THE PREVENTION OF ARTERIAL RESTENOSIS USING ENDOVASCULAR PHOTODYNAMIC THERAPY

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ABSTRACT

Atherosclerosis is the commonest aetiology in deaths arising from cardiovascular disease. It is characterised by the build up of an intraluminal plaque leading to arterial stenosis. Balloon angioplasty offers a minimally invasive method of dilating such stenoses in both peripheral and coronary arteries. However, despite very favourable immediate results, 15-40% of arteries restenose within 3-6 months following angioplasty with obvious clinical and resource implications. Restenosis is caused by a combination of neointimal hyperplasia (NIH) and negative geometric remodelling, the combined effect of which results in luminal narrowing.

The aim of this thesis was to investigate a method of inhibiting restenosis using photodynamic therapy (PDT). PDT involves the interaction of light of a specific wavelength with a pre-administered photosensitiser to produce cell death by oxygen-dependent cytotoxic mediators.

Experimental project

Preliminary studies established the pharmacokinetics of the chosen photosensitiser 5-aminolaevulinic acid (ALA) in a swine model. From these experiments the optimum drug-light interval was calculated and in a second study, PDT was applied to normal porcine iliac and coronary arteries using an endovascular light source. Depletion of medial vascular smooth muscle cells (VSMC) was seen at 3 and 14 days and was found to be partially dependent on the drug-light interval. Finally, iliac and coronary arteries were balloon injured and then treated with PDT or sham illumination. Histomorphometric studies following harvest at 28 days showed less NIH and less negative remodelling in the group treated with PDT.

Clinical project

A clinical pilot study of angioplasty with adjuvant PDT was commenced. Patients deemed to be at high risk of restenosis underwent femoral angioplasty followed by endovascular PDT and were followed up by duplex and digital subtraction angiography at 6 months. The results of this small study would suggest that PDT is successful in inhibiting restenosis, but this now needs to be confirmed in a randomised controlled trial.

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This work is dedicated to my wife

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ABBREVIATIONS

ABPI	Ankle-brachial pressure index
ACE	Angiotensin converting enzyme
ALA	5-aminolaevulinic acid
AlS_2Pc	Aluminium di-sulphonated phthalocyanine
Ang I	Angiotensin I
Ang II	Angiotensin II
ANOVA	Analysis of variance
b FGF	Basic fibroblast growth factor
Cx	Circumflex coronary artery
DHE	Di-haematoporphyrin ether
ECM	Extracellular matrix
EEL	External elastic lamina
EVG	Elastin van Gieson
H&E	Haematoxylin and eosin
HPF	High power field
HPD	Haematoporphyrin derivative
IEL	Internal elastic lamina
IQR	Interquartile range
iv	Intra-venous
iv DSA	Intra-venous digital subtraction angiogram
IVUS	Intravascular ultrasound
MI	Myocardial infarction
MMP	Matrix metalloproteinases
MRA	Magnetic resonance angiography
NIH	Neointimal hyperplasia
NLB	Nuclei lysis buffer
NO	Nitric oxide
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction

PDGF	Platelet derived growth factor
PDT	Photodynamic therapy
POBA	Plain old balloon angioplasty
PpIX	Protoporphyrin IX
PSVR	Peak systolic velocity ratio
PTA	Percutaneous transluminal angioplasty
PTCA	Percutaneous transluminal coronary angioplasty
SFA	Superficial femoral artery
SLB	Sucrose lysis buffer
TGF-β	Transforming growth factor β
TIMP	Tissue inhibitors of matalloproteinases
VEGF	Vascular endothelial growth factor
VSMC	Vascular smooth muscle cell

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CHAPTER 1 ATHEROSCLEROSIS AND ANGIOPLASTY

1.1 ATHEROSCLEROSIS

1.1.1 Introduction

Atherosclerosis has existed as a named entity for less than a century (Marchand, 1904), but a pathological process akin to atherosclerosis is known to have occurred in the time of the pharaohs of Egypt (Shattock, 1909 and Ruffer, 1911). During a symposium at the Royal Society of Medicine in 1960, Crawford defined atherosclerosis as:

"...the widely prevalent arterial lesion characterised by patchy thickenings comprising accumulations of fat and layers of collagen-like fibres, both being present in widely varying proportions."

This morphological definition holds true today, but perhaps fails to make clear the fact that although atherosclerosis is widespread in anatomical terms, its pattern of distribution is predictable. Although it may affect all arteries down to a diameter of 2mm, it tends to remain localised to precise regions, namely: the carotid, coronary and first few centimetres of the mesenteric arteries, the abdominal aorta, iliac and lower limb arteries. This distribution manifests itself in the well known clinical entities of cerebrovascular, peripheral vascular and ischaemic heart disease, which together constitute the main cause of death in almost half the people dying in the Western world today.

1.1.2 Prevalence and Mortality

One of the first population studies to assess the prevalence of both peripheral and coronary artery disease amongst apparently asymptomatic subjects was the Basle study. This documented the prevalence of each condition amongst 6,400 employees at four pharmaceutical companies (Widmer et al, 1964). In the 50-59 year age group, up to 3% and in the 60-69 year group, 5% of men showed

signs of lower limb atherosclerosis with a similar proportion showing signs of heart disease. In the UK the Whitehall study of 18,388 civil servants over the age of 40 yielded rates of 1% with claudication and 5% with angina (Reid et al, 1974) and although both studies could be criticised as to the rigour applied to the assessment, similar figures have since been reported in more objective studies (Hughson et al, 1978; Fowkes et al, 1991).

Cardiovascular disease accounts for more deaths in England and Wales than deaths from all types of cancers combined. The 1995 report from the Office for National Statistics (formerly the Office of Population Censuses and Surveys) revealed that diseases of the circulatory system accounted for the deaths of 243,390 individuals in England and Wales. This figure represented 43% of all deaths occurring in that year (The Office for National Statistics, Mortality statistics, 1995). Of these, the majority died from ischaemic heart disease (55%) or stroke (25%) with deaths solely attributable to peripheral vascular disease constituting a small percentage. It is important to appreciate however that the three diseases often coexist, a fact borne out by the expected mortality of patients with the most benign manifestation of peripheral vascular disease - intermittent claudication. From studies in a number of countries it has been estimated that the expected 5, 10 and 15 year mortality for a claudicant is 30, 50 and 70% respectively (Eickhoff, 1978; Reunanen et al, 1982; McDaniel and Cronenwett, 1989; Davey Smith et al, 1990). This is only slightly more optimistic than Allen's original estimate that half of all claudicants would be dead within 5 years (Allen et al, 1955). Moreover it has been shown that severe coronary artery disease (detected angiographically and requiring surgical revascularisation) is present in approximately 30% of patients undergoing surgery for peripheral vascular disease (Hertzer et al, 1984) and remains the commonest cause of death amongst claudicants accounting for between 40-60% of all deaths in this group (Bloor, 1961; Kannel et al, 1970; Reunanen et al, 1982).

The American Heart Association has estimated that 1 out of every 4.6 deaths in the USA in 1993 was as a result of coronary artery disease (Stone, 1996). The estimated annual cost of treating heart disease in the USA has spiralled from $128 billion in 1994 to >$151 billion in 1996 and is still rising (Stone, 1996). However, demographic data from the USA suggest that the mortality from atherosclerosis has reached a peak and is now on the wane (Kannel and Thom, 1984; Stone, 1996). The reasons for this remain obscure and with an ageing population, the treatment of atherosclerosis and its associated diseases will remain one of the major health challenges and resource allocations into the next millennium.

1.1.3 Pathology

The atheromatous plaque which constitutes the macroscopic manifestation of atherosclerosis ('athere', porridge and 'sclerotic', hard) is focal, raised above the intima and consists of a lipid-rich soft core covered by a harder fibrous cap. Microscopically, the soft core is seen to contain proliferating VSMCs, lipid-laden foam cells, extracellular lipids, cholesterol crystals and areas of necrosis.

The origin of atheromatous plaques is unclear and although lipid deposits in the form of fatty streaks are widespread within the aorta of children at autopsy, they do not correlate with the distribution of atheromata in later life. Furthermore, fatty streaks are observed in infants from a wide variety of races and geographical locations in marked contrast to the geographical distribution of atherosclerosis in later life which is predominantly a disease of the Western world. It is likely that some fatty streaks regress whilst others progress to mature atheromatous plaques and studies in primates (Faggiotto et al, 1984) would suggest that a high cholesterol diet is important in this process which may explain the geographical discordance between the two lesions.

In adult life atherosclerosis may be isolated, but is more likely to be widespread and tends to have a predilection for certain sites which is manifest in the

clinical presentation of a number of clinical conditions, all of which are secondary to atheromatous plaques at different anatomical sites. The most commonly affected regions are the:

- Circle of Willis
- Vertebral and carotid arteries
- Coronary arteries
- Thoracic aorta
- Abdominal aorta and its visceral branches
- Iliac, femoral, popliteal and crural arteries,

which are affected by aneurysmal and occlusive disease to varying degrees.

1.1.4 Clinical manifestations

The basic atheromatous plaque can lead to three distinct pathological processes: lumenal stenosis, embolisation and aneurysm formation and although all plaques are capable of all three, the degree to which one predominates and its clinical relevance varies between anatomical sites. For example, in a large elastic artery such as the aorta, aneurysm development is far commoner than stenosis, but in the coronary circulation stenosis is of prime importance in the aetiology of ischaemic heart disease whereas it is embolisation from the internal carotid artery which is responsible for the development of some strokes. Even amongst a given pathology, its site may alter the importance of the clinical sequelae as evidenced by the fact that an aneurysm of the abdominal aorta tends to present by rupturing whilst an aneurysm of the popliteal artery tends to give rise to symptoms by embolising or thrombosing.

Perhaps the commonest manifestation of atherosclerosis in terms of need for treatment is the raised plaque which gives rise to lumenal narrowing and hence reduction in the flow of blood distal to the plaque. This gives rise to relative

ischaemia of the end organ at times when a greater blood flow is needed, familiar to cardiologists as angina on exercise, and to vascular surgeons as intermittent claudication. An increase in the severity of lumenal narrowing gives rise to constant symptoms at normal flow rates; so called unstable angina and critical limb ischaemia. Depending on the presence of a collateral circulation or not, total occlusion will lead to infarction of the end organ, which is the inevitable result in end arteries such as the coronaries. Although the process as outlined above usually occurs chronically, thrombosis consequent on plaque rupture may lead to acute occlusion of a vessel (Davies and Thomas, 1984) and therefore myocardial infarction for example, could be the first manifestation of coronary artery disease.

1.1.5 Risk factors

From the early interim analysis of results in the Framingham study, it was obvious that certain factors were related to an increase in cardiovascular events: smoking, hypertension and hypercholesterolaemia (Kanel and Shurtleff, 1971). Later analysis revealed the strong association between diabetes and claudication and to a lesser extent ischaemic heart disease (Kannel and McGee, 1979). The above risk factors have since been confirmed by many groups including the Edinburgh Artery Study (Fowkes et al, 1992) and numerous other risk factors identified: plasma viscosity, fibrinogen and homocysteine (Graham et al, 1997), water hardness, obesity, exercise, family history and alcohol intake (Fowkes, 1996). Underlying risk factors include age and the male sex (a five-fold preponderance), but the difference between the sexes seems to become less important with increasing age (Lindop and Dargie, 1992). However, the number of males living into the ninth decade is much smaller than the number of females, and may represent a selected group which is not strictly comparable, therefore artificially skewing the sex ratio data for this age group.

1.1.6 Therapeutic Interventions

1.1.6.1 Prevention

Prevention of advanced disease by early modification of risk factors is the logical conclusion from knowledge gained from the above epidemiology studies. Unfortunately, some risk factors cannot be modified (age, sex, and family history) and the single most modifiable factor, smoking, despite patient education, remains a habit amongst the majority of claudicants seen in arterial clinics. At present, optimisation of blood pressure, control of diabetes, dietary advice and weight control provide the mainstay of preventative medicine in atherosclerosis. Aspirin, although not a treatment for atheroma per se, by virtue of its anti-thrombotic properties, has become widely prescribed for both coronary and peripheral vascular patients.

Recently, control of serum lipid levels has become possible using a new group of drugs, the statins. In a large scale randomised double blind trial of 4444 patients with ischaemic heart disease, lowering serum cholesterol by 25% resulted in a reduction in all-cause mortality (with a relative risk of death of 0.70) in the group taking simvastatin (Scandinavian Simvastatin Survival Study Group, 1994). Within the study group, the relative risk of a coronary death was 0.58 and there was a 37% reduction in the need for coronary revascularisation procedures. This data, together with increasing evidence that hypercholesterolaemia may be the most important risk factor above all others (Sniderman et al, 1997), may allow the real possibility of prevention of atherosclerosis in the future.

1.1.6.2 Treatment

Treatment has two main objectives: to control symptoms and limit disease progression. Conservative treatment concentrates on drugs to reduce the workload of the heart and reduce peripheral resistance in patients with angina, and exercise programmes for claudicants.

In the peripheral vascular system surgical intervention has been the mainstay of treatment of aneurysmal disease (exclusion), symptomatic severe internal carotid artery stenosis (endarterectomy) and occlusive disease (bypass). Over the last decade or so, occlusive disease of the iliac and femoral arteries has increasingly been treated by percutaneous transluminal angioplasty (PTA) and recently, aortic aneurysm by endovascular repair. Angioplasty is less invasive than open surgery, is better tolerated by the patient and has the advantage of not requiring a general anaesthetic or an inpatient stay. In selected cases the early results are comparable with bypass, but long-term, angioplasty requires a higher rate of reintervention to maintain patency. It would be naive however, at least in the periphery, to consider PTA and bypass surgery as competitors, as they both have a place in the treatment of symptomatic peripheral artery occlusive disease.

Likewise, in coronary artery disease, although bypass surgery and PTA may be equally therapeutic in the short term, meta-analysis of randomised trials has shown that patients undergoing bypass surgery were less likely to require reintervention at one year (Pocock et al, 1995).

1.2 PERCUTANEOUS TRANSLUMINAL ANGIOPLASTY

1.2.1 History

In 1963, Charles Dotter inadvertently performed the first percutaneous passage through an occluded artery when he passed a catheter retrogradely through an iliac artery in order to perform a diagnostic aortogram. However, he had the forethought to see the technique's potential for therapeutic purposes as can be seen from his comments on the matter at the time:

"Perhaps it is wishful thinking, but in any event I am convinced that the relief of the atheromatous obstruction in small arteries can best be accomplished by catheter techniques. A flexible guide introduced percutaneously into an artery proximal to an area of atheromatous narrowing can be manipulated so as to traverse the obstruction. A mechanical attack upon the lesion would then become feasible, perhaps by gradual direct dilatation....." (Dotter, 1965).

Soon after this, Dotter performed the first intentional arterial dilatation using a system of coaxial catheters and after 11 cases published his results (Dotter and Judkins, 1964). Although largely ignored in the United States, the technique was used and refined throughout Europe with variable success. The blunt catheter tip was modified to a tapered design by Staple (Staple, 1968), but the catheter shaft remained stiff and inflexible which made crossing tortuous vessels both difficult and dangerous. The next major advance came in the shape of a balloon expandable device which increased flexibility and reduced the necessary catheter circumference and hence arteriotomy size (Porstmann, 1973).

However, it is Andreas Gruntzig who should be singled out as being responsible for transforming angioplasty from a haphazard and variable procedure to the reliable and safe technique we know today. Working closely with a fledgling medical instrument manufacturer, Schneider, Gruntzig

developed a double lumen balloon, initially made of polyvinyl chloride and later, less elastomeric, polyethylene (Gruntzig and Hopff, 1974). Gruntzig was a true pioneer and very soon moved on to dilatation of coronary arteries (Gruntzig 1978), which prompted the USA to take notice and rapidly accept the technique: percutaneous transluminal coronary angioplasty (PTCA) was born.

1.2.2 Technique and Uses

Today's procedure owes as much to advances in technology as to improved techniques. It is based on a percutaneous arterial puncture technique described by Seldinger, (Seldinger, 1953) in which a guidewire is passed through the core of a needle used to puncture the artery. Access to the arterial system is gained usually via the femoral artery at the groin, or via a cutdown to the brachial artery should the groins be hostile to puncture. A sheath with a self-sealing port is introduced 'over the wire' and a soft-tipped guidewire advanced in an antegrade or retrograde direction under fluoroscopic control until the stenosed segment or occlusion is crossed. Following systemic heparinisation, a balloon catheter, appropriately sized for the artery in question is introduced over the wire through the sheath and manipulated until its radio-opaque marker corresponds to the stenosed area. It is inflated with a dilute solution of contrast in order that deployment can be visualised and 'waisting' of the balloon seen as it conforms to the shape of the stenosis. Following the procedure, completion angiography is performed to assess the result and distal circulation. Unless otherwise contraindicated patients are prescribed an anti-platelet drug, usually aspirin, for life.

Adaptation of the above technique has occurred to suit the particular needs of certain anatomical areas. For coronary angioplasty, a guide-catheter is positioned within the aortic root and balloon catheters directed by guidewires as small as 0.014 inches advanced into the coronary artery to be treated. Steerable and high-torque guidewires have made manipulation through tortuous arteries both safer and more successful.

It is probably true to say that PTA has been attempted in almost every artery in the body, but is now established in:

- Coronary arteries
- Subclavian arteries
- Iliac, femoral and popliteal arteries
- Renal and mesenteric arteries
- Vein graft and anastomotic stenoses

and although used, is not yet substantiated in the treatment of internal carotid and crural vessel disease.

