Coronary Artery Remodelling, Atherosclerosis and Vascular Function

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Abstract

Objectives: The aims of the thesis were to assess coronary artery remodelling and plaque load, and to determine whether this influences vascular and endothelial function *in vivo* in man.

Methods: Coronary artery remodelled segments were categorised using intravascular ultrasound (IVUS). Plaque type was characterised directly from spectral analysis of the radiofrequency ultrasound signal. Central arterial stiffness was assessed using non-invasive measures of arterial stiffness obtained by applanation tonometry of the radial, carotid and femoral artery. Coronary artery plaque volume was determined following computerised three-dimensional reconstruction of IVUS images obtained during a motorised pullback device. Coronary vessel area, arterial stiffness and vasomotor responses were determined using IVUS and Doppler Flow measurement and endothelial fibrinolytic responses by coronary sinus sampling during selective intracoronary infusions.

Results: ***Plaque characteristics*** Positively remodelled segments had a larger vessel area (16.5 ± 1.1 mm^2 vs. 8.7 ± 0.9 mm^2, $p<0.01$) and plaque area (7.3 ± 1.1 mm^2 vs. 4.4 ± 0.8 mm^2, $p=0.05$) than negatively remodelled segments. Both positive and negatively remodelled segments had a greater percentage of fibrous plaque ($p<0.01$) than calcified or lipid rich plaque. Comparing positively and negatively remodelled segments, there was no significant difference between the proportion of fibrous, calcified and lipid rich plaque. ***Comparisons with non-invasive measures*** Plaque volume positively correlated with carotid-radial pulse wave velocity ($r=0.47$, $p=0.008$) and appeared to correlate with carotid-femoral pulse wave velocity ($r=0.34$, $p=0.07$). Aortic augmentation ($r=0.24$, $p=0.16$), augmentation index ($r=0.3$, $p=0.08$), and pulse pressure ($r=0.22$, $p=0.2$) did

not significantly correlate with proximal coronary artery plaque volume.

Structure and function In comparison to non- and positively remodelled segments, negatively remodelled segments had a higher stiffness index (67±16 *vs.* 33±5 and 38±8 respectively; $p<0.02$). A significant degree of preservation of vasodilatation to 10^{-6} M acetylcholine was evident in positively remodelled compared with negatively remodelled segments ($p<0.05$). Coronary blood flow increased with both substance P and sodium nitroprusside infusions ($p<0.001$), although coronary sinus plasma t-PA antigen and activity concentrations increased only during substance P infusion ($p<0.006$ for both). There was a significant inverse correlation between coronary artery plaque burden and the release of active t-PA ($r=-0.61$, $p=0.003$).

Conclusions: Pulse wave analysis may be a useful non-invasive surrogate marker for the extent of coronary atherosclerosis. Atherosclerotic risk factors and coronary plaque load are associated with impaired vasomotor and endogenous fibrinolytic function. Though plaque type was similar in remodelled types, negative remodelling was associated with more pronounced local vascular and endothelial dysfunction. These findings collectively suggest an important local interrelationship between coronary vascular structure and function that has implications for the pathophysiology of ischaemic heart disease.

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Declaration

This thesis represents research undertaken in the Department of Cardiology in both the Western General Hospital and the Department of Cardiology at the Royal Infirmary, Edinburgh. The substantial part of the work described has been my own and carried out during the period between 1998 and 2001 whilst I was a Research Fellow in Cardiology. The study constituting Chapter 5 was carried out in collaboration with Dr D. Newby (Senior Lecturer, Royal Infirmary Edinburgh). I was involved in the intravascular ultrasound / doppler flow wire assessment and Dr D. Newby was involved in the Fibrinolysis assessment. The work constituting Chapters 5 and 6 has been published in peer reviewed journals: see Bibliography, with the work constituting Chapters 3 and 4 being currently in press. The thesis has not been accepted in any previous applications for a degree and all sources of information have been acknowledged.

Ethics

All studies were conducted with the approval of the Lothian Research Ethics Committee and with the written, informed consent of each subject. All studies were performed in accordance with the guidelines set out in the revised Declaration of Helsinki 1964.

Lists of Tables and Figures

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Acknowledgements

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I am thankful to all the patients that agreed to take part in the studies. I would like to acknowledge the assistance of the cardiac technicians, the nursing staff and radiographers in the Cardiology Departments at the Western General Hospital and the Royal Infirmary of Edinburgh. I would also like to thank Sister Laura Flint who was involved with the coordination of patient studies and blood samples at the Royal Infirmary of Edinburgh Hospital and Dr Robin Watson who performed the coronary plaque characterisation work.

Abbreviations

ACh	acetylcholine
ANOVA	analysis of variance
AUC	area under curve
bpm	beats per minute
CI	confidence intervals
CSA	cross sectional area
CT	computed tomography
DCA	directional coronary atherectomy
DBP	diastolic blood pressure
ECG	electrocardiograph
HR	heart rate
ia	intra-arterial
ICAM-1	intra-cellular adhesion molecule
iv	intravenous
IVUS	intravascular ultrasound
MCP-1	monocyte chemotactic protein-1
MDU	motor drive unit
MRI	magnetic resonance
NO	nitric oxide
PDGF	platelet-derived growth factor
PTCA	percutaneous transluminal coronary angioplasty
PWA	pulse wave analysis
RF	radiofrequency
SBP	systolic blood pressure
SEM	standard error of the mean

TGF-ß	transforming growth factor-beta
VCAM	V-cellular adhesion molecule

Chapter 1

Background

1.1 Coronary Artery Remodelling

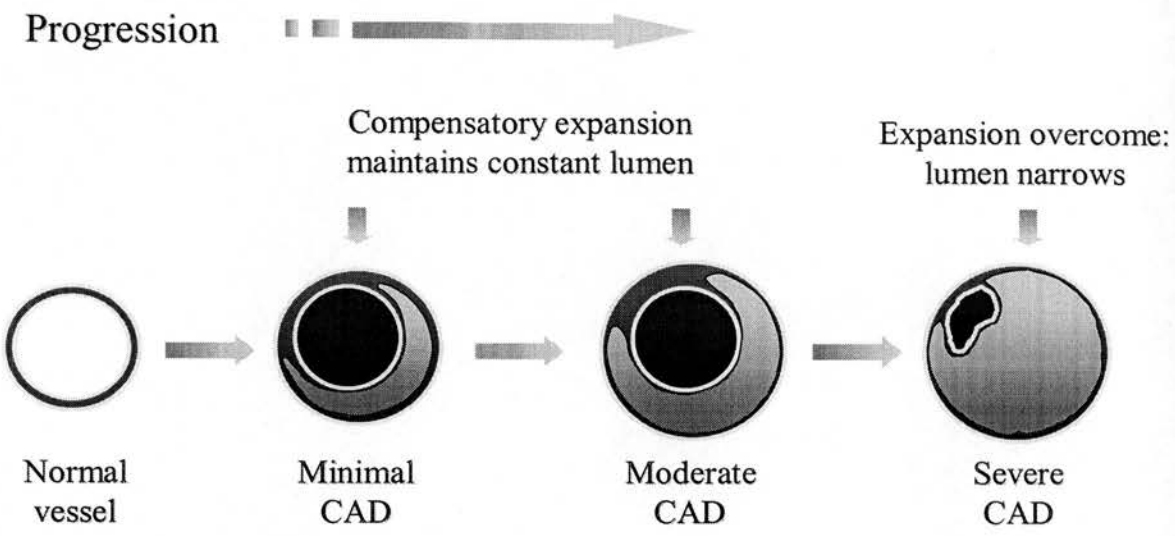
1.1.1 Historical Perspective

The ability of an artery to expand to accommodate plaque was first noted, in 1972, from an autopsy series of Masai tribesman where significantly enlarged coronary arteries on external inspection were noted to contain a substantial atherosclerotic plaque burden (Mann et al 1972). This plaque load, with a concomitant increase in lumen area, was greater than seen in matched North American subjects.

Further observational studies by Glagov and colleagues (Glagov et al 1987; Zarins et al 1988), confirmed the ability of coronary arteries to expand to accommodate increasing atherosclerotic plaque (Clarkson et al 1994; McPherson et al 1991). From analysis of human left main stem histological segments, it was concluded that coronary arteries enlarge with the increase in the lesion area and only when plaque constituted 40% of the total vessel area did luminal compromise occur. This process was termed compensatory enlargement (Glagov et al, 1987)(Figure 1.1).

Subsequent intravascular ultrasound (IVUS) and post mortem studies have shown that the extent of remodelling in a given artery may be highly variable with segments of positive remodelling (vessel expansion), no change, and negative remodelling (vessel shrinkage) with encroachment on lumen area (Pasterkamp et al 1995; Nishioka et al 1996).

Figure 1.1 Glagov's Coronary Artery Remodelling Hypothesis



Adapted from Glagov et al, *N Engl J Med* 1987;316:1371-1375

1.1.2 Pathophysiology

The term remodelling is now used in de novo atherosclerosis, restenosis and transplant vasculopathy, though the underlying mechanisms may be different.

Remodelling and de novo atherosclerosis

Remodelling is a dynamic process with an artery responding to changes in shear stress and wall tension (Langille et al 1996; Ward et al 2000). Previous work has suggested that with increasing plaque load, vascular response to shear stress is preserved (Krams et al 1998; Dirksen et al 1998; Smedby et al 1997). Increased shear stress may potentiate positive remodelling through endothelial production of nitric oxide (Tronc et al 1996) with an increase in matrix metalloproteinase activity (Lee et al 1996; Sasaki et al 1998) and apoptosis of smooth muscle cells (Cooke et al 1997). Furthermore, shear stress leads to enhanced expression of adhesion molecules (ICAM-1 and VCAM) (Walpola et al 1995) with increased inflammatory cell infiltration into the plaque, and a local increase in macrophages and T-cell activity (Dirksen et al 1998). In contrast, with low flow states, production of growth factors predominate (TGF-ß, PDGF), with a resultant increase in smooth muscle cells and collagen deposition which potentially mediates negative remodelling (Mondy et al 1997; Bassiouny et al 1998).

Remodelling and restenosis

After coronary intervention, there appears to be an acceleration of the remodelling process, as described by intravascular ultrasound. Di Mario et

al described immediate and late changes in 34 patients after balloon angioplasty (PTCA) and directional coronary atherectomy (DCA) (Di Mario et al 1995). A reduction in plaque area was the main operative mechanism of DCA accounting for 66% of acute lumen enlargement, compared to balloon angioplasty where this accounted for 52% of the increase in lumen area. There was also a significant increase in total vessel area in both groups thus accounting for 48% and 34% of the acute lumen gain in PTCA and DCA groups, respectively. After 6 months, in the DCA group, an increase in plaque area was the predominant mechanism of late lumen loss (92%) whereas this accounted for only 33% of loss after PTCA, the remainder coming from a reduction in total vessel area (late recoil or negative remodelling).

In another study, 209 patients underwent serial ultrasound and angiography pre- and post-intervention at mean 6 months follow-up (Mintz et al 1996). A total of 73% of the reduction in lumen area from post-intervention to follow-up was due to a decrease in vessel size with the remainder due to an increase in plaque area. As expected, this negative remodelling process was most pronounced in those patients with angiographic restenosis (defined as a diameter stenosis >50% by angiography). During follow-up, 22% of lesions showed an increase in total vessel area complementing an increase in plaque area (positive remodelling). These lesions had no overall change in the lumen area although 49% of them demonstrated a late lumen gain with a restenosis rate of 26% compared to that of 62% in lesions showing a decrease in total vessel area.

The relationship between acute lumen gain and late lumen loss was analysed in a prospective serial ultrasound and angiographic study after balloon angioplasty or DCA (Kimura et al 1996a). Late lumen loss correlated closely with the reduction in vessel area and increase in plaque area, although only the increase in vessel area was related to the acute gain suggesting that arterial remodelling but not changes in plaque volume was indicative of a generalised response to injury (Kimura et al 1996b). This was subsequently confirmed in a porcine model of deep wall injury, where although the degree of injury correlated with neointimal formation, the final lumen size was more closely related to the final vessel size confirming that arterial remodelling was more important than neointimal hyperplasia, in determining late luminal narrowing (Anderson et al 1996).

The response of the adventitia to vascular injury is an attractive hypothesis towards the development of negative remodelling. An early correlation was noted between the degree of adventitial inflammation and the severity of disease in the overlying arterial wall (Schwartz et al 1962). Given the barotrauma of coronary intervention, the adventitia proliferates within two days from the stretch/pressure stimulus gradually abating between one and two weeks from the event, even supplying proliferating cells to the neointima (Scott et al 1996). Work defining the three-dimensional network of the (adventitial) vaso vasorum using microscopic computer tomography confirms neovascularisation after balloon injury with an increase in circumferential blood vessel density and an association with lesion severity (Kwon et al 1998). It has been hypothesised that the degree of barotrauma and endothelial disruption at the time of intervention accelerates the negative remodelling process through an inability of adventitia to

accommodate the residual plaque load. The reduced restenosis rates with the antioxidant probucol, attributed to preserved positive remodelling in the presence of an increased plaque load, may thus be due to a relative preservation of endothelial function. However, it is not known, whether endothelial function in a segment of a diseased vessel modulates the ability of adventitia to expand to accommodate an increasing plaque load in the absence of intervention.

Remodelling in transplant vasculopathies

Both positive and negative remodelling has been described in transplanted hearts. Though haemodynamic stimuli exist as in the native coronary circulation, the effect of denervation on remodelling is unknown. Previous improvements in stenosis severity by angiography with HMG-CoA reductase inhibitors has been attributed following IVUS studies to a reduction in intimal growth as opposed to positive remodelling. An in-vivo IVUS study investigated 27 patients with minimal or no epicardial transplant coronary artery disease during their annual angiographic follow up (Schwarzacher et al 2000). The degree of coronary remodeling (as estimated by the ratio of total vessel area / [total vessel area – lumen area]) was significantly greater in eccentric lesions compared to concentric lesions, despite similar intimal-medial areas, suggesting that plaque topography may influence the remodelling response. Eccentric lesions also had significantly greater compliance suggestive of preserved endothelial function (or a section of dynamic vasomotor function in the less diseased arc) though whether this determines the remodelling response is unknown.

The absence of longitudinal studies has led to speculation as to whether positive and negative remodelling is part of the same pathological process. With different types of remodelling in the same vessel it suggests that lesion specific factors for example plaque type, degree of inflammation, shear stress, age of a lesion and vessel angulation may be more important than patient specific factors.

The mechanisms determining why the vessel can only accommodate a certain amount of plaque before positive remodelling is exhausted is unknown. Previous work using quantitative coronary angiography in patients with normal angiograms or mild coronary artery disease has suggested that positive remodelling may occur early in the development of atherosclerosis (Lerman et al 1998). Loose connective tissue and lipid accumulation have been associated with early development of atherosclerosis (Di Mario et al 1992), with fibrotic and calcified lesions evident at a later stage (Stary et al 1995). Calcification has been associated with negative remodelling (Mintz et al 1997; Sabate et al 1999), as well as unrelated to the form of remodelling (Weissman et al 1999). However, the hypothesis that negative segments represent a late stage of atherosclerosis, where the plaque segment underwent positive remodelling first, is difficult to justify in the absence of longitudinal studies. Longitudinal studies, however, in view of the slow development of stable atherosclerotic plaque makes this impractical, expensive, and ethically difficult. However with on-going development of noninvasive methods of coronary imaging such as MRI and ultrafast CT, it is possible that serial studies in humans may become more feasible.

1.1.3 Clinical Relevance

Coronary lesions with positive remodelling, as opposed to lesions with negative remodelling have a greater plaque volume by 3D reconstruction of IVUS images (Von Birgelen et al 1998). Plaque rupture in an acute coronary syndrome occurs at sites where vessels show a greater degree of positive remodelling than in stable lesions (Schwarzacher et al 1998; Jeremias et al 2000; Shoenhagen et al 2000). Indeed patients presenting with stable angina were more likely to present with culprit lesions which are negatively remodelled (Shoenhagen et al 2000) often with more fibrous and calcified plaques (Kearney et al 1996). In a detailed histological study, immunohistochemical staining of post mortem atherosclerotic arteries demonstrated more markers of plaque vulnerability (increased macrophages and lymphocytes, less collagen and smooth muscle cells) in sections with a larger plaque to vessel area, i.e. positively remodelled vessels (Pasterkamp et al 1998). Ironically, this suggests that although positive remodelling allows the vessel to expand to avoid lumen encroachment and the potential for coronary flow-limitation, it may lead to instability in the plaque increasing its likelihood for erosion and rupture. Following lipid-lowering treatment in atherogenic rabbits, there is evidence for plaque stabilisation with reduced accumulation of macrophages and expression of metalloproteinases, along with greater collagen accumulation (Aikawa et al 1998; Pasterkamp et al 1998). Whether the increased risk for instability in positively remodelled arteries is modulated by changes in the integrity or function of the overlying endothelium is unknown.

1.2 Limitations of Coronary Angiography

Since the late 1950s, the gold standard for the anatomical assessment of coronary arteries was selective coronary angiography (Judkins et al 1967; Sones et al 1972). However, although becoming widely applicable for the assessment and management of coronary artery disease, limited information is available from angiography about plaque load. Images are taken at different angles, with the image illustrating the minimum lumen diameter taken as the index picture of the greatest lesion severity, which may not be the case. Angiography describes a two-dimensional silhouette of a contrast-filled lumen, however, there are many limitations both with respect to image quality and the inability to examine the arterial wall directly. Angiography depends on adjacent segments to characterise lesion severity but the normal reference segment is frequently diseased due to the diffuse nature of atherosclerosis (Mintz et al 1995), leading to an underestimation of disease severity. Accurate quantitative angiography requires computer-assisted edge detection and in specific situations such as in eccentric or ostial lesions, and with vessel fore-shortening and overlap, there is a difficulty in interpretation with wide interobserver variability. Problems with accurate angiographic quantitation are frequently accentuated after coronary intervention due to plaque rupture and dissection with the subsequent appearance of a hazy lumen of indeterminate severity. The absolute resolution of image intensifiers is also limited by radiation dose considerations and by rapid coronary artery motion, leading to an image blur of up to 0.5 mm (Topol et al 1995).

In addition to issue with image resolution and appearance, the inability of angiography to detect this occult disease may also be due to the presence of positive remodelling and the diffuse nature of disease throughout the entire vessel length in many patients. Furthermore, important pathological features not demonstrated by angiography include plaque composition, in particular the presence of a lipid-rich or fibrous plaque, and thus an indication of the stability/vulnerability of the plaque. As a result, the degree of stenosis has been shown to be a weak indicator as to whether plaque will eventually cause a myocardial infarction (Falk et al, 1995).

As a consequence of these differences between the two imaging modalities, significant discrepancies have been found between IVUS and angiography: lesions were classified more frequently as eccentric by ultrasound than by angiography, 77% vs. 33%. Target lesion calcification was documented in 62% of cases by ultrasound but only 35% of angiograms and after angioplasty, fracture or dissection of plaque was described in 49% of lesions by ultrasound compared to 22% of angiograms (Fitzgerald et al, 1993).

