use. In the first method conventional Palmaz-Schatz stents were ion bombard in a cyclotron to emit low dose beta and gamma radiation from radioisotopes Co55, 56, 57, Mn52 and Fe55 with half lives between 17.5 hours and 2.7 years [57]. The radiation is predominantly short range and homogeneously distributed over the length and circumference of the stent and being absolutely fixed to the metal. Because of this the stents do not require a license from the International Atomic Authority. An intimal surface dose rate of 4mGray/h results in an integral dose of 180mGray after a period of 100 days.

The low dose radioactive stents were found to markedly inhibit neointimal hyperplasia in rabbits. Endothelialisation of the radioactive stents was found to be delayed with macrophages being located on top of the radioactive stent struts until endothelialisation was complete. Although the degree of neointimal hyperplasia was reduced it was found, paradoxically, that extracellular matrix production increases after radioactive stent implantation [57].

The second method is though the use of beta Particles [58, 59]. Beta particles (free electrons) may represent the ideal means of local irradiation. P32 is an excellent candidate for local delivery as the maximal range of beta particles is 3-4mm in tissue. It has a desirable half life of 14.3 days and once implanted after balloon angioplasty, there is no detectable radiation by 4 months. P32 or other Beta emitter and also be implanted directly onto the stent wire.

Such P32 impregnated stents have been now fabricated. In vitro work suggests that very low beta particle activity levels inhibit smooth muscle cell growth preferentially within 5-7 mm of P32 coated stent wires, while endothelial cells appear to be much more radio resistant. In vivo animal testing in a porcine restenosis model using low dose rate P32 stents have demonstrated inhibition of neointimal growth [58].

A P32 coated stent with doses similar to those described should be safe in man. Total body dose to the patient would be less than 1/1000 that of fluoroscopy during angioplasty. Furthermore the radiation would be local and would not reach any mediastinal tissues. Additionally, the radiation to the interventionalist will be much less than fluoro scatter.

Conclusions

The stent is the second wind of coronary angioplasty. It is for every physician a predictable therapy for bailout, improving a suboptimal result and reducing the risk of restenosis. As a result of these advantages over 40% of coronary interventional procedures in the current year will be by stent implantation. Currently available stents, however, have a number of limitations which are currently being addressed. Drug eluting stents to address the problem of the small vessel and the low flow situation are on the horizon. Covered stents where a membrane acts as an isolating barrier passivating the lesion, minimising local thrombus formation and delivering local drug therapy are promising. Radioactive stents to inhibit local neointima formation are another promising avenue of investigation. Biodegradable stents and composite stents are also being actively investigated. The future is bright indeed for coronary stenting.

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Chapter 18

Conclusion

Principal findings of the current thesis

Restenosis after percutaneous coronary revascularisation remains a major problem limiting the long term efficacy of these techniques. Accumulated evidence suggests that intimal thickening secondary to the migration and proliferation of vsmc is the main cause. We investigated the problem using a combination of experimental and clinical approaches.

Coronary atherectomy provides a unique opportunity to obtain plaque tissue from a wide variety of clinical syndromes. In chapter 2 we used directional coronary atherectomy to percutaneously retrieve tissue from patients with clinically and angiographically documented coronary artery disease and examined the hypothesis that vsmc cultured from restenotic lesions differ in extracellular matrix synthesis from primary atherosclerotic cells. We demonstrated that restenotic cells synthesise significantly more collagen and sulfated aminoglycans than primary cells. This would suggest that extracellular matrix synthesis may play a far greater role in restenosis than previously thought and may be one potential avenue of treatment.

Although extracellular matrix production is undoubtedly of importance in defining the volume of the neointimal lesion, the proliferative and migratory potential of the smooth muscle cell must also play a substantial role. In Chapter 3 we investigated the relationship between the clinical and histopathological substrate of tissue retrieved during directional coronary atherectomy and the proliferative and migratory potential of smooth muscle cells as judged from successful outgrowth during cell culture. We found that although cell outgrowth was not influenced by any of the clinical variables assessed, it was influenced however by the presence of organising thrombus in the atherectomy specimen. Thus the presence of organising thrombus in the retrieved tissue appeared to facilitate smooth muscle cell outgrowth suggesting an enhanced proliferative and migratory potential for the smooth muscle cell in coronary syndromes where mural thrombosis is likely to be present.