A recent advance has allowed the recanalisation of long occlusions of the superficial femoral artery (SFA), which traditionally have been outwith the scope of conventional PTA. Subintimal angioplasty involves manipulation of the guidewire from the lumen into the subintimal space proximal to an occlusion, traversing the occlusion within this space, and then re-entering the lumen distally. The occluded segment is then ballooned to create a new lumen, which is continuous with the native lumen proximally and distally. Although some have claimed impressive results (Bolia et al, 1990) and used the technique to salvage cases which have been complicated by perforation (Nasim et al, 1996), its current use is limited to one or two centres and confirmation of its reproducibility is awaited.

1.2.3 Mechanism of PTA

Originally, it was assumed that angioplasty merely compressed the atheromatous plaque against the sidewall of the artery, thus enlarging the lumen. However, it has since been established by post mortem studies lead by Fogarty's group, that the true mechanism involves plaque rupture and vessel wall disruption with simple compression accounting for only 1-1.5% of the effect (Kinney et al, 1984). Histological studies have shown that dehiscence of

the intima from the media occurs and at the point of maximal dilatation, limited arterial dissection is evident (Zarins et al, 1982). These findings have since been confirmed by a recent post mortem study involving 130 patients who died between one day and one month post PTCA (Waller et al, 1996) and by in vivo intra-vascular ultrasound imaging (IVUS) (Tenaglia et al, 1992). This technique has greatly increased our understanding of the morphological events occurring after angioplasty.

1.2.4 Results of PTA

Before considering the results of PTA, one has to define the end point against which the outcome is judged. Unfortunately, the literature is riddled with different assessments of PTA outcomes; results are often not presented as a life table analysis; and all-comers not included in an intention-to-treat manner. The peripheral vascular literature is further disadvantaged by inadequate follow up, often relying on subjective and clinical outcome parameters, whereas angiographic follow up is the norm following PTCA. This probably reflects the lack of an 'end organ' for cardiologists to assess and the notorious difficulties in correlating chest pain with ischaemia. However in the periphery, clinicians have been tempted to attach too much importance to the presence or absence of pulses in the 'end organ' (foot) and the measurement of claudication distance. Both these parameters are affected as much by changes in the inflow and run off vessels as they are by changes at the PTA site.

In the early 1990's it became obvious that it was impossible to compare results from different series, which prompted the Standards of Practice Committee of the Society of Cardiovascular and Interventional Radiology (1990) and Robert Rutherford to lay down guidelines for reporting the results of peripheral PTA (Rutherford, 1991; Rutherford and Becker, 1991). The following recommendations were made:

1. Patency should be defined according to the need for re-intervention. *Primary patency* was described as a period of uninterrupted patency without further intervention. *Secondary patency* of a procedure was defined as the final outcome following one or more re-interventions necessary to restore blood flow subsequent to occlusion of the original PTA or graft. An intermediate classification of *Assisted primary patency* was included to accommodate the situation whereby a failing PTA or graft was diagnosed (often by duplex surveillance) and its patency maintained by timely intervention.

2. Patients described in any series should include the whole cohort treated within the time span studied in an intention-to-treat manner without exclusion of initial technical failures and deaths from the final results.

3. Patients should be classified according to their presenting symptoms:

Grade I	Mild to severe claudication
Grade II	Ischaemic rest pain
Grade III	Tissue loss.

4. Outcome measures should conform to certain objective assessments of patency including one or more of the following: angiography, duplex and maintenance of improvement in the segmental pressure index.

1.2.4.1 Iliac PTA

Iliac angioplasty is perhaps the least disputed indication for a percutaneous procedure due to the good long term results and favourable morbidity in comparison with the surgical alternative - aorto-bifemoral bypass. Initial technical success rates approach 90%, but are lower for occlusions compared with stenoses, a difference which is amplified after 5 years of follow up (Johnston et al, 1987). The other variables which have been found to favourably influence the outcome are site of PTA (common rather than external); the presence of good runoff; the absence of a procedural

complication; one dilatation rather than many; and the absence of diabetes (Johnston et al, 1987).

Table 1.1 summarises the results of some of the more comprehensive series in the literature. It is instantly apparent that patency rates at 5 years for treatment of stenotic disease vary between 92% at best and 53% at worse. This is more likely to represent differences in assessment of outcome and inclusion of all patients on an intention-to-treat basis than real differences in PTA outcome.

1.2.4.2 Femoro-popliteal PTA

For the vast majority of reported series, this means PTA of the superficial femoral artery (SFA). Compared with iliac PTA, the initial technical success rate is lower and the overall long term result worse: from 8 series since 1981, cumulative patency at 3 years in the iliac segment was 75% compared with 62% in the femoral (Eikelboom et al, 1992). In most series, stenoses respond better than occlusions, with long occlusions (>15cm) doing particularly badly. This has prompted some (Eikelboom et al, 1992) to conclude that long occlusions should be treated surgically, but with on going technical advances, this statement may now be viewed as conservative. Although Vroegindeweij reports better results than most with a 44% 3 year patency rate following intervention in 62 patients with femoral occlusions, the definition of restenosis used was a "peak systolic velocity ratio greater than or equal to 3.0" which probably underestimates the true incidence of restenosis (Vroegindeweij et al, 1997). Table 1.2 illustrates the outcome of PTA in the SFA segment including the most recent series which shows that stents at this location do not improve patency rates (Gray et al, 1997).

1.2.4.3 Coronary PTA (PTCA)

As in the periphery, most series conclude that results are better for short discrete stenoses than long occlusions and results from the treatment of single vessel disease are slightly superior to those of multiple (Pocock et al, 1995).

Further analysis of results between series is difficult because, despite the fact that the majority of series report patency rates based on angiographic follow up, the definition of patency and restenosis varies. When Beatt reviewed the reporting of results of PTCA in 1994 he highlighted such inconsistencies in the literature by finding 6 different definitions of restenosis used in 11 reported series (Beatt KJ et al, 1990). Moreover, the constraints imposed by angiographic assessment mean that follow up periods are short, the majority limited to less than 6 months. Table 1.3 summarises the results of the largest reported series.

1.2.5 Complications of PTA

Complications occur either at the PTA site, the distal circulation or the arterial puncture site, the latter being common to both peripheral PTA and PTCA. The overall complication rate following 9627 peripheral PTA's as reviewed by Eikelboom (Eikelboom et al, 1992) was 7.7% minor and 4.1% major, with 2.5% requiring surgery. In the largest single series assessing the need for acute surgery, 123 (2.8%) of 4380 PTA's resulted in surgery (Fraedrich et al, 1987). Of these, 69% were related to problems at the puncture site (haematoma and false aneurysm), 26% to PTA site problems (thrombosis and perforation) and 15% were secondary to distal embolisation. PTA is however a safe procedure - the overall procedural mortality rate from 6 individual series comprising the 9627 patients mentioned above was 0.28% (Eikelboom et al, 1992). Long-term complications include progression of disease and restenosis, which will be discussed in detail in Chapter 2.

PTCA has a similar number of puncture site complications, but a higher percentage of major complications and a higher mortality rate that reflects the more serious consequence of dilatation site problems in the heart. In a review of the progress of PTCA, Landau documented a myocardial infarction rate of 3-5%; need for urgent bypass surgery rate of 3-7%; and mortality rate of 0-2% (Landau et al, 1994). Abrupt vessel closure due to dissection, thrombosis or

vasospasm accounts for the majority of the early morbidity and mortality. In the last few years, an adjunct to PTCA, stenting, has revolutionised the management of abrupt vessel closure, but also encouraged cardiologists to tackle more and more difficult lesions. The incidence of abrupt vessel closure has recently been shown to be significantly reduced by blockade of the platelet glycoprotein IIb/IIIa receptor (EPILOG Investigators, 1997) which should further impact on the acute complication rate after PTCA.

1.2.6 Alternative and Adjunctive Percutaneous Procedures

The profusion of new devices and recent vogue for using stents in the majority of PTCAs has lead to the relegation of the term PTA to POBA (plain old balloon angioplasty) amongst many authors.

1.2.6.1 Stents

Although described as long ago as 1983, (Dotter et al, 1983 and Cragg et al, 1983) it is only over the last decade that stent use has become widespread. However, their perceived efficacy is such that it has recently been estimated that up to 80% of all coronary artery dilatation procedures performed in the UK and Europe in 1997 involved stents (Horrigan, 1997). Stenting was originally conceived with two main aims in mind: to provide an immediate repair of severe dissections to circumvent acute vessel shutdown and to reduce long term restenosis. It has now been employed as a prophylactic measure for difficult lesions and to achieve satisfactory post-procedural results where POBA alone was limited.

Stents can be classified according to their mechanism of action into two basic forms: balloon expandable and self-expanding. The former rely on a radial force produced by balloon inflation to deploy them and once deployed are relatively rigid and are capable of resisting radial forces. There are now many types on the market, but most experience has been gained with the Palmaz

(Johnson & Johnson, New Jersey, USA), Strecker (Medi-Tech, Mass. USA) and the Gianturco-Roubin stents (Cook, Indiana, USA). Self-expanding stents depend either on the resumption of a certain configuration triggered by a thermal memory at body temperature or by a spring mechanism consequent on being unloaded from a constraining sheath. The Wall stent (Medinvent SA, Lausanne, Switzerland) has found a wide application in both iliac and coronary segments, its major advantage being the ease of deployment and its inherent longitudinal flexibility. Nitinol (nickel titanium naval ordinance laboratory) has a thermal memory to which it conforms at body temperature and several manufacturers have exploited this unique property to construct stents that expand only when placed within the body cavity.

The efficacy of stenting is now undisputed in iliac (Sullivan et al, 1997) and coronary lesions (Serruys et al, 1994), but is limited in the SFA (Gray et al, 1997) due to thrombotic problems and restenosis. It is likely that the poor performance in this location is secondary to the reduced flow rate in the SFA compared with other arterial segments because size alone would not account for the difference as coronary stents remain patent at much smaller diameters. Despite the impressive results of stenting and their undeniable usefullness acutely, their impact on long term restenosis rates remains limited and 'in-stent restenosis' is an increasing problem. Moreover, there are many trials to assess the safety and efficacy of individual stents and comparisons between stents, but none that address important issues such as which lesions and which patients should be stented (Serruys and Kutryk, 1996).

1.2.6.2 Recanalisation Devices

Such devices were proclaimed to allow occlusions to be crossed which were resistant to the passage of a simple guidewire. Unfortunately, both the Kensey and Rotacs devices were complicated by a high perforation rate and have now become unpopular. Laser probes (hot tips) which work either by their direct thermal effects or by a combination of thermal and direct laser energy were

similarly disadvantaged by arterial perforation and failure to penetrate calcified plaques. In a controlled trial between laser and balloon recanalisation of occluded femoral segments, laser devices were found to be no more effective than guidewires and balloons alone (Jeans et al, 1990).

One of the problems of laser and other recanalisation methods is that the new channel created was insufficiently large on its own. Therefore, adjunctive balloon dilatation was also necessary and its inherent injurious nature lead to high restenosis rates. Advances in guidewire design including hydrophilic coated and steerable wires have allowed complex lesions to be crossed safely and rendered many mechanical and thermal recanalisation devices obsolete. Although the rise and fall of the laser as an important player in the mainstream treatment of cardiovascular disease has already been expertly reviewed (Cross, 1992; Mitchell et al, 1994), it may still have a role under certain favourable circumstances.

1.2.6.3 Atherectomy Devices

Plaque debulking prior to, or instead of, POBA offers theoretical advantages especially in the treatment of calcified and eccentric lesions. Many devices exist on the market including the Simpson AtheroCath, Auth Rotablator, Transluminal Extraction Catheter (TEC), Trac-Wright Catheter and Excimer Laser, all of which offer impressive immediate radiological results with difficult lesions. The introduction and initial investigation of these devices was heralded with much promise (Waller, 1989), but the long and medium term results have been dismal (Ahn and Concepcion, 1996). Both the CAVEAT (Coronary Angioplasty Versus Excisional Atherectomy Trial) (Elliott et al, 1995) and a recent randomised trial of excimer laser, rotablation and balloon angioplasty for complex coronary lesions (ERBAC study), have shown no benefit in late outcomes compared with angioplasty alone (Reifart et al, 1997). In the periphery, many randomised trials have shown no additional benefit from

excimer laser ablation (Lammer et al, 1992) or hot tip laser ablation (Fisher et al, 1996) over conventional angioplasty alone.

Trials of more aggressive debulking are awaited, but it is unlikely that they will offer any superior long-term performance and may well increase the acute complication rate.

1.2.6.4 Laser Balloon Angioplasty

True laser balloon angioplasty as opposed to other devices using laser energy, refers to a balloon which transmits laser energy in an attempt to weld dissection flaps against the arterial wall. It was proposed that this would seal any intimal dissection which would provide a smoother luminal surface less prone to restenosis (Spears, 1987). However, this has not been borne out in experimental studies. In a rabbit iliac model, histology following laser angioplasty showed evidence of sealing in only 1/10 arteries and although there was a trend in improved lumenal diameter in the laser group (postulated to be secondary to abolition of elastic recoil), this improvement was not significant (Alexpoulos et al, 1994). Despite initial enthusiasm and satisfactory acute results, its long-term efficacy has not been realised in clinical practice (Reiss et al, 1991).

1.3 SUMMARY

Atherosclerosis is one disease but by affecting the whole arterial tree becomes multi-organ necessitating management by individual specialities. Population studies have identified risk factors, but the relative importance of individual factors and how they interact remains to be elucidated. Although the incidence in the Western World is declining, it remains one of the biggest killers of our time.

Future treatment must be directed at primary prevention, but at present a large sector of the population requires treatment for established disease. PTA has revolutionised the treatment of occlusive atherosclerotic disease, offering day case treatment with a lower morbidity than, and equivalent short-term results to, open surgery. With the exception of stents, newer devices have failed to show any appreciable long-term advantage over conventional balloon angioplasty, the efficacy of which however, is limited by restenosis.

Author	Year	No. Patients	No. PTA's	% Stenosis	% Occlusion	% Critical	Technical success %	% Stented	Assessment	Intention to treat	% Patency(yrs) 1	3	5	7
van Andel	1980	48	51	100	0	0	100	0	ABPI	No		88		
Spence	1981	131	160	100	0	14	93	0	Clinical	No		79		
van Andel	1985	154	194	100	0	10	96	0	Clinical / ABPI	No	98	94	92	90
Johnston	1987	902	684	81	-	}13	99	0	ABPI	Yes			53	
				-	19		82						39	
Sullivan	1997	288	510	90	10	28	89	100	ABPI	No	93			

Table 1.1 Major series reporting results of iliac angioplasy

Author	Year	No. Patients	No. PTA's	% Stenosis	% Occlusion	% Critical	Technical success %	% Stented	Assessment	Intention to treat	% Patency(yrs) 1	2	3	5
Greenfield	1980	70	70	-	-	55	81	0	ABPI	No			84	
Martin	1981	46	67	0	100	54	76	0	Clinical / ABPI	Yes	46			
Krepel	1985	129	164	77	23	10	84	0	ABPI / Angio	No	81			70
Murray	1987	162	193	60	-	}34	}81	0	ABPI	No *	72			54
				-	40						86			73
Capek	1991	152	217	-	23	26	80	0	ABPI/ Angio	No	81	61		58
Johnston	1992	236	254	62	38	20	96	0	ABPI	Yes	63	50		38†
Matsi	1994	106	208	66	34	0	88	0	ABPI	Yes	47	42†		
Stanley	1996	176	200	53	47	26	93	0	ABPI	Yes	46†			
Gray	1997	55	131	11	89	50	-	100	Duplex / Angio	Yes	22†			
Vroegindeweij	1997	62	62	0	100	-	-	0	Duplex / Angio	Unclear			44°	

Table 1.2 Results of angioplasty in the femoro-popliteal segment. *** = 67% Follow up ° = PSVR > 3**

† =Primary patency

Author	Year	No. Patients	% Angio Follow up	Restenosis criterion	% Restenosis (months)
Holmes	1984	665	84	NHBLI 4	34 (6)
Leimgruber	1986	1758	57	NHBLI 4	30 (7)
Serruys	1988	400	85	> 0.72mm	26 (4)
Nobuyoshi	1988	229	96	NHBLI 4	43 (3)
Arora	1990	723	93	-	41 (4)

Table 1.3 Coronary angioplasty.

NHBLI = National Heart, Lung and Blood Institute

CHAPTER 2 RESTENOSIS

2.1 INTRODUCTION TO CLINICAL RESTENOSIS

From Chapter 1, it is evident that PTA is a successful treatment for coronary and peripheral atherosclerotic stenosing lesions. Durable efficacy is however limited, and in over one third of cases restenosis occurs within six months. Restenosis is the subject of this thesis.

2.1.1 Assessment and Definition

Restenosis is a mechanical obstruction to flow that occurs after an initially successful vascular intervention. It occurs following angioplasty, stenting and surgical bypass and its incidence varies depending on the method of detection. An objective definition is difficult as many authors report their restenosis rates based on different criteria and follow up regimens. In general, follow up after coronary intervention tends to be angiographic, but limited to six months, whereas after peripheral intervention, assessment is often clinically based, but may continue for years.