Angiography by virtue of being a lumenogram is still unable to allow complete assessment of coronary plaque and yet is widely used in many coronary atherosclerosis trials. In several small plaque regression trials (FATS, STARS), little difference was seen in coronary stenosis severity using quantitative angiography at follow-up despite large differences in clinical outcome (Brown et al 1993). This suggests that the ability to study relatively mild (<50% diameter stenosis by angiography) but potentially unstable stenoses by intravascular ultrasound may give additional

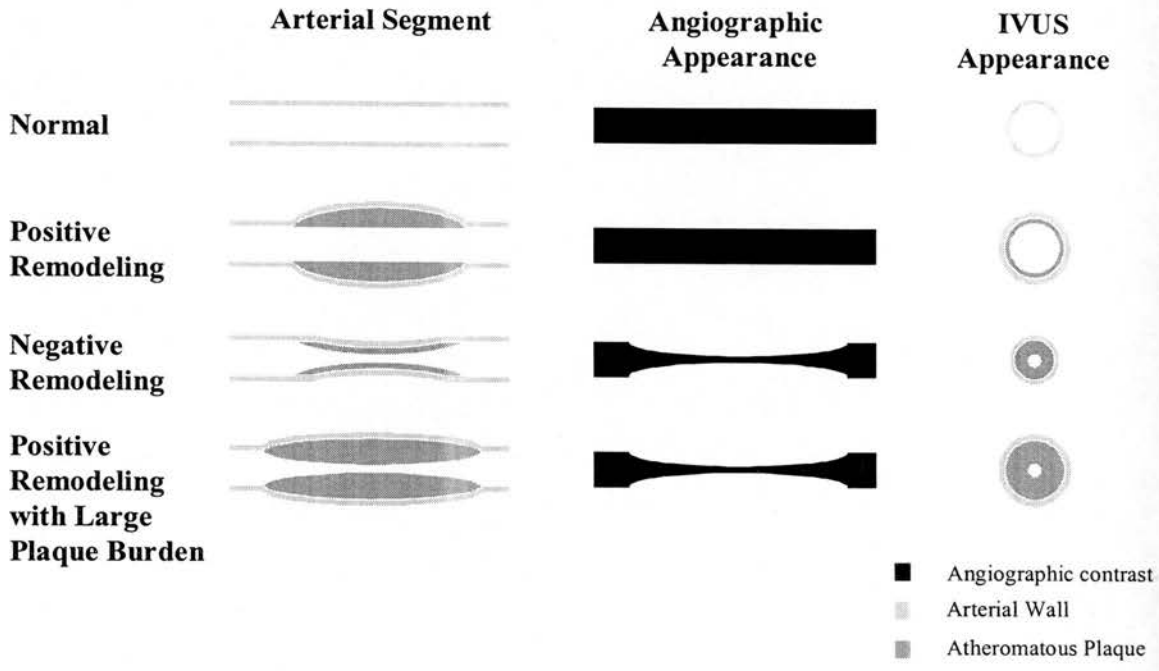
information regarding clinical outcome and emphases the need for complementary methods of imaging coronary arteries.

1.3 Intravascular Ultrasound (IVUS)

1.3.1 Introduction

The technology to image coronary plaque in vivo only became available in the late 1980s with the development of catheter based intravascular ultrasound which has revolutionized our understanding of atherosclerotic coronary artery disease (Yock et al 1989). As previously discussed angiography is limited in its ability to assess the disease process accurately, and to define plaque composition and extent. Advances in miniturisation has led to ultrasound catheters as small as 2.6F (c. 0.9 mm) diameter, without loss of image quality. IVUS gives two-dimensional information regarding the arterial layers in cross-section, providing, both a visual and quantitative assessment of plaque burden with the potential to reconstruct this information three-dimensionally off-line (Figure 1.2).

Figure 1.2 Coronary arterial remodelling associated with atherosclerosis: angiographic and intravascular ultrasound views



1.3.2 Plaque characterisation

IVUS provides information about plaque composition (Nishimura et al 1990; Tobis et al 1991). Classification of plaque by the IVUS subgroup of the Working Group of Echocardiography of the European Society of Cardiology defined plaque characteristics as homogenous or mixed. Homogenous related to soft (echolucent or low echoreflectivity), fibrous (similar echoreflectivity to adventitia), or calcific plaque (high echoreflectivity with shadowing respectively). Mixed plaque constituted the following combinations: soft/fibrous, soft/calcific and fibrocalcific (Di Mario et al, 1998) (Table 1.1).

IVUS permits accurate quantification of coronary atherosclerotic plaque. Only a small degree of inter-observer variability has been confirmed in several studies as lateral dropout and distortion of the image can cause a small difference between the interpretation of different observers (R=0.93-0.98). Catheter movement during the cardiac cycle has not been substantiated as a significant cause of error (Kearney et al, 1995a).

Table 1.1 Qualitative ultrasound definitions

Plaque Echographic Characteristics	**Histology**
echoreflectivity < adventitia	fibrofatty tissue, thrombus
echoreflectivity = adventitia	fibrous tissue
echoreflectivity > adventitia	dense fibrous tissue
high echoreflectivity + shadowing	calcium

Plaque Characteristics

Homogenous*	**Mixed†**
soft	soft / fibrous
fibrous	soft / calcific
calcific‡	fibrocalcific

* >80% area constituted by the same plaque components. No calcium or focal calcium deposits (arc of calcium < 10 degrees)

† presence of multiple plaque components not matching the 80% criterion of prevalence

‡ total calcific arc > 180 degrees

Modified from Di Mario et al, *Eur Heart J*;19:207-229.

Radiofrequency analysis

Previous methods in characterising atheromatous plaque include both visual analysis and comparison of intima with the echo-reflectivity of the adventitia (Gussenhoven et al 1989; Siegel et al 1992; Di Mario et al 1992) and videodensitometric analysis (Peters et al 1994; Rasheed et al 1995) of a grey scale level. These methods, however, are difficult to reproduce in view of the poor resolution provided by the video image along with various post-processing operations that are applied to the ultrasound signal such as the gain settings of the image (Kearney et al 1995b).

The unprocessed radio-frequency (RF) signal from ultrasound scanners provides a more reproducible source of data. Plaque characterisation using the power spectral analysis from raw ultrasound information has been described in both coronary (Dixon et al 1997; Spencer et al 1997; Moore et al 1998; Watson et al 2000; Nair et al 2002) and carotid arteries (Noritomi et al 1997a, 1997b).

In an animal model of allograft rejection, RF analysis of unprocessed intravascular ultrasound signal "backscatter" was found to be more sensitive in characterising tissue than visual or quantitative grey-scale analysis (Jeremias et al 1999). Watson et al selected 299 regions-of-interest (ROI) from eight post-mortem coronary arteries using a 30MHz IVUS scanner. Predominant plaque composition of the vessel wall was identified by routine histology and staining. A suture needle inserted in the vessel wall throughout the IVUS pullback showed clearly on the IVUS image and allowed alignment of the IVUS data with histology. With RF

analysis defined in terms of spectral features, 86% of coronary plaque was correctly classified into one of three categories (mixed fibrous tissue, lipid-rich plaque and calcified plaque) (Watson et al 2000).

1.4 Endothelial Function in Coronary Artery Disease

The endothelium plays a central role in the maintenance of normal vascular function and homeostasis including the control of blood flow, coagulation, fibrinolysis and inflammation. Consequently, the maintenance and regulation of tissue perfusion critically depends upon the integrity of endothelial function and the release of various endothelium-derived factors.

1.4.1 Endothelial Dysfunction

The endothelium-dependent regulation of peripheral vascular tone is impaired by many of the risk factors associated with atherosclerosis, such as hypercholesterolaemia (Chowienczyk et al 1992; Stroes et al 1995; Heitzer et al 1996a), diabetes mellitus (Calver et a*l* 1992), a familial history of atherosclerosis (Celermajer et al 1992), hypertension (Vanhoutte 1996) and smoking (Celermajer et al 1996; Heitzer et al 1996a+b; Newby et al 1999a]. This dysfunction appears to be generalised with demonstrable impairment of endothelial function in the coronary circulation of patients with coronary artery disease (Ludmer et al 1986) and its risk factors (Vita et al 1990) including smoking (Zeiher et al 1995). Moreover, the greatest constrictor response to the endothelium-dependent vasodilator, acetylcholine, was described in those with most risk factors (Vita et al 1990)

1.4.2 Plaque Load and Endothelial Dysfunction

Endothelial dysfunction may be the earliest manifestation of the atherosclerotic disease process and account for many of the abnormalities documented in regional tissue perfusion that occur before the development of obstructive coronary artery disease (Uren and Crake 1996).

With the development of atheroma, endothelial-dependent relaxation to a variety of different vasoactive agents is impaired. Acetylcholine infusion may even cause paradoxical vasoconstriction in patients with coronary artery disease (Ludmer et al 1986). Coronary artery disease is a diffuse process with more than one discreet lesion affecting some or all of the main epicardial coronary arteries along with a variable degree of endothelial dysfunction. Concomitantly, the atherosclerotic process appears to have a similar effect on coronary vasomotor tone in epicardial vessels and resistive vessel function in man (Uren and Crake 1996).

Normal epicardial vessels dilate in response to increasing flow induced by the effect of a vasodilator such as intracoronary papaverine on the resistive vessels (Drexler et al 1989). However, flow-dependent coronary vasodilatation is reduced in atherosclerotic arteries. Zeiher et al studied the coronary vasomotor responses to three different endothelium-mediated stimuli; acetylcholine, increasing blood flow, and sympathetic activation by cold pressor testing in different patients (Zeiher et al 1991). All three stimuli dilated angiographically normal coronary arteries in subjects with no cardiovascular risk factors. Subjects with normal arteries but hypercholesterolaemia sustained vasoconstriction to acetylcholine.

Patients with a normal artery by angiography but with disease elsewhere showed both vasoconstriction to both acetylcholine and cold pressor, with preservation of flow-dependent vasodilatation. Patients with angiographic wall irregularities showed abolition of vasodilatation to all three stimuli, thus indicating a hierarchy of impairment on endothelial-dependent vasodilatation in epicardial arteries with progression of the atherosclerotic process. In the microcirculation, the dilator response to acetylcholine was markedly reduced in patients with angiographically normal epicardial arteries and hypercholesterolaemia, compared to the other groups. Patients with angiographically normal arteries but disease elsewhere also showed a reduced vasodilator response to acetylcholine, but this was found in those with a raised LDL-cholesterol. However, the epicaridial vasodilator response to papaverine (flow-dependent vasodilatation) was lower only in those with angiographic disease of the study artery compared to the control group. Thus, there is progressive impairment in endothelium-dependent vasodilatation in patients at different stages of early atherosclerosis. In the IVUS era, it is interesting to speculate that the hierarchy of response in vessels defined by their angiographic appearance probably reflects total atheroma plaque burden by 3D IVUS reconstruction.

1.4.3 Endogenous t-PA and Endothelial Dysfunction

Whilst endothelial-dependent vasomotion is an important measure of cell function, Newby and co-workers, described an in vivo model to measure endothelial derived tissue plasminogen activator (t-PA) release, in the assessment of the fibrinolytic capacity of the human forearm, using the

Furthermore a reduction in t-PA release was demonstrated after inducing experimental 'endothelial dysfunction' with nitric oxide synthase inhibition (Newby et al 1998).

Interestingly, the endothelium-dependent vasodilator acetylcholine does not induce t-PA release despite an increase in forearm blood flow (Brown et al 1999). Endothelial fibrinolytic dysfunction, as manifest by an impairment of endothelial t-PA release, appears to be a feature of cigarette smoking and atherosclerosis (Newby et al 1999) but not hypercholesterolaemia or subsequent lipid lowering therapy (Newby et al 2002). This is in contrast to endothelial function as assessed by vasomotion following lipid-lowering therapy (Treasure et al 1995; Anderson et al 1995). This would indicate that endothelial dysfunction can be manifest in separate distinct pathways depending upon the nature of the insult.

1.4.4 Coronary Artery Remodelling and Endothelial Function

The variable response of coronary arteries to endothelium-dependent vasodilatation may be largely caused by the extent of local plaque load and at what stage a given segment is in the remodelling process. Results based on quantitative angiography define the atherosclerotic plaque load poorly and do not take account of coronary remodelling. In effect, the relationship between the degree of endothelial dysfunction and the remodelling process in coronary arteries is not well-defined.

1.5 Pulse Wave Analysis

The arterial pressure waveform alters with progression down the arterial tree. This is due to local variations in vascular stiffness as well as superimposition of the reflected pressure waveform (O'Rourke et al 1993). The latter phenomenon occurs when the anterograde pressure wavefront encounters the main points of impedance mismatch, such as the transition from small to resistance arteries. This causes a reflected pressure waveform to return to the central arteries and aorta in diastole, thereby enhancing coronary perfusion pressure. Progressive arterial stiffening, as occurs with ageing, causes an increase in the aortic pulse pressure due to the combination of local aortic stiffness and an increase in both pulse wave velocity and wave reflection. These stiffness-related effects produce an increase in central aortic pressure and cardiac afterload, and a reduction in coronary perfusion pressure due to the movement of the reflected wave into systole (Nichols et al 1990; London et al 1990).

1.5.1 Arterial Stiffness and Cardiovascular Risk

Arterial stiffness increases with age (Avolio et al 1983), hypertension (Nichols et al 1990), and in subjects with diabetes mellitus (Lehmann et al 1992), atherosclerosis (Wada et al 1994) and end-stage renal disease (London et al 1990). An increase in arterial stiffness may have several important consequences. Elevation of central aortic pressure promotes the development of left ventricular hypertrophy (Lakatta et al 1990; Gatzka et al 1998) and is associated with an increase in mortality (Levy et al 1990). Stiffness of both the common carotid aorta and abdominal aorta has been

found to progressively increase with 1, 2 and 3 vessel coronary artery disease and in subjects with previous myocardial infarction (Hirai et al 1989).

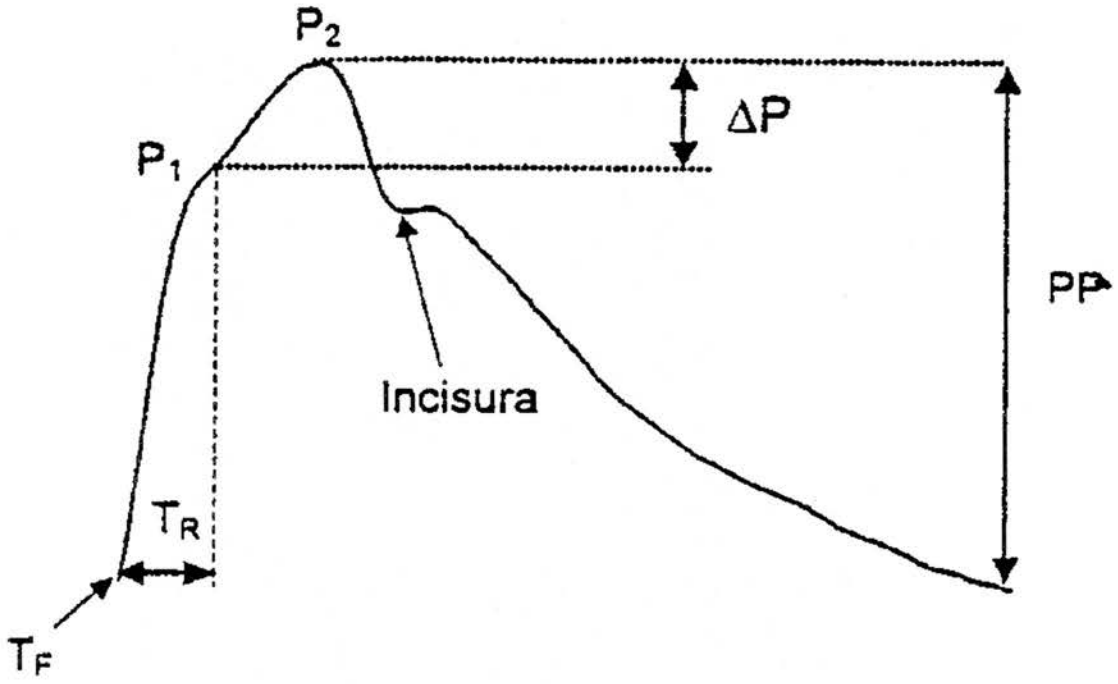
Increased pulse pressure augments atherogenesis in primate models (Lyon et al 1987) and may increase the likelihood of atherosclerotic plaque rupture in humans (Cheng et al 1993). Moreover, in clinical studies, elevations in pulse pressure and aortic stiffness are associated with an increase in the future risk of coronary events (Mitchell et al 1997; Blacher et al 1998; Gatzka et al 1998; Lehmann et al 1998) and overall mortality (Benetos et al 1998; Domanski et al 1999; Blacher et al 1999).

1.5.2 Applanation Tonometry

The peripheral pulse pressure waveform (pulse wave analysis) and the velocity of its propagation through the arterial tree (pulse wave velocity) can be determined non-invasively using a technique called applanation tonometry (O'Rourke et al 1996, Kelly et al 1989). Arterial stiffness creates increased pulse wave velocity and the systolic peak is augmented in view of the early reflection of the pressure wave from the periphery (Latham et al 1985, O'Rourke et al 1993). A central aortic waveform is derived from the peripheral waveform using a validated transfer function (Karamanoglu et al 1993, O'Rourke et al 1995) (Figure 1.3) Surrogate markers of atherosclerosis have been associated with increased pulse wave velocity, namely the aortic calcification index (Blacher et al 2001), carotid intima-medial thickness (Taniwaki et al 1999) and the ankle/brachial systolic pressure index (Duprez et al 2001). No studies to date have been

performed using applanation tonometry combined with intravascular ultrasound of the coronary arteries *in vivo* in man, to investigate the relationship between coronary plaque burden and systemic arterial stiffness.

Figure 1.3 A Central Aortic Pressure Waveform



The difference between the two systolic peaks, P_1 and P_2 (ΔP), define the augmentation index which is expressed as a percentage of the pulse pressure (PP). The ejection duration is defined as the time from the foot of the pressure wave to the incisura. T_R provides a measure of the timing of the reflected wave.

1.6 Aims

The aims of the thesis were the following in patients with coronary artery disease:

- to characterise and to identify the predominant plaque type *in vivo* using unprocessed radio-frequency (RF) intravascular ultrasound backscatter, in remodelled segments of human atherosclerotic coronary arteries (Chapter 3).
- to examine the relationship between invasive measures of coronary artery plaque volume using intravascular ultrasound and non-invasive measures of arterial stiffness using applanation tonometry (Chapter 4).
- to establish the relationship between the volume of proximal coronary artery atheroma, the presence of associated risk factors and local coronary and endothelial function (Chapter 5).
- to assess whether the pattern of vascular remodelling influences the physical and vasomotor responses of the coronary arteries *in vivo* in man (Chapter 6).

1.7 Hypothesis

In defining the aims of the thesis, several related hypotheses were generated and are as follows:

- Positively remodelled segments have a greater proportion of lipid-rich plaque than negatively remodelled segments.
- Coronary artery plaque load correlates positively with aortic stiffness as measured by applanation tonometry.
- The extent of coronary atheroma will influence the vascular / endothelial response.
- Type of plaque remodelling will determine local vascular and endothelial function.

Chapter 2.

Methodology

2.1 Introduction

Coronary angiography is limited both with respect to image quality and the inability to examine the arterial wall directly. Intravascular ultrasound, in particular, allows a more detailed functional and morphological assessment of the epicardial coronary circulation. The coronary Doppler flow wire provides a further means of assessing the coronary flow response. Applanation tonometry and pulse wave analysis provide a means of assessing arterial stiffness non-invasively.

Subselective intracoronary infusions have the advantage of assessing the heart and coronary circulation in relative isolation without invoking systemic effects. This is particularly important for the assessment of cardiac and coronary function which is heavily dependent on changes in the systemic vasculature and the haemodynamic status. In addition, relatively high doses of investigational agent can be administered locally which may be important for the desired physiological or therapeutic effect.