One of the limitations of cell culture is that it does not take into account the normal anatomical relationships of the different cellular components of the vessel wall or the cell to cell interactions which may modulate growth. Furthermore cell culture from atherectomy specimens has a low yield and it can take a substantial amount of time before adequate numbers of cells can be harvested for further investigation. Therefore in chapter 4 an in vitro organ culture of human coronary artery subjected to balloon angioplasty was developed. In this system intimal smooth muscle cell proliferation occurred reproducibly but because of the diffuse nature of the disease it was difficult to assess any differential effects balloon angioplasty may have. We were however able to demonstrate quantitatively that balloon angioplasty results in substantial wall damage which recovers after culture.

The above in-vitro model relies on the use of serum to maintain tissue viability and does not therefore allow us to assess the role that growth factors may play in the in-vivo situation. To determine this an in-vivo porcine model of restenosis was developed, in which intimal proliferation occurs reproducibly within 4 weeks of coronary angioplasty and can be easily quantified (Chapter 5). The integrity of the internal elastic lamina appears to be of critical importance in minimising neointimal thickening and smooth muscle cell migration and proliferation in this model. Using this model and a serum free adaptation of the previously developed organ culture technique we were able to show that growth factors may be released by the vessel wall, suggesting that growth factor activity by cells intrinsic to the angioplasty site may regulate vsmc proliferation in vivo.

Local drug delivery by perfusion catheters may be a means of delivering high concentrations of active drug, such as growth factor antagonists, into the vessel wall. Pressure mediated trauma and duration of balloon inflation have, until now, been key limitations of these devices. In chapter 6 we investigated a new delivery catheter which promises to overcome both these restrictions on normal porcine coronary arteries. Inflation duration of up to 60 minutes did not result in any signs of ischaemia or haemodynamic compromise. Furthermore intracoronary infusion of adenosine with the catheter inflated demonstrated coronary flow velocities similar to controls. Macroscopic examination demonstrated that compounds ranging from 0.3-70 kDa could be successfully infused into the coronary wall with only localised endothelial damage and an intact internal elastic lamina and media. Our data thus suggest that prolonged balloon inflation is feasible with this device and that local drug infusion occurs with minimal trauma to the vessel wall. The device may have potential for ameliorating restenosis post coronary intervention. Human studies are currently under way although preliminary data from the ITALIC study suggest that local delivery of antisense oligonucleotides, despite positive animal results, may not reduce clinical and angiographic restenosis.

Although experimental models can provide insight into the pathophysiology of restenosis they can never however replace the clinical situation. Intracoronary ultrasound imaging allows direct visualisation of the vessel wall in vivo. In the second part of this thesis we looked at what role this may play in our understanding of the pathophysiological mechanisms involved in the mechanism of acute luminal gain and subsequent restenosis after coronary intervention. In chapter 8 we were able to compare the coronary luminal dimensions obtained from intracoronary ultrasound with both geometric and videodensitometric quantitative angiography (the current gold standard) pre and post intervention. We were able to show that intracoronary ultrasound may be better at assessing the complex morphological changes induced by intervention. Chapter 9 summarises our initial clinical experience with a new device combining intravascular ultrasound imaging with balloon angioplasty thus obviating the need for repeated catheter exchanges and allowing on line imaging during the procedure. We were able to show that intracoronary imaging provides information additional to that provided by contrast

angiography in a large proportion of cases and that this information can, on occasion, influence clinical management. In chapter 10 we used intracoronary ultrasound to assess the influence of the underlying de-novo vessel remodeling mode on the mechanism of acute luminal gain and long term restenosis following coronary balloon angioplasty and directional coronary atherectomy. We were able to show that the mode of vessel remodelling may contribute to the efficacy of the interventional device (vessel stretch or debulking) and may play a role of the mechanism of restenosis. This would be important in the optimal use of new devices. For example our study suggests that, in a vessel with paradoxical shrinkage, stent implantation, to act as a scaffold for the vessel wall and prevent late vessel recoil, may be the optimal device to prevent restenosis whereas in a vessel with compensatory enlargement, plaque reduction (debulking), during the initial interventional procedure and concomitant medical treatment to prevent the growth of intimal hyperplasia using local drug delivery or endovascular radiation may be the most useful way of reducing restenosis.