A quoted incidence of restenosis is therefore irrelevant without specifying the method of assessment and the definition employed. Unfortunately, most definitions tend to be arbitrary, and are inevitably specified at a given time point of follow up. Therefore an ongoing dynamic process is expressed in terms of whether it attains a certain criterion at a given time point. The assessment and definition of restenosis will therefore be considered together.

2.1.1.1 Peripheral Restenosis

Assessment of the peripheral vascular system lends itself to more subjective clinical methods of evaluation when compared with the coronary circulation. Consequently, assessment is less invasive and therefore applicable to a larger

number of patients for a longer duration even outside the scope of clinical trials. It is however, less accurate.

Clinical evaluation. This involves documentation of symptoms (essentially walking distance for claudicants) and examination of peripheral pulses. Walking distance as a method of monitoring restenosis has the advantage that it has traditionally been used as a benchmark of severity of claudication, often with a cut off of <100m determining the need or otherwise for initial intervention. Despite all the criticisms levelled at this rather artificial approach, not least the inaccuracy of estimating walking distance (Watson et al, 1997), as a means of assessing restenosis for a given individual, it has some merit as it offers a longitudinal measurement and expresses the need for re-intervention based on symptomatic criteria.

Likewise the palpation of pulses distal to the treated arterial segment. It is clear that if an absent pulse prior to treatment, returns following treatment and then disappears on follow up, a further event has occurred within that arterial segment. What is not clear however, is whether this event has occurred at the PTA site or represents a new lesion within the same segment or one influencing either inflow or run-off. Moreover, this is a subjective assessment and the ability of clinicians to palpate pulses varies and tends only to be accurate in differentiating between a patent and an occluded segment. It is likely that methods based solely on clinical criteria greatly underestimate the true incidence of restenosis.

In an attempt to introduce some reproducibility, quantitative pressure measurements based on the ratio of the lower and upper limb systolic blood pressure have been used. The ABPI (ankle-brachial pressure index) or more specific segmental pressure index allows the documentation of an objective measurement. A rise in the ABPI of >0.1 is regarded as a mark of a successful PTA and therefore restenosis in this context can be defined as a fall by >0.15 from the immediate post-treatment value (Rutherford and Becker, 1991). The

sensitivity of such measurements can be increased by comparing pre- and post-exercise values, but this is often limited by the patient's cardiopulmonary reserve. Regardless of their objectivity and reproducibility, the major disadvantage of the above whole limb assessments is that they are not lesion specific and are therefore unable to differentiate between restenosis at the angioplasty site and new disease elsewhere.

Duplex. Duplex has the advantage of being able to localise the angioplasty site and quantify the degree of stenosis. Duplex is a non-invasive test which involves the integration of a pulsed Doppler signal with B-mode ultrasound which gives haemodynamic information on flow and visualises the lesion in question. Duplex is thus able to demonstrate a stenosis (or restenosis) by showing narrowing of the lumen (in transverse or longitudinal format) and by estimating the alteration in flow velocity consequent to the stenosis. This is achieved by considering the change in the arterial waveform and increase in the peak systolic velocity either side of a stenosis. In practice, it is the ratio of the peak systolic velocity (PSVR) across the stenosis and that proximal to the stenosis which is used to estimate the degree of stenosis. A PSVR of >2 with loss of the reflux wave represents a stenosis (or restenosis) of >50% and is the most commonly used criterion to define a lesion as significant (Jäger et al, 1996).

It must however be emphasised that duplex is user-dependant and the accuracy obtained depends on the skill and experience of the sonographer. Nevertheless, excellent sensitivity and specificity figures were reported even from early studies (Kohler et al, 1987; Moneta and Strandness, 1987) and these now exceed 90% compared with the gold standard of angiography (Aly et al, 1998a). The limitations of duplex are now also established and signal fall off due to calcified lesions and at certain anatomical locations (for example the SFA at the adductor hiatus) appreciated. Although once regarded as being inaccurate in the face of multiple stenotic lesions, new evidence suggests that

duplex can attain a sensitivity and specificity of greater than 90% even with stenotic disease within more than one anatomical segment (Aly et al, 1998c).

Recently, advances in resolution have enabled colour-coded duplex to quantify plaque morphology and differentiate between primary atherosclerotic and restenotic lesions (Baumgartner I et al, 1996a). Plaque characterisation is an exciting prospect as the natural progression of this is the ability to classify lesions as high or low risk for subsequent event (Aly et al, 1998b). Such a concept could revolutionise the treatment of asymptomatic carotid artery disease.

Nyamekye et al have stressed the importance of visualising the angioplasty site when assessing limbs for evidence of restenosis (Nyamekye et al, 1996b). The objectivity of this is unequivocal especially when used to evaluate the results of a new vascular intervention, but its clinical usefulness must be questioned. Demonstrating restenosis per se is different from demonstrating the need for re-intervention due to symptomatic restenosis which has been shown to be no better predicted by duplex than ABPI and clinical modalities (Tielbeek et al, 1996). Similar conclusions have now also been reached from graft surveillance programmes which result in better patency rates, but do not seem to influence amputation rates (Golledge et al, 1996; Beattie et al, 1997). However, a recent duplex method of estimating collateral blood flow may be of benefit in predicting which patients are likely to be symptomatically compromised should restenosis occur (Hussain et al, 1996).

Angiography. Peripheral arteriography has long been regarded as the "gold standard" investigation for arterial disease. It is a user-friendly medium presenting a map of arterial disease and allows easy comparison of before and after views. However, its usefulness as a follow up examination to detect restenosis is limited by its invasiveness. It is also disadvantaged by the difficulty in detecting eccentric lesions with a single plane view and, without pressure measurements, represents a purely anatomical assessment.

Restenosis may be defined angiographically in terms of percentage diameter stenosis. The 3 commonest definitions in use are:

- Loss of 50% of the gain in diameter achieved by angioplasty
- Greater than 50% stenosis (when PTA resulted in <50% residual stenosis)
- An increase in percentage diameter stenosis from the post PTA value by 30%.

Such definitions may however give a false representation of specificity as the degree of stenosis is estimated by reference to an adjacent artery that may not be entirely normal. Moreover, the calculation assumes the stenosis to be concentric and is therefore inaccurate when applied to eccentric lesions. One of the commonest used definitions, (>50% stenosis) better defines the significance of a de novo lesion than restenosis. It is based on historical physiological data from animal coronary experiments where this value was found represent blunting of the hyperaemic response (Gould and Lipscombe, 1974).

For comparison at different time points arteriograms must also be standardised for the volume and rate of contrast injection as this will influence the definition (and even diameter) of the outlined lumen. Arteriography is rarely used simply for follow up information and never used outside clinical trials in asymptomatic patients. Intra-venous digital subtraction angiography (ivDSA) is sometimes used as an alternative because it does not require an arterial puncture, but the images produced are not as good as arteriography. ivDSA also depends on a large volume of contrast and adequate right heart function and is therefore unsuitable for patients with severe renal impairment or valvular heart disease.

Intravascular ultrasound (IVUS). Intravascular ultrasound is a relatively new development, first demonstrated in 1989, (Yock et al, 1989) capable of providing a cross-sectional ultrasound image from a probe within the arterial

lumen. It can thus measure the area of the lumen and provides information on the constituents of the plaque and arterial wall (Waller et al, 1992). It is not suitable for use in routine follow up as it is invasive and prohibitively expensive, but is superlative in supplying information on the behaviour of stenoses following angioplasty. It has also resulted in increased knowledge of the vessel wall changes and mechanism of restenosis following angioplasty (Mintz et al, 1996) and stenting (Hoffmann et al, 1996).

Magnetic resonance angiography (MRA). This relatively new modality is likely to replace conventional contrast and X-ray angiography for diagnostic imaging within the next decade. Its major advantage is that it does not require an arterial puncture or catheterisation of a vessel and avoids the side effects of radiographic contrast and radiation dosage. Initially, picture quality was poor and the time required to image excessive, but new technology has improved both image quality and speed. Used in combination with duplex, it is already becoming established for carotid imaging.

2.1.1.2 Coronary Restenosis

The majority of reported coronary series involve angiographic assessment of restenosis, but most are limited to less than a year and the many different definitions of restenosis used make comparison difficult (Beatt et al, 1990). Other methods of assessing restenosis are limited.

Clinical and non-invasive investigations. A history of angina post intervention is an important symptom of cardiac ischaemia, but may be a poor indication of restenosis as it does not localise the artery let alone the stenosis. In a comprehensive review of this subject it has been estimated that 60-70% of patients with recurrent angina and 10-20% of asymptomatic patients have restenosis (Califf et al, 1991). Electrocardiography may be used to localise regional ischaemia and give some indication of which arterial territory is involved. Monitoring during exercise increases the specificity of detection

(Rosing et al, 1984). Radionuclide perfusion and exercise thallium scans (Wijns et al, 1985) are impressive in revealing regional perfusion defects, but their sensitivity is limited and are obviously more unreliable in multivessel disease. In terms of predictive value, a positive result from any of the above tests is only moderately accurate in predicting stenosis, but a negative result is a much better indication of the absence of restenosis.

Cardiac catheterisation and IVUS. A coronary angiogram gives information on the function of the left ventricle and demonstrates coronary anatomy. Like peripheral arteriography, it is invasive and has a demonstrable, but small mortality rate (<1%). It is however, the only way to visualise individual stenoses and their response to intervention and remains the reference standard for the study of restenosis (Holmes et al, 1991). Many different definitions of restenosis based on angiography have been used in the major restenoses studies (see Table 1.3) reported in the literature:

- Loss of at least 50% of the initial gain achieved at angioplasty (criterion 4 of the National Heart, Lung and Blood Institute)
- A return to within 10% of the pre-angioplasty stenosis diameter
- An immediate post-angioplasty stenosis diameter of <50% that increases to ≥ 50% at follow-up
- As above, but for a stenosis diameter ≥ 70% at follow-up
- An increase of ≥30% from the immediate post-angioplasty stenosis diameter
- Deterioration of 0.72mm or greater in minimal luminal diameter from immediately post-angioplasty to follow-up
- Deterioration of 0.5mm or greater in minimal luminal diameter from immediately post-angioplasty to follow-up.

All definitions use one of two methods of calculating restenosis: a percentage change based on a reference diameter or an absolute measured change. Neither of these methods is ideal. Firstly, subjective choice of the reference segment

can bias the result and secondly, it would be naive to assume that the reference segment remains constant. The reference diameter could increase as a consequence of angioplasties proximally or decrease as a result of progression of disease, both of which will influence the calculated restenosis rate even if the treated segment remains unchanged. The concept of a definition based on a change in absolute minimal diameter was introduced to counteract some of the above problems. The cut-off value in defining restenosis (0.72 or 0.5mm) is based on double the expected variability in measuring the same lesion on two occasions. This can also be criticised, as the definition is not related to the size of the vessel. Thus the haemodynamic impact of such a change would vary greatly between for example, a left main stem lesion in a male and a more distal lesion in a much smaller artery of a diabetic female.

One of the other drawbacks to angiography, is the information obtained is limited to the lumen; it is in fact a "luminogram" and tells us little of events occurring in the arterial wall. IVUS is superior in this respect and has become the "gold standard" for assessing new coronary interventions (Waller et al, 1992).

2.1.2 Other Factors influencing reported Restenosis Rates

From the above definitions of restenosis one can see that the adequacy of the initial angioplasty will influence some, but not all definitions. More importantly however is whether series include all cases or exclude those that fail immediately. Including cases on an intention-to-treat basis will automatically increase the reported restenosis rate as can be seen from Table 1.1 where only one series is reported on this basis (Johnston et al, 1987). In Table 1.2, again one can see that series reported on an intention-to-treat basis (Martin et al, 1981; Johnston et al, 1992; Matsi et al, 1994; Stanley et al 1996 and Gray et al, 1997) report patency rates of almost half the series not reported in this way.

There is also variability in the definition of the patency rate used as an outcome measure. Primary patency rates will invariably be worse than reported rates where one or more further interventions are employed to maintain patency. This can be seen in Table 1.2.

2.2 PATHOBIOLOGY OF RESTENOSIS

2.2.1 Introduction

Restenosis is a diverse process. It occurs as a consequence of a combination of neointimal hyperplasia and geometric remodelling. The relative importance of each is often disputed, but probably varies as a result of different interventions - remodelling is more important after PTA, but NIH predominates after stenting.

2.2.2 Restenosis Theories

Although I will consider individual theories in isolation for clarity, it must be remembered that each process probably takes place concurrently, which might explain the failings of some therapies directed at a single process.

2.2.2.1 Neointimal Hyperplasia

Clinical evidence. NIH, sometimes referred to as myointimal, fibrocellular or simply intimal hyperplasia was first recognised as causing restenosis clinically in 1971 (Grondin et al, 1971). It results from a combination of VSMC proliferation and migration and, until the early 1990's, was believed to be the sole cause of restenosis. The origins of this belief stem from postmortem studies that identified VSMC accumulation within the intima of some primary atherosclerotic lesions (Haust et al, 1960). Furthermore, analysis of the

angioplasty site of patients dying at various intervals following PTCA revealed the presence of an abundance of VSMCs within a large mass of extracellular matrix (Austin et al, 1985). The development of atherectomy offered the unique opportunity to sample histological material from restenotic and primary lesions *in vivo.* Johnson et al, in a study of 170 primary peripheral stenoses and 48 restenoses demonstrated a fundamental histological difference between the lesions. The majority of primary lesions consisted of atherosclerotic plaque alone (see Chapter 1), but restenotic lesions were characterised by intimal hyperplasia due to smooth muscle proliferation within a loose fibrous stroma (Johnson et al, 1990).

The above findings were confirmed by other atherectomy studies and additional evidence for the proliferative role of VSMCs was gained from *in vitro* work with explants of atherectomy specimens. The outgrowth kinetics of samples of VSMCs from restenotic lesions were found to be consistent with proliferative lesions both in terms of yield and timing (Pickering et al, 1992). Moreover, markers of cell proliferation such as proliferating cell nuclear antigen (PCNA) showed a much higher percentage of proliferating cells from restenotic lesions than primary ones (Pickering et al, 1993).

Animal model evidence. The above findings from studies in humans were thought to confirm the role of VSMC proliferation and extracellular matrix deposition in the development of restenosis. More stringent audit of cases undergoing percutaneous and open revascularisation procedures increasingly revealed them to be limited by restenosis and therefore much research effort was directed towards "a cure". Based on the above clinical evidence, it was only natural to turn to animal injury models of VSMC proliferation as a model to investigate the stages and control of NIH. Therefore much of our knowledge of the biology and pharmacology of NIH is based on the response of animal injury models rather than the response of a human atherosclerotic lesion to PTA.

In recent years this subject has been extensively reviewed in the cardiovascular literature (Thyberg et al, 1990; Forrester et al, 1991; Reidy et al, 1992; Casscells, 1992; Davies and Hagen, 1993; Davies and Hagen, 1994; Schwartz et al, 1995). The best studied animal model is the rat carotid artery and its response to balloon injury which has been shown to involve three distinct phases or waves:

First Wave. Arterial injury causes loss of the endothelium and VSMC death in the media. Within 24 hours this is followed by medial VSMC proliferation which may be related to the release of basic fibroblast growth factor (bFGF) from dying VSMCs (Lindner and Reidy, 1991). It has also been shown that inhibition of this phase (using anti-sense agents directed at the genes controlling the cell cycle) can diminish the final amount of NIH production (Simons et al, 1994).

Second Wave. Smooth muscle cell migration across the internal elastic lamina (IEL) occurs from day 4 onwards for at least two weeks. It is probably mediated by a number of molecules including platelet derived growth factor (PDGF), transforming growth factor-ß (TGF- ß), bFGF and angiotensin II (Ang II).

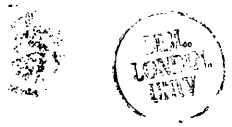
Third Wave. Replication of VSMCs within the neointima is present from day 7 to approximately 1 month following injury. It is unclear which mitogens control this phase, but infusion of a number of molecules (including TGF- ß, bFGF and Ang II) can result in further replication at this stage which rather artificially has been termed a *fourth wave.*

In terms of a target for intervention (which will be discussed in more detail later), two important observations stand out. Firstly, that inhibition of the first wave by antibodies to bFGF can influence the whole cascade (Lindner and Reidy, 1991), and secondly that regeneration of the endothelium inhibits

further proliferation of underlying VSMCs (Clowes et al, 1983). It is unclear exactly how the endothelium exerts its effect, but extracellular matrix substances such as type V collagen and certain glycoproteins are known to be important. It might also be mediated via nitric oxide (NO) production which is known to inhibit proliferation of many cells. Paradoxically however, NO synthase activity has been shown to increase following balloon injury (Joly et al, 1992), and based on *in vitro* work, has even been put forward as a mechanism for delaying re-endothelialisation (Sarkar et al, 1995). In summary, the longer that endothelial denudation persists for, the longer VSMCs are allowed to replicate unmodulated (Clowes et al, 1983) and the more NIH is produced.

Concentrating on the VSMC itself, it is apparent from cell culture work that two distinct phenotypes exist: contractile and synthetic. Contractile cells are characterised by abundant actin and myosin filaments whereas synthetic cells contain few myofilaments, but a prominent endoplastic reticulum and many mitochondria. It has been suggested that the above phenotypes may represent the extremes of a number of intermediate stages in differentiation (Chamley-Campbell and Campbell, 1981), although the extremes are easier to identify. In normal uninjured arteries the contractile phenotype predominates, but following injury there is a change to the synthetic phenotype (beginning during the *first wave*) which leads to extracellular matrix production.