2.2 General

2.2.1 Ethical Considerations

All studies were undertaken in accordance with the Declaration of Helsinki (1989) of the World Medical Association and with the approval of the Lothian Research Ethics Committee. The written informed consent of each subject or patient was obtained before entry into all of the studies reported in this thesis.

2.3 Spectral Analysis

2.3.1 Data Acquisition

During the motorised IVUS pullback (Boston Scientific Corporation, USA), a full frame of RF data was collected once per cardiac cycle by ECG triggering, with a full frame made up of 256 radial scan lines, corresponding to 360 degree rotation of the transducer. Each scan line was digitised at a rate of 250 Msamples/s for a depth of approximately 6 mm resulting in 2048 samples per line. Data was captured at 8-bit amplitude resolution by an ADC card (GageApplied CS8500, Canada) installed in a personal computer.

Frames of RF data were demodulated and scan-converted to produce images equivalent to the standard IVUS video images (Research Systems Inc., Boulder, CO, USA). These images were used to select frames of interest, corresponding to the matched frame on s-VHS tape. Selected frames had their regions of plaque marked out manually by tracing an inner and an outer contour line on the scan-converted image.

The unprocessed RF data corresponding to the selected plaque regions were divided into smaller regions of interest (ROI) of size 64 samples by 5 scan-lines, with an overlap of 16 samples and 2 scan-lines. Each 5-line ROI was analysed by taking a Fourier transformation of each scan-line and averaging over the 5 scan-lines. The resultant power spectrum was normalised to the power spectrum obtained from the plane glass reflector that measures the frequency response of the system and allows a suitable

correction to be made to the ROI data for the response of the transducer-machine combination (Spencer et al, 1997; Watson et al, 2000).

2.4 Pulse Wave Analysis

2.4.1 Applanation Tonometry

With the subject lying supine, blood pressure was measured twice using an Omron automated sphygmomanometer (HEM-705CP; Omron Corporation, Japan) and the mean taken. Pulse wave analysis and velocity were determined by applanation tonometry using a high-fidelity micromanometer (SPC-301; Millar Instruments, TX, USA) and the Sphygmocor™ system (Sphygmocor BPAS; PWV Medical, Sydney, Australia). Triplicate pulse pressure waveforms were recorded from the radial, femoral and carotid arteries and gated to the R wave of the electrocardiogram. Pulse pressure waveform transit times were determined by calculating the time between the foot of the pressure wave and the inflection point, which provides a measure of the timing of the reflected wave (Mackenzie et al 2002). Separation of the pulse waveforms was defined as the difference between the distances from the sternal notch to the radial head, the inguinal ligament and the thyroid cartilage. Data were recorded on-line and the tonometer was repositioned to maximise the quality of the trace and to minimise artefact. All measurements were made in duplicate and mean values used in subsequent data analysis. Systolic and diastolic variability during recordings in excess of 5% were excluded and the measurement repeated.

2.5 Intracoronary Ultrasound

2.5.1 Coronary Artery Cannulation

Following diagnostic coronary angiography, patients with left main stem disease or a minimal luminal diameter of <2 mm in the index epicardial coronary artery were excluded because of the potential for the IVUS catheter to impede anterograde coronary blood flow. A non-tortuous, non-branching segment of artery with a reference luminal diameter of □3.0 mm and luminal irregularity (diameter stenosis of (20-70%) was then identified for IVUS assessment. All patients received 5,000 IU of intravenous heparin and, through an 8 F haemostatic sheath (Cordis®, Cordis Europa N.V., Roden, The Netherlands), the left and right coronary arteries were cannulated with a 7 F guide catheter (Cordis®).

2.5.2 Morphometric Assessment: Intravascular Ultrasound

Following insertion of a 0.014 inch 12.5 MHz Doppler wire (Flowire™, Endosonics, Rancho Cordova, CA), across the arterial segment under study, a 3.2 F Ultracross™ 30 MHz IVUS imaging catheter (Boston Scientific Corporation, Maple Grove, MN) was advanced over the Doppler wire. At the time of the IVUS catheter insertion, an intracoronary bolus of 200 μg glyceryl trinitrate was delivered through the guide catheter. The imaging probe was retracted within the imaging catheter sheath at 0.5 mm/s using a motorised pullback device (Boston Scientific Corp.). All IVUS images were recorded on high fidelity s-VHS videotape for later off-line quantitative analysis.

2.5.3 Ultrasound Measurements

Two-Dimensional Assessment

Vessel cross-sectional area was measured by computer-assisted planimetry, identifying the border between the hypo-echoic media (low collagen content) and the echoreflective adventitia (high collagen content). Lumen area was defined by planimetry of the lumen-intima interface. In the calculations of intimal area, the media is incorporated because it can not always be distinguished separately from the external elastic lamina which lends itself to a reliable boundary for planimetry (Von Birgelen et al 1996). This does not have major implications for calculation of plaque area as the media constitutes only a small fraction of the total area (200 μm in thickness). Thus, the cross sectional area of intima + media (or plaque area) represents the two-dimensional measurement of atheroma.

Plaque Area = Vessel CSA - Lumen CSA

Three-Dimensional Assessment

Three-dimensional computerised reconstruction of the two-dimensional IVUS images was performed using the Toltec™ system (TomTec GmbH, Munich, Germany). This provides frame-by-frame measurements of vessel, lumen and plaque area along with total vessel, lumen and plaque volumes. The proximal atheromatous plaque volume was calculated using a well-validated edge detection algorithm (Von Birgelen et al, 1996; Von Birgelen et al, 1997). Two longitudinal images were automatically reconstructed with contours corresponding to lumen-intima and media-adventitia interfaces. These were checked visually and corrected manually

if necessary. Subsequent contour detection of the planar images was performed with the axial location indicated by a cursor. This was used to scroll through each image with any necessary manual correction. The plaque area was defined as the region between the lumen border and the echogenic interface of the external elastic lamina as previously described (Di Mario et al, 1998; Von Birgelen et al, 1996). Intraobserver variability in plaque volume acquired from a motorized pullback has previously been reported with a mean (SD) of –0.3 ±1.0 % (Von Birgelen et al, 1996).

Volume measurements of the lumen, total vessel and plaque were calculated with the adaptation of Simpson's rule:

$$\text{Volume} = \sum_{I=1}^{N} \text{cross sectional area } I * H$$

Where N is the number of images and H is the thickness of each slice.

2.5.4 Intracoronary Drug Infusion

The IVUS imaging catheter was positioned at the index segment. Selective coronary artery drug infusion was attained by infusion through the flush port of the IVUS catheter [Schwarzacher *et al* 2000]. The Doppler guide wire was retracted to the tip of the imaging catheter (to be as close to the IVUS image as possible) and maintained in a stable position by the short monorail segment of the IVUS catheter (Schwarzacher et al 2000; Newby et al 2000). All drugs were given as 2 ml boluses, followed by a 2 ml 0.9% saline flush via the guide catheter (Newby et al 2000). The IVUS images and Doppler ultrasound were recorded simultaneously to allow the measurement of luminal area and coronary blood flow velocity for two minutes following each drug bolus. The average peak velocity of the Doppler signal was recorded onto S-VHS tape using the Flomap™ (Cardiometrics, Endosonics, Rancho Cordova, CA) Doppler velocimeter.

2.5.5 Coronary Compliance

IVUS permits real-time visualisation of coronary arteries. With cross-sectional imaging, this permits measurement of vessel area and the phasic changes in systole (when the vessel expands) and diastole. The images were gated to the electrocardiogram and measurements were made at the onset of the QRS complex (end-diastole) and the offset of the T-wave (end-systole). Absolute compliance was determined from the resultant pressure changes:

$$\textbf{Absolute Compliance} = (dA/dP) * 10$$

where dA and dP represented change in total vessel area and coronary pressure during one cardiac cycle.

Normalised Compliance corrects for vessel area differences:

$$\textbf{Normalised Compliance} = ([dA/A_{diastole}]/dP)\ mmHg^{-1} * 10^3$$

To eliminate the influence of varying systemic blood pressure, a stiffness index (ß) is derived by normalising the dimension change to the diastolic mean diameter (Nakatani et al, 1995):

Stiffness Index (ß) = [Ln(SBP/DBP]/(dD/diastolic mean diameter)

Here, the systolic and diastolic mean diameters are calculated from those areas with the assumption that the cross section was circular, hence, diameter = $[2 * (area/\Pi)^{1/2}]$, where Π=3.142

2.5.6 Coronary Blood Flow Measurement

The coronary artery cross-sectional area was measured using computerised planimetry (Clearview™, Boston Scientific Inc) of the IVUS vessel luminal area. The images were gated to the electrocardiogram and measurements were made at the onset of the QRS complex. Blood flow velocity was determined using average peak velocity of the Doppler signal (Flowmap™ (Cardiometrics, Endosonics). Blood flow in the index coronary artery was defined according to Doucette and co-workers as [Doucette *et al* 1992]:

$$\textbf{Coronary blood flow (mL/min)} = \textbf{CSA} * \frac{\textbf{APV}}{\textbf{2}} * \frac{\textbf{60}}{\textbf{100}}$$

where CSA = cross-sectional area (mm^2) and APV = average peak velocity (cm/s).

Chapter 3

Classification of Arterial Plaque by Spectral Analysis in Remodelled Human Atherosclerotic Coronary Arteries

McLeod AL, Watson RJ, Anderson T, Uren NG, Newby DE, Northridge DB, McDicken WN. Classification of Arterial Plaque by Spectral Analysis in Remodelled Atherosclerotic Coronary Artery.

Ultrasound Med Biol; in press

3.1 Abstract

We aimed to characterise and to identify the predominant plaque type *in vivo* using unprocessed radio-frequency (RF) intravascular ultrasound backscatter, in remodelled segments of human atherosclerotic coronary arteries. 16 remodelled segments (9 positive, 7 negative) were identified using a 30 MHz IVUS scanner in-vivo. Spectral parameters (maximum power, mean power, minimum power and power at 30 MHz) were calculated and applied to a previous histologically validated classification scheme. Plaque type was characterised as fibrous, calcified or lipid rich. Positively remodelled segments had a larger vessel area (16.5±1.1 mm^2 vs. 8.7±0.9 mm^2, $p<0.01$) and plaque area (7.3±1.1 mm^2 vs. 4.4±0.8 mm^2, $p=0.05$) than negatively remodelled segments. Both positive and negatively remodelled segments had a greater percentage of fibrous plaque ($p<0.01$) than calcified or lipid rich plaque. Comparing positively and negatively remodelled segments, there was no significant difference between the proportion of fibrous, calcified and lipid rich plaque. We have been able to characterise and to identify plaque composition *in vivo* in human atherosclerotic coronary arteries. Our data suggest that remodelled segments are predominantly composed of fibrous plaque although plaque composition is similar irrespective of the remodelling type.

3.2 Introduction

Coronary artery remodelling describes the response of the arterial wall to the presence of atherosclerotic plaque. In many cases the arterial wall expands (positive remodelling) to accommodate the developing plaque to reduce the encroachment on the lumen (Glagov et al 1987; Zarins et al 1988; McPherson et al 1991; Clarkson et al 1994). However there may be shrinkage of the arterial wall (negative remodelling) in the presence of atherosclerotic plaque with resultant luminal narrowing (Pasterkamp et al 1995; Nishioka et al 1996; Mintz et al 1997). Intravascular ultrasound (IVUS) can provide reliable measurements of vessel dimensions and is superior to coronary angiography in assessment of coronary artery remodelling (von Birgelen et al 1996).

It has been suggested that discreet coronary artery lesions only become apparent angiographically when the accumulation of plaque above a threshold of 40% of total vessel area overcomes the ability of the vessel to expand any further (Kakuta et al 1989; Post et al 1994; Currier et al 1995). The inability of angiography to detect this occult disease is due to the presence of positive remodelling and the diffuse nature of disease throughout the entire vessel length in many patients. Furthermore, important pathological features are not demonstrated by angiography including plaque composition and the presence of a lipid-rich core, which may indicate plaque stability or vulnerability. As a result, the degree of luminal stenosis has been shown to be a weak indicator as to whether plaque will eventually cause a myocardial infarction (Falk et al 1995).

Plaque rupture has been shown to occur at sites where vessels show a greater degree of positive remodelling than seen in stable lesions (Schwarzacher et al 1998; Jeremias et al 2000). In a detailed histological study, immunohistochemical staining of post mortem atherosclerotic arteries demonstrated more markers of plaque vulnerability (increased macrophages and lymphocytes, less collagen and smooth muscle cells) in sections with a larger plaque to vessel area, i.e. positively remodelled vessels (Pasterkamp et al 1998).

The unprocessed radio-frequency (RF) signal from ultrasound scanners is not subject to operator-dependent settings or to machine-dependent processing, and allows use of frequency-domain techniques (Urbani et al 1993; Spencer et al 1997), and hence may provide a more accurate means of characterizing plaque in coronary artery remodelled segments than previous visual and videodensitometric analysis of a grey scale level (Gussenhoven et al 1989; Siegel et al 1992; Di Mario et al 1992; Peters et al 1994; Rasheed et al 1995).

Plaque characterization using spectral features from IVUS images has been described in both coronary arteries (Dixon et al 1997; Spencer et al 1997; Moore et al 1998; Watson et al 2000; Nair et al 2002) and carotid arteries (Noritomi et al 1997a, 1997b). Watson et al selected 299 regions-of-interest (ROI) from eight post-mortem coronary arteries using a 30MHz IVUS scanner. Predominant plaque composition of the vessel wall was identified by routine histology and staining. A suture needle inserted in the vessel wall throughout the IVUS pullback showed clearly on the IVUS image and allowed alignment of the IVUS data with histology. With RF

analysis in terms of spectral features, 86% of coronary plaque was correctly classified into one of 3 categories (mixed fibrous tissue, lipid pool and calcified plaque) (Watson et al 2000).

The aim of the present study was to characterize the predominant plaque type *in vivo* using RF IVUS backscatter, in remodelled segments of human coronary arteries.

3.3 Methods

3.3.1 Patient Selection

Ten patients (8 male) aged 55 to 68 years with angiographic evidence of mild to moderate coronary artery disease were recruited at the time of coronary angiography in two centres (Royal Infirmary and Western General Hospital, Edinburgh, UK). Patients were excluded if they had severe left main stem disease, left ventricular hypertrophy or significant concurrent illness. The study was undertaken with the approval of the local research ethics committee, in accordance with the Declaration of Helsinki, and the written informed consent of each subject.

3.3.2 Study Protocol

All patients discontinued their medication on the study day, attended fasted and underwent diagnostic coronary angiography. Standard diagnostic images were taken using the Judkin's technique with a non-ionic contrast agent (Niopam™ 340, MERCK Pharmaceuticals, Middlesex, UK). A non-tortuous, non-branching segment of artery with a reference luminal diameter of 3.0 mm and luminal irregularity (diameter stenosis of up to 70%) was then identified for IVUS assessment.

A 7 French guiding catheter was used to cannulate the left or right coronary artery. Following a 5,000 IU intravenous bolus of heparin (Leo Laboratories Ltd., Princes Risborough, UK) and 200μg intracoronary bolus of nitroglycerin (Nitrocine™, SCWARZ Pharma Ltd, Chesham, UK), a 3.2

F Ultracross™ 30 MHz IVUS imaging catheter (Atlantis SCIMED®, Boston Scientific Corporation, Maple Grove, MN) was advanced into the coronary artery. The IVUS examination of the proximal artery was performed at 0.5 mm/s using a motorized pullback device (Boston Scientific Corporation). All IVUS images were recorded on high fidelity s-VHS videotape for later off-line quantitative analysis. Subsequent three-dimensional computerized reconstruction of the two-dimensional IVUS images was performed using the TomTec™ system (TomTec GmbH, Munich, Germany).

Assessment of Remodelled Segments

During the automated pullback, potential regions of interest (remodelled segments) were identified. Segments were selected for inclusion in the study if there was optimal image quality without rotational, angular or image artifacts; clear demarcation of the endoluminal and the external elastic laminal borders; and calcification of less than 180 degrees. Segment remodelling was defined according to existing criteria based on the total vessel area (external elastic laminal border) at the index site relative to normal or near-normal proximal and distal reference segments (Jeremias et al 2000). This enabled classification of the remodelled segment by calculating relative total vessel area – the ratio of the total vessel area at index segment to the mean of total vessel area at proximal and distal reference segments. Categorization of segments was defined as follows: positively remodelled segments with a ratio of >1.05 and negatively remodelled segments with a ratio of <0.95 (Jeremias et al 2000) (Figure 3.1).

3.3.3 Data Analysis

Analysis of the IVUS data was performed using software written in IDL (Research Systems Inc., Boulder, CO, USA). Frames of RF data were demodulated and scan-converted to produce images equivalent to the standard IVUS video images. These images were used to select frames of interest, corresponding to the matched frame on s-VHS tape. Selected frames had their regions of plaque marked out manually by tracing an inner and an outer contour line on the scan-converted image.

The unprocessed RF data corresponding to the selected plaque regions were divided into smaller regions of interest (ROI) of size 64 samples by 5 scan-lines, with an overlap of 16 samples and 2 scan-lines. Each 5-line ROI was analysed by taking a Fourier transformation of each scan-line and averaging over the 5 scan-lines. The resultant power spectrum was normalised to the power spectrum obtained from the plane glass reflector that measures the frequency response of the system and allows a suitable correction to be made to the ROI data for the response of the transducer-machine combination (Spencer et al, 1997; Watson et al, 2000).

Classification

Features describing the normalised power spectrum were selected and fed into a minimum-distance classification scheme. The four selected features were the mean power over the (18-40) MHz bandwidth of the transducer; the maximum power over the bandwidth; the spectral slope over the bandwidth; and the intercept of the spectral slope with the 0-Hz axis. The Mahalanobis distance was used as the distance metric, and the reference

data used was that obtained from an earlier *ex vivo* study (Watson et al 2000).

Each ROI was classified into one of fibrous, calcified or lipid rich plaques. The total area of fibrous, calcified or lipid in the selected plaque region was calculated. The relative contribution of fibrous, calcified and lipid rich plaque as a percentage of the overall plaque area (as defined by total vessel area – lumen area) was also calculated

3.3.4 Statistical Analysis

All results are expressed as mean +/- standard error of the mean. Continuous variables were analysed using the two-tailed Students *t*-test. Probability (p) values <0.05 were considered statistically significant.

3.4 Results

RF data was acquired from 10 patients involving 16 remodelled segments (9 positively and 7 negatively remodelled).

Positively remodelled segments had a larger total vessel area (16.5±1.1 mm^2 vs. 8.7±0.9 mm^2, $p<0.01$) and plaque area (7.3±1.1 mm^2 vs. 4.4±0.8 mm^2, $p=0.05$) than negatively remodelled segments. Both positively and negatively remodelled segments had a greater percentage of fibrous plaque (92% and 80% respectively) than calcified (7% and 6%) or lipid rich (8% and 30%) plaque. Comparing positively and negatively remodelled segments, there was no significant difference between the proportion of fibrous, calcified and lipid rich subtypes.

3.5 Discussion

In this study using spectral features of IVUS signals, we have found that fibrous plaques predominate and that there appears to be no difference in plaque characteristics between positively and negatively remodelled coronary artery segments, suggesting plaque stability is similar in both remodelled types.

Positive remodelled segments have been found to be associated with unstable coronary syndromes (Schwarzacher S et al 1998; Jeremias et al 2000), though our findings in this small study suggest that this is independent of plaque type. Positive remodelled segments were found to have a larger plaque burden that may be a factor in unstable lesions in addition to other pathophysiological mechanisms such as inflammation, shear stress and vascular compliance. We have however only studied patients with stable coronary artery disease and our findings may not be applicable in patients with acute coronary syndromes.