The clinical literature on restenosis is confusing and conflicting because of the lack of a readily accepted definition of restenosis and widespread differences in the methodology used in different trials. Based on the pioneering work of Serruys and Nobuyoshi however it is likely that restenosis is one end of a continuous spectrum and that all lesions deteriorate over time, with around 30% deteriorating to such an extent by six months that they are said to have restenosed. A number of studies have attempted to define the role that patient, lesion and procedural related characteristics may play in predisposing to restenosis. These studies are, however, flawed by inconsistencies in the definition of restenosis, the angiographic follow up rate and the method of angiographic assessment used. For any study to accurately reflect the incidence of restenosis it should involve angiographic follow up of all patients, at a predetermined interval, and a well validated system of analysis with known accuracy and variability. The logistics, amount of work and expense involved however in organising a major restenosis trial are such that no single investigator or group of investigators can possibly mount such a study. In the third part of the thesis we used data pooled from 4 major restenosis trial with a high angiographic follow up rate and where the angiographic criteria were standardised with one central angiographic core lab performing the quantitative angiographic analysis in all studies to assess what clinical procedural and angiographic variables may influence restenosis.

The objective of our study in Chapter 11 was to examine the relationship between serum cholesterol, a known risk factor for atherosclerosis, and long term restenosis. We found the restenosis rate to be similar in patients with and without hypercholesterolaemia. Additionally there was no difference in either the absolute or relative loss, between patients with, and without, hypercholesterolaemia. Conversely the total serum cholesterol in patients with restenosis (using the categorical definition) was similar to those without restenosis. Dividing the population into deciles according to total cholesterol and examining the categorical reste-

nosis rate, as well as the absolute and relative loss, again revealed no significant differences between deciles. There was also no differences in the categorical restenosis rate, or the absolute or relative loss between deciles according to LDL, HDL, or LDL:HDL ratio suggesting no influence by these cholesterol subfractions on restenosis. Our results thus indicate that there is no association between cholesterol and restenosis, suggesting that measures aimed at reducing total cholesterol, although highly desirable in this high risk group, are unlikely by themselves, to significantly influence post angioplasty restenosis. This has been subsequently borne out by a number of studies looking at cholesterol lowering therapy post angioplasty, including the Flare study, all of which have been negative.
Another major risk factor for atherosclerosis is smoking. The influence of smoking on post angioplasty restenosis, however is unclear. In our study population we found substantial differences in the underlying demographic characteristics between smokers and non-smokers (Chapter 12). Smokers were less likely to be diabetic or hypertensive but were younger, more likely to be male, to have peripheral vascular disease, have sustained a previous myocardial infarction and be in a worse anginal class. Their lesions were more likely to be located in the right coronary artery and to require a longer duration of inflation at higher pressures. There was, however, no significant difference in either categorical or continuous measures of restenosis at 6 months. Thus smokers require coronary intervention earlier than non smokers but their short term, six month outcome is similar to non smokers.

Our experimental work in Chapter 3 suggested that he presence of organising thrombus in retrieved atherectomy tissue facilitates smooth muscle cell outgrowth suggesting an enhanced proliferative and migratory potential potential in these lesions. Therefore in Chapter 13 we examined whether the presence and subsequent organisation of local thrombus may influence post angioplasty restenosis. We found the categorical restenosis rate and both the absolute and relative loss to be significantly higher in lesions containing thrombus. The higher restenosis in lesions containing thrombus was primarily due to an increased incidence of occlusions at follow up angiography in this group. When these were excluded from the analysis the categorical restenosis rate and the absolute and relative loss were similar in the two groups. Our results thus indicate that thrombus is associated with higher restenosis but that this reflects an increased risk of occlusion rather than long term luminal renarrowing, suggesting that although thrombus may be of importance in this, it is unlikely to play an important role in long term intimal hyperplasia after PTCA.