A proliferative response to balloon injury has also been demonstrated in the porcine model. Schwartz and colleagues have been responsible for much of the pioneering work, and established a reliable injury response to balloon expanded wire coils in the pig coronary (Schwartz et al, 1990). The same authors went on to quantify the response and develop an injury score based on the penetration of metal coils into the arterial wall:



Score 0	Endothelium denuded; IEL intact: media compressed.
Score 1	IEL lacerated: media compressed, but not lacerated.
Score 2	IEL lacerated; media lacerated; external elastic lamina (EEL) intact.
Score 3	Media disrupted; EEL lacerated.

Furthermore, a linear relationship was found between the injury score and neointimal thickness. This was also true for percentage area stenosis and to a lesser extent, total neointimal area (Schwartz et al, 1992a). Other injury scoring systems based on balloon injury exist (Guzman et al, 1996), but most are more complex than the above system and offer no advantage.

The time course of VSMC proliferation and neointimal response following injury has also been studied in the porcine coronary model. Carter et al have demonstrated that VSMC proliferation (measured by the percentage of PCNA-positive cells) was maximum at day 7, and was followed by the greatest increase in neointimal area between day 7 and 14. Neointima formation was almost complete by day 14 and increased only slightly to day 28 (Carter et al, 1994).

2.2.2.2 Thrombosis

Platelet accumulation readily occurs on the exposed subendothelial surface following balloon injury in large animal models (Steele et al, 1985; Lam et al, 1986). Steele et al used a balloon injury pig carotid model and observed complete endothelial denudation for up to 4 days following injury. Platelet deposition was monitored by ^{111}Indium labelling and shown to occur from 1 hour after injury. By 4 days, platelet deposition was significantly reduced which coincided with partial endothelial regrowth. Intimal proliferation of VSMCs was seen to begin at day 7 and reach a peak at day 14 and persist until day 60. From this data, the authors proposed two mechanisms for restenosis:

1. Platelet deposition on the damaged arterial wall could form a mural thrombus, organisation of which could itself result in luminal narrowing.

2. Release of PDGF by platelets accumulating at the angioplasty site could induce VSMC migration to and proliferation in the intima. Formation of a neointima could then cause obstruction of the lumen.

Schwartz has proposed a similar theory based on the unequivocal evidence of the early involvement of platelets which has been confirmed by many subsequent studies (Schwartz et al, 1992b). Severe mechanical injury to a normal porcine artery was suggested to result in a 3 phase response:

Stage 1. Rapid thrombus formation consisting of platelets at the site of deepest injury (white thrombus) covered by a fibrin and red cell component (red thrombus).

Stage 2. Cellular recruitment from the lumen surface begins from day 3 with re-endothelialisation followed by macrophage and lymphocyte attraction.

Stage 3. A proliferation phase is initiated from day 7-9 beginning as a thin "cap" of cells (probably VSMCs) beneath the endothelium. As the cap thickens, thrombus is resorbed and replaced by mature neointima.

A direct relationship between thrombus volume and eventual neointimal volume was suggested and fibrin thrombus implicated as playing a central role in the whole process. Against this hypothesis however is animal data from another group (using a similar model) and evidence from clinical therapeutic studies. Carter et al also measured thrombus area and found it to be minimal at 24 hours and although similar in volume to the neointima at day 7, thereafter neointimal area increased significantly without any further thrombus accumulation (Carter et al, 1994). Moreover, if thrombosis was as important as suggested, anti-platelet drugs would be expected to reduce the restenosis rate

after clinical angioplasty. Numerous clinical trials however have shown aspirin, dipyridamol, heparin and warfarin all to be ineffective in reducing restenosis rates (Thornton et al, 1984; Ellis et al, 1989).

2.2.2.3 Remodelling

Despite the above evidence for a proliferative cause for restenosis, reanalysis of some of the clinical data originally proposing to support such a theory may suggest otherwise. In one of the first atherectomy studies, intimal hyperplasia was found at only 36 out of 48 restenotic lesions (Johnson et al, 1990), suggesting that intimal hyperplasia may not be the only cause of restenosis. This was further illustrated by a unique study by Isner and colleagues who gained histological material (by atherectomy) from 18 primary atherosclerotic coronary lesions and compared it with further material from the same lesions when they restenosed (Isner et al, 1993). VSMC proliferation was observed in only 13 out of 18 cases of restenosis, the remaining 5 apparently having no obvious cause.

It was only when the dimensions of the whole artery were assessed and compared with an untreated reference segment that changes in the dimensions of the entire artery following balloon angioplasty were noted. These dimensional changes were referred to by Post as 'remodelling'. He demonstrated that angiographic late loss following balloon injury in a pig and rabbit model correlated better with dimensional changes than neointimal thickness (Post et al, 1994). Although there were certain methodological problems with the above paper (mainly concerning the use of angiography in assessment and the lack of objective quantification of the injury produced) as outlined by Isner (Isner, 1994), it was a seminal observation and rapidly confirmed by a profusion of abstracts. More recently, more exacting studies in the rabbit (Kakuta et al 1994; Guzman et al, 1996) and the pig (Anderson et al, 1996) have confirmed the importance of remodelling in the restenosis process.

The term remodelling is now often used to refer to the reduction in cross sectional area (of all arterial dimensions) that may occur following angioplasty, a process which should be termed negative geometric remodelling.

Furthermore, it has been revealed that the degree of injury correlates with neointimal area, which in turn correlates with the EEL area, i.e. the greater the intimal area , the larger the whole arterial dimension (Kakuta et al 1994; Guzman et al, 1996). However, the final histological lumen area was determined by the IEL and EEL area in both the above studies. Using a novel chain-encircled balloon injury method in the pig, Anderson et al, also demonstrated that neointimal area correlated with the degree of injury and overall vessel size (ie EEL area), but not with lumen area (Anderson et al, 1996). By analysing consecutive transverse coronary arterial sections they were able to build up a longitudinal picture of arterial dimension changes over the course of the injured segment which also demonstrated the presence of adventitial thickening proximal to, and at the injury site. This however did not correlate with the overall vessel size at the site of maximal stenosis, but nevertheless prompted the authors to suggest that neoadventitial formation may have a role in restenosis by "strangulating" the artery. Changes in the adventitia have also been observed by other workers (Shi et al, 1996) who have demonstrated greater cellular proliferation in the adventitia than in the media after injury.

Two other observations underline the importance of remodelling and suggest that it is not merely a concept confined to animal models. Firstly, it has already been established that both coronary (Glagov et al, 1987) and peripheral (Losordo et al, 1994) arteries increase in overall dimension in response to increasing plaque burden in the progression of atherosclerosis. It is therefore not very surprising that dimensional changes can occur after angioplasty and not inconceivable that arteries can remodel in response to a number of stimuli. Secondly, clinical serial IVUS studies have demonstrated that coronary restenosis is unequivocally determined by the direction and magnitude of

remodelling (Mintz et al, 1996). The only word of caution comes from Pasterkamp who has pointed out the inherent bias in using pooled data from different vessels which may mask changes in individual vessels. In support of this he has shown that in the majority of 25 femoral artery segments studied by IVUS and histomorphometry, individual plaque area did not correlate with IEL area, although there was a significant correlation when the data was pooled (Pasterkamp et al, 1996). Although this was post-mortem data, it does show how certain methods of analysis can dramatically influence results and also reveals how arterial behaviour may vary amongst individuals which is in keeping with the fact that restenosis is not universal after angioplasty.

The mechanism behind remodelling remains to be elucidated, but it is probable that the endothelium plays an important role in the signalling process. Remodelling itself is probably mediated by many matrix components, especially collagen and elastin and regulated by a host of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) as proposed by a number of reviewers (Gibbons and Dzau, 1994; Post et al, 1995). It has also been suggested that blood flow post intervention may be a crucial factor in determining whether a vessel restenoses or not (Glagov, 1994). This may explain the poor response of crural arteries to PTA compared with comparably sized coronaries and the fact that PTA at areas of disturbed flow (e.g. arterial ostia) invariably does badly.

The exact proportion of restenosis attributable to NIH and remodelling is far from being unequivocally defined. Some workers using the same animal model as Guzman above, have in contrast to the findings of Guzman, shown that remodelling may not be the principal cause of restenosis (Gertz et al, 1994). Moreover, in an exacting study using IVUS, quantitative angiography and histomorphometry (in a minipig model), late lumen loss was found to correlate with geometric remodelling after balloon injury, but NIH after stenting (Post et al, 1997). In clinical series it has also been demonstrated with IVUS that following stent insertion, restenosis was exclusively secondary to NIH

(Hoffman et al, 1996), and in diabetics, NIH and remodelling were equally important even in unstented lesions (Kornowski et al, 1997). In addition, a recent clinical coronary IVUS study has presented evidence to suggest that the neointimal thickness was less in patients with a good early result, but thicker in patients who chronically exhibited gain or less late lumen loss (De Lezo et al, 1997). It is likely that the exact type of injury and vessel microenvironment have an important bearing on the mechanism of the resulting restenosis and in reality both are important and probably interrelated.

One could therefore postulate a mechanism for restenosis which is dependent on the degree of vessel injury produced by the intervention and the resulting blood flow dynamics. Arterial injury would lead to VSMC proliferation and NIH, and the degree of remodelling would depend on flow, perhaps sensed by the endothelium and mediated via changes in collagen and elastin. The balance of forces would determine the final lumen dimensions and whether vessel patency is maintained at its post procedure level.

2.2.2.4 Extracellular Matrix

Increasing evidence has accumulated to suggest that the extracellular matrix (ECM), far from being an inert scaffolding, is a dynamic system which may have a pivotal role in restenosis. In some restenotic lesions, the ECM is the most abundant component and constitutes up to 70% of the total volume of the lesion. Rather than an alternative theory, the role of the ECM should perhaps be seen as the vehicle through which changes in the arterial wall are exerted. The composition of the matrix determines the physical properties of the arterial wall via changes in collagen, elastin, proteoglycans and glycoproteins and homeostasis is probably achieved by interaction of the plasminogen activator system and the MMPs.

In support of the above, there is evidence available that VSMCs can themselves produce enzymes capable of degrading the ECM (Galis et al, 1994) and that

inhibitors of the MMP cascade can cause a significant reduction in VSMC migration in the rat (Bendeck et al, 1994). MMPs may also modulate collagen turnover (Strauss et al, 1996), but the role of collagen in restenosis remains unclear. Despite evidence that a dramatic increase in collagen synthesis occurs following angioplasty (Strauss et al, 1994), restenotic vessels have been shown to contain a significantly decreased collagen content compared with nonrestenotic ones in an atherosclerotic rabbit model (Coats et al, 1997). Furthermore, these authors found a positive correlation between collagen content and luminal area which is counterintuitive to the dogma that arterial restenosis results from neointimal accumulation of ECM containing collagen. To further complicate matters, a new analysis technique based on the birefringent properties of collagen has revealed an extensive network of collagen, albeit disordered, in restenotic human atherectomy specimens (Pickering et al, 1996). Clearly more work is needed in this area, as the role of collagen and interventions that affect it may have an important bearing on the prevention of restenosis.

2.3 THERAPIES DIRECTED AT PREVENTION OF RESTENOSIS

The problems associated with the diagnosis, assessment and interpretation of restenosis studies were highlighted in an editorial in early 1992 (Ellis and Muller, 1992). Recommendations were made regarding the investigation of new interventions aimed at preventing restenosis. The suggested strategy involved initial tests on cell culture systems, followed by at least two animal species prior to safety and efficacy testing in a small-scale clinical trial. Only after the above would a full scale clinical trial of 500-1000 patients be justified. In reality however, even this strategy is inadequate as the animal species are not specified and must include swine or non-human primate to be meaningful. It is also now clear that a large-scale clinical series per se does not possess the

power of a randomised controlled trial which is mandatory to prove the worth of any new intervention.

2.3.1 Systemic Therapies

Numerous substances have proved to be beneficial in reducing VSMC proliferation *in vitro* and NIH in small animal models *in vivo* as seen in Table 2.1 (modified from Davies and Hagen, 1994). Based on the knowledge gained from animal work, the majority of agents tested clinically have had anti-proliferative or anti-thrombotic properties or have attempted to influence the function of the endothelial cell. Few however have been efficacious in improving restenosis rates with the exception of certain trials of dietary fish oils (Olsson, 1989) and lipid lowering agents (Sahni et al, 1991). Unfortunately, for every such trial reporting a positive result there is one with a negative conclusion (Reis and Pasternak, 1990) and it seems probable that most effects are mediated by influencing the progression of disease rather than any specific action on restenosis.

Table 2.1 Systemic therapies proposed to reduce restenosis

Generic Therapy	Specific examples
Antiplatelet drugs	Aspirin, dipyridamole, thromboxane synthetase inhibitors, ticlopidine.
Anticoagulants	Heparin, hirudin, coumarin.
Anti-inflammatories	Dexamethasone, methylprednisolone.
Antioxidants	Probucol, lazaroids.
Antibodies	Antiplatelet, anti-CD 18, anti-PDGF, anti-FGF.
ACE inhibitors	Captopril, cilazapril.
Calcium antagonists	Verapamil, nifedipine, diltiazem.
Immunosuppressants	Azathioprine, cyclosporin.
Matrix modulators	Collagen synthesis inhibitors.
Endothelial modulators	L-arginine, prostacyclin analogues.
Peptides	TGF-ß, interferon γ, somatostatin.
Receptor antagonists	Prazocin, ketanserin, losartan.

There are a number of feasible explanations as to why the majority of the above agents have been effective in animal models, but ineffective in clinical trials. Firstly, normal arteries in small animal models are obviously histologically distinct from their atheromatous counterpoints in man. Secondly, the sole endpoint of VSMC proliferation and the assessment of specific inhibitory agents may be inappropriate in the light of more recent knowledge of the multi-factorial causes of restenosis. Thirdly, many of the doses used in trials of the above agents have exceeded those permissible in man and in other cases the timing of delivery has not corresponded with animal trials.

Recently the effectiveness of calcium antagonists has been reassessed however. Although all trials prior to 1995 had been negative, a prospective randomised double-blind trial of 189 patients has recently shown that diltiazem significantly reduces the restenosis rate after coronary angioplasty (Unverdorben et al, 1996). Furthermore, a meta-analysis of 5 previous trials (which were individually unconvincing) has demonstrated a 30% reduction in the chance of restenosis in patients receiving a calcium antagonist (Vahanian and Iung, 1996; Thaulaw and Jorgensen, 1997). This has prompted the initiation of a new trial, the CAPARES (Coronary Angioplasty Amlodipine in Restenosis) trial, the results of which are awaited.

A further exception to the aforementioned lack of reproducibility between animal and clinical work is a recent trial of antioxidants (Tardif et al, 1997). Again in a double-blind randomised trial (of 317 patients), this group have shown that by commencing treatment one month prior to PTCA, probucol significantly reduces the restenosis rate. The only criticism is that numbers were small as it contained 4 subgroups and confirmation in a larger trial is still awaited. However, it does underline the care needed in anticipating the exact mechanism of action of interventions and in designing trials accordingly.

2.3.2 Local Therapies

2.3.2.1 Stenting

The mechanistics of stenting has already been discussed in Chapter 1. Suffice as to reiterate that stenting influences the outcome of angioplasty by reducing the acute complication rate (secondary to dissections and intimal flaps) and by limiting negative remodelling. Stents are however a stimulant for intimal hyperplasia and are used as such in certain animal models to create a neointima (Carter et al, 1994). In-stent restenosis is a well-recognised complication of stenting and has been shown by angiography (Gordon et al, 1993) and IVUS to result from NIH; the latter modality demonstrating an even distribution along the length of the stent (Hoffmann et al, 1996).

Several trials of coronary stenting have reported high restenosis rates: BElgian and NEtherlands STENT I (Benestent-I) study - 22% (Serruys et al, 1994); STent REStenosis (Stress) study - 31.6% (Fischman et al, 1994); and Intracoronary Stenting and Antithrombotic Regimen (ISAR) - 26.8% for the antiplatelet arm and 28.9% for the anticoagulation arm (Kastrati et al, 1997). It is difficult to compare these results as in the former two studies only simple lesions were included, but the latter included patients following MI and with unstable angina in addition to patients with complex lesions and multivessel disease. It is also clear that such rates may not accurately reflect the restenosis rates expected in conventional clinical practice where stents tend to be reserved for more difficult lesions.

More recent trials using heparin-coated stents followed by aspirin and ticlopidine have managed to lower restenosis rates (Serruys et al, 1996), but again this probably represents the best possible rather than the average outcome of stenting. The take home message from such studies is that platelet and thrombus control is an essential part of therapeutic stenting and an effective agent is likely to have a profound effect on the results from stenting (Willerson, 1997).

The rapid acceptance of stenting especially in coronary arteries (which now accounts for up to 70% of all coronary artery procedures in Europe) should perhaps prompt a re-evaluation of some of the NIH-directed therapies which failed to show any benefit after PTA alone. Because of its benefit in the acute situation, stenting will certainly continue to play a major role in the percutaneous management of coronary disease and prevention of in-stent restenosis therefore remains an increasingly important goal.