Grey-scale interpretation limits the IVUS assessment of heterogeneous plaque commonly found in unstable lesions. Combined with spectral analysis, the ability to characterise plaque composition accurately may have important implications in both the identification and treatment of vulnerable plaques. This study allowed *in vivo* assessment of quantitative plaque composition. Previous *ex vivo* studies with histological validation have revealed that spectral analysis of IVUS RF data can reveal information regarding plaque characteristics (Watson et al 1997; Spencer et al 1997; Moore et al 1998). RF data provide multiple parameters and,

with classification trees, prediction can be improved by autoregressive classification schemes as opposed to classical Fourier transformations (Nair et al 2002).

Our study classified each ROI into 3 broad sub-groups: calcified, fibrous or lipid rich (Watson et al, 2000). The study by Nair and colleagues has also reported a high accuracy of identifying calcified necrotic and fibrolipid areas *ex vivo* with histological validation (Nair et al, 2002). Watson et al previously collected the reference *ex vivo* data on a different IVUS system (HP SONOS) from that used in the present *in vivo* study (Boston Scientific CVIS). However, the calibration technique should correct for any differences between the two systems. The reference data were also obtained by scanning vessels through saline whereas the present remodelling data were obtained *in vivo* and hence through blood. The effects of blood on the backscatter information have not yet been characterized.

There exists a tissue-dependent variation of backscatter with angle of incidence. The reference data were collected with the catheter located approximately centrally in the vessel, where any angle-dependent effects would be small. The difficulty of controlling the catheter location during *in vivo* data collection means that some angle-dependent effects cannot be excluded.

The effects of attenuation due to overlying tissue have not been considered here, although the analysed sites were close to the surface of the vessel. Spencer et al (Spencer et al 1997) investigated the variation of spectral

features with depth (i.e. distance into the vessel wall) up to 1.5mm and found that variations due to tissue characteristics were considerably greater than any visible variation with depth.

Although the present data were collected on a different system and under different conditions from that of the classifier reference data, the plane-glass reflector data should in principle be able to correct for any system-dependent effects.

This study was conducted in the necessary clinical setting of patients with a combination of risk factors and concomitant therapies undergoing diagnostic coronary angiography. The small sample size means that this study lacked sufficient power to address the influence of the individual variables associated with coronary artery disease. Remodelled sites were limited to 1 or 2 per patient in view of procedure duration and single vessel data acquisition. Multiple sites per patient ideally would have been required to help assess the influence of both local and patient factors on determining the type of remodelling. Our findings may not be representative of all coronary artery atherosclerotic plaques. Heavily calcified lesions were excluded because the resultant acoustic shadow makes it very difficult to measure vessel areas and to characterise the deeper plaque. In addition, small vessels and significant stenoses were also excluded because of the potential for vessel occlusion with the IVUS catheter.

Conclusions

In this small *in vivo* study, using the novel technique of using unprocessed RF intravascular ultrasound backscatter, remodelled segments were identified by RF analysis to be predominantly composed of fibrous plaque. Plaque composition was similar irrespective of whether there was positive or negative arterial remodelling.

Table 3.1 Cross sectional area (CSA) measurements with intravascular ultrasound.

	Positive remodelling	Negative Remodelling	P value
Number	9	7	
Vessel CSA (mm^2)	16.5±1.1	8.8±0.9	<0.01
Lumen CSA (mm^2)	9.2±1.4	4.5±0.6	0.05
Plaque CSA (mm^2)	7.3±1.1	4.4±0.8	NS

Values are mean±SEM

Table 3.2 Plaque classification in segments with positive and negative remodelling.

Plaque characteristic	Positive remodelling	Negative Remodelling	P value
Fibrous (%)	92.1±1.6	80.4±6.5	NS
Calcified (%)	6.6±1.4	5.7±0.6	NS
Lipid rich (%)	8.0±1.9	30.0±8.2	NS

Values are mean±SEM

Chapter 4

Non-invasive Measures of Pulse Wave Velocity Correlate with Coronary Arterial Plaque Load in Humans

Non-invasive Measures of Pulse Wave Velocity Correlate with Coronary Arterial Plaque Load in Humans.

McLeod AL, Uren NG, Wilkinson IB, Webb DJ, Maxwell SR Northridge DB, Newby DE

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4.1 Abstract

Arterial stiffness is an emerging major risk factor for cardiovascular morbidity and mortality. The aim of the present study was to assess if coronary artery plaque load correlates with non-invasive measures of arterial stiffness. In 35 patients (61±2 years), coronary artery plaque burden was assessed using a 30 MHz intravascular ultrasound catheter during an automated pullback. Proximal coronary artery plaque volume was determined using a validated edge detection algorithm following three-dimensional computerised reconstruction. Central arterial stiffness was assessed in each patient using applanation tonometry to radial, carotid and femoral pulses, with derivation of aortic pressure augmentation and pulse wave velocity using pulse wave analysis. Proximal coronary arterial plaque volume was 5.9±0.6 mm^3 per mm of vessel. Plaque volume positively correlated with carotid-radial pulse wave velocity (r=0.47, p=0.008) and appeared to correlate with carotid-femoral pulse wave velocity (r=0.34, p=0.07). Aortic augmentation (r=0.24, p=0.16), augmentation index (r=0.3, p=0.08), and pulse pressure (r=0.22, p=0.2) did not significantly correlate with proximal coronary artery plaque volume. Non-invasive measures of pulse wave velocity correlate with the extent of coronary artery plaque volume and may be a useful non-invasive surrogate marker for the extent of coronary atherosclerosis. Our findings are consistent with the suggestion that central aortic stiffness may promote the development of coronary atherosclerosis and ischaemic heart disease.

4.2 Introduction

The arterial pressure waveform alters with progression down the arterial tree. This is due to local variations in vascular stiffness as well as superimposition of the reflected pressure waveform (O'Rourke et al 1993). The latter phenomenon occurs when the anterograde pressure wavefront encounters the main points of impedance mismatch, such as the transition from small to resistance arteries. This causes a reflected pressure waveform to return to the central arteries and aorta in diastole, thereby enhancing coronary perfusion pressure. Progressive arterial stiffening, as occurs with ageing, causes an increase in the aortic pulse pressure due to the combination of local aortic stiffness and an increase in both pulse wave velocity and wave reflection. These stiffness-related effects produce an increase in central aortic pressure and cardiac afterload, and a reduction in coronary perfusion pressure due to the movement of the reflected wave into systole (Nichols et al 1990; London et al 1990).

An increase in arterial stiffness may have several important consequences. Elevation of central aortic pressure promotes the development of left ventricular hypertrophy (Lakatta et al 1990; Garzka et al 1998) and is associated with an increase in mortality (Levy et al 1990). Increased pulse pressure, a measure of arterial stiffness, augments atherogenesis in primate models (Lyon et al 1987) and may increase the likelihood of atherosclerotic plaque rupture in humans (Cheng et al 1993). Moreover, in clinical studies, elevations in pulse pressure and aortic stiffness are associated with an increase in the future risk of coronary event events

(Mitchell et al 1997; Blacher et al 1998; Gatzka et al 1998; Lehmann et al 1998) and overall mortality (Benetos A et al 1998; Domanski et al 1999; Blacher et al 1999).

The peripheral pulse pressure waveform (pulse wave analysis) and the velocity of its propagation through the arterial tree (pulse wave velocity) can be determined non-invasively using applanation tonometry (O'Rourke et al 1996; Kelly et al 1989). This simple, reproducible and well-validated technique (Karamanoglu et al 1993; Chen et al 1997; Fetics et al 1999; Wilkinson et al 1998) of assessing central arterial stiffness has widespread clinical and research applications (Mackenzie et al 2002).

Surrogate markers of atherosclerosis have been associated with increased pulse wave velocity (van Popele et al 2001). However, the relationship between arterial stiffness and coronary artery plaque load is currently unknown. The quantitation of coronary artery plaque load is difficult because standard quantitative coronary angiography has several inherent limitations and inaccuracies that occur because it can only assess the arterial lumen and is unable to take account of arterial remodelling. In contrast, intravascular ultrasound (IVUS) permits an accurate and reproducible quantification of coronary atherosclerotic plaque in man (von Birgelen et al 1996).

The aim of the present study was therefore to examine the relationship between invasive measures of coronary artery plaque volume using intravascular ultrasound and non-invasive measures of arterial stiffness using applanation tonometry.

4.3 Methods

4.3.1 Patient Selection

Thirty-five patients with angiographically normal coronary arteries or mild to moderate coronary artery disease were recruited at the time of elective coronary angiography in two centres (Royal Infirmary and Western General Hospital, Edinburgh, UK). Patients were excluded if they had severe left main stem disease, left ventricular hypertrophy or significant concurrent illness. All patients had their coronary risk factors determined by standard clinical criteria. Hypercholesterolemia was defined as a fasting total cholesterol >190 mg/dL (>5.0 mmol/L) prior to initiation of lipid lowering therapy. The study was undertaken with the approval of the local research ethics committee, in accordance with the Declaration of Helsinki, and the written informed consent of each subject.

4.3.2 Study Protocol

Coronary Angiography

All patients discontinued their medication on the study day, attended fasted and underwent diagnostic coronary angiography. Standard diagnostic images were taken using the Judkin's technique with a non-ionic contrast agent (Niopam™ 340, MERCK Pharmaceuticals, Middlesex, UK).

Intravascular Ultrasound and Plaque Volume Assessment

A non-tortuous, non-branching segment of artery with a reference luminal diameter of 3.0 mm and luminal irregularity (diameter stenosis of up to 70%) was identified for IVUS assessment. A 7 French guiding catheter was used to intubate the left or right coronary artery. Following a 5,000 IU intravenous bolus of heparin (Leo Laboratories Ltd., Princes Risborough, UK) and 200 μg intracoronary bolus of nitroglycerin (Nitrocine™, SCWARZ Pharma Ltd, Chesham, UK), a 3.2 F Ultracross™ 30 MHz IVUS imaging catheter (Atlantis SCIMED®, Boston Scientific Corporation, Maple Grove, MN) was advanced into the coronary artery on an 0.014" guide-wire.

The IVUS examination of the proximal artery was performed at 0.5 mm/s using a motorized pullback device (Boston Scientific Corporation). All IVUS images were recorded on high fidelity s-VHS videotape for later off-line quantitative analysis. The plaque area was defined as the region between the intima-lumen interface and the external elastic lamina (von Birgelen et al 1996; Newby et al 2001). Subsequent three-dimensional computerized reconstruction of the two-dimensional IVUS images was performed using the TomTec™ system (TomTec GmbH, Munich, Germany) with plaque volume derived from a well-validated edge detection algorithm (von Birgelen et al 1996; Newby et al 2001). Plaque volume was calculated per mm of coronary artery length.

Pulse Wave Analysis

With the subject lying supine, blood pressure was measured twice using an Omron automated sphygmomanometer (HEM-705CP; Omron Corporation, Japan) and the mean taken. Pulse wave analysis and velocity were determined by applanation tonometry using a high-fidelity micromanometer (SPC-301; Millar Instruments, TX, USA) and the Sphygmocor™ system (Sphygmocor BPAS; PWV Medical, Sydney, Austrailia). Triplicate pulse pressure waveforms were recorded from the radial, femoral and carotid arteries and gated to the R wave of the electrocardiogram. Pulse pressure waveform transit times were determined using the foot-to-foot methodology (Mackenzie et al 2002). Separation of the pulse waveforms was defined as the difference between the distances from the sternal notch to the radial head, the inguinal ligament and the thyroid cartilage. Data were recorded on-line and the tonometer was repositioned to maximise the quality of the trace and to minimise artefact. All measurements were made in duplicate and mean values used in subsequent data analysis. Systolic and diastolic variability during recordings in excess of 5% were excluded and the measurement repeated.

After acquiring a series of consecutive waveforms, the mean peripheral and central arterial waveforms were recorded and the augmentation index (AIx) derived. The AIx was calculated as the difference between the first and second peaks of the central aortic waveform expressed as a percentage of the pulse pressure.

4.3.3 Statistical Analysis

All results are expressed as mean ± standard error of the mean. Data were analysed, where appropriate, using analysis of variance (ANOVA), Student's *t*-test and regression analysis. Probability (p) values <0.05 were considered statistically significant.

4.4 Results

4.4.1 Patient Dermographics

Thirty-five patients (26 male) were assessed with an average age of 61 years (range 46-78). Baseline characteristics are shown in Table 4.1. The procedure was well tolerated and there were no significant adverse effects.

4.4.2 Plaque Volume

Following IVUS assessment, plaque was quantified in the left anterior descending (28), right (3) and left circumflex (4) coronary arteries. The mean length of vessel analysed was 32±1mm (range 7 – 40mm). Average plaque volume was 5.9 ± 0.6 mm^3 per mm of vessel (range 0.9 - 13.7 mm^3/mm; Table 4.2). Plaque volume appeared to be lower in the female subgroup but this was not statistically significant.

4.4.3 Pulse Wave Analysis

Pulse wave analysis of the study population is given in Table 4.3. Coronary artery plaque volume correlated with carotid-radial pulse wave velocity (r=0.47, p=0.008; Figure 4.1) with a trend in the relationship with carotid-femoral pulse wave velocity (r=0.34, p=0.07). There was no significant association between plaque volume and aortic augmentation (r=0.24, p=0.16), augmentation index (r=0.3, p=0.08), and pulse pressure (r=0.22, p=0.2). Carotid-radial pulse wave velocity was significantly greater in males than females (8.4± 0.4ms^{-1} vs. 6.8± 0.4ms^{-1}; p<0.001) and

there was a trend towards a greater carotid-femoral pulse wave velocity ($9.4\pm0.4ms^{-1}$ vs. $8.5\pm0.5ms^{-1}$; p=NS). There was no significant difference in aortic augmentation, augmentation index and pulse pressure between male and female subgroups (Table 4.2).

4.5 Discussion

We have shown that the underlying coronary artery plaque burden correlates with pulse wave velocity but not other measures of pulse wave analysis. This association is likely to reflect the common aetiological factors involved in the development of aortic and coronary atheroma. However, our findings are also consistent with the suggestion that central aortic stiffness may promote the development of coronary atherosclerosis and ischaemic heart disease

Consistent with our findings, stiffness of both the thoracic aorta and abdominal aorta progressively increases in patients with single, two and three-vessel disease as well as those with previous myocardial infarction (Hirai et al 1989). Interestingly, exercise stress testing in patients with angiographic evidence of moderate coronary artery disease has also shown a correlation between time to ischaemia and large artery stiffness as assessed by carotid applanation tonometry (Kingwell et al 2002). This suggests that aortic stiffness is not only associated with structural atherosclerotic disease, but also the functional consequences.

It is now recognised that arterial stiffness is associated with both cardiovascular risk (Benetos et al 1998, Mitchell et al 1997; Franklin et al 1999) and a strong predictor of myocardial infarction (Boutouyrie et al 2002). Our demonstration of an association between coronary artery plaque volume and arterial stiffness raises questions of cause-and-effect. Longitudinal studies would be necessary to document whether coronary artery disease develops in subjects with increased arterial stiffness.

However, it is interesting to note that young children with hypercholesterolaemia (Iannuzzi et al 1999; Aggoun et al 2000), marked obesity (Tounian et al 2000) or low birth weight (Martin et al 2000) have increased arterial stiffness at a young age before the development of widespread atheroma.

Our study population had a combination of risk factors for coronary artery disease that will contribute to arterial stiffness (Cockcroft et al 1991). [31] Hypercholesterolaemia is associated with endothelial dysfunction and impaired endogenous nitric oxide release (Chowienczyk et al 1992; Stoes et al 1995). Arterial stiffness is increased by both nitric oxide synthase inhibition and hypercholesterolaemia (Iannuzzi et al 1999; Wilkinson et al 2002). Furthermore treatment with lipid lowering therapy improves endothelial function and increases the bioavailability of nitric oxide (Stroes et al 1995; Leung et al 1993). Thus, the association between coronary artery plaque volume and aortic stiffness may, in part, reflect the common aetiological factors that will inevitably exist in this clinical population.

Study Limitations

Because of ethical and technical considerations, we did not assess patients with severe coronary artery disease, tight luminal stenoses and a very high plaque load. Moreover, we assessed a single coronary artery segment and we cannot be certain that this was representative of the entire coronary circulation in each patient. However, intravascular ultrasound is a very precise method of determining plaque load with excellent reproducibility (von Birgelen et al 1996; Hagenaars et al 2000).

By necessity, many of the study patients were established on concomitant medical therapy. Treatments such as β-adrenergic receptor blockade and angiotensin-converting enzyme inhibition have been shown to influence aortic stiffness (Ting et al 1991; Breithaupt-Grogler et al 1996). Whilst such treatments may affect the functional aspects of arterial stiffness, the structural component of arterial stiffness will be unaffected and we were still able to demonstrate a significant association.

We did not find an association between other markers of arterial stiffness and coronary plaque volume. However, this is likely to reflect a lack of power as the overall trend was in the same direction for all measures. Moreover, it is recognised that pulse wave velocity is one of the more robust and sensitive measures of arterial stiffness and accounts for its closer association (Mackenzie et al 2002).

Conclusions

The association between coronary artery plaque burden and pulse wave velocity provides further evidence that central aortic stiffness may promote the development of coronary atherosclerosis and ischaemic heart disease.

Table 4.1 Baseline patient characteristics.

Number	35
Age (mean, range)	60± 1.5
Sex (male:female)	26:9
Risk factors	
Hypercholesterolaemia	25 (71%)
Smoking habit:	
Ex-smoker	11 (31%)
Current	8 (23%)
Hypertension	13 (37%)
Family history	10 (29%)
Diabetes mellitus (type II)	4 (11%)
Concomitant drug therapy	
Aspirn	32 (91%)
B-Blocker	23 (66%)
Calcium Antagonist	18 (51%)
Long-acting Nitrate	14 (40%)
Lipid Lowering Therapy	18 (51%)
ACE Inhibition	3 (9%)

Table 4.2 Pulse wave analysis and arterial stiffness in male and female subgroups.