Total occlusions are an interesting subgroup of the angioplasty population. Although a number of factors are known to influence the acute success rate relatively little accurate information is available regarding long term restenosis. In chapter 14 we evaluated restenosis in total occlusions and compared this with the control population of stenoses. We found restenosis to be significantly higher in occlusions using both the categorical and continuous approach. The higher reste-

nosis in these lesions was again predominantly due to an increased number of occlusions at follow up angiography in this group. There were no significant differences within the occlusions group between total and functional occlusions, in either long term restenosis or re-occlusion. These results thus indicate that successfully dilated coronary occlusions have a higher rate of angiographic restenosis at 6 months, chiefly due to a higher rate of re-occlusion at follow up angiography. Measures aimed at reducing restenosis following successful dilatation of coronary occlusion should therefore be focused in this direction.

Experimental and human intravascular ultrasound studies suggest that unfavourable vascular remodelling may be important in restenosis after coronary angioplasty. In chapter 16 we evaluated whether favourable vascular remodelling, ie luminal enlargement, may also occur following successful balloon angioplasty and which factors may influence this. Within our study population 27.3% of lesions underwent luminal enlargement in the angiographic follow up period. Multivariate analysis suggested that diuretic therapy, MLD and diameter stenosis pre PTCA were positively related whilst age, heparin therapy, number of vessels diseased, lesion length, Lad location, total inflation time and relative gain were negatively related to luminal enlargement at follow up angiography. Thus luminal enlargement may occur after successful coronary angioplasty and can be substantially influenced by clinical, angiographic and procedural variables.

Future interventional perspectives for these findings

Restenosis remains a major limitation of percutaneous coronary revascularisation detracting from both the clinical and economic advantages of the techniques. In this thesis both in-vivo and in-vitro models of intimal hyperplasia after balloon angioplasty were developed and the pathophysiological mechanisms involved studied. In addition the epidemiology of restenosis was investigated and lesions with a higher rate of angiographic restenosis identified.

In many ways however the current thesis marks the end of one era and the beginning of another. With the landmark publication of the Benestent/Stress studies and subsequent refining of the stents themselves as well as the deployment technique, coronary stenting has finally overcome some of the major limitations of coronary angioplasty in terms of both acute occlusion and long term restenosis. Coronary stenting has in many ways moved the goal posts further back allowing us to dilate lesions previously thought difficult or impossible, with relative impunity. Although coronary stenting has overcome some of the mechanisms involved in restenosis, such as acute/chronic recoil and remodeling, in-stent restenosis still occurs and in fact, if we look at absolute loss as a biological marker of intimal hyperplasia, is probably greater after stent implantation than it is after balloon angioplasty. The basic science factors discussed in the first part of this thesis now become even more relevant, as in-stent restenosis is predominantly neointimal

hyperplasia. Furthermore coronary stenting by encasing the vessel in a metallic constraint, may prevent favourable arterial remodeling and hence any long term luminal enlargement which, as discussed in Chapter 15, may occur in up to 27% of lesions. Thus the interventional cardiologist has finally created the disease he initially thought was responsible for a large percentage of restenosis after angioplasty and may have inadvertently also minimised any possibility of favourable vessel remodeling.