2.3.2.2 Local Drug Delivery and Gene Therapy.

The potential advantage of localised delivery of an active agent is the ability to use higher doses without systemic toxicity. Much effort has been directed at developing and perfecting delivery systems which allow delivery of an agent targeted to the angioplasty site (Wilensky et al, 1993; Gonschior et al, 1995a; Gonschior et al, 1995b). Interestingly, Gonschior used the delivery of a photosensitiser, Photofrin and the arterial fluorescence produced to compare devices and was able to demonstrate delivery to all three arterial layers. The benefits of adventitial delivery, both in terms of its influence on remodelling and its ability to act as a reservoir to supply the arterial wall via the vasavasora have recently been highlighted (Huehns et al, 1996).

Many problems with control of release and retention at the proposed site of action remain to be solved and, to date, no clinical trials have shown any benefit from localised drug delivery. A safe and effective agent to be delivered is also lacking although it has recently been demonstrated that microporous balloon delivery of Taxol is beneficial in a rabbit model (Axel et al, 1997).

Gene therapy in the prevention of restenosis, as in many other areas of medicine, holds great promise for the future, but at present remains at the experimental stage (Isner and Feldman, 1995). It has already been demonstrated that genetic material can be delivered via a catheter using the following vectors: adenoviruses, retroviruses, plasmid-liposome complexes,

and naked DNA coated balloons (Nabel et al, 1990). Retroviral vectors have a good stability and safety profile, but transfection efficiency remains low, while adenoviruses are much more efficient vectors, but their toxicity is unknown. Antisense oligonucleotides are single strands of DNA which are synthesised to complement a specific mRNA sequence and can be targeted against various cell cycle proteins (eg. c-myb, c-myc). They have been shown to suppress intimal thickening following injury in a rat carotid model (Simons et al, 1992), but are currently disadvantaged by their instability and relatively short half life.

Another approach being investigated is the local implantation of genetically engineered endothelial cells (Nabel et al, 1989) which if transferred in large enough numbers could allow early re-endothelialisation and termination of the restenosis process before it has been properly initiated. The ability to induce endothelial seeding along synthetic bypass grafts would undoubtedly increase their longevity, but despite promising *in vitro* work, has yet to be realised effectively *in vivo*. Vascular endothelial growth factor (VEGF) has now been investigated clinically in patients with peripheral vascular disease (Isner et al, 1995), but the reality of routine gene therapy to modify cardiovascular disease may be a long way off.

2.3.2.3 Ionising Irradiation.

External beam irradiation has not been shown to be effective in reducing NIH in a porcine coronary model (Schwartz et al, 1992c), but intra-coronary brachytherapy using gamma- (Wiedermann et al, 1994) and beta- emitters (Waksman et al, 1995) has been shown to significantly decrease the percentage arterial stenosis produced in a similar model. Logistic problems with using γ-emitters for clinical use are however enormous, as it would require the patient to be left unattended for over 30 minutes during delivery (King, 1996). In contrast, ß-irradiation is absorbed within a few millimetres of tissue and is therefore a more practical source for use in the catheter laboratory as it does not require any additional screening for staff involved. ß-emitting radioactive stents

have also been investigated in the swine model where NIH was inhibited at low and high doses, but was exacerbated at doses in the medium range (Carter et al, 1996). This would suggest that dosimetry is crucial and the therapeutic window may be quite narrow, both of which have serious implications for successful clinical application.

Although γ-emitters have been used in the periphery following femoral stenting (Liermann et al, 1994), the first clinical study of intra-coronary brachytherapy used a ^{90}Y ß-emitting source (Verin et al, 1997). The results from this were far from encouraging with 6 out of 15 patients developing restenosis at 6 months, prompting some to speculate that ß-irradiation does not produce a sufficient depth of penetration to be effective (Tierstein, 1997a). Other groups have now used γ-sources (^{192}Ir) and reported beneficial effects in terms of reduced late lumen loss at a median of 8 months follow up (Condado et al, 1997). However, careful examination of these results reveals them to be not as satisfactory as portrayed: out of 21 patients, 2 occlusions and 2 pseudoaneurysms were seen on angiography, and at 1 year, 7 were symptomatic with angina, 2 had had infarcts and 4 a further PTCA. An editorial in the same issue of Circulation concluded that intra-coronary γ-irradiation was feasible, but probably neither safe nor efficacious (Serruys and Levendag, 1997)!

The above evidence may be seen as enough evidence to condemn brachytherapy were it not for a further series, also using an ^{192}Ir source, which reports much more promising results (Tierstein et al, 1997b). This study of a randomised group of 55 restenotic patients has demonstrated an unequivocal benefit in terms of minimal luminal diameter in the brachytherapy group compared with balloon or stented controls at 6 months. Moreover there were no complications attributable to the procedure and clinical endpoints of death, MI, and need for further revascularisation were significantly reduced in the brachytherapy group.

The case for radiotherapy to prevent restenosis is far from proven as safety and long term efficacy concerns remain. It should not be forgotten that the first trial of external beam irradiation produced a significant increase in NIH thickness in the irradiated group (Schwartz et al, 1992). Perhaps more worrying is the late vascular disease caused by radiotherapy for other conditions (Corn et al, 1990), which has been shown to significantly increase the risk of cancer patients (who received mediastinal radiotherapy) developing coronary artery disease (Stewart et al, 1995). Furthermore, once developed, radiotherapy-induced peripheral arterial occlusive disease is both difficult to treat and has a high complication rate (Phillips et al, 1992; Melliere et al, 1997). Finally, the risk of radiation-induced carcinogenesis should not be totally dismissed on the grounds of the specificity of delivery and low doses used as the targeting ability of centering devices may be deformed by eccentric plaques. It should not be forgotten that there is little long-term information available on the effects of irradiation delivered in this way.

2.4 SUMMARY.

Restenosis is the major limiting factor of percutaneous transluminal angioplasty and other interventional endovascular techniques. Its incidence is dependent on the methods employed in the post-operative assessment of patients and is very much higher with direct visualisation of the angioplasty site.

The mechanism of restenosis, despite much research, remains unclear and many theories abound. Much of the evidence comes from animal models where an over-distension or denudation balloon injury in a normal artery has been used to mimic the response of an atherosclerotic human artery to angioplasty. Despite the limitations of such experimental models, many of the findings have been confirmed clinically both from human atherectomy material and by IVUS. There is evidence to show that a thrombotic, proliferative and remodelling

process occurs and rather than thinking of them as competing individual theories, they should be thought of as components of one process. It is true however that the relative importance of each process is dependent on the intervention used and will be different following angioplasty, atherectomy, stenting or bypass grafting. This may explain the apparently contradictory results obtained in certain studies and thus the origins of distinct theories.

Following on from this, one can appreciate the failure of specific "magic bullet" approaches to intervention, as targeting one component is unlikely to have a profound influence on the whole process. Future success probably entails finding the "trigger" and signalling mechanism (the endothelium and MMPs look promising suspects) and modulating these, perhaps genetically. For the time being however, research must continue into safe and effective methods of limiting the restenosis process, once initiated, as it remains an important clinical and economic problem.

CHAPTER 3 PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) is a photochemical reaction involving the interaction of light and a pre-administered photosensitiser which results in the production of reactive intermediates which are cytotoxic.

3.1 HISTORY

The first documented evidence that the toxicity of certain compounds could be influenced by light comes from the observations of Marccaci who noticed that quinine was more toxic to enzymes and frog's eggs in the light than in the dark (Marccaci, 1888). However, the significance of these observations was lost to both Marccaci and the rest of the scientific world. It was the medical student, Oscar Raab, working with Professor Hermann von Tappeiner in Munich, who was the first to scientifically investigate the interplay of light and certain compounds. By sheer chance, Raab had noticed that the toxicity of acridine to a certain protozoan was influenced by the intensity of sunlight in the laboratory. He went on to show that a low concentration of acridine was harmless in the dark, but caused rapid protozoan killing in the light and that other compounds including quinine and eosin also exhibited this phenomenon (Raab, 1900).

Tappeiner suggested as early as 1900 that such reactions may have a therapeutic use in dermatology (Tappeiner, 1900) and together with Jesionek, used the combination of eosin and light to treat many skin conditions which included herpes, psoriasis and molluscum contagiosum (Tappeiner and Jesionek, 1903) and six cases of skin cancer (Jesionek and Tappeiner, 1905). Following the discovery that many different compounds could, under the influence of light and in the presence of oxygen, exert toxicity on protozoans, fungi and proteins, Tappeiner coined the term "photodynamische wirkung" (photodynamic action) to describe the process (Tappeiner and Jodlbauer, 1904).

Like many investigators of the day the first human experiment was carried out on the investigator himself. Following an injection of haematoporphyrin, Meyer-Betz experienced a sunburn reaction and remained sensitised for over six weeks (Meyer-Betz, 1913). The therapeutic potential of PDT in the treatment of malignancy was intimated by a series of experiments in which intra-peritoneal injection of haematoporphyrins were shown to preferentially accumulate in mouse tumours and embryonic tissue (Figge et al, 1948). Moreover, in a similar mouse model, exposure to light following haematoporphyrin injection resulted in increased tumour necrosis (Auler and Banzer, 1942). In 1967, a clinical case was reported describing the use of haematoporphyrin-sensitised PDT in the treatment of breast cancer (Lipson et al, 1967), but it was the work of Dougherty in the 1970's which established PDT with haematoporphyrin derivatives as an experimental modality for the treatment of malignancy (Dougherty et al, 1975 and Dougherty et al, 1978).

3.2 PRINCIPLES OF PDT

The photochemical reaction which follows the interaction of non-thermal light and non-toxic light-absorbing chemicals results in a cytotoxic tissue effect, the magnitude of which is dependent on a number of variables. The type and dose of sensitiser; energy and wavelength of the light; and delay between the sensitisation and light exposure (drug-light interval) all influence the final tissue effect.

3.2.1 Mechanism of Action

The prerequisites for a photodynamic reaction are the simultaneous existence of a photosensitiser and light of a specific wavelength which interact in the presence of oxygen. The sensitiser, which is normally in the ground state is converted into an excited state following absorption of light energy. There are two classes of excited state: the singlet and triplet, the former of which can return to the ground state or be converted to the triplet state. The triplet state of the sensitiser can react directly with the tissue substrate itself (type I reaction)

producing hydroxyl radicals and superoxide anions or with oxygen (type II reaction) to produce a highly reactive singlet oxygen species which has a lifespan measured in microseconds (Foote, 1968). Oxygen has been shown to be important both *in vitro* (Moan and Sommer, 1985) and *in vivo* (Bown et al, 1986).

Type I and II reactions may occur simultaneously, but despite the difficulty in measuring singlet oxygen production, there is indirect evidence to suggest that type II reactions are responsible for the majority of tissue damage produced by PDT (Henderson and Dougherty, 1992). The ratio of the two reactions is influenced not only by the oxygen concentration, but by the target tissue, sensitiser and sensitiser-tissue binding. It is likely therefore that the predominant reaction type will vary in an individual tissue depending on which sensitiser is used, and for a given sensitiser, the reaction type may differ from tissue to tissue. As PDT progresses, induction of hypoxia, within a tumour for example, may shift the mechanism of action from type II to type I (Ochsner, 1997).

The reactivity of singlet oxygen limits its diffusion distance and lifetime amongst cells (Moan, 1990), thereby restricting its activity very close to the site of its production. The prime target appears to be the cell membrane (Kessel, 1977) and the membranes of certain intracellular organelles including lysosomes and mitochondria (Robinson et al, 1987). Although PDT can result in DNA damage, the extent of damage is less than that secondary to ionising irradiation, and is thought not to be lethal, implying a recovery in the mechanisms responsible for DNA, RNA and protein synthesis (Kvam and Moan, 1990). The actual mode of cell death is either by necrosis or apoptosis and depends on both the cell type and sensitiser used. The available data on mode of death is complicated by different methods of identifying apoptosis, although the majority of authors use electron microscopy or the detection of 'DNA ladders' (as reviewed by Moore et al, 1997).

In addition to the direct cellular mechanisms outlined above, PDT exerts an action in some tissues (and tumours) *in vivo* by a 'vascular effect' (Reed et al, 1988). In experimental tumour models, microvascular occlusion secondary to platelet aggregation occurs within seconds of light exposure and is followed by blood stasis and tissue oedema. This can be seen within the arterioles of the hampster cheek-pouch preparation (Hermann, 1983), and evidence for the role of platelets in the occlusion process is further enhanced by the fact that aspirin can delay occlusion for hours (Stern et al, 1992). PDT may also affect the vascular endothelium and this may lead to direct vasoconstriction perhaps by impairment of nitric oxide release. Damage to the vascular endothelium may be the initiator of a whole cascade of events which eventually leads to vessel occlusion (Fingar, 1996).

It is therefore likely that tissue and tumour destruction occurs due to a combination of processes at different time intervals following light exposure. This would reconcile the prompt PDT-induced apoptosis seen in *in vitro* studies with the gross necrosis observed at 24 hours in animal models. The latter is probably ischaemic in origin and hence the vascularity of the target tissue may well influence the mode of its destruction.

3.2.2 Photosensitisers

The theoretical concept of specific targeting of tumours with sparing of normal tissues underpins the rationale for PDT. However, such a concept is dependent on the uptake of sensitisers into tumour tissue being higher than into normal tissue. In reality however most sensitisers are distributed to, and retained by, normal as well as malignant tissue. The ratio of uptake to tumour/normal tissue varies between sensitisers, tissue type and animal models. For example, ratios of 8:1 were obtained in colorectal adenocarcinoma with haematoporphyrin derivative (HPD) in man (Wooten et al, 1989) compared with only 2:1 in the rat colonic cancer model with Aluminium phthalocyanines (AlSPc) (Barr et al,

1991). Interpretation of such data is however fraught with pitfalls. Firstly, the majority of animal tumour models involve implanted tumours that may not accurately represent the clinical situation of a tumour arising from surrounding normal tissue. Secondly, fluorescence excitation as a measure of sensitiser uptake only represents the difference in fluorescence between the surface of the tumour and normal tissue and does not impart any knowledge as to the concentration of sensitiser in the body of the tumour. Of therapeutic interest is the observation that in a given tissue, the grade of malignant change may also influence uptake. In the rat bladder tumour model, photofrin was found in ratios of 1.3:1, 2.0:1 and 3.5:1 for hyperplasia, non-invasive cancer and invasive cancer respectively (Baumgartner R et al, 1992).

The mechanism behind the preferential uptake of sensitisers by tumours is unclear, but may involve a number of factors. At a simple physiological level, the increased permeability of tumour capillaries and the impaired lymphatic drainage of many tumours may allow sensitisers to be retained within the tumour. Specific uptake mechanisms have also been suggested and low-density lipoprotein (LDL) implicated as a carrier for Photofrin (Kessel et al, 1986). Since many tumours have an increased number of LDL receptors it was postulated that this would increase endocytosis of Photofrin, but there is now evidence to the contrary (Korbelik et al, 1990). However, it is clear that hydro- and lipo-philicity in addition to polarity and aggregating properties of sensitisers may be capable of influencing their uptake into cells (Moore et al, 1997).

The important message from a large number of animal studies with a variety of systemically administered sensitisers is that *relative* selectivity is produced by differential sensitiser uptake and therefore only *relative* sparing of normal tissue occurs (Moore et al, 1997). In clinical practice, selectivity is enhanced by light irradiation concentrated on tumour rather than normal tissue. This could not be illustrated better than by a recent paper describing the use of Photofrin as a sensitiser in the treatment of small squamous cell cancers of the oral cavity

(Grant et al, 1997). Following surface illumination the cancers were excised together with a cuff of normal tissue and histological examination revealed non-selective necrosis of both tumour and normal tissue.

3.2.2.1 Porphyrins

The porphyrins are the best studied of all photosensitisers and their *in vitro* pharmacodynamics established by a number of groups. Haematoporphyrin derivative (HPD) is probably the most commonly used sensitiser clinically and Photofrin® is the only sensitiser to be licenced by the FDA (Food and Drug Administration) for PDT in the USA, where it is being used as an experimental therapy for oesophageal cancer. Photofrin or di-haematoporphyrin ether (DHE) is an aggregate of two haematoporphyrin molecules linked by ether bonds produced by the fractionation of HPD.

Photofrin absorbs long wavelength (red) light necessary to produce any depth of effect with PDT rather poorly and is therefore by no means the ideal sensitiser for PDT. Its other major drawbacks are its prolonged skin photosensitivity (of the order of 6 weeks) and the 3 day drug-light interval necessary prior to treatment. These make photofrin a cumbersome drug to use clinically and will inevitably limit its use to malignant conditions not amenable to other forms of therapy.

3.2.2.2 Phthalocyanines

Phthalocyanines are synthetic porphyrins whose central pyrrol ring has been extended by condensation with extra benzene rings bridged by aza nitrogens rather than methine groups. This phthalocyanine macromolecule can chelate with a large number of elements and the complexes made with certain metal ions can alter the phototoxicity by extending or shortening the lifetime of the active triplet state. Thus the diamagnetic ions, aluminium and zinc enhance photoactivity and paramagnetic ions, copper, cobalt, iron and lead reduce it. To

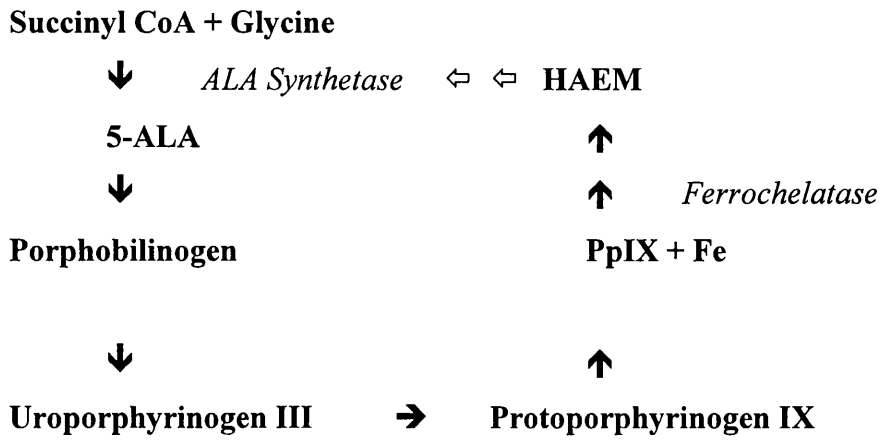
achieve water solubility, essential for the use in biological systems, hydrogen ions on the benzene ring are substituted with sulphonate groups.