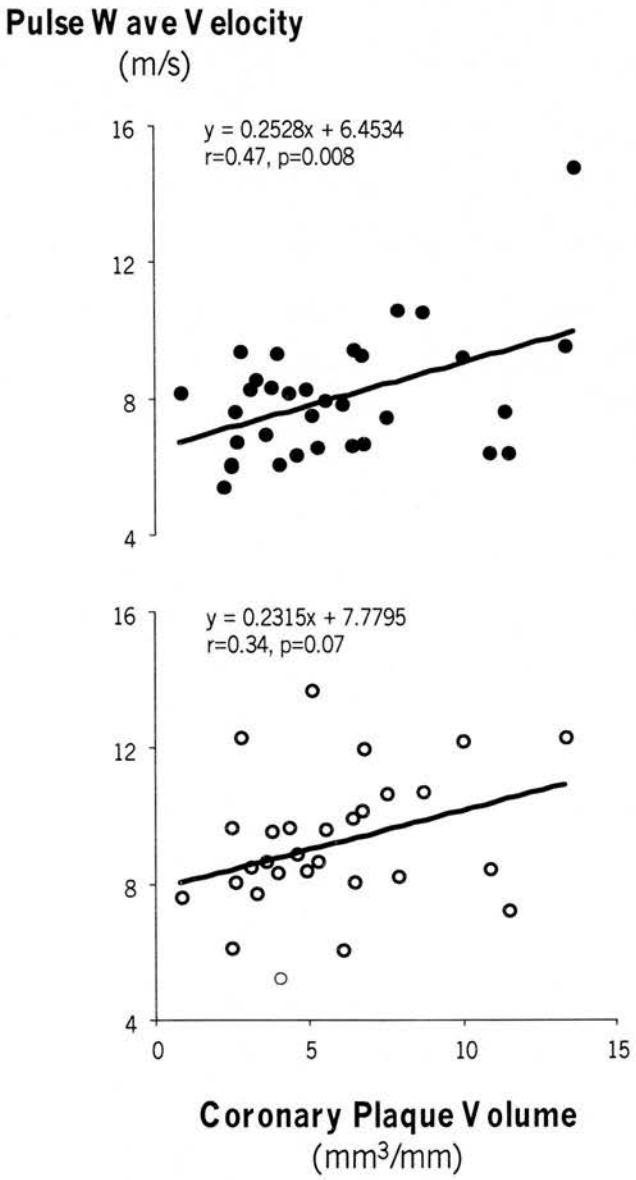
	Male	Female	
Coronary plaque volume	6.6 ± 0.6	3.9 ± 1.2	(mm^3/mm)
Carotid-radial velocity	8.4 ± 0.4	6.8 ± 0.4*	(ms^{-1})
Carotid-femoral velocity	9.4 ± 0.2	8.5 ± 0.5	(ms^{-1})
Aortic augmentation	12 ± 1.7	15 ± 2.9	(mmHg)
Augmentation index	25 ± 1.9	26 ± 3.6	(%)
Pulse pressure	54 ± 4	62 ± 6	(mmHg)

*$p<0.001$ Student's *t*-test

Table 4.3 Pulse wave analysis and velocity.

Pulse	68 ± 2	(/min)
Brachial blood pressure		
Systolic	145 ± 4	(mmHg)
Diastolic	79 ± 2	(mmHg)
Pulse Pressure	62 ± 4	(mmHg)
Aortic blood pressure		
Systolic	135 ± 4	(mmHg)
Diastolic	80 ± 2	(mmHg)
Pulse Pressure	55 ± 3	(mmHg)
Augmentation	12 ± 2	(mmHg)
Augmentation Index	25 ± 2	(%)
dP/dt_{max}	865 ± 48	(mmHg/s)
Buckberg SVR	152 ± 5	Wood units
Ejection duration	314 ± 10	(ms)
Pulse wave velocity		
Carotid-radial	8.0 ± 0.3	(m/s)
Carotid-femoral	9.2 ± 0.4	(m/s)

Figure 4.1 Correlation between coronary artery plaque volume and carotid-radial (closed circles; upper panel, n=33) and carotid-femoral (open circles; lower panel, n=29) pulse wave velocity.



Chapter 5

Coronary Atherosclerosis and Cigarette Smoking Impair Coronary Tissue Plasminogen Activator Release: Direct Association between Endothelial Dysfunction and Atherothrombosis

Newby DE, McLeod AL, Uren NG, Flint L, Ludlam CA, Webb DJ, Fox KAA, Boon NA. Impaired coronary fibrinolytic capacity is associated with coronary atherosclerosis and cigarette smoking: direct association between endothelial dysfunction and atherothrombosis. *Circulation* 2001;**103**:1936-1941

5.1 Abstract

The objective of the study was to establish the relationship between the volume of proximal coronary artery atheroma, the presence of associated risk factors and the stimulated release of tissue plasminogen activator (t-PA) from the heart. Following diagnostic coronary angiography in 25 patients, the left anterior descending artery (LAD) was instrumented and the proximal LAD plaque volume was determined using intravascular ultrasound (IVUS). Blood flow and fibrinolytic responses to selective LAD infusion of saline, substance P (10-40 pmol/min) and sodium nitroprusside (5-20 μg/min) were measured using intracoronary IVUS and Doppler, combined with arterial and coronary sinus blood sampling. Mean plaque burden was 5.5 ± 0.8 mm^3 per mm of vessel (range 0.6-13.7). LAD blood flow increased with both substance P and sodium nitroprusside ($p<0.001$), although coronary sinus plasma t-PA antigen and activity concentrations increased only during substance P infusion (p□0.006 for both). There was a significant inverse correlation between the LAD plaque burden and release of active t-PA ($r=-0.61$, $p=0.003$). Cigarette smoking, but no other risk factor, was associated with an impairment of coronary release of active t-PA (smokers, 42 ± 21 *versus* nonsmokers, 202 ± 73 $IU.min^{-1}$; $p=0.01$). We have found an association between both the coronary atheromatous plaque burden and smoking habit with the acute local fibrinolytic capacity of the heart. These important findings potentially provide a direct link between endogenous fibrinolysis, endothelial dysfunction and atherothrombosis.

5.2 Introduction

The endothelium plays a vital role in the control of blood flow, coagulation, fibrinolysis and inflammation. Consequently, the maintenance and regulation of tissue perfusion critically depends upon the integrity of endothelial function and the release of potent endothelium-derived factors. Following the seminal work of Furchgott and Zawadski (Furchgott & Zawadski 1980), it has been widely recognised that an array of mediators can influence vascular tone through endothelium-dependent actions. There is an extensive body of evidence to show that endothelium-dependent vasomotion is abnormal in patients with atherosclerosis (Ludmer et al 1986) and its associated risk factors (Celermajer et al 1992; Chowienczyk et al 1992; Celermajer et al 1996). However, whilst endothelium-dependent vasomotion is an important indicator of endothelial cell function, it is an indirect surrogate measure of the central pathophysiological role of the endothelium in atherothrombosis.

Acute rupture or erosion of a coronary atheromatous plaque and subsequent coronary artery thrombosis causes the majority of sudden cardiac deaths and myocardial infarctions (Burke et al 1997; Davies 2000). Small areas of denudation and thrombus deposition are a common finding on the surface of atheromatous plaques and are usually sub-clinical (Davies 2000). However, in the presence of an imbalance in the fibrinolytic system, such microthrombi may propagate, ultimately leading to arterial occlusion (Rosenberg & Aird 1999). Indeed, genetic murine models indicate that t-PA deficiency is associated with myocardial

necrosis and the development of regional wall motion abnormalities (Christie et al 1999).

In the Northwick Park Heart Study (Meade et al 1993), low basal plasma fibrinolytic activity was a leading determinant of the risk of sustaining a myocardial infarction or sudden cardiac death in younger men. Moreover, a reduction in exercise induced release of t-PA is associated with an increased incidence of major adverse cardiac events in patients with stable angina pectoris (Held et al 1997). Recently, Rosenberg and Aird (Rosenberg & Aird 1999) have postulated that vascular-bed-specific defects in haemostasis exist, and that coronary thrombosis critically depends on the local fibrinolytic balance. However, there have been no clinical studies to date which have directly assessed the acute local fibrinolytic capacity of the coronary vascular bed in patients with coronary artery disease.

Using forearm venous occlusion plethysmography and the endothelium-dependent agonist, substance P, we have recently developed and characterised a novel model of assessing the acute release of endogenous t-PA *in vivo* in man (Newby et al 1997b). This has enabled us to demonstrate that cigarette smoking is associated with an impairment of acute t-PA release (Newby et al 1999a) although this has yet to be confirmed in the coronary circulation. The aims of the present study were: first, to apply this approach to the coronary circulation and thereby establish a method of assessing acute coronary t-PA release; second, to determine the relationship between the extent of coronary artery atheroma, quantified by intravascular ultrasound (IVUS), and the acute fibrinolytic

capacity of the coronary vascular bed; and third, to determine if cigarette smoking impairs coronary as well as forearm t-PA release.

5.3 Methods

5.3.1 Patient Selection

Patients undergoing elective coronary angiography were recruited unless they had significant aortic stenosis, severe left ventricular dysfunction or recent (<3 months) myocardial infarction. Following angiography, patients were excluded if they had significant left main stem disease or a minimal luminal diameter of less than 2 mm in the proximal left anterior descending coronary artery. All patients had their risk factor profile determined by standard clinical criteria: smoking habit (current/ex-smoker or non-smoker), family history of premature (male: <50 years, female: <55 years) coronary artery disease, and a history of hypertension (systolic blood pressure > 160 mmHg or diastolic blood pressure >90 mmHg on 3 or more occasions), diabetes mellitus (fasting blood glucose concentration >7.8 mmol/L or >11.1 mmol/L 2 hours after 75 g glucose load) or hypercholesterolaemia (total serum cholesterol concentration >5.0 mmol/L). Since many patients discontinued a regular smoking habit just prior to angiography, current and ex-smokers were considered as a single group. The study was undertaken with the approval of the local research ethics committee and in accordance with the Declaration of Helsinki. The written informed consent of each subject was obtained before entry into the study.

5.3.2 Study Protocol

All patients discontinued their medication on the study day, attended fasted and underwent diagnostic coronary angiography at 08.00 h. Following angiography, patients fulfilling the entry criteria were entered into the study and received 5,000 IU of intravenous heparin. Cannulation of the coronary sinus was established from the femoral vein using a preformed specific 6 F catheter (modified Simmons Torcon NB catheter, HNB6.0-NT-100-PW-2S-112393-BH) (Katritsis & Webb-Peploe 1997). To avoid atrial blood mixing, the catheter was advanced, sometimes with the assistance of a guide wire, deep into the ostium and beyond the posterior interventricular vein. Stable and selective cannulation of the coronary sinus was achieved in all but three subjects. Arterial samples were obtained through an 8 F haemostatic sheath placed in the right femoral artery.

The left coronary artery was cannulated with a 7 F guiding catheter and a 0.014 inch 12.5 MHz Doppler wire (Flowire™, Cardiometrics, Endosonics, Rancho Cordova, CA, USA) was passed into the left anterior descending coronary artery. A 3.2 F Ultracross™ 20 MHz IVUS imaging catheter (SCIMED®, Boston Scientific Corporation, MN, USA) was advanced into the left anterior descending coronary artery over the guide wire. The IVUS examination of the proximal artery was performed at 0.5 mm/s using a motorised pullback device (Boston Scientific Corp) whilst recording the ultrasound images onto S-VHS tape using the Clearview™ ultrasonogram (Boston Scientific Corp). Following the pullback examination, the IVUS imaging catheter was repositioned just distal to the ostium of the left anterior descending artery. The Doppler guide wire was

retracted to the tip of the imaging catheter and maintained in a stable position by the short monorail segment of the IVUS catheter [Schwarzacher et al 2000]. The average peak velocity of the Doppler signal was recorded onto S-VHS tape using the Flomap™ (Cardiometrics) Doppler velocimeter.

5.3.3 Drug Administration

Pharmaceutical-grade substance P (Clinalfa AG, Läufelfingen, Switzerland), an endothelium and neurokinin type 1 receptor dependent vasodilator [Newby et al 1999c], and sodium nitroprusside (David Bull Laboratories, Warwick, U.K.), an endothelium independent vasodilator, were administered following dissolution in saline (0.9%: Baxter Healthcare Ltd, Thetford, UK). Five minute infusions were administered using an IVAC P1000 syringe pump (IVAC Ltd, Basingstoke, UK) at 1 mL/min via the IVUS catheter flush port (Schwarzacher et al 2000). The agents were given in the following order: saline, substance P 10 pmol/mL, substance P 20 pmol/mL, substance P 40 pmol/mL, sodium nitroprusside 5 μg/mL and sodium nitroprusside 20 μg/mL. Due to limitations in procedural time, the sodium nitroprusside responses were determined in 14 patients only.

5.3.4 Measurement of Plaque Volume and Coronary Blood Flow

Computerised three dimensional reconstructions of the proximal left anterior descending coronary artery were performed off-line by a single blinded operator using the TomTec computer system (Echoscan, TomTec Imaging Systems, Unterschleissheim, Germany). The proximal atheromatous plaque volume was calculated using a well validated edge detection algorithm (von Birgelen et al 1996a; von Birgelen et al 1997). The planar contours were checked by the operator and, if necessary, edited. The plaque area was defined as the region between the luminal border and the echogenic interface of the external elastic lamina as previously described (Di Mario et al 1998; von Birgelen et al 1996b).

The left anterior descending coronary artery cross-sectional area was measured using computerised planimetry (Clearview™, Boston Scientific Inc) of the vessel lumen. The IVUS images were gated to the electrocardiogram and measurements were made at the onset of the QRS complex. Blood flow velocity was determined using average peak velocity of the Doppler signal (Flopmap™, Cardiometrics). Blood flow in the left anterior descending coronary artery was determined from the mean of five measurements made in the final minute of each infusion period and was defined as (Doucette et al 1992) Coronary blood flow (mL/min) =

$$CSA \cdot \frac{APV}{2} \cdot \frac{60}{100}$$

where CSA = cross-sectional area (mm^2) and APV = average peak velocity (cm/s).

5.3.5 Blood Sampling and Plasma Assays

Ten mL of arterial and coronary sinus blood were obtained simultaneously at the end of each infusion period, collected into acidified buffered citrate (Biopool® Stabilyte™, Umeå, Sweden) and citrate (Monovette®, Sarstedt, Nümbrecht, Germany) tubes, and kept on ice before being centrifuged at 2,000 *g* for 30 minutes at 4°C. Platelet free plasma was decanted and stored at -80°C before assay. Coronary sinus oxygen saturations were determined at the end of each infusion period using an automated oximeter (Oxicam™ 300, Watco Services, Basingstoke, UK).

5.3.6 Data Analysis and Statistics

Coronary t-PA release was defined as the product of the left anterior descending artery plasma flow (based on the haematocrit, Hct and the left anterior descending coronary blood flow, CBF) and the plasma arterial ($[t\text{-}PA]_{Art}$) and coronary sinus ($[t\text{-}PA]_{Ven}$) concentration differences.

$$\text{Coronary t-PA release} = CBF \times \{1\text{-}Hct\} \times \{[t\text{-}PA]_{Ven} - [t\text{-}A]_{Art}\}$$

In order to compare vasomotor and fibrinolytic responses with proximal atheromatous plaque volume, the area under the curve (AUC) was calculated for each response: coronary blood flow, plasma arterial and coronary sinus t-PA concentration differences, and estimated net t-PA release.

Data were examined by analysis of variance (ANOVA) with repeated measures, two tailed paired and unpaired Student's *t*-test, and univariate and multivariate regression analysis using StatView *v*5.0.1 (SAS Institute Inc., Cary, North Carolina, USA). All results are expressed as mean ± standard error of the mean. Statistical significance was taken at the 5% level.

5.4 Results

Baseline patient characteristics are shown in Table 5.1. In keeping with the anticipated profile of patients undergoing coronary angiography, the study population was predominantly middle-aged, male and had a combination of risk factors. Throughout the study there were no significant changes in heart rate, mean arterial pressure or haematocrit (0.40 ± 0.01).

5.4.1 Plaque Volume and Blood Flow Responses

The proximal 29 ± 1 mm of the left anterior descending coronary artery were reconstructed and found to contain 160 ± 24 mm^3 of atheromatous plaque: a plaque burden of 5.5 ± 0.8 mm^3 per mm of vessel (range, 0.6 to 13.7). There was a significant linear correlation between the plaque burden and the serum total cholesterol: HDL-cholesterol ratio ($r=0.55$, $p=0.004$).

Left anterior descending coronary artery blood flow increased with both substance P and sodium nitroprusside infusion ($p<0.001$, ANOVA; see Table 5.2). There was a significant linear correlation between the percentage increase in coronary sinus oxygen saturations and left anterior descending coronary artery flow ($r=0.46$, $p<0.001$). However, there was no correlation between the plaque burden and the AUC for the coronary blood flow responses to substance P or sodium nitroprusside infusion. In contrast, there was an association between the number of risk factors for atherosclerosis and the coronary blood flow responses to substance P (Figure 5.1: $r=-0.42$, $p<0.05$).

5.4.2 Plasma Fibrinolytic Parameters

There was a significant rise in plasma t-PA antigen and activity concentrations from the coronary sinus during substance P infusion (Table 5.2: ANOVA, $p<0.001$ and $p<0.006$ respectively) but not during sodium nitroprusside infusion. There was a significant inverse correlation between the plaque burden and the AUC for active t-PA release (Figure 5.2: $r=-0.61$, $p=0.003$), and a trend for the AUC for t-PA antigen release ($r=-0.34$, $p=0.15$). There was also an inverse linear correlation between the basal coronary sinus plasma t-PA antigen concentration and the AUC for active t-PA release ($r=-0.58$, $p<0.005$).

Ex- and current cigarette smokers had a higher basal plasma t-PA antigen concentration and an impaired active t-PA response to substance P infusion in comparison to non-smokers despite similar plasma PAI-1 concentrations and coronary arterial plaque burden (Table 5.2). Subgroup analysis demonstrated that current smokers (n=4) had similar release of active t-PA compared to ex-smokers (n=13): 49 ± 32 and 40 ± 28 IU/min respectively. Hypercholesterolaemia, hypertension, diabetes mellitus and a family history of premature coronary artery disease had no influence on t-PA release.

There were no significant changes in plasma PAI-1 antigen and activity concentrations throughout the study (Table 5.2). Basal coronary sinus plasma PAI-1 antigen concentrations correlated positively with plaque burden ($r=0.47$, $p<0.03$) and negatively with release of active t-PA ($r=-0.44$, $p=0.04$).

Multivariate regression analysis identified plaque burden and basal coronary sinus t-PA antigen concentrations as the independent variables that were significantly associated with release of active t-PA ($p<0.02$ for both).

5.5 Discussion

For the first time, we have shown a direct relationship between both the coronary atheromatous plaque burden and smoking habit with the acute fibrinolytic capacity of the heart. These important findings suggest that both atherosclerosis and smoking habit influence the local fibrinolytic balance in the coronary circulation, and provide a direct link between endothelial dysfunction, atherothrombosis and myocardial infarction.

The clinical importance of endogenous t-PA release is exemplified by the high rate of spontaneous reperfusion in the infarct related artery after acute myocardial infarction; occurring in one third of patients within the first 12 hours (Wood et al 1980; Armstrong et al 1989; Rentrop et al 1989).The present study is the first to attempt directly to assess the acute release of t-PA in the coronary circulation of patients with stable coronary artery disease. Although thrombin and activated coagulation Factor X may be more physiologically relevant to acute t-PA release than substance P, we have used the latter because its vascular actions are endothelium-dependent [Gross et al 1994] and its administration intra-arterially is safe and well tolerated (Crossman et al 1989; Newby et al 1997a]). Using this approach, we have been able to stimulate the fibrinolytic activity of the coronary vascular bed and demonstrate that this response appears to be sensitive to the presence of atheroma: a rapid decline in release of active t-PA associated with an increasing plaque burden. This reduction in acute fibrinolytic capacity appears to reflect both an impairment of acute t-PA release and an elevation in plasma PAI-1 concentrations. The mechanisms underlying this relationship remain to be established but are likely to

involve chronic endothelial cell injury and an impairment of the L-arginine:nitric oxide pathway (Newby et al 1998). In addition, this association may reflect the chronic stimulation and up-regulation of basal t-PA release secondary to the presence of atheroma (Steins et al 1999) and arterial denudation. The subsequent depletion of endothelial cell t-PA stores, the associated increases in PAI-1 concentrations and the overall reduction of the acute dynamic fibrinolytic response, would potentially be detrimental. This hypothesis is consistent with the epidemiological observations of a positive correlation between plasma t-PA and PAI-1 antigen concentrations and future coronary events (Hamsten et al 1985; Meade et al 1986; Jansson et al 1993; Meade et al 1993; Ridker et al 1993a; Thompson et al 1995; Thögersen et al 1998) as well as our findings of inverse correlations between the release of active t-PA, and basal coronary sinus t-PA and PAI-1 antigen concentrations.