Balloon angioplasty

Balloon angioplasty has been left in the doldrums since the widespread uptake of coronary stenting. Nonetheless a substantial number of coronary interventions are currently being performed with stand alone balloon angioplasty and potentially, by using ultrasound guidance for lesion characterisation and selection with subsequent optimal balloon sizing excellent long term results can be obtained using standalone angioplasty. Another potential avenue for optimising balloon angioplasty is through Doppler flow guidance to achieve a haemodynamically excellent acute result. Two further avenues of potential expansion of balloon angioplasty are brachytherapy and local drug/therapeutic agent delivery. Ionizing radiation is known to have an inhibitory effect on cellular proliferation and is widely used in the treatment of both neoplastic and non neoplastic conditions. In addition brachytherapy has also been demonstrated to reduce restenosis in peripheral and coronary arteries in animal models and restenosis after stent implantation in femoral-popliteal arteries in man. More recently a number of groups have been able to demonstrate that coronary brachytherapy is both feasible and safe in man and preliminary analysis suggests a reduction in late loss. The efficacy and safety of the different sources and procedures however have still not been well established and neither has their long term safety. As more data becomes available, if the efficacy and safety of brachytherapy is confirmed it is likely to lead to increasing endovascular revascularisation and a resurgence of balloon angioplasty. Brachy therapy and, as discussed below, the local delivery of therapeutic agents may thus be the second wind of coronary angioplasty.

Stent implantation

It is also feasible that coronary stenting which reduces recoil and vascular remodeling may also be married to radiotherapy in the form of radioactive stents to reduce neointimal proliferation. These would provide endovascular scaffolding to the vessel but also deliver very low dose ionising radiation (up to 10,000 times lower than activity levels of sources used for catheter-based vascular brachytherapy) whilst at the same time allowing uniform dose distribution and precise dosi

metry. Initial animal work is encouraging demonstrating potent inhibition of neointimal hyperplasia. One caveat however is that stent endothelialisation appears to be delayed; this may have important clinical implications with regard to stent occlusion.

Intravascular imaging

Intracoronary ultrasound imaging has seen a major advance in our understanding of the mechanisms involved in the acute results of percutaneous revascularisation and the long term restenosis. As imaging transducers become smaller, and are married with balloon/stent/atherectomy devices they are likely to become an integral part of the armamentarium of the interventional cardiologist. As well as providing tomographic imaging of the vessel wall intravascular ultrasound has also been used recently for assessing flow. Using analysis of the intravascular radiofrequency echo signals and a decorrelation based method, extraction of cross sectional velocity profiles and quantification of volume blood flow is performed which allows a unique opportunity to simultaneously assess physiologic and anatomic parameters. What impact this may have on clinical decision making still has to be demonstrated.

Local drug delivery

Locally deliveredGene therapy is emerging as a potential strategy for the treatment of restenosis after angioplasty. Experimental studies have already demonstrated that vascular smooth muscle proliferation and lesion formation can be prevented by the blockade of genes regulating cell cycle progression and that therapeutic effects can also be achieved by the in vivo transfer of gene(s) whose product(s) (eg nitric oxide) exert a paracrine effect on the vessel wall. Adenoviral vectors have been recently used to demonstrate that therapeutic genes encoding both cytotoxic and cytostatic products successfully inhibit smooth muscle cell proliferation and related intimal hyperplasia. Major technical issues however include the toxicity of first generation adenoviral vectors, inefficient transduction of atherosclerotic arteries, and the risk of extra-arterial transfection. These issues remain to be addressed before gene therapy can be applied to clinical restenosis.

Therapeutic angiogenesis

Diffuse coronary disease remains a problem for both the interventional cardiologist and the cardiac surgeon. A promising approach to the treatment of such patients is therapeutic angiogenesis, the induction of new capillaries and other blood vessels thereby by-passing the epicardial vessels. The first clinical study on

angiogenic therapy for ischaemic tissue was the administration of vascular endothelial growth factor to patients with severe peripheral vascular disease demonstrating improved collateral blood flow with a corresponding increase in both resting and maximal blood flow in the treated extremity. More recently the first angiogenic therapy of human coronary artery disease has been demonstrated. Shumacher and colleagues were able to show that neovascularisation occurs, extending out from the relevant area, 12 weeks after injection of fibroblast growth factor-1 directly into the myocardium, at the time of coronary artery bypass grafting. A number of issues remain to be clarified however. These include defining what is the optimal agent and the optimal route, what is the optimal dosing regimen, what is the duration of therapy or action, and whether therapy can be targeted towards ischaemic tissues, thus minimising the potential for undesired angiogenesis.