The above synthetic modifications have endowed the phthalocyanines with properties which make them potentially superior sensitisers compared with HPD and DHE. They are stable compounds with intense light absorption at the red end of the spectrum, and their preferential tumour retention and minimal skin photosensitivity allow superior targeting with reduced complications.

3.2.2.3 5-Aminolaevulinic acid induced Protoporphyrin IX

5-aminolaevulinic acid (ALA) is an endogenous compound formed from glycine and succinyl CoA in the first step of the biosynthesis of haem. In the final stage, haem itself is formed by incorporation of iron into protoporphyrin IX (PpIX), catalysed by the enzyme ferrochelatase within mitochondria (see Figure 3.1). By adding exogenous ALA to this system, the negative feedback loop whereby haem regulates glycine and succinyl CoA conversion to ALA is bypassed leading to accumulation of haem precursors. The next rate-limiting step in the production chain is the limited capacity of ferrochelatase and therefore PpIX is formed in excess. PpIX is an efficient photosensitiser. The above is a simplified description of how PpIX can be created in excess in some cells, but it must be appreciated that the rate limiting steps within the pathway vary from cell to cell, and in certain cells other haem precursors (with varying photosensitising properties) may accumulate in excess.

Figure 3.1 Pathway of haem biosynthesis.



Kennedy was the first to use ALA in a clinical capacity for PDT (Kennedy et al, 1990). Topical administration followed by light activation 3-6 hours later lead to a 90% complete response rate in 80 treated basal cell carcinomas, but only a partial response in thicker lesions. This illustrates the limited tissue penetration of red light which at 635nm (the optimal wavelength for excitation of PpIX fluorescence) has been estimated to penetrate the human hand by only 2mm (Peng et al, 1997). In fact, light at 410nm in the Soret band gives the maximum fluorescence intensity with PpIX, but the penetration ability of such light restricts its use to very superficial lesions. Wavelength of light and tissue penetration per se is not the complete story however, as photofrin excited by 630nm light can produce 5mm of necrosis. There are now a number of series showing the efficacy of topical ALA-induced PpIX in the treatment of cutaneous lesions (Peng et al, 1997), but its usefulness in the treatment of other malignancies remains questionable.

Systemic administration of ALA leads to localisation of PpIX fluorescence within the mucosal layer of the gastrointestinal tract and other hollow organs which is a further mechanism by which the depth of penetration of ALA PDT is limited as compared with other photosensitisers. Following oral administration of ALA in patients with gastrointestinal malignancies, light application produced only superficial necrosis, although selective tumour

uptake of PpIX could be demonstrated, (Regula et al, 1995). This has also been demonstrated in the oral cavity (Grant et al, 1993), where ALA-PDT has been found to be a satisfactory treatment for dysplasia, but not invasive cancer (Fan et al, 1995,1996). In conclusion, the role of ALA PDT in the treatment of malignancy is, at present, limited to the treatment of mucosal conditions such as Barrett's oesophagus and small superficial cancers of the gastrointestinal tract (Gossner et al, 1995).

Another potentially useful application of ALA-induced PpIX fluorescence is for tumour identification and mapping of mucosal field change. This exploits the relatively favourable uptake of PpIX by cancers and rapidly proliferating cells as shown by fluorescence when illuminated. This has aided clinicians in identifying cancerous and pre-cancerous lesions in the bladder after intra-vesical ALA installation (Kriegmair et al, 1994) and in the respiratory tract after inhalation of ALA (Baumgartner R et al, 1996).

ALA-induced PpIX as a sensitiser has a number of properties that can be taken advantage of clinically. Firstly, it can be administered both topically and orally; has a drug-light interval of a few hours and a skin photosensitivity limited to 24-48 hours. It also has a favourable tumour : normal tissue fluorescence pattern which together with its rapid photobleaching properties allows limitation of damage to normal tissue even for extended light treatments. The serious limitation of ALA for the treatment of invasive carcinoma is the restricted depth of necrosis achievable. This partly reflects the limited depth of penetration of 635nm light, but also the preferential expression of PpIX by superficial mucosal layers compared with deeper muscular layers as demonstrated in small animal studies (Bedwell et al, 1992, Loh et al, 1992). This however is advantageous for the treatment of conditions limited to superficial layers and further enhances the targeting ability of ALA-PDT.

Increasing the efficacy of ALA without reducing its selectivity is currently being investigated. The use of inhibitors of ferrochelatase and

protoporphyrinogen oxidases would potentially increase PpIX accumulation (Kennedy and Pottier, 1992, Peng et al, 1997) and promoting iron chelation has been shown to have the same effect (Chang et al, 1997). Various long chain ALA ester derivatives have recently been shown to lead to an increase in bioavailability (Kloek and van Henegouwen, 1996) and intravenous ALA administration would allow higher doses to be used without increasing hepatic toxicity by reducing the effect of first pass metabolism.

3.2.3 Light Delivery and Tissue Distribution

Light fluence in tissues is attenuated by optical absorption of both the target tissue (mainly due to haemoglobin), the sensitiser itself and by optical scattering. Therefore light penetration will vary amongst tissues, essentially dependent on their haemoglobin content. Moreover, although in general, absorption by the sensitiser itself is much less than tissue absorption, for some sensitisers (for example the phthalocyanines) light absorption can be quite high leading to a phenomenon known as "self-shielding".

The behaviour of light within a given tissue is also wavelength dependent as higher scatter occurs at lower wavelengths. Light penetration is therefore also wavelength dependent and of the order of 1-3mm at 630nm and double that at 700-850nm (Wilson et al, 1985). The depth of effect gained from sensitisers which absorb at longer wavelengths is therefore greater.

Numerous different light sources may be used for PDT. The only absolute requirement is that the light is of the relevant wavelength for the photosensitiser used. Both laser and non-laser sources are used clinically, each with specific advantages and disadvantages. Non-laser devices such as fluorescent tubes and incandescent lamps are cheaper and more portable than lasers. However their application is limited to surface treatment and the light generated is not wavelength specific. This means that a significant amount of

infra-red light is also generated and unless this is filtered out, hyperthermia can occur (Stringer, 1995). (Fluences must be kept below 200mW/cm^2 to avoid thermal reactions occurring in addition to photodynamic reactions). The broader band available from lamp sources may have an advantage for use with ALA as one of the protein-derived photoproducts produced when PpIX is exposed to light is also a photosensitiser (Moan et al, 1996). Photo-protoporhyrin is a chlorine and absorbs light at 670nm, a wavelength which would be included in the emission from a lamp, but not from a laser set up for PpIX at 635nm.

A laser is often the light source of choice for PDT as it is possible to emit light of a defined wavelength. The real advantage of laser as a light source is the ability to direct the high intensity output to a distant target by using fibreoptics. The draw back is the cost and the skill and time required to maintain metal vapour lasers. Metal vapour lasers (gold, copper, argon) are restricted to a fixed wavelength. However, when combined with a dye laser, a tuneable laser is produced which can be tuned to the desired wavelength for a given sensitiser. The metal vapour laser acts as an energy source and the beam produced from the dye lasing medium is tuned by means of a diffraction grating to a particular wavelength. Recently, compact diode lasers have been developed, which if sufficient power can be produced at suitable wavelengths, will probably replace dye and metal vapour lasers currently used for PDT.

Over the last few years evidence has accumulated to suggest that a break in light transmission increases the magnitude of the PDT effect in small animal models (Messman et al, 1995, Vangeel et al, 1996). This has been termed 'fractionation' and its efficacy may be based on the re-accumulation of oxygen and PpIX depending on the length of the break in light transmission. A fractionation period of minutes would allow oxygen to be replenished at the target site and a longer period may allow re-accumulation of PpIX. Animal data would suggest that the optimum time for fractionation is after 20% of the light dose and that if given too late is of no benefit probably because microvascular

thrombosis renders the fractionation mechanism ineffective (Messman et al, 1995). In a small clinical series of patients with oesophageal carcinoma, light fractionation has been attempted and reported as improving the effectiveness of PDT (Messman et al, 1997), but obviously needs to be confirmed in a larger series.

3.3 CLINICAL TARGETS FOR PDT

3.3.1 Tumours and PDT

Frank malignancy and dysplastic processes form the bulk of indications for PDT. In general most reported series can be divided into the palliative treatment of cancers not amenable to conventional therapy and attempted curative treatment of small tumours or pre-malignant conditions which are difficult to treat without radical surgery. The literature is however bedevilled by anecdotal reports, small series, inconsistent response definitions and the absence of randomised controlled trials. Even in the largest reported series of 540 cases of malignancy (Li et al, 1990), this included a heterogenous group which involved the treatment of not less than 13 different tumour types.

The main difficulty in achieving adequate necrosis of large solid tumours is the limited penetration of light to the centre of the tumour and the relative hypoxia of the environment in the centre. The oncological soundness of PDT for malignancy must also be questioned. As highlighted by Bown, the crux of long term efficacy must be dependent on the treatment of what surgeons and histopathologists term the 'resection margin', on which there is precious little information (Bown, 1990). Although absolute selectivity is often seen as the elusive goal of PDT, it could be argued that this would be undesirable as PDT is never likely to be sensitive enough to effect a complete cancer cell kill. There would therefore be a risk of viable tumour cells remaining at the tumour / normal tissue interface. Afterall, it has been well established over decades that in the surgical resection of malignant tumours an adequate margin of normal tissue is required to prevent local recurrence.

Despite the above reservations, PDT has been used successfully in the treatment of many superficial malignancies including non-melanotic skin cancers (reviewed by Peng et al, 1997); oral dysplasia (Fan et al, 1996); Barretts oesophagus (Overholt and Panjehpour, 1997); small oesophaeal and lung cancers (Hayata et al, 1996) and superficial bladder cancer (Kriegmair et al, 1994). The use of PDT for larger solid tumours has been less successful however (Regula et al, 1995) and some of the early bladder and pulmonary cases were complicated by complications of contraction and fatal haemorrhage respectively. A novel use of PDT in haematological malignancies may allow reduction in tumour cell contamination in autologous bone marrow transplantation (as reviewed by Mulroney et al, 1994).

3.3.2 Vascular disease and PDT

3.3.2.1 Sensitisation of Arteries

The first report on the sensitisation of arteries was based on the realisation that atheroma, like neoplastic tissue, had a higher rate of proliferation compared with normal tissue. Using the same concept as had been used for tumour localisation, Spears showed that atheroma in rabbits and various other animals including a primate, showed selective fluorescence following systemic HPD administration (Spears et al, 1983). Soon after it was demonstrated that atheromatous lesions in the aorta of human post mortem subjects also exhibited selective fluorescence with HPD (Kessel and Sykes, 1984). At the time, the impetus for continuing investigation came from the need for experimental models to mimic neoplastic disease and the belief that fluorescent mapping of atheroma may potentially aid a future treatment modality. It was later proposed as a method of computer assisted guidance for laser angioplasty in an effort to reduce the complication of vessel wall perforation.

In the same way that Spears has demonstrated relative selectivity between atheromatous plaque and normal arterial wall, it has also been shown that

sensitiser accumulates in hyperplastic arterial tissue to a greater extent than in the uninjured arterial wall. With phthalocyanine sensitisation in the rat carotid model, preferential fluorescence partitioning has been demonstrated in injury-induced NIH compared with uninjured arterial wall where there was a 60% reduction in fluorescence (LaMuraglia et al, 1993). This would suggest that some degree of targeting diseased segments of the arterial tree may be possible with PDT.

3.3.2.2 Arterial PDT

Again using HPD sensitisation, PDT was initially applied *in vivo* to an atherosclerotic rabbit model (Litvak et al, 1985), but it is difficult to draw any conclusions from this work as light was delivered from a laser fibre in flowing blood. Reduction in the size of a rabbit aortic atherosclerotic plaque by PDT was also demonstrated using Photofrin, (Neave et al, 1988), but it is unclear whether adjacent 'control' plaques were adequate controls or also received light as well.

At this time the VSMC had been highlighted as an important cell in primary stenotic arterial lesions and implicated as playing a pivotal role in the emerging problem of restenosis (after balloon angioplasty). In a timely publication, Dartsch demonstrated that VSMCs from both stenotic and restenotic lesions (obtained from human atherectomy specimens) could be killed by incubation with Photofrin *in vitro* even without light application (Dartsch et al, 1990a). These cells were also found to be more sensitive than VSMCs from normal arteries and this effect was even more pronounced with Photofrin and ultra-violet light (Dartsch et al, 1990b). This latter paper went on to suggest that PDT may be potentially beneficial in the prevention of vascular restenosis after arterial recanalisation or angioplasty.

This work spawned numerous publications on the effect of PDT on intimal hyperplasia *in vivo.* Intimal hyperplasia was generated by endothelial

denudation of the carotid artery following the passage of a Fogarty balloon. In a rabbit model, Eton showed that PDT using Photofrin (5mg/Kg) as a sensitiser and 7.6J/cm^2 red light could significantly reduce the development of intimal hyperplasia at 5 weeks (Eton et al, 1992). However, in this series PDT was applied 9 days following the injury, and surprisingly, Photofrin alone and light alone were also seen to reduce intimal hyperplasia compared with controls. Although this difference was not significant, the difference between these groups and the PDT group was also not significant. The implication of this is that Photofrin may have some innate cytotoxicity *in vivo* as well as *in vitro* (Dartsch et al 1990a) and that red light alone may also be therapeutic even in non-thermal doses. [Red light has recently been shown reduce intimal hyperplasia following stent implantation in a porcine coronary model (DeSchreerder et al, 1997)].

Ortu et al, using a phthalocyanine sensitiser and 100J/cm^2 (675nm) light showed that PDT administered 2 and 7 days post injury reduced intimal hyperplasia in a rat carotid model (Ortu et al, 1992). Moreover, the reduction in intimal hyperplasia at 14 days was seen to correlate with VSMC depletion from the media. In the same model and using the same parameters this effect has been shown to be sustained at 16 weeks (La Muraglia et al, 1994).

Not all studies have shown a sustained benefit of PDT. Eton, using a rabbit carotid model, has since reported that PDT with AlSPc (Eton et al, 1995) and Photofrin (Eton et al, 1996) produced an acute cytotoxic effect, but no benefit in terms of neointima reduction at 6 weeks. This work however may be flawed for two reasons. Firstly, two different scenarios were studied with both the prevention of neointima after injury and the treatment of established neointima assessed. Secondly, the injury imposed resulted in a high rate of vessel occlusions, which were then excluded from the analysis. The authors attributed this to the small size of rabbit carotids, but against this is the fact that occlusion does not seem to be such a problem in the rat which is even smaller. It may also

be true that one or more of the many variables involved in the efficacy of PDT may be critical to the outcome.

All the above studies relied on light application to arteries 1-9 days following balloon injury. Nyamekye et al demonstrated that it was not necessary to delay PDT following balloon injury and therefore the effect was not dependent on VSMCs being stimulated (Nyamekye et al, 1995). In fact, Hsaing et al have shown convincingly that a higher degree of selective uptake is achieved if the sensitiser is given immediately after injury rather than delayed (Hsaing et al, 1995a). In the rat carotid model, Nyamekye demonstrated VSMC depletion in normal arteries and a reduction in intimal hyperplasia in balloon injured arteries with ALA (Nyamekye et al, 1995) and aluminium disulphonated phthalocyanine (AlS_2Pc) (Nyamekye et al, 1996a). A dose dependent response was seen from 50-200mg/Kg ALA and 0.5-5mg/Kg AlS_2Pc at a dose of $50J/cm^2$ 630 and 675nm light respectively. The response was still evident, but the effect reduced, even at 26 weeks.

The interesting observation from all the above reports, using different sensitisers, is the consistent histological appearence of arteries following PDT. All authors commented that VSMC depletion was not accompanied by an inflammatory cell infiltrate. Re-endothelialisation was seen to occur by specific staining at 2 weeks (Nyamekye et al, 1995) and by electron microscopy at 4 weeks (LaMuraglia et al, 1994). Grant et al, studied the response of normal rat femoral arteries to PDT with ALA and AlS_2Pc sensitisation (Grant et al, 1994). He also found an absence of an acute inflammatory infiltrate and confirmed re-endothelialisation at 2 weeks. The striking revelation of this paper was that in the ALA group, VSMC depletion persisted even at 6 months. Moreover this was accompanied by a significant increase in the lumenal cross sectional area without evidence of aneurysm formation.