Questions of cause and effect cannot be resolved by the present study but our observations are also consistent with a reduced fibrinolytic activity causing enhanced atherogenesis. Detailed post-mortem studies have shown that plaque growth is induced by episodic subclinical plaque disruption and thrombus formation (Mann & Davies 1999). The prolonged presence of residual thrombus over a disrupted or eroded plaque will provoke smooth muscle migration and the production of new connective tissue, leading to plaque expansion (Galis et al 1997; Nomura et al 1999; Davies 2000). This is consistent with the enhanced macrovascular fibrin deposition and atherogenesis seen in genetic murine models of t-PA and plasminogen deficiency (Xiao et al 1997; Christie et al 1999).

In agreement with our previous work in the peripheral circulation (Newby et al 1999a), we have also observed an elevated basal plasma t-PA antigen concentration and an impairment in coronary release of active t-PA in cigarette smokers. These observations suggest that the increased risk of coronary thrombosis seen in smokers (Burke et al 1997) relates to impaired endogenous fibrinolysis and the propagation of thrombus that would otherwise undergo lysis and remain sub-clinical. Although cigarette smokers have a higher overall mortality from myocardial infarction than non-smokers (Håheim et al 1993), the in-hospital mortality is lower (Mueller et al 1992; Barbash et al 1993; Zahger et al 1995). This so-called "smokers' paradox" can be explained by the observation that the infarct related artery is more than twice as likely to become patent in current smokers compared to non-smokers following thrombolytic therapy for acute myocardial infarction (Gomez et al 1993; Zahger et al 1995; De Chillou et al 1996). Indeed, it has been suggested that thrombolytic therapy should only be given to smokers, and that alternative strategies such as primary angioplasty used in non-smokers (Bowers et al 1996). Our findings may account for these observations since it might be anticipated that patients with impaired endothelial cell t-PA release would benefit most from thrombolytic therapy whilst those with a normal endogenous fibrinolytic capacity are more likely to have coronary thrombus resistant to fibrinolysis.

Previous studies (Zeiher et al 1991; Kuga et al 1995) have suggested that there is a direct association between coronary atherosclerosis and endothelium-dependent vasodilatation. These studies have assessed the vasomotor responses and the extent of atherosclerosis using quantitative

coronary angiography (QCA). There are several inherent limitations and inaccuracies of QCA (Newby 2000b) which permits only crude estimates of plaque load (De Feyter et al 1991; Topol & Nissen 1995) and underestimates the coronary artery luminal diameter and cross-sectional area (De Scheerder et al 1994; Escaned et al 1996; Moussa et al 1999). These inaccuracies occur because QCA can only assess the arterial lumen and the arterial wall is extrapolated from a reference segment and does not take account of "Glagovian" remodelling (Glagov et al 1987; Escaned et al 1996). In contrast, IVUS provides a more accurate assessment of intracoronary plaque volume that has been extensively validated (Hausmann et al 1994; von Birgelen et al 1997). Using this methodology, we have failed to detect a direct association between the atherosclerotic plaque burden and substance P induced vasodilatation. This is, in part, likely to reflect the independent influence of atherosclerotic risk factors on endothelium-dependent vasomotion and is borne out by the correlation of the substance P vasodilatation and the prevalence of these risk factors.

Study Limitations

The proportionate increase in coronary plasma t-PA concentrations appears to be more modest than that obtained from the forearm circulation in healthy volunteers (Newby et al 1997b) and is likely to reflect a number of factors including the direct influence of atherosclerosis. Because the coronary sinus also drains regions of the heart outwith the left anterior descending coronary artery territory, the current methodology will also tend to underestimate the net coronary release of t-PA. Moreover, regional and visceral differences in t-PA release do exist such that the upper limbs release four times the amount of the lower limbs (Keber 1988), and unlike

the systemic circulation, the liver and kidney do not acutely release t-PA when challenged with desmopressin (Brommer et al 1988).

Since the estimated net t-PA release was defined as a product of the arteriovenous difference and plasma flow, impaired endothelial vasodilatation may contribute to the observed reduction in net release of active t-PA. However, this is unlikely for two reasons. First, there was an independent correlation between the arteriovenous difference and the atheromatous plaque burden. Theoretically, smaller increases in blood flow would be associated with greater increases in the arteriovenous concentration differences and obscure this latter association. Second, we failed to detect a significant correlation between the atheromatous plaque burden and substance P induced vasodilatation.

A clear incremental dose response to substance P infusion was not observed. The doses of substance P were chosen on the basis of the effective end-organ concentrations associated with t-PA release in the forearm (Newby et al 1999a). Given that the left anterior descending coronary artery blood flow is approximately 4-5-fold forearm blood flow, we utilised substance P at doses of 10-40 pmol/min (c.f. 2-8 pmol/min in the forearm) which are at the limit of breakthrough systemic effects (Crossman et al 1989). These intracoronary doses have previously been shown to have a similar plateau of vasodilatation responses (Crossman et al 1989) and are consistent with our own findings.

In conclusion, we have demonstrated, for the first time, a direct association between both the coronary atheromatous plaque burden and smoking habit with the acute local fibrinolytic capacity of the coronary circulation. These important findings may provide a direct link between endothelial dysfunction and atherothrombosis. Interventions targeted at the enhancement of the local fibrinolytic capacity will potentially be of major importance.

Table 5.1 Patient Characteristics.

Number		25	
Age		56 ± 2	years
Sex		17	male
Body Mass Index		28 ± 1	kg/m^2
Risk Factors	**Current/Ex-smoker**	17	
	Hypertension	6	
	Diabetes Mellitus	3	
	Hyperlipidaemia	19	
	Family History	13	
Serum Lipid Profile	**Total Cholesterol**	5.8 ± 0.3	(mmol/L)
	LDL Cholesterol	3.2 ± 0.2	(mmol/L)
	HDL Cholesterol	1.3 ± 0.1	(mmol/L)
	Total/HDL Cholesterol Ratio	4.9 ± 0.5	
	Triglycerides	2.3 ± 0.1	(mmol/L)
Medical therapy	**Aspirin**	22	
	ß-Adrenergic Blockade	18	
	Calcium Antagonism	12	
	Long-acting Nitrate	8	
	Lipid Lowering Therapy	16	
	ACE Inhibition	3	
	Diuretics	3	
Previous Myocardial Infarction		6	
Non-Invasive Testing	**Low Risk**	9	
	High Risk	12	
	Not Performed	4	
Angiographic Data	**Good Left Ventricular Function**	24	
	Normal/Mild Disease	8	
	Single Vessel Disease	9	
	Two Vessel Disease	6	
	Three Vessel Disease	2	

Table 5.2 Haemodynamics, coronary blood flow and fibrinolytic parameters.

		Saline	Substance P (pmol/min)			Saline	Sodium Nitroprusside (μg/min)	
			10	20	40		5	20
Heart Rate	(/min)	65 ± 2	64 ± 3	64 ± 3	64 ± 3	69 ± 3	69 ± 3	68 ± 4
Mean Arterial Pressure	(mmHg)	98 ± 3	99 ± 3	98 ± 3	98 ± 3	94 ± 3	96 ± 5	90 ± 4
Coronary Sinus Oxygen Saturation	(%)	44 ± 2	48 ± 2	48 ± 3	46 ± 2	44 ± 3	-	54 ±4
Intravascular Ultrasound and Doppler								
Luminal Cross-sectional Area	(mm^2)	15.7 ± 1.0	16.4 ± 1.0‡	16.5 ± 1.0‡	16.9 ± 1.0‡	14.9 ± 1.1	15.4 ± 1.1‡	16.1 ± 1.3†
Average Peak Velocity	(cm/s)	21.4 ± 1.6	27.3 ± 2.2‡	27.5 ± 2.4‡	28.0 ± 2.3‡	22.7 ± 2.2	27.0 ± 2.2†	47.1 ± 4.6‡
Absolute Coronary Blood Flow	(mL/min)	103 ± 12	138 ± 18‡	140 ± 19‡	143 ± 18‡	109 ± 18	131 ± 20†	242 ± 42‡
Change in Coronary Blood Flow	(%)	0	36 ± 6‡	37 ± 7‡	46 ± 10‡	0	29 ± 9†	140 ± 24‡
Plasma t-PA Antigen								
Arteriovenous Concentration Difference	(ng/mL)	0.3 ± 0.2	0.8 ± 0.3	0.7 ± 0.3	1.0 ± 0.3*	0.0 ± 0.2	-	-0.1 ± 0.4
Estimated Net Release	(ng/min)	12 ± 11	22 ± 31	19 ± 36	74 ± 24†	1 ± 15	-	23 ± 82
Plasma t-PA Activity								
Arteriovenous Concentration Difference	(IU/mL)	0.1 ± 0.1	0.6 ± 0.2†	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	-	-0.0 ± 0.0
Estimated Net Release	(IU/min)	6 ± 3	45 ± 15†	15 ± 8	27 ± 14	12 ± 7	-	-10 ± 9
Plasma PAI-1 Antigen								
Coronary Sinus Concentration	(ng/mL)	69 ± 8	68 ± 7	67 ± 7	66 ± 7	68 ± 11	-	72 ± 12
Arterial Concentration	(ng/mL)	69 ± 8	69 ± 8	66 ± 7	66 ± 7	70 ± 12	-	72 ± 12
Plasma PAI-1 Activity								
Coronary Sinus Concentration	(AU/mL)	19 ± 4	19 ± 4	17 ± 4	16 ± 4	18 ± 8	-	17 ± 8
Arterial Concentration	(AU/mL)	18 ± 3	18 ± 3	18 ± 4	18 ± 3	15 ± 6	-	18 ± 8

n=14 for sodium nitroprusside responses; n=22 for fibrinolytic parameters (except PAI-1 activity, n=13); *p<0.05; †; p<0.01; ‡p<0.001 (*versus* baseline, paired *t*-test)

Table 5.3 Influence of smoking status on the tissue plasminogen activator (t-PA) release, plasminogen activator inhibitor type 1 (PAI-1) and endothelium-dependent increases in coronary blood flow.

	Smokers	Non-Smokers	
Plaque Burden	5.8 ± 0.8	4.5 ± 1.6	(mm^3/mm
Coronary Blood Flow			
Basal Blood Flow	104 ± 16	93 ± 15	(mL/min)
Percentage Increase*	129 ± 28	125 ± 42	(%)
Plasma t-PA Antigen			
Basal Coronary Sinus Concentration	8.7 ± 0.6	6.3 ± 0.7†	(ng/mL)
Basal Arterial Concentration	8.3 ± 0.6	6.3 ± 0.8‡	(ng/mL)
Arteriovenous Concentration Difference*	2.8 ± 1.0	2.6 ± 1.2	(ng/mL)
Estimated Net Release*	98 ± 108	190 ± 85	(ng/min)
Plasma t-PA Activity			
Basal Coronary Sinus Concentration	0.5 ± 0.1	0.6 ± 0.2	(IU/mL)
Basal Arterial Concentration	0.4 ± 0.1	0.5 ± 0.2	(IU/mL)
Arteriovenous Concentration Difference*	0.6 ± 0.3	2.8 ± 1.1†	(IU/mL)
Estimated Net Release*	42 ± 21	202 ± 73†	(IU/min)
Plasma PAI-1 Antigen			
Basal Coronary Sinus Concentration	73 ± 9	59 ± 14	(ng/mL)
Basal Arterial Concentration	73 ± 9	60 ± 15	(ng/mL)
Plasma PAI-1 Activity			
Basal Coronary Sinus Concentration	17 ± 5	24 ± 8	(AU/mL)
Basal Arterial Concentration	17 ± 4	23 ± 8	(AU/mL)

*Area under the curve

†$p<0.02$; ‡$p=0.07$ (current/ex-smokers *versus* non-smokers, unpaired *t*-test)

Figure 5.1 Prevalence of risk factors and influences on coronary blood flow responses to substance P infusion.

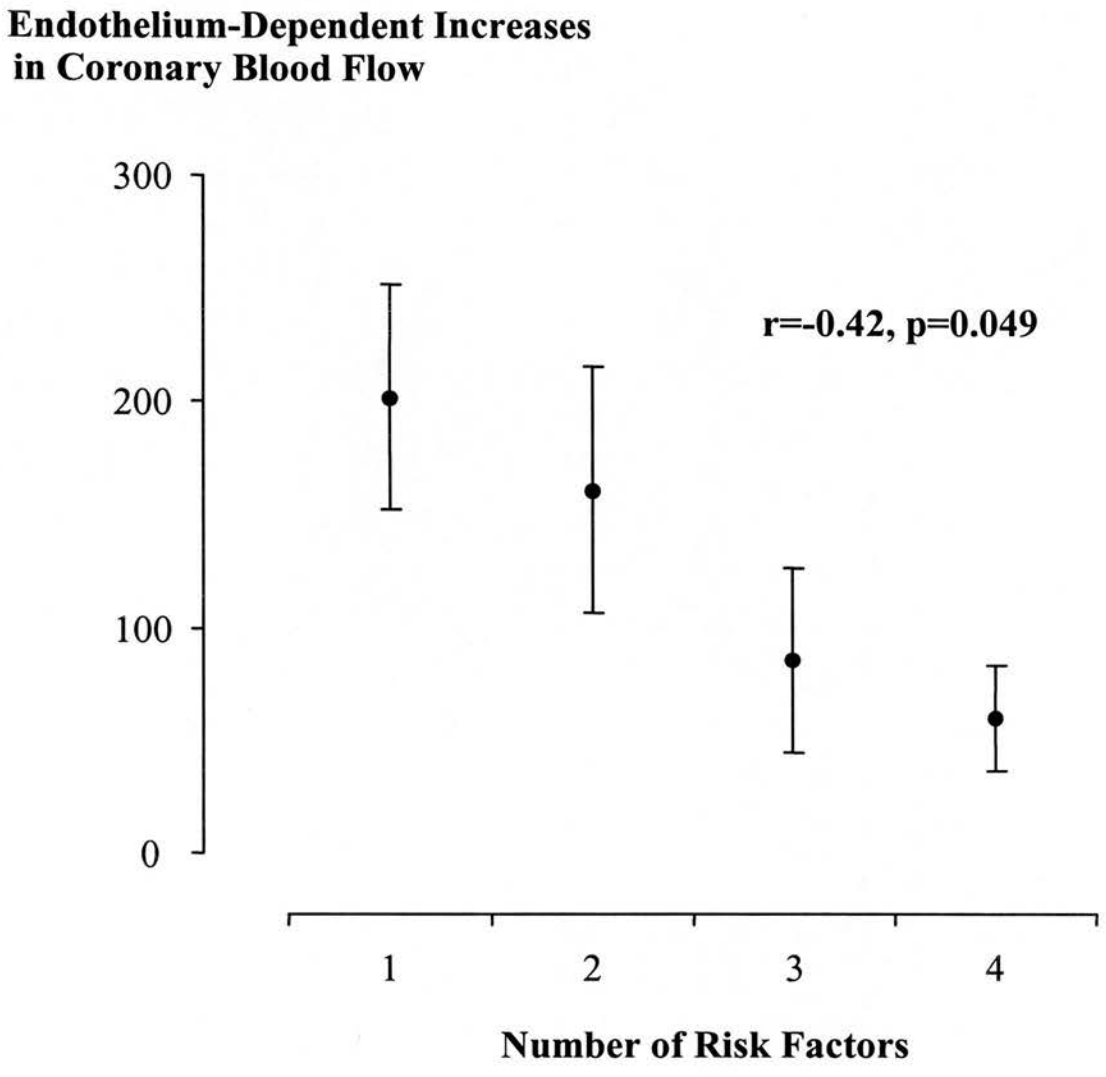
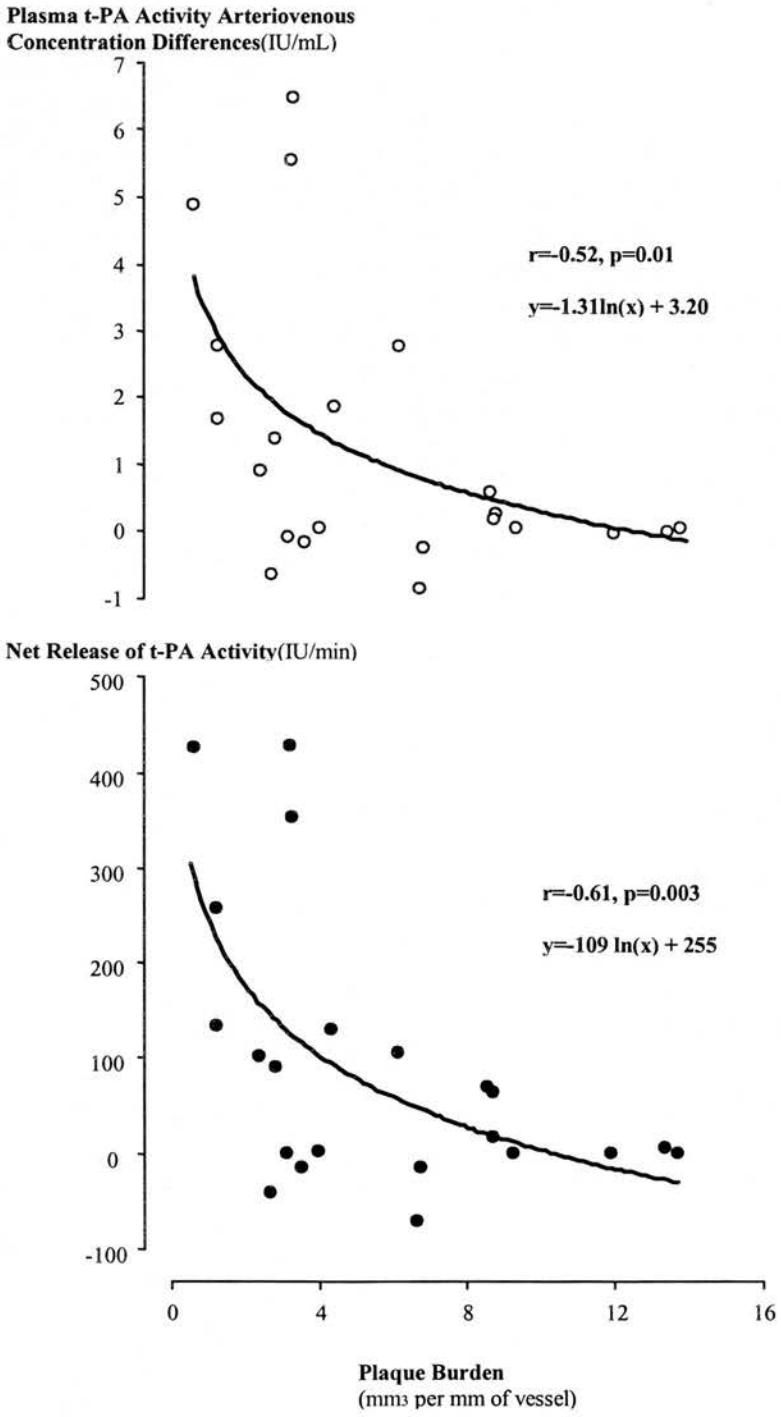


Figure 5.2 Correlation of the plaque burden of the left anterior descending coronary artery with the AUC for the arteriovenous concentration differences (upper panel) and the estimated net release (lower panel) of tissue plasminogen activator (t-PA).



Chapter 6

Influence of Differential Vascular Remodelling on the Coronary Vasomotor Response

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Influence of Differential Vascular Remodelling

on the Coronary Vasomotor Response.