Transmyocardial revascularisation

Transmyocardial revascularisation is based on the premise underlying the original Vineberg procedure, first proposed more than 40 years ago, that ischaemic myocardium may be directly supplied with oxygenated blood. In the case of TMR this is directly from the left ventricle, via the creation of a series of small transmyocardial channels. Although the mechanism of benefit by TMR remains unclear preliminary clinical studies have demonstrated clear improvement in anginal symptomatology; this however has come at the cost of substantial perioperative mortality. Whether the less invasive, percutaneous, methods of TMR currently undergoing clinical trials will result in equivalent benefit at substantially lower mortality remains to be answered.

Final thoughts

Percutaneous revascularisation has finally come of age, 21 years after Andreas Gruentzig's seminal procedure. We can now tackle almost any lesion in the coronary tree with relative impunity, and even small centres, with a limited selection of balloons and stents can offer their patients percutaneous revascularisation with little risk and a high chance of long term success. We are finally entering an era in which percutaneous revascularisation can replace open heart surgery for the majority of patients and lesions. The only cloud on the horizon, is in-stent restenosis. If this can be eliminated by stent coating, local drug therapy, or brachytherapy the future will be bright indeed for coronary revascularisation.

Chapter 19

Samenvatting

PTCA is een techniek die wijd verbreid is voor de behandeling van vernauwingen in de kransslagaders. Een beperking is echter, dat in 25 tot 30% van de gevallen restenose optreedt. Pathologisch-anatomisch onderzoek suggereert dat de belangrijkste oorzaak van restenose hyperplasie van de intima tengevolge van proliferatie van gladde spiercellen in de vaatwand is. Ons inzicht in het probleem wordt momenteel beperkt door een gebrek aan adequate laboratioriummodellen waarin het proces verder onderzocht kan worden en door een gebrek aan klinische en epidemiologische gegevens van goede kwaliteit. Wij onderzochten het restenoseprobleem, gebruikmakend van elkaar aanvullende experimentele en klinische benaderingen.

In het eerste deel (hoofdstuk 2 t/m 6) van dit proefschrift worden laboratioriummodellen en -technieken geëvalueerd die inzicht kunnen verschaffen in de onderliggende pathologische processen. Bijvoorbeeld synthese van extracellulaire matrix door gladde spiercellen (gsc) in de vaatwand kan een belangrijk mechanisme zijn voor restenose.
In hoofdstuk 2 onderzochten we het vermogen van gsc om extracellulaire matrix te produceren in vitro, waarbij we gsc gebruikten die gekweekt waren uit primaire vernauwingen en uit restenose weefsel, dat in vivo verkregen was door middel van directional coronary atherectomy (DCA). We toonden aan dat cellen uit restenose-weefsel significant meer collageen en gesulfateerde aminoglycanen synthetiseerden dan cellen uit primaire vernauwingen. Dit suggereert dat synthese van extracellulaire matrix een veel grotere rol kan spelen bij restenose dan in het verleden werd gedacht.
In hoofdstuk 3 onderzochten we de relatie tussen klinische factoren en het histopathologische substraat van via DCA verkregen weefsel aan de ene kant, en het proliferatief en migratorisch vermogen van gladde spiercellen, zoals bepaald op basis van succesvolle groei bij celkweek aan de andere kant. We vonden dat de aanwezigheid van een zich organiserende trombus in het verkregen weefsel de groei van gladde spiercellen faciliteert, hetgeen een toegenomen proliferatief en migratorisch potentieel suggereert van de gsc in deze lesies.
Celkweek houdt echter geen rekening met de normale anatomische verbanden van de verschillende cellulaire componenten van de vaatwand of de cel-cel interacties die de groei kunnen moduleren. Daarom ontwikkelden we in hoofdstuk 4 een orgaankweek van menselijke coronair-arterie die ballon angioplastiek had ondergaan teneinde de cellulaire en moleculaire basis van intima-proliferatie te onderzoeken in een opzet die de anatomische verbanden van de vaatwand intact liet.
We vonden dat intima-proliferatie optrad in orgaancultuur van menselijke coronair arterie die ballon-angioplastiek ondergaan had, en dat de gladde spiercellen in de neointimale laag het resultaat zijn van zowel migratie als proliferatie.
In hoofdstuk 5 gebruikten we een serumvrije aanpassing van de orgaancultuur techniek in combinatie met een in vivo varken model van restenose om te bepalen of het vrijkomen van groeifactoren van cellen intrinsiek aan de vaatwand een rol speelt bij de ontwikkeling van restenose. We vonden dat de lamina elastica interna een sleutelrol speelt bij het minimaliseren van neointimale verdikking en smc