The authors went on to investigate the mechanical integrity of arteries that had received PDT in a rabbit carotid model (Grant et al 1995). Animals sacrificed

at 3, 7 and 21 days after PDT showed no reduction (and even an increase at 21 days) in the hydrostatic pressure required to burst the treated carotid artery compared with controls. The authors speculated that this may result from the preservation of collagen which has been shown to occur in the rat colon following PDT (Barr et al, 1987). Furthermore there is evidence that collagen may undergo cross-linking following PDT which may explain the increased strength despite cellular depopulation (Verweij et al, 1981; Spikes, 1993).

Some insights into the apparent contradiction of VSMC eradication accompanied by a healing response and re-endothelialisation, all mediated by a cytotoxic modality, come from *in vitro* work on the ECM (Adili et al, 1996). Using bovine aortic cultures of endothelial and smooth muscle cells, PDT (using AlSPc) directed at the ECM was found to inhibit attachment and proliferation of smooth muscle cells, but potentiate that of endothelial cells. This work highlights the complex interaction of different cells with the ECM and suggests that PDT produces a different response in binding sites for different cells. Recent evidence, from the same laboratory, suggests that this response may be mediated by cytokine inhibition, specifically transforming growth factor ß, known to have a role in tissue repair (Statius van Eps and LaMuraglia, 1997). The only caveat to this intriguing work is its application to the *in vivo* situation.

3.4 SUMMARY

Photodynamic therapy is a cytotoxic treatment which has been used in the management of benign proliferative and malignant conditions. Its efficacy is dependent on a number of variables which makes dosimetry complex and in some cases unpredictable. Nevertheless, PDT has gained acceptance in the treatment of certain conditions that have proved difficult to treat by other means. It has not however, become widely established which is probably as a result of the cumbersome nature of some of the older generation

photosensitisers that have not found favour with patients and clinicians alike. Newer sensitisers with skin photosensitivity periods limited to days rather than weeks allied to convenient diode laser light sources may improve the logistic problems associated with PDT. The major block to acceptance by the medical scientific world is the lack of therapeutic proof from randomised controlled trials without which PDT will remain a largely unproven treatment modality.

Intra-arterial PDT to prevent restenosis has promise because it offers a new approach to an important and unsolved clinical problem and can be conveniently applied at the same time as angioplasty. The mechanism of action in the arterial wall is still unclear, but research to date has focused only on its anti-proliferative role, and accurate morphometric assessment of its affect on arteries has not been studied.

CHAPTER 4 AIMS OF THESIS

4.1 DEFICIENCIES OF ARTERIAL PDT WORK TO DATE

4.1.1 Animal Models

The advantages, disadvantages and limitations of various animal models of restenosis have been extensively reviewed (Muller et al, 1992; Höfling and Huehns, 1996; Mehta et al, 1996; Pratt and Dzau, 1996). From this work, one can conclude that there is a wide variation amongst species in cardiovascular morphology, physiology and the response to arterial injury.

The rat model has been extensively used in many interventional studies aimed at influencing the response to arterial injury, including much of the early PDT work (Ortu et al, 1992; La Muraglia et al, 1994; Nyamekye et al, 1995; Nyamekye et al, 1996a). The rat is cheap to purchase, easily supplied and housed and relatively easy to handle. However, it is seriously disadvantaged by its small size in comparison to humans both in terms of the difference in arterial size for interventional purposes and the vast weight and hence drug dose differential. Even more important is the fact that the response to injury is limited to abundant smooth muscle proliferation which appears to be overly sensitive to pharmacological and perhaps other forms of therapy. Rabbit models have also been used for PDT work (Eton et al, 1992; Eton et al, 1995; Eton et al, 1996) and although the size differential is better than the rat (the rabbit iliac artery is similar to the human coronary) and hence suitable for percutaneous intervention, the response to injury tends to be very concentric, unlike the eccentric plaques characteristic of human atherosclerosis.

The porcine model has many advantages compared with tha above small animal models. The arterial morphology and physiology closely resemble the human and the size and weight allow the use of percutaneous techniques and similar drug doses to those used in man. Despite this, swine have been used by relatively few investigators working with PDT (Hsiang et al, 1994; Hsiang et

al, 1995b; Gonschior et al, 1996) and where they have been used, they have not been used to their best advantage in terms of method of injury, appropriateness of photosensitiser and percutaneous interventional methodology.

4.1.2 Method of Injury

The response to injury depends not only on the animal model used, but also on the type of injury inflicted. Recent work has highlighted the different response obtained by endothelial denudation and balloon over-distension (Doornekamp et al, 1996) and by thermal injury and stent implantation (Staab et al, 1997). Endothelial injury produces an intimal hyperplastic response due to smooth muscle proliferation, but a deeper injury, perhaps involving the adventitia (Staab et al, 1997) is required to produce any change in arterial dimension ie. remodelling.

Previous arterial PDT work in the rat, rabbit and swine models has relied on endothelial denudation as a method of injury and hence been limited to the study of NIH alone. Although endothelial denudation does occur during angioplasty, it is limited to the segment ballooned and does not occur over the extensive length produced by the rather artificial denudation injury induced by some authors. Excessive endothelial injury over and above the length of the treated segment has been postulated by some (Nyamekye, 1996a) to be responsible for the re-emergence of NIH during follow up. Work carried out in Post's laboratories has recently confirmed that re-endothelialisation occurs faster after a 2.5cm injury than a 5cm one (Doornekamp et al, 1997). In this study however the difference in re-endothelialisation between the two lesions did not influence NIH development. The authors concluded that this was perhaps explained by a dysfunctional phenotype of the regenerated endothelium, but an alternative explanation would be that there was insufficient difference in the length of the lesions produced and a better comparison would have been made between a 2.5cm and 15cm lesion. In many species including rat and man, re-endothelialisation stops spontaneously after

8-12 weeks (Clowes and Reidy, 1991) leaving the central portion of a long length of denudation uncovered, but the reasons behind this remain elusive.

In contrast, balloon angioplasty used to produce an injury in an animal model closely resembles the therapeutic situation in clinical treatment of stenoses and has the added advantage of using identical balloon catheters. The pathophysiology of the histological response to balloon angioplasty has been closely studied in the pig (Steele et al, 1985) and this is probably the closest model to the clinical situation notwithstanding the diseased nature of atherosclerotic arteries. It produces a proliferative cellular response and mechanical arterial wall disruption and allows the assessment of both NIH and remodelling.

4.1.3 Photosensitisers

The majority of arterial PDT work has used systemic Photofrin (Neave et al, 1988; Eton et al, 1992; Hsiang et al, 1995; Eton et al, 1996) or phthalocyanines (Ortu et al, 1992; Nyamekye et al, 1996a) as sensitisers. Both have prolonged skin sensitivity periods, which would make them impractical for clinical use in the context of restenosis prevention. Local drug delivery of Photofrin has been investigated as a means of eliminating skin photosensitivity (Gonschior et al, 1996), but this introduces the technical and equipment complexities of local drug delivery prior to light delivery.

The other major disadvantage of the above sensitisers is their depth of effect is greater than the thickness of the arterial wall and therefore there is a risk of an extra-arterial PDT effect extending beyond the arterial wall. ALA however, produces a response limited to a few millimetres and is therefore an ideal sensitiser for arterial work, but to date has only been used in the rat endothelial denudation model (Grant et al, 1994; Nyamekye et al, 1995).

4.1.4 Light Delivery

Only three papers have reported the use of percutaneous light delivery devices (Hsiang et al, 1994; Hsiang et al, 1995c; Gonschior et al, 1996), the remainder used an open approach and external light source. Both the above authors describe intra-arterial light sources as cylindrical probes of a fixed diameter consisting of a 200μm fibre and a diffuser. The main difficulty with such devices is the layer of blood which would collect between the device and the arterial wall which has been shown to reduce light delivery (Vincent et al, 1991). Even though Gonschior et al recommend saline flushing, failure to use an inflatable catheter-based light delivery system deployed flush to the arterial wall would inevitably have resulted in a variable and unpredictable amount of light being absorbed by blood.

4.1.5 Methodology

The assessment of the results of arterial PDT has, in the vast majority of studies, been compromised by focusing exclusively on NIH area or diameter. A few studies also measured either lumen or medial area, but none looked at complete morphometry and were thus unable to assess any remodelling response. Subsequent knowledge regarding the role of remodelling has exposed this as a serious oversight in the investigation of arterial PDT as a method of preventing restenosis.

4.2 THESIS AIMS

4.2.1 Experimental Work

Previous work has established the concept that PDT can reduce the neointimal hyperplastic response that results from arterial injury. In this thesis my objective is to make arterial PDT a practical therapy which could conceivably be used as an adjuvant treatment to conventional angioplasty. My hypothesis is that light for PDT can be delivered percutaneously and that it influences the

development of intimal hyperplasia and arterial remodelling following angioplasty.

With this in mind, a photosensitiser (ALA) with a skin photosensitivity period limited to 24-36 hours was chosen. An experimental large animal model - the juvenile domestic swine was proposed as representing the optimum model, both in terms of arterial size and structure and response to angioplasty. The first series of experiments were designed to calculate the pharmacokinetics of ALA in the porcine arterial system. Based on the timing of maximal fluorescence, the next series of experiments aimed to develop a system of intra-arterial light delivery using a catheter-based laser fibre. Normal, uninjured arteries in sensitised animals and appropriate controls were exposed to light percutaneously, and recovered. On culling the relevant sections were harvested and histological sections examined to assess the effect of PDT, the end point being VSMC depletion.

Using data from the above experiments, intra-arterial PDT was delivered after arterial injury to assess its effectiveness in preventing the development of a stenotic lesion. Accurate morphometry was performed to measure both the neointimal area and total arterial dimensions of injured segments with and without PDT.

4.2.2 Clinical Work

One of the problems with the introduction of a therapy to combat the development of restenosis post angioplasty is the accurate identification of the "at risk" group. Restenosis occurs in approximately one third of patients following an uncomplicated first time angioplasty and thus exposure of all patients to an adjuvant treatment would result in over treatment of two thirds. No method currently exists to identify those patients likely to restenose, but the recent identification of polymorphism of the ACE gene offers some hope. The

DD allelle of this gene has been associated with both an increased risk of developing atherosclerosis and a poor response to intervention.

A series of patients was investigated with respect to their outcome following vascular intervention (PTA and bypass) and their genotypes analysed. A correlation between restenosis and the DD genotype will be sought.

As the above large animal experiments were successful, a pilot series of clinical patients underwent arterial PDT following femoral angioplasty. This series was limited to patients who had already restenosed after conventional angioplasty and were therefore at heightened risk of a further poor outcome after repeat angioplasty. They were closely monitored for 6 months to assess the safety and efficacy of the technique.

4.3 STATEMENT OF ORIGINALITY

The vast majority of the work comprising this thesis was my own. In collaboration with my supervisors, I wrote the grant application to secure funding for the experimental part of this work and designed the protocols for the investigation of PDT in the swine model. The experimental work was performed by myself and I acknowledge the valuable assistance of Dr Jean McEwan with the coronary angioplasty technique. I am indebted to Biological Services at University College London (UCL) for their skillful animal care and to the Imperial Cancer Research Fund for their expert processing of all histological material. All data was collected and analysed by myself.

I wrote the clinical protocol for the investigation of the ACE genotype/restenosis relationship and was responsible for all patient recruitment and investigation. The genotyping was performed by Dr Saul Meyersin under the direction of Dr Hugh Montgomery in Professor Steve Humphries laboratory at UCL.

I was responsible for the organisation of the clinical PDT trial and assisted Dr Maurice Raphael with the angioplasty procedure. Duplex surveillance was performed in conjunction with the technologists at the Vascular laboratory, Middlesex Hospital. Again, data collection and analysis was solely my responsibility.

CHAPTER 5 PHARMACOKINETICS OF ALA IN THE SWINE MODEL

5.1 INTRODUCTION

The aim of these experiments was to develop a temporal fluorescence profile of ALA-derived PpIX in the porcine arterial wall. The only previous work with ALA in the arterial system (Nyamekye et al, 1995; Grant et al, 1994) in the rat model had produced conflicting results with respect to the timing of maximal fluorescence. This together with the different histological structure of the porcine arterial wall made these preliminary experiments essential before embarking on PDT studies.

5.2 METHODS

5.2.1 Animals

All animal studies were carried out under licence (Animals [Scientific Procedures Act] 1986) and with the co-operation of the named veterinary surgeon. Large White/Landrace crossbred pigs weighing 15-20Kg were used for all experiments. Animals were housed in straw covered pens and fed and watered with a standard pig diet. Following photosensitisation they were kept in subdued lighting for over 24 hours.

5.2.2 Photosensitiser

ALA, *Levulan* (Dusa Pharmaceuticals, Valhalla, New York, USA) was obtained in a purified powder form and made up into an intravenous preparation by the addition of water for injection in the Pharmacy department of University College London Hospitals. In conjunction with pharmacists at Imperial College London, 8.4% sodium bicarbonate was titrated to obtain the optimum balance of product stability and pH. Buffering to a pH of 4.8 was

sufficiently alkaline to be tolerated by the animals and acidic enough to be stable for over 8 hours. ALA was prepared as an intravenous (iv) preparation at a concentration of 20mg/ml and given as a bolus injection over a 5 minute period via an ear vein.

5.2.3 Anaesthetic

Animals were anaesthetised with inhaled halothane following pre-medication with intra-muscular medetomidine hydrochloride at 10-20 ug/Kg. They were intubated and maintained under spontaneous respiration with a mixture of 0.5 L/min nitrous oxide, 4 L/min oxygen and 1.5% halothane. For the purpose of iv photosensitisation alone, intra-muscular sedation with medetomidine was used.

5.2.4 Experimental Protocol

In all, 6 animals were sensitised, 3 with 60 mg/Kg and 3 with 120mg/Kg. Animals were cleaned and draped under sterile conditions in the conventional manner. In 4 animals, 2 at each dose, arterial biopsies were taken prior to sensitisation (time zero) and then sequentially to 8 hours (at 30 minute intervals to 2 hours and then hourly). In another 2 animals, one at each dose, 24 hour samples were taken following sensitisation under sedation the day before. Biopsies were taken via longitudinal groin and neck approaches for access to femoral, external iliac and carotid arteries respectively and were randomised with respect to biopsy site and time point between animals. 1 cm transverse sections of arteries were excised between ligatures, snap frozen in pre-cooled isopentane and stored in liquid nitrogen. Care was taken to ensure that sequential biopsies were taken from progressively more proximal sites which remained perfused throughout. Prior to termination, a biopsy of the proximal segment of the left anterior descending coronary artery was taken via a median sternotomy. Animals were not recovered, but were culled with a lethal dose of sodium pentobarbitone on termination of the experiment.

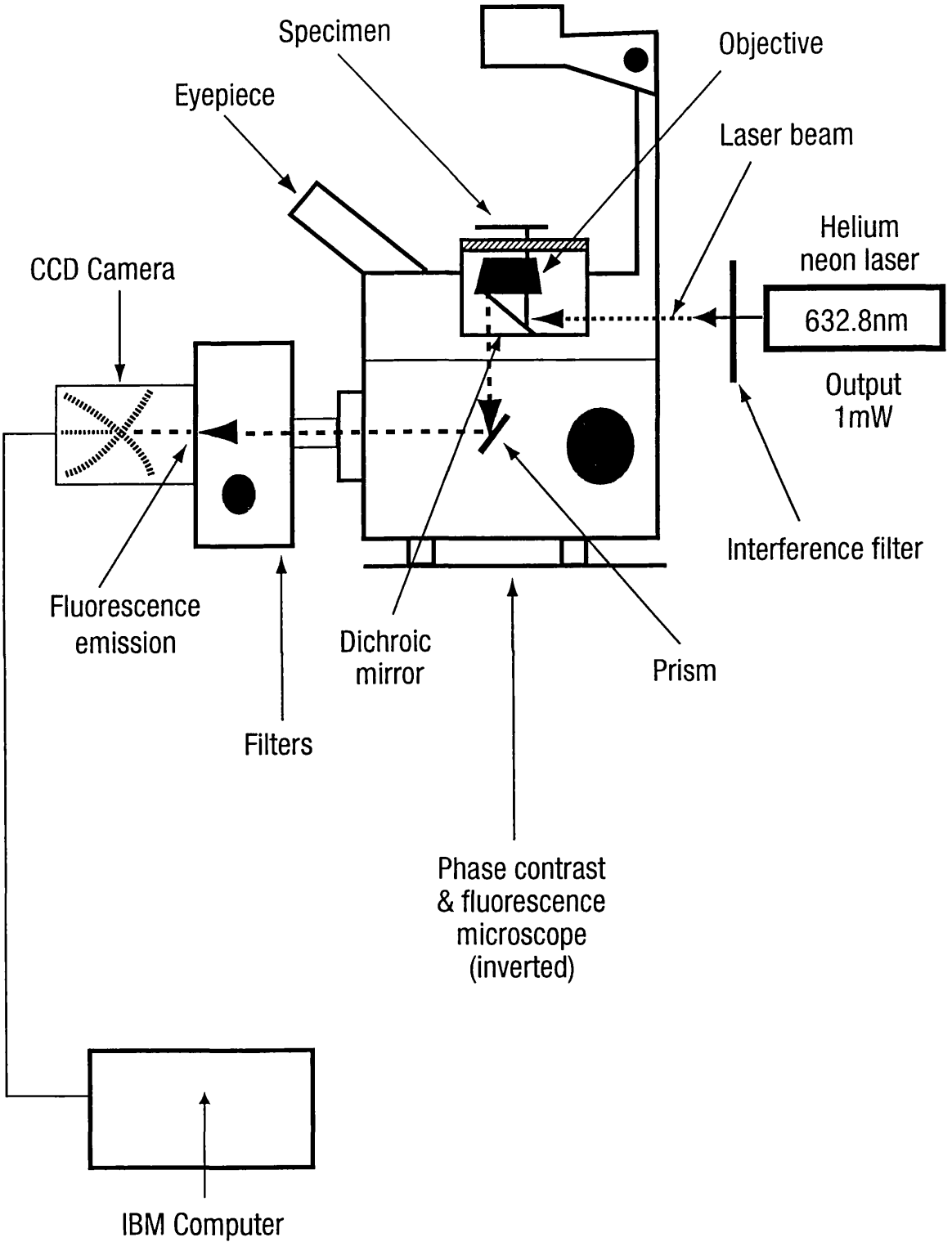


Figure 5.1 Fluorescence microscope and CCD image system.