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6.1 Abstract

Arterial remodelling may increase or decrease the luminal encroachment of atherosclerotic plaques in the coronary circulation. However, the factors determining the nature and consequences of the remodelling process remain poorly characterized. The study aims were to assess whether the pattern of vascular remodelling influences the physical and vasomotor responses of the coronary arteries *in vivo* in man. Coronary vessel area, distensibility and stiffness were determined in positively, negatively and non-remodelled arterial segments using intravascular ultrasound and Doppler flow measurement. Epicardial vasomotor responses were determined following intracoronary boluses of acetylcholine (10^{-6}M and 10^{-4}M), adenosine (24-30μg) and nitroglycerin (200μg). Fifty-six coronary arterial segments were studied in 25 patients. In comparison to non- and positively remodelled segments, negatively remodelled segments had a higher stiffness index (67±16 *vs.* 33±5 and 38±8 respectively; $p<0.02$) and appeared to have lower compliance and distensibility (0.66±0.17 *vs.* 1.65±0.54 and 0.94±0.18 /mmHg; p=NS). Non-remodelled segments had a greater change in vessel area with 10^{-6} M acetylcholine (4.9±0.8%), compared to positively and negatively remodelled segments (0.6±1.8% and -4.9±1.8% respectively, $p<0.05$). A significant degree of preservation of vasodilatation to 10^{-6} M acetylcholine was evident in positively remodelled compared with negatively remodelled segments ($p<0.05$). Nitroglycerin caused greater vasodilatation in non-remodelled segments (7.2±3.8%) than either positively or negatively remodelled segments (4.7±0.9% and 3.7±0.6% respectively, $p<0.05$). Vascular remodelling is an important and major determinant of local epicardial vasomotor responses.

Both structural and functional abnormalities are associated with negative remodelling that may contribute to the adverse effects of such lesions.

6.2 Introduction

Coronary arterial remodelling is described by the response of the arterial wall to the presence of an atherosclerotic plaque. In many cases, the artery expands to accommodate the developing plaque burden such that there may be little or no luminal encroachment: so-called positive remodelling (Glagov et al 1987). However, this vascular expansion may be absent (non-remodelled) or reversed (negatively remodelled) leading to more rapid luminal encroachment and the development of significant flow-limiting stenoses. The remodelling process can consequently have a profound influence on the outcome of atheroma deposition with the same plaque burden leading to either an absent (positive remodelling) or significant (negative remodelling) luminal stenosis and myocardial ischemia. To date, the factors determining the nature and consequences of the remodelling process remain poorly characterized.

Since the seminal work of Furchgott and Zawadski, it has been widely recognized that the endothelium can influence vascular tone through an array of mediators (Furchgott and Zawadski 1980). Endothelial stimulation results in the rapid release of vasodilator mediators, including prostacyclin, nitric oxide and endothelium-derived hyperpolarising factor, as well as vasoconstrictor mediators, such as angiotensin II and endothelin-1. Indeed, both nitric oxide and endothelin-1 are released continuously by the endothelium to regulate basal vascular tone and blood pressure (Vallance et al 1989; Haynes et al 1993; Haynes et al 1996). Thus, there is an inextricable interplay between these endothelium-derived vasodilator and

vasoconstrictor mediators that have a local counter-regulatory paracrine action on the adjacent vascular smooth muscle (Verharr et al 1998).

The endothelium responds to physical changes within the vascular lumen, such as leucocyte adhesion and alterations in shear stress, and the vessel wall, such as barotrauma and lipid deposition. Given these interactions, the integrity of endothelial function may profoundly influence the local vessel wall response to lipid deposition and atheroma formation (Newby et al 2001), and thereby determine the resultant pattern of vascular remodelling (Lerman et al 1998; Sudhir et al 1995). However, the exact relationship between endothelial function and the direction of vascular remodelling in atherosclerotic coronary arteries is unclear.

The purpose of the present study was to assess whether the pattern of vascular remodelling influenced the physical and vasomotor responses of the coronary arteries *in vivo* in man. Using intravascular ultrasound (IVUS) and Doppler flow velocity measurement, the resting physical characteristics of vascular remodelling were determined, and the endothelium-dependent and -independent vasomotor function was characterized in remodelled coronary artery segments.

6.3 Methods

6.3.1 Patient selection

Twenty-five patients (20 male, mean age 59±2 years) with angiographic evidence of mild to moderate coronary artery disease were recruited at the time of coronary angiography in two centres (Royal Infirmary and Western General Hospital, Edinburgh, UK). Patients were excluded if they had severe left main stem disease, left ventricular hypertrophy or significant concurrent illness. All patients had their coronary risk factors determined by standard clinical criteria. Hypercholesterolemia was defined as a fasting total cholesterol >200 mg/dL prior to initiation of lipid lowering therapy. The study was undertaken with the approval of the local research ethics committee, in accordance with the principles outlined in the Declaration of Helsinki, and the written informed consent of each subject.

6.3.2 Study Protocol

All patients discontinued their medication on the study day, attended fasted and underwent diagnostic coronary angiography. Standard diagnostic images were taken using the Judkin's technique with a non-ionic contrast agent (Niopam™ 340, MERCK Pharmaceuticals, Middlesex, UK). A non-tortuous, non-branching segment of artery with a reference luminal diameter of >3.0 mm and luminal irregularity (diameter stenosis of 20-70%) was then identified for IVUS assessment. Where percutaneous coronary intervention was planned, the study was performed in an adjacent artery prior to the interventional procedure.

A 7 French guiding catheter was used to cannulate the left or right coronary artery and, following a 5,000 IU intravenous bolus of heparin (Leo Laboratories Ltd., Princes Risborough, UK), a 0.014 inch 12.5 MHz Doppler wire (Flowire™, Endosonics, Rancho Cordova, CA) was passed across the arterial segment under study. A 3.2 F Ultracross™ 30 MHz IVUS imaging catheter (Atlantis SCIMED®, Boston Scientific Corporation, Maple Grove, MN) was advanced over the Doppler wire (Nitrocine™, SCWARZ Pharma Ltd, Chesham, UK).

The IVUS examination of the proximal artery was performed at 0.5 mm/s using a motorized pullback device (Boston Scientific Corporation). All IVUS images were recorded on high fidelity s-VHS videotape for later off-line quantitative analysis. Subsequent three-dimensional computerized reconstruction of the two-dimensional IVUS images was performed using the TomTec™ system (TomTec GmbH, Munich, Germany).

6.3.3 Assessment of Remodelled Segments

During the automated pullback, potential regions of interest (remodelled segments) were identified. Segments were selected for inclusion in the study if there was optimal image quality without rotational, angular or image artifacts; clear demarcation of the endoluminal and the external elastic laminal borders; and less than 180 degrees of calcification. Segment remodelling was defined according to existing criteria based on the vessel area at the index site relative to normal or near-normal proximal and distal reference segments. IVUS pullbacks were acquired to define these regions. This enabled classification of the remodelled segment by

calculating the relative vessel area as the ratio of the vessel area at the index segment to the mean of the vessel area at proximal and distal reference segments. Categorization of segments was defined as follows: non-remodelled segments = vessel area ratio of >0.95 and <1.05; positively remodelled segments = ratio of >1.05; and negatively remodelled segments = ratio of <0.95.

6.3.4 Drug Administration

Following the pullback examination, the IVUS imaging catheter was repositioned at the index segment. The Doppler guide wire was retracted to the tip of the imaging catheter and maintained in a stable position by the short monorail segment of the IVUS catheter (Schwarzacher et al 2000; Newby et al 2000). Acetylcholine at a dose of 10^{-6} M and 10^{-4} M (Miochol™, OMJ Pharmaceuticals Inc.), adenosine at a dose of 24-30 μg (Sanofi Winthrop Ltd, Guildford, UK) and nitroglycerin at a dose of 200 μg (Schwarz Pharma Ltd) were given as 2 mL boluses, followed by 2 mL 0.9% saline flush via the guide catheter (Newby et al 2000). The IVUS images and Doppler ultrasound were recorded simultaneously to allow the measurement of luminal area and blood flow velocity for two minutes following each drug bolus.

6.3.5 Ultrasound Measurements

Coronary artery cross-sectional area (vessel and luminal area) was measured using computerized planimetry (Clearview™, Boston Scientific Corporation.) of the vessel lumen at the onset of the QRS complex

(diastole) and the offset of the T wave (systole) (Jeremias et al 2000). Blood flow velocity was determined using average peak velocity of the Doppler wire signal. Coronary artery blood flow was previously defined as half the product of the average peak velocity and the diastolic luminal cross-sectional area (Newby et al 2000; Doucette et al 1992). The atheromatous plaque volume was calculated using a well-validated edge detection algorithm as previously described and was expressed as mm^3 per mm of vessel (Newby et al 2000; von Birgelen et al 1996; von Birgelen et al 1997). The distensibility index of the coronary artery was defined as $[(\wedge A/A)/ \wedge P] \times 10^3$/mm Hg; where $\wedge A$ represents the luminal area change between systole and diastole, A the smallest luminal area in diastole, and $\wedge P$ the difference in systolic and diastolic blood pressure (Jeremias et al 2000; Nakatani et al 1995; Yamagishi et al 1997). The stiffness index, β, was calculated using the following equation: $\beta = [\ln(P_{sys}/P_{dias})]/(\wedge D/D)$, where P_{sys} represents the systolic pressure, P_{dias} the diastolic pressure, $\wedge D$ the difference between systolic and diastolic mean lumen diameters, and D the diastolic mean luminal diameter (Jeremias et al 2000; Hirai et al 1989).

6.3.6 Data Analysis and Statistics

Data were examined by analysis of variance (ANOVA) with repeated measures and Student's *t*-test using StatView *v*5.0.1 (SAS Institute Inc., Cary, NC). Where ANOVA demonstrated significant differences in responses, post-hoc comparisons were made using the Fisher protected least significant difference test (StatView *v*5.0.1). All results are expressed as mean ± standard error of the mean. Statistical significance was taken at the 5% level.

6.4 Results

Baseline patient characteristics are shown in Table 6.1. The majority of patients had at least one risk factor for coronary artery disease; in particular, a history of hypercholesterolemia (19/25). A maximum of three segments were selected in each artery to give a total of 56 segments: 28 non-remodelled, 15 positively remodelled and 13 negatively remodelled. The left anterior descending artery was studied in 12 patients, right coronary artery in 6 patients, and left circumflex coronary artery in 7 patients. Heart rate and mean arterial pressure were stable throughout the study in all subjects. There were no procedural or post-procedural in-hospital complications.

6.4.1 Characteristics of Vessel Segments

Non-remodelled segments (4.4 ± 0.8 mm^2) had significantly less plaque cross-sectional area than either positively remodelled (8.0 ± 1.1 mm^2, $p<0.05$) or negatively remodelled (7.1 ± 0.6 mm^2, $p<0.01$) segments. Total vessel cross-sectional area was similar for the three types of vessel remodelling (Figure 6.1). Negatively remodelled segments had a higher stiffness index in comparison to non- and positively remodelled segments (67±16 *vs.* 33±5 and 38±8 respectively; $p<0.02$) and with a trend towards a lower compliance and distensibility (0.66±0.17 *vs.* 1.65±0.54 and 0.94±0.18/mmHg; p=NS).

6.4.2 Vasomotor Responses

Resistance Vessels

Acetylcholine, adenosine and nitroglycerin all caused coronary resistance vessel vasodilatation and an increase in coronary artery blood flow (p□0.002; Figure 6.2) with adenosine causing the largest increase ($p<0.004$ vs. other agents). There were no differences in the resistance vessel responses between the three remodelling groups.

Conduit Vessels

All vessel segments vasodilated to adenosine and nitroglycerin, and vasoconstricted to 10^{-4} M acetylcholine ($p<0.05$ for all; Figure 6.3). The vasomotor response to acetylcholine 10^{-6} M varied according to the category of vascular remodelling ($p<0.001$; Figure 6.3). Non-remodelled segments had a greater change in vessel cross-sectional area with 10^{-6} M acetylcholine (4.9±0.8%), compared to positively and negatively remodelled segments (0.6±1.8% and -4.9±1.8% respectively, $p<0.05$). A significant degree of preservation of vasodilatation to 10^{-6} M acetylcholine was evident in positively remodelled compared with negatively remodelled segments ($p<0.05$). There was a trend towards greater adenosine-induced vasodilatation in non-remodelled segments (increase in vessel cross-sectional area of 6.2 ± 1.0 %) compared with positively (3.7 ± 0.8 %) and negatively (3.9 ± 0.9%) remodelled segments ($p=0.14$, Figure 6.3). Similarly, nitroglycerin caused greater vasodilatation in non-remodelled segments (increase in vessel cross-sectional area of 7.2 ± 3.8 %) than either positively (4.7 ± 0.9 %) or negatively (3.7 ± 0.6%) remodelled segments ($p<0.01$, Figure 6.3).

6.5 Discussion

We have demonstrated that, in keeping with a more fibrotic and vasospastic response to atheroma deposition, negatively remodelled coronary arterial segments are stiffer and less distensible. Moreover, we have shown that the type of vascular remodelling determines the local epicardial vasomotor response to both endothelium-dependent and -independent vasodilators. In particular, negatively remodelled segments have an exaggerated vasoconstrictor response to acetylcholine and impaired endothelium-independent vasodilatation. We conclude that negative remodelling is associated with more pronounced local vascular and endothelial dysfunction.

Endothelial Function

At the lowest concentration of acetylcholine, there was preservation of endothelium-dependent vasodilatation in non-remodelled segments and, to a lesser extent, positively remodelled segments. However, in negatively remodelled segments, low dose acetylcholine caused vasoconstriction that was equivalent to that observed with high dose acetylcholine in all segments. The phenomenon of paradoxical epicardial vasoconstriction during acetylcholine administration in atherosclerotic coronary arteries was first described 15 years ago (Ludmer et al 1986). It has been attributed to the direct muscarinic vasoconstrictor action of acetylcholine on the vascular smooth muscle cells in the absence of functional endothelium-dependent vasodilatation. Consistent with previous work (Nishimura et al 1995), our observation of low dose acetylcholine-induced vasoconstriction cannot be directly attributed to the atherosclerotic plaque burden since both

positively and negatively remodelled segments had a similar plaque load but distinct vasomotor responses. It may, in part, be explained by the reduced vasodilatation response to the endothelium-independent vasodilator, nitroglycerin, suggesting impaired vascular smooth muscle sensitivity to nitric oxide in remodelled segments. However, this cannot be the only explanation since the reduced response to nitroglycerin was observed in both positively and negatively remodelled segments but only negatively remodelled segments demonstrated vasoconstriction to low dose acetylcholine. This suggests that negative remodelling is associated with endothelial dysfunction or alterations in muscarinic receptor responsiveness.

Vascular Remodelling

The current finding of an association between negative remodelling and vascular dysfunction does not address the question of cause and effect. It is tempting to speculate that localized vascular and endothelial dysfunction may lead to a more vasospastic artery that would cause increased vessel stiffness and negative remodelling. Alternatively, these features may have a common etiology with the potentially more fibrotic and inflammatory reaction to atheroma formation not only leading to negative remodelling but also vascular and endothelial dysfunction. In this regard, longitudinal studies would be of value to monitor the changes in vascular function and vessel remodelling over time in order to determine which is the dominant factor in determining these vascular effects. This would also determine if vessels negatively remodel from the beginning or go through an initial phase of positive remodelling which becomes exhausted through progressive endothelial dysfunction and fibrosis (Ward et al 2000). In a

hypercholesterolemic rabbit model, endothelial dysfunction was associated with negative remodelling and restenosis after balloon injury implicating endothelial dysfunction in the causation of negative remodelling (Lafont et al 1999).

Irrespective of the causative relationship, the present study suggests that there are functional abnormalities associated with coronary artery remodelling in addition to anatomical and structural changes. There has been only one previous study to address this issue. Lerman and colleagues used quantitative coronary angiography to determine epicardial responses to 10^{-4} M acetylcholine and nitroglycerin in the left anterior descending coronary artery (Lerman et al 1998). This study used a single dose of acetylcholine and employed a different classification of vascular remodelling. However, consistent with our current findings, Lerman and co-workers suggested that positive remodelling is associated with endothelial dysfunction. We have now extended these observations by providing more detailed assessments of remodelled segments, and demonstrating that negative remodelling is associated with more pronounced endothelium-dependent and -independent vasomotor abnormalities.

Study Limitations

This study was conducted in the necessary clinical setting of patients with a combination of risk factors and concomitant therapies undergoing diagnostic coronary angiography. The modest sample size means that this study lacks sufficient power to address the influence of all the individual variables associated with coronary artery disease. The study was also

performed at a single time point and temporal changes in the remodelling process are unknown.

We defined the remodelling segment with reference to proximal and distal segments. Because of the clinical setting of this study, some of these segments will have incorporated some mild degree of atherosclerotic plaque: the so-called 'near-normal' segments. Because this could theoretically affect the classification of the remodelling segments, we avoided arterial segments where the remodelling classification was unclear. It should also be recognised that our findings may not be representative of all coronary artery atherosclerotic plaques. Heavily calcified lesions were excluded because the resultant acoustic shadow makes it very difficult to measure vessel areas. In addition, whilst we excluded segments with rotational, angular or image artifacts, we cannot completely eliminate these effects and recognise this as a potential limitation.

Conclusions

Vascular remodelling is an important and major determinant of epicardial vasomotor responses. Both structural and functional abnormalities are associated with negative remodelling that may contribute to the adverse effects of such lesions.

Table 6.1 Patient characteristics (n=25).

	N	%
History of hypercholesterolaemia (>200 mg/dL)	19	76
Lipid Profile (mg/dL)		
Total cholesterol	181±6	
LDL cholesterol	110±6	
HDL cholesterol	44±3	
Triglycerides	168±19	
Smoking habit		
Non-smoker	9	36
Ex-smoker	7	28
Current smoker	9	36
Hypertension	12	48
Family history of premature vascular disease	10	40
Type 2 diabetes mellitus	3	12
Previous myocardial infarction	7	28
Drug therapy		
Aspirin	22	88
ß-blocker	14	56
Calcium antagonist	13	52
Long-acting nitrate	13	52
Lipid lowering agent	15	60
ACE Inhibitor	1	4
Coronary Angiography		
Normal/ mild disease	5	20
Single vessel disease	6	24
Two vessel disease	7	28
Three vessel disease	8	32

ACE - angiotensin-converting enzyme; LDL - low density lipoprotein; HDL - high density lipoprotein.

Figure 6.1 Baseline physical characteristics of non- (white bars), positively (gray bars) and negatively (black bars) remodelled segments. ANOVA for comparison of remodelled segment types.