proliferatie in dit model en vonden direct bewijs voor groeifactor activiteit van cellen intrinsiek aan de plaats van angioplastiek, die smc proliferatie kunnen reguleren. Karakterisering van deze factoren kan ons helpen de mechanismen te begrijpen die een rol spelen bij restenose en richting geven aan de ontwikkeling van nieuwe therapieën die tot doel hebben de incidentie ervan te verlagen.
Een van de manieren waarop men hoopt restenose te kunnen reduceren is lokale toediening van geneesmiddelen via perfusie catheters. Trauma door de druk veroorzaakt en duur van de ballon inflatie zijn echter belangrijke beperkingen van deze techniek. In hoofdstuk 6 evalueerden we een nieuwe lokale afgifte catheter, de Dispatch, die ontworpen is om beide beperkingen te boven te komen. We vonden dat langdurige ballon-inflatie haalbaar is met dit instrument, en dat lokale drug-infusie optreedt met minimale beschadiging van de vaatwand. Het instrument biedt dus grote mogelijkheden voor het lokaal toedienen van geneesmiddelen die acute occlusie en restenose na coronaire interventie kunnen verminderen.

Hoewel experimentele modellen inzicht kunnen verschaffen in de pathofysiologie van restenose, kunnen ze nooit de klinische situatie vervangen. In het tweede deel van dit proefschrift (hoofdstuk 7 t/m 10) onderzochten we een nieuwe techniek, intravasculair ultrageluid (IVUS), die van revolutionair belang kan zijn voor ons begrip van de mechanismen die een rol spelen bij de gebeurtenissen tijdens coronair angioplastiek en de daarop volgende restenose in de klinische situatie.
Hoofdstuk 7 geeft een beknopt overzicht van het onderwerp terwijl hoofdstuk 8 de met IVUS aan het vaatlumen verrichte metingen vergelijkt met de gouden standaard tot nu toe, kwantitatieve coronair angiografie.
Hoofdstuk 9 geeft een samenvatting van onze eerste klinische ervaringen met een nieuw instrument, waarbij beeldvorming door intravasculair ultrageluid gecombineerd wordt met ballon angioplastiek, waardoor on-line beeldvorming gedurende de procedure mogelijk wordt en de behoefte aan herhaald verwisselen van catheters vervalt. We vonden dat intravasculare beeldvorming in meer dan 50% van de gevallen extra informatie opleverde bovenop de informatie die verkregen werd door contrast angiografie. Deze informatie speelde in ruim 10% van de gevallen een doorslaggevende rol bij het bepalen van de behandeling van de patiënt.
In hoofdstuk 10 gebruikten we intracoronair ultrageluid om te zien wat de rol is van het zich aktief omvormen van de vaatwand (vascular remodelling) bij de mechanismen van vergroting van het vaatlumen tijdens PTCA en DCA, en bij de lange termijn restenose erna.