3 transverse frozen sections (10 μm thick) were cut from each block and then stored at -20°C before being thawed just prior to fluorescence microscopy. Fluorescence of PpIX was excited by an 8 mW helium-neon laser at 633nm and the signal detected between 665 and 710 nm by a slow scan CCD (charge-coupled device) camera (Wright instruments, model 1) fitted to the fluorescence microscope. Cryogenic cooling of the CCD sensor (model P8603, EEV Ltd) was performed to minimise background thermal noise. CCD camera operation, processing and image analysis were controlled by an IBM computer and images stored on optidiscs (see Figure 5.1).

False colour images were generated and quantitative analysis performed by measuring the mean arbitrary count per pixel for each arterial layer. Fluorescence images were compared with conventional light microscopy images from specimens fixed in formalin and stained with haematoxylin and eosin (H&E) (see Figures 5.2-5.6). 3 sections per animal per time point were analysed and 9 readings taken from each section; 3 from each of the adventitia, media and intima. Background counts were periodically checked to confirm adequate equipment cooling and autofluorescence from control (time zero) sections subtracted from each count. Mean counts for each layer were then plotted for each time point.

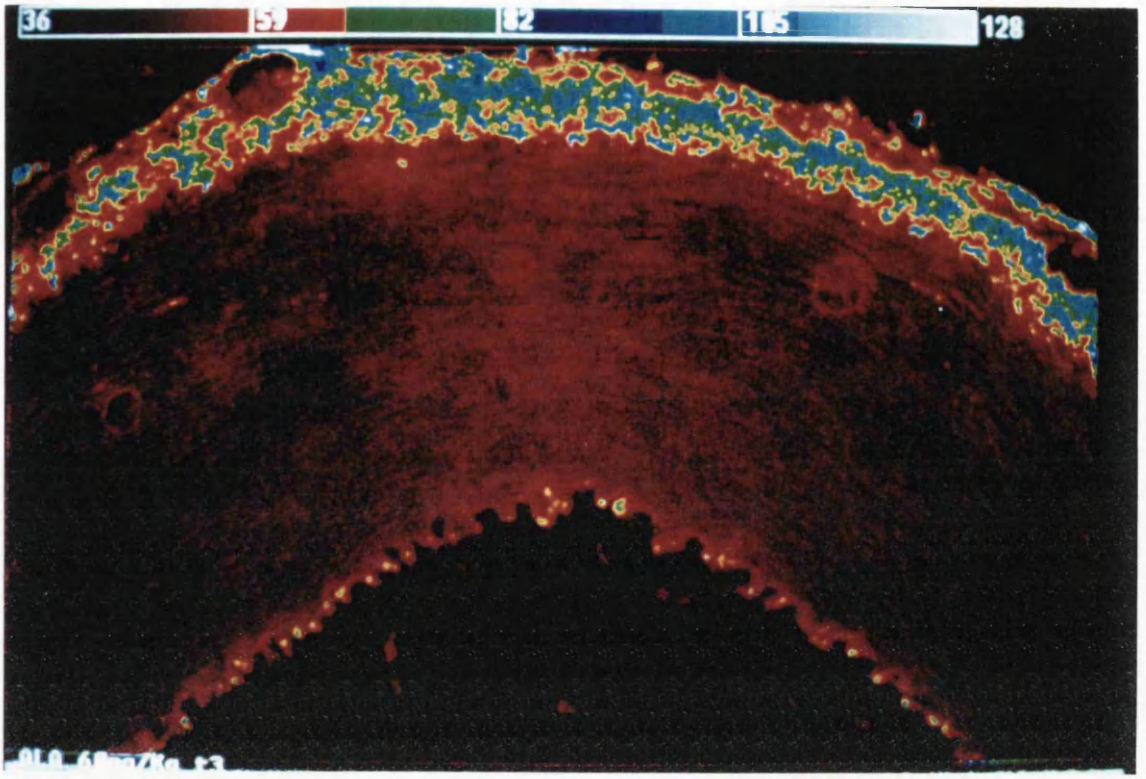
5.3 RESULTS

Arterial wall fluorescence was observed following iv ALA administration as seen in Figures 5.2-5.6. Objective measurement of PpIX fluorescence intensity, expressed as counts per pixel, increased twofold by doubling the dose of ALA given. However the temporal profile for individual arterial wall layers remained remarkably constant between the two doses (see Figures 5.6 and 5.7). Counts within the intima increased gradually to 4 hours before plateauing and tailing off. Medial fluorescence increased at approximately the same rate, but continued increasing to peak at 6 hours when counts per pixel were approximately double those in the intima and adventitia at each dose. Counts

per pixel within the adventitial layer rose steeply to initially peak between 1.5-2 hours, remained in a trough at 2-3 hours and then increased to reach a second peak at 4 hours before declining. By 24 hours fluorescence in all layers at both doses had decreased to 20 counts per pixel (equivalent to background levels).

Fluorescence counts observed in coronary biopsies were no different from those in peripheral arteries at the same time point (see Table 5.3).

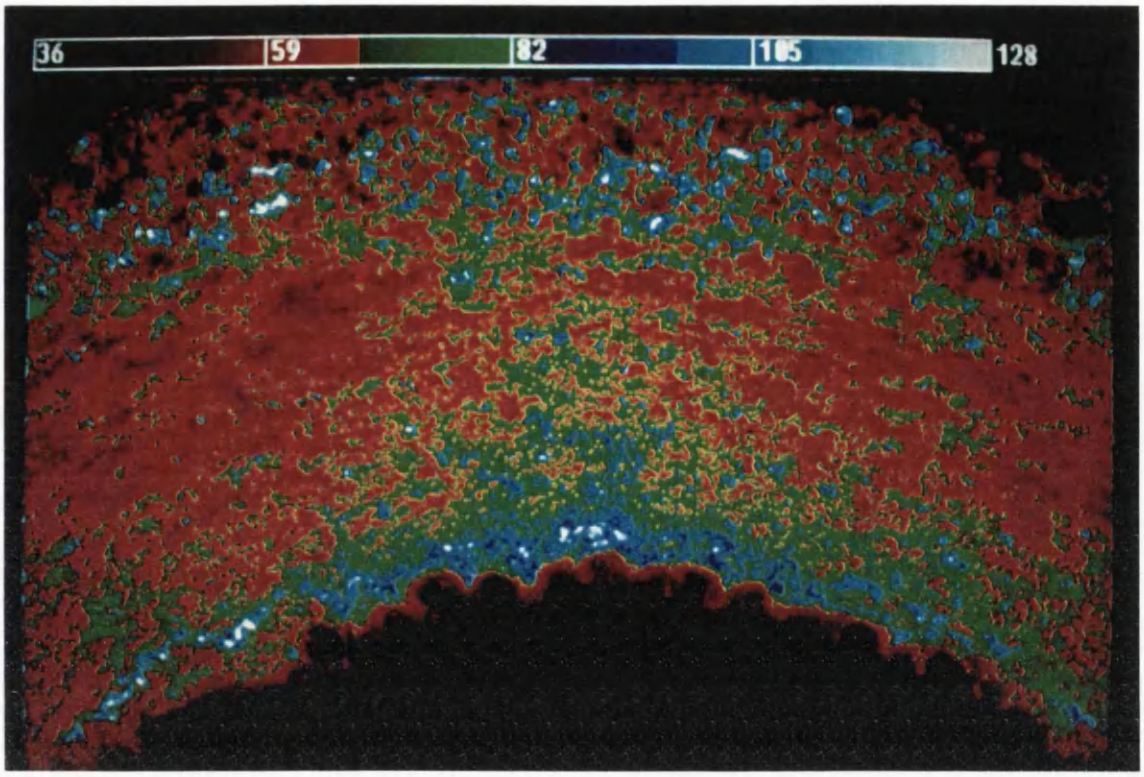
(a)



(b)

Figure 5.2 Transverse arterial sections showing (a) false colour fluorescence image (60 mg/Kg ALA at 1.5 hours) and (b) corresponding histological section (H&E).

(a)



(b)

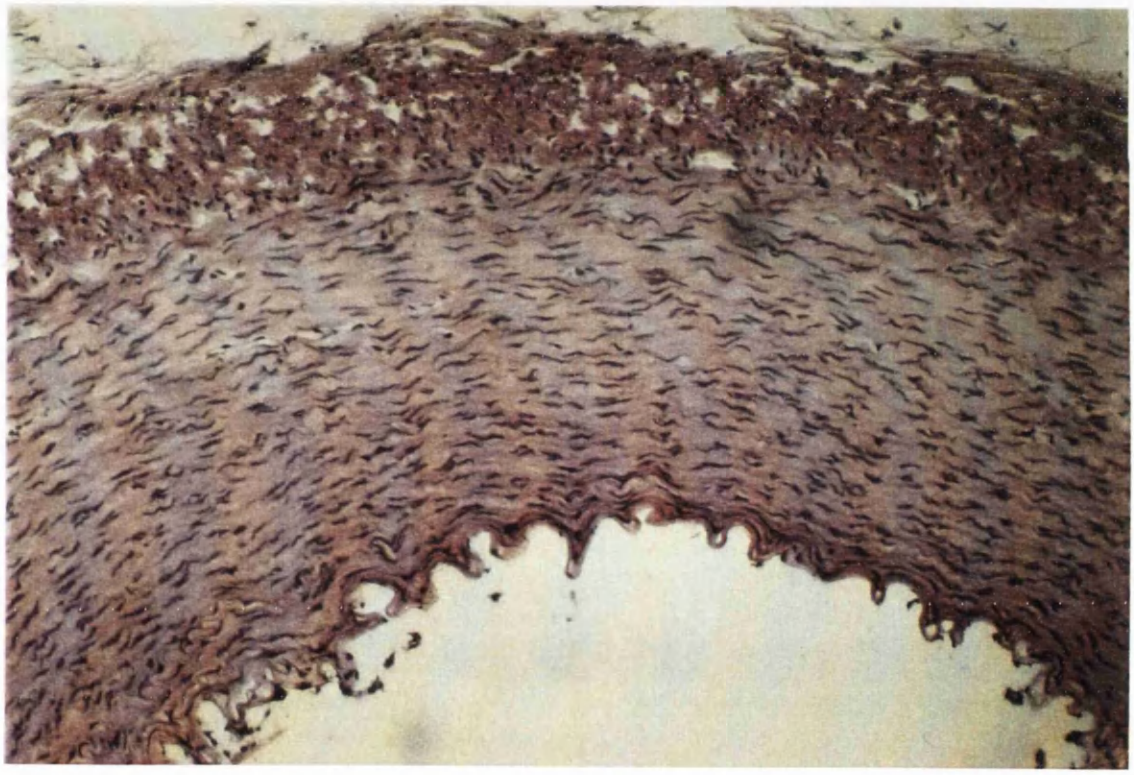
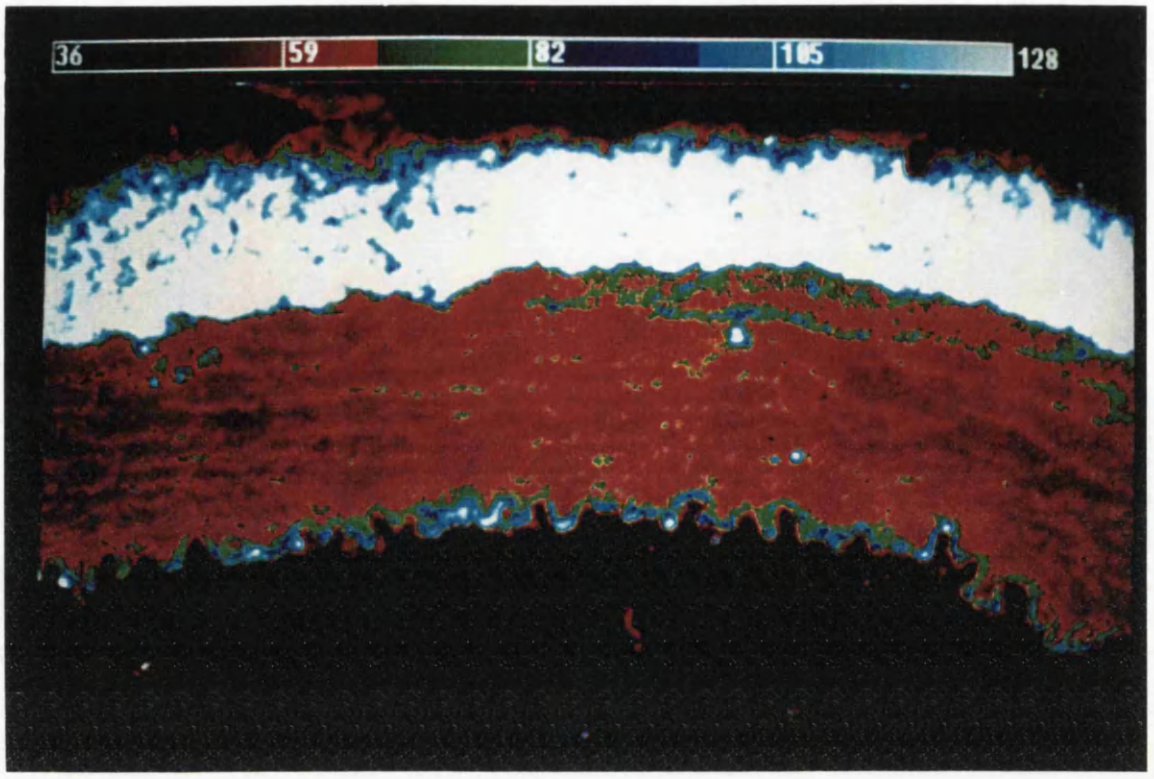


Figure 5.3 Transverse arterial sections showing (a) false colour fluorescence image (60 mg/Kg ALA at 4 hours) and (b) corresponding histological section (H&E).

(a)



(b)

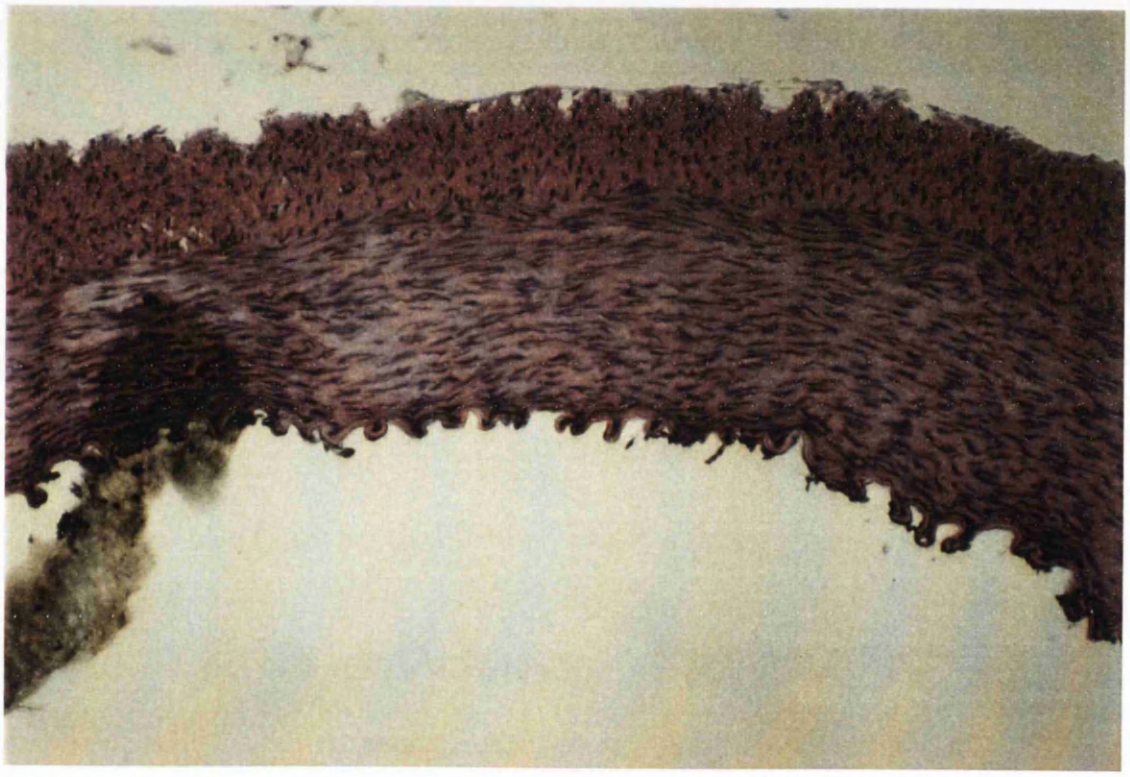