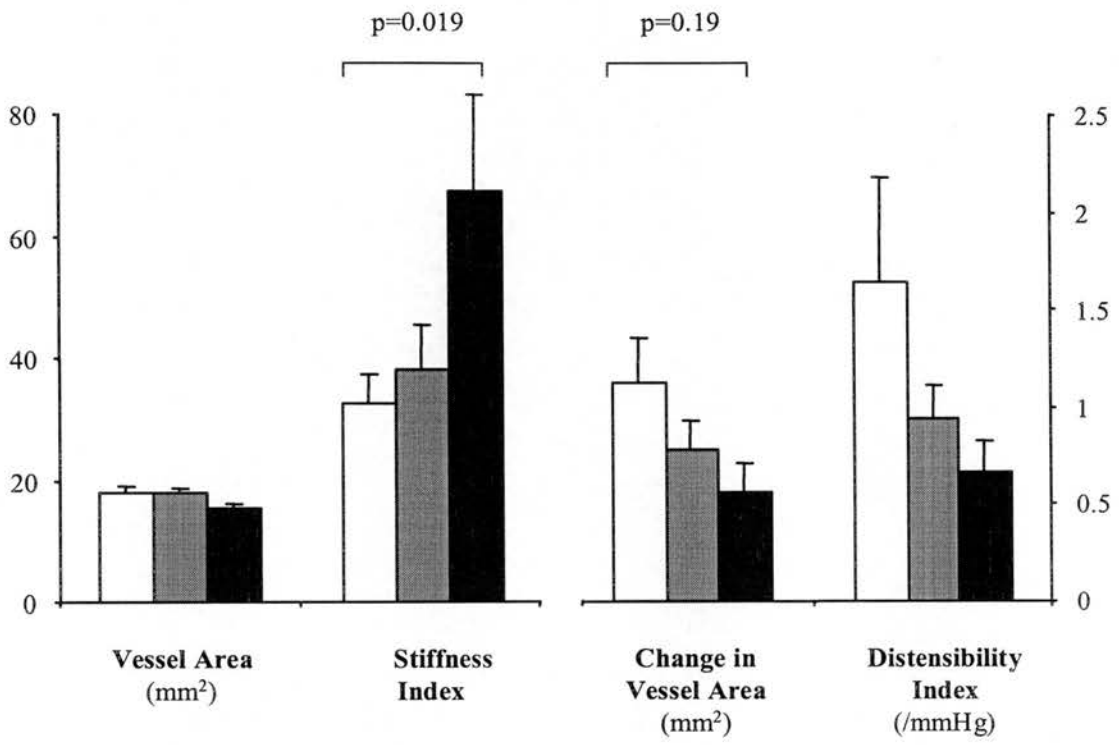
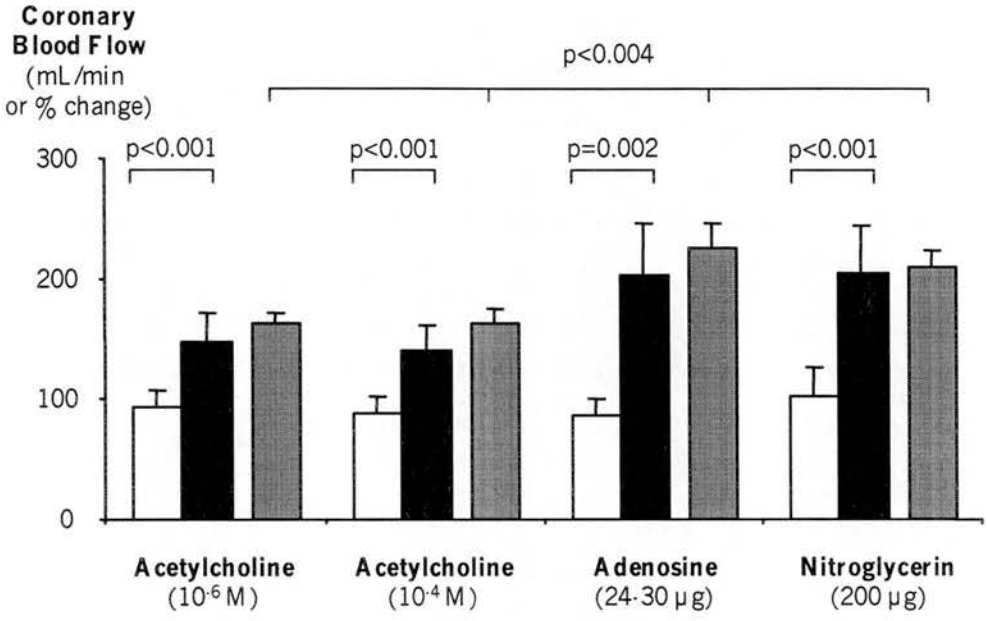


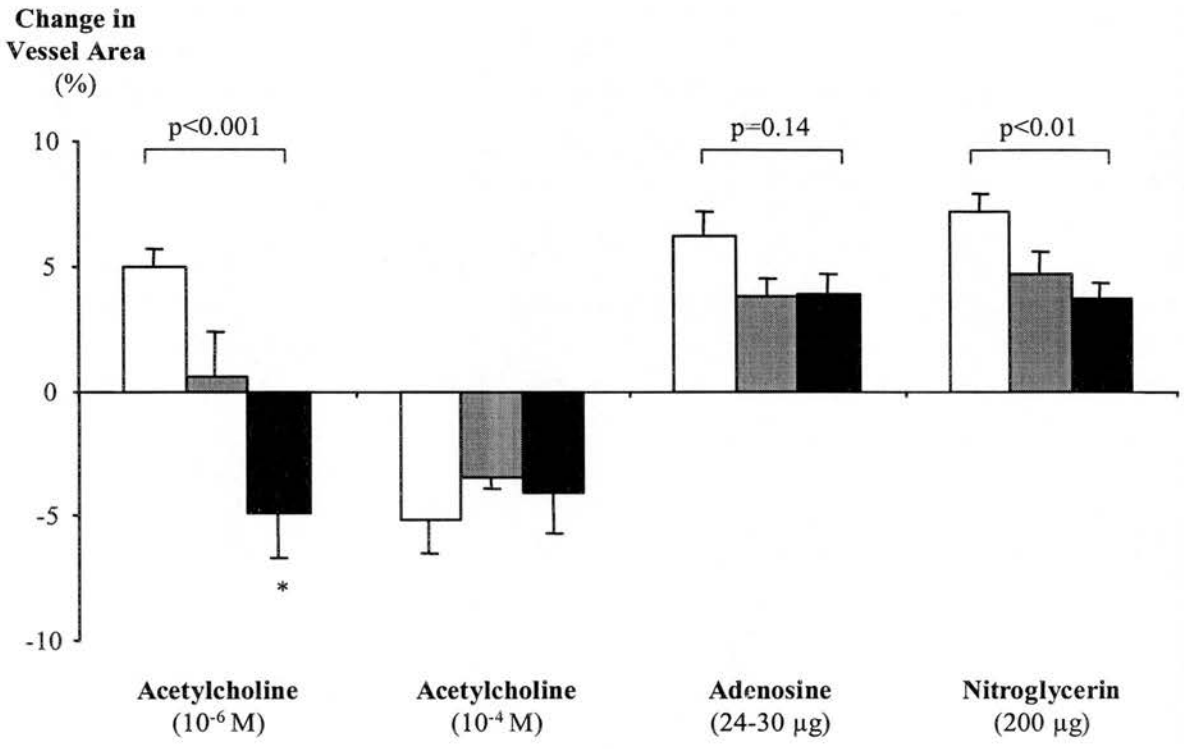
Figure 6.2 Baseline (white bars) and peak (black bars) coronary blood flow (percentage change, gray bars) to intracoronary acetylcholine, adenosine and nitroglycerin.



ANOVA for comparison of percentage increase in blood flow for the 4 drug boluses.

Paired *t*-test for comparison of baseline and peak blood flow for each drug bolus.

Figure 6.3 Changes in vessel area following intracoronary acetylcholine, adenosine and nitroglycerin in non- (white bars), positively (gray bars) and negatively (black bars) remodelled segments.



ANOVA for comparison of remodelled segment types.

Chapter 7

Conclusions and Future Directions

7.1 Conclusions

7.1.1 Coronary Artery Remodelling and Spectral Analysis of Plaque.

With negative remodelling predisposing to increasing luminal stenosis and myocardial ischaemia, positively remodelled segments have been associated with unstable lesions (Schwarzacher et al 1998; Jeremias et al 2000, Shoenhagen et al 2000). Using spectral analysis of *in vivo* radiofrequency intravascular ultrasound backscatter, plaque composition was similar with fibrous plaque predominant in both remodelled types, suggesting this is not an integral determinant of unstable lesions. Positively remodelled segments were found, however, to have a larger plaque burden and that may be a factor in unstable lesions in addition to other pathophysiological mechanisms such as shear stress, vascular compliance, matrix metalloproteinase activity and inflammation. We have however only studied patients with stable coronary artery disease and our findings may not be applicable in patients with acute coronary syndromes. The ability to characterise plaque composition, using spectral analysis of radiofrequency intravascular ultrasound backscatter may have important implications in both the identification and in monitoring the response of plaque to therapy such as lipid-lowering medication.

7.1.2 Coronary Plaque Load and Non-Invasive Assessment of Aortic Stiffness.

We have demonstrated using intravascular ultrasound and applanation tonometry that coronary artery plaque load correlates with carotid-radial

pulse wave velocity and appears to correlate with carotid-femoral pulse wave velocity. Other measures reflecting aortic stiffness (aortic augmentation and augmentation index) did not reveal positive associations with plaque load.

Our study population had a combination of risk factors for coronary artery disease that contributes to arterial stiffness (Cockcroft et al 1991). Hypercholesterolaemia is associated with endothelial dysfunction and impaired endogenous nitric oxide release (Chowienczyk et al 1992; Stoes et al 1995). Arterial stiffness is increased by both nitric oxide synthase inhibition and hypercholesterolaemia (Iannuzzi et al 1999; Wilkinson et al 2002). Furthermore treatment with lipid lowering therapy improves endothelial function and increases the bioavailability of nitric oxide (Stroes et al 1995; Leung et al 1993).

There was no positive association demonstrated with both aortic augmentation and augmentation index with plaque load. With the modest sample size, the study lacked sufficient power to address the influence of all the individual variables associated with coronary artery disease which potentially could create a type II error. By necessity, many of the study patients were established on concomitant medical therapy. Treatments such as β-adrenergic receptor blockade and angiotensin-converting enzyme inhibition have been shown to influence aortic stiffness (Ting et al 1991; Breithaupt-Grogler et al 1996). However, whilst such treatments may affect the functional aspects of arterial stiffness, the structural component of arterial stiffness will be unaffected. Furthermore because of ethical and technical considerations IVUS was not performed in vessels with a severe

luminal stenosis nor was it performed on all three epicardial vessels which may have underestimated the overall plaque burden.

The association between coronary artery plaque volume and aortic stiffness may, in part, reflect the common aetiological for atherosclerosis that will inevitably exist in the clinical population. However, the results suggest that pulse wave velocity may be a useful non-invasive surrogate marker for the extent of coronary atherosclerosis.

7.1.3 Coronary Plaque Load and Endothelial Function

Previous studies (Zeiher et al 1991; Kuga et al 1995) have suggested that there is a direct association between coronary atherosclerosis and endothelium-dependent vasodilatation. These studies have assessed the vasomotor responses and the extent of atherosclerosis using quantitative coronary angiography (QCA). There are several inherent limitations and inaccuracies of QCA (Newby 2000b) which permits only crude estimates of plaque load (De Feyter et al 1991; Topol & Nissen 1995) and underestimates the coronary artery luminal diameter and cross-sectional area (De Scheerder et al 1994; Escaned et al 1996; Moussa et al 1999). These inaccuracies occur because QCA can only assess the arterial lumen and the arterial wall is extrapolated from a reference segment and does not take account of coronary artery remodelling (Glagov et al 1987; Escaned et al 1996). In contrast, IVUS provides a more accurate assessment of intracoronary plaque volume that has been extensively validated (Hausmann et al 1994; von Birgelen et al 1997). Using this methodology, we have failed to detect a direct association between the atherosclerotic

plaque burden and substance P induced vasodilatation. In contrast, there was an association between the number of risk factors for atherosclerosis and the coronary blood flow responses to substance P, suggesting an independent influence of atherosclerotic risk factors on endothelium-dependent vasomotion.

Endothelial function as assessed by endothelial derived t-PA release, in the coronary circulation, correlated inversely with plaque load. The impaired coronary release of active t-PA in cigarette smokers suggests that in the presence of atheroma, this adversely influences the local fibrinolytic balance in the coronary circulation and may provide a link between endogenous fibrinolysis, endothelial dysfunction and athero-thrombosis. With endothelial fibrinolytic dysfunction appearing to relate to cigarette smoking and plaque load and not to hypercholesterolaemia (Newby et al 2002), this would indicate that endothelial dysfunction can be manifest in separate distinct pathways depending upon the nature of the insult.

7.1.4 Coronary Artery Remodelling and Vascular/Endothelial Function.

Coronary artery remodelled segments were defined using IVUS and combined with an assessment of the epicardial response to vasoactive agents this provided functional information in terms of local vascular and endothelial dysfunction.

Negatively remodelled segments were found to be stiffer and less distensible, suggesting a less dynamic segment in vasoactive terms. In

contrast the greater distensibility in positively remodelled segments was in keeping work by Jeremias et al who postulated that this was reflective of increased pulsatile stretch and preserved endothelial function with nitric oxide release (Jeremias et al 2000).

We have shown that the type of vascular remodelling determines the local epicardial vasomotor response to both endothelium-dependent and -independent vasodilators. In particular, negatively remodelled segments have an exaggerated vasoconstrictor response to acetylcholine and impaired endothelium-independent vasodilatation. With similar plaque loads between positively and negatively remodelled types and similar changes in vessel area to nitrogylcerine, this suggests that negative remodelling is associated with a greater degree of endothelial dysfunction or alterations in muscarinic receptor responsiveness (to acetylcholine).

With the absence of longitudinal studies it is unclear as to whether positive and negative remodelling are parts of the same pathological process, but at different time points of development. Different types of remodelling were present in the same vessel suggesting that lesion specific factors for example plaque type, degree of inflammation and shear stress were more important than patient-specific factors. Ideally, longitudinal studies would be of value in determining which factors are dominant in determining the remodelling type, though the use of reference segments to define the extent of remodeling assumes that the reference site reflects the lesion site at an earlier time and also it becomes less accurate with diffuse disease.

We have shown that negatively remodelled segments are more vasoconstricted segments and are associated with endothelial dysfunction in the presence of similar plaque loads, compared with positively remodelled segments. This would suggest that in negatively remodelled segments there is impairment of smooth muscle relaxation and release of nitric oxide compared to positively remodelled segments. The increased resistance to pulsatile stretch and nitric oxide release would imply less fluidity in the matrix skeleton of the vessel wall.

As a consequence, the remodelling process can consequently have a profound influence on the outcome of atheroma deposition with a plaque burden leading to either positive remodelling with potential for unstable lesions, or negative remodelling with luminal stenosis and resultant myocardial ischaemia. Lipid lowering therapy and ACE inhibition have been associated with improvement in endothelial function (Treasure et al 1995; Anderson et al 1995; Lippy et al 2003) and plaque stabilisation (Hornig et al 1997, Dzau et al 2002; Lippy et al 2003). Although speculative, with preservation or enhancement of endothelial dysfunction this may protect against negative remodelling and resultant luminal encroachment in the presence of plaque load.

7.2 Future Directions

Intravascular ultrasound offers a unique opportunity to assess the coronary atherosclerotic burden by providing information regarding the severity, composition, and distribution of atheroma. With IVUS, positive remodelling and larger plaque areas have been shown to be more prevalent in unstable lesions (Pasterkamp et al 1998; Schwarzacher et al 2000; Shoenhagen et al 2000), whereas patients who presented with stable angina were more likely to present with negative remodelling (Shoenhagen et al 2000)

The disparity between angiographic dimensions and clinical outcome seen in the multicentre lipid-lowering trials (Brown et al 1993)) suggests that the ability to study relatively mild (<50% diameter stenosis by angiography) but potentially unstable stenoses by intravascular ultrasound may give additional information regarding clinical outcome. With longitudinal studies, the interaction between the atheroma load and the change in vessel and lumen dimensions can be accurately assessed in vivo. Schartl and colleagues compared the effects of 12 months aggressive lipid lowering therapy with standard lipid lowering therapy in 131 patients using serial 3D intracoronary ultrasound (Schartl et al 2001). Whilst no effect was seen on plaque volume, there was a significant change in the apparent composition of the atherosclerotic plaque. Aggressive LDL cholesterol reduction was associated with an increase echogenicity of the atherosclerotic plaque indicating a reduction in lipid content and greater plaque stability.

The recently reported REVERSAL study compared the effects of intensive versus moderate lipid lowering on changes in atheroma burden measured by intravascular ultrasound. The primary efficacy parameter was the percentage change in atheroma volume over 18 months. Whilst there was a significant 2.7% increase in plaque volume in the moderate lipid lowering arm there was no change in the intensive arm, suggesting a greater reduction in atherogenic lipoproteins (Nissen et al 2003).

With on-going development of non-invasive imaging of coronary arteries with MRI and ultrafast CT, it is possible that that serial studies in humans may become more feasible. Furthermore, further anticipated reductions in the size of IVUS catheters and higher frequency catheters as well as the high resolution (20 μm) offered by optical computed tomography (see below) which is also catheter-based, may allow the accurate and reproducible plaque characterisation and an endpoint of plaque vulnerability assessment. Recent advances in intravascular imaging have provided several additional modalities to characterise atheroma with the ability to identify vulnerable plaque in vivo by using various features of plaque vulnerability as methods of identification.

IVUS Elastography.

IVUS permits real-time visualisation of coronary arteries and resultant changes in systole and diastole. IVUS elastography combines ultrasound radiofrequency data with images of local strain. In-vitro work using intravascular elastography to characterise fibrous, fibro-lipid and lipid plaque types revealed higher strains in the lipid plaque types indicating its potential for determining plaque type (de Korte et al 2000). A positive

correlation was demonstrated, ex vivo, with strain measurements and macrophage presence, with an inverse relation to the amount of smooth muscle cells, demonstrating potential to identify vulnerable plaque (Schaar et al 2002). De Korte et al recently described an in vivo animal study validating IVUS elastographic criteria for identifying lipid-rich plaque, demonstrating a sensitivity and specificity of 92% in identifying the presence of macrophages at foci of increased strain within the plaque (de Korte 2002).

Optical Coherence Tomography (OCT).

Optical Coherence Tomography (OCT) is a catheter-based imaging modality which measures backscattered infrared light directed at the arterial wall. With ex-vivo histological correlation, plaque morphology was identified with sensitivity and specificity of 92% and 94%, respectively for lipid rich plaque; 95% and 100% for fibrocalcific plaque; and 87% and 97% for fibrous plaque (Yabushita et al 2002). A small in-vivo study, demonstrated that OCT was equivalent to IVUS in detecting plaque and differentiating between echolucent regions, fibrous plaques and macrocalcifications in coronary atheroma (Jang et al 2002). OCT has also been recently shown to be able to detect and quantify macrophage infiltration within the fibrous cap (Tearney et al 2003). Combining other modalities with OCT includes light polarisation imaging, elastography and spectroscopy with biochemical analysis of plaque types.

Further Areas.

Other developing invasive imaging modalities include intravascular magnetic resonance imaging, direct visualisation of the arterial wall with

angioscopy, intracoronary thermography, where micro-elevations in temperature reflecting inflammatory activity and potential vulnerability can be detected.

Developing pharmacological interventions include targeting specific receptor activation and cell metabolism. Magnetic nanoparticle based contrast agents have been developed that display enzyme specific fluorescence detectable by optical or fluorescence-mediated tomography (Josephson et al 2002). Alternatively, using intravascular ultrasound, antibody labeled and contrast loaded microspheres have been developed that bind directly to selected antigens on cell surfaces (BHF RG/2000014). These techniques have the potential to target specific components of the atherosclerotic plaque *in vivo*, for example, specific vascular cell proteins, receptors or inflammatory cells and to locally deliver drug therapies. This would provide the prospect of relating the structural characteristics of the endothelium and vascular wall to its *in vivo* function

With characterisation of coronary plaques and identification of vulnerability, this would be an important diagnostic contribution to the clinician in both risk assessment, targeting therapies and in monitoring the response of plaque to current pharmacological therapies such as statin, ACE inhibitors, angiotensin receptor blockers and newer therapies such as peroxisomal proliferator activator receptor agonists.

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