In het laatste deel van dit proefschrift (hoofdstuk 11 t/m 17) werden gegevens van vier grote Europese en Amerikaanse trials gebruikt om de invloed van klinische, angiografische en procedure-gerelateerde factoren op restenose te onderzoeken.
Hoofdstuk 11 onderzoekt de rol van cholesterol en cholesterol subfracties op restenose, terwijl hoofdstuk 12 de invloed van het roker of ex-roker zijn op restenose na angioplastiek nagaat. We vonden geen verband tussen cholesterol (inclusief subfracties) en restenose bij een categoriale noch bij een continue benadering. Dus maatregelen die uitsluitend gericht zijn op het verlagen van het totale cholesterol

zullen waarschijnlijk geen significante invloed hebben op het optreden van restenose na PTCA. We vonden geen verband tussen rookgewoonten en restenose, met een categoriale noch met een continue benadering, hetgeen suggereert dat maatregelen gericht op het terugdringen van roken waarschijnlijk geen significante invloed zullen hebben op restenose na PTCA.
Hoofdstuk 13 gaat verder in op een aantal van onze bevindingen en bepaalt de rol van lokale trombus vorming en -incorporatie op restenose. We vonden dat trombus geassocieerd is met meer restenose. Het mechanisme hiervoor staat in verband met occlusie tijdens follow-up angiografie, hetgeen suggereert dat maatregelen die gericht zijn op het verbeteren van het resultaat bij deze patiënten toegespitst moeten worden in deze richting.
Hoofdstuk 14 gaat in op totale occlusies en de kans dat bij deze lesies restenose optreedt, terwijl hoofdstuk 15 de rol onderzoekt van gunstige vaatwand-omvorming (gedefinieerd als negatief verlies van lumen) na PTCA.
Hoofdstuk 16 geeft een kritisch overzicht van de grote multi-centre onderzoeken waarop de vorige onderzoeken gebaseerd waren en bespreekt manieren waarop sommige van de beperkingen ervan gereduceerd zouden kunnen worden in toekomstige onderzoeken.
Tenslotte vatten we in hoofdstuk 17 het huidige spectrum van technologieen samen, dat de interventionele cardioloog ter beschikking staat bij zijn strijd tegen restenose.

Restenose blijft een belangrijke beperking van angioplastiek van de kransvaten, die afbreuk doet aan zowel de klinische als economische voordelen van de techniek. In dit proefschrift werden zowel in vivo als in vitro modellen van intimale hyperplasie na ballon angioplastiek ontwikkeld en de pathofysiologische mechanismen die een rol spelen bestudeerd. Daarnaast werd de epidemiologie van restenose onderzocht en werden lesies waarbij restenosen vaker optreedt geïdentificeerd.

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Curriculum Vitae

Dr AG Violaris was born in Paphos, Cyprus, on January 31st 1961. His parents emigrated to London in 1970, where he received his secondary education. He graduated in Medicine from Sheffield University in 1984, where he also did his internship and gained his initial experience in Cardiology with Dr JS Fleming. In 1987 he became a member of the Royal College of Physicians and moved to Brighton where he gained further experience in Cardiology with Drs DA Chamberlain and R Vincent. The post subsequently rotated to the Cardiology department at King's College Hospital, London where he received further clinical and invasive training under Drs D. Jewitt, G Jackson, C Bucknall and PJ Richardson. In 1990 he moved to the department of Cardiology, Northern General Hospital, Sheffield as a Research Fellow. There he gained further clinical and interventional experience but also received training in research, working with Drs DC Cumberland, S Campbell and Mr GD Angelini, on clinical and experimental aspects of post angioplasty restenosis. He was subsequently awarded the MD degree from Sheffield University for this work. In September 1992 he was awarded an International Travelling Fellowship by the Welcome Trust to investigate experimental and clinical aspects of restenosis and moved to the Thorax Centre, Erasmus University Rotterdam, The Netherlands. In 1994 he was made a member of the British Cardiac Society. He is currently Lecturer/Senior Registrar in Cardiology at St Mary's Hospital, Paddington, London.

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Junior Cardiac Club Research Prize, London, UK, 1996

Wellcome International Research Fellowship, 1992-95

Pfizer Academic Travel Award, 1992-93

Vezey Strong Scholarship, 1979-1982

Grimwade Prize, 1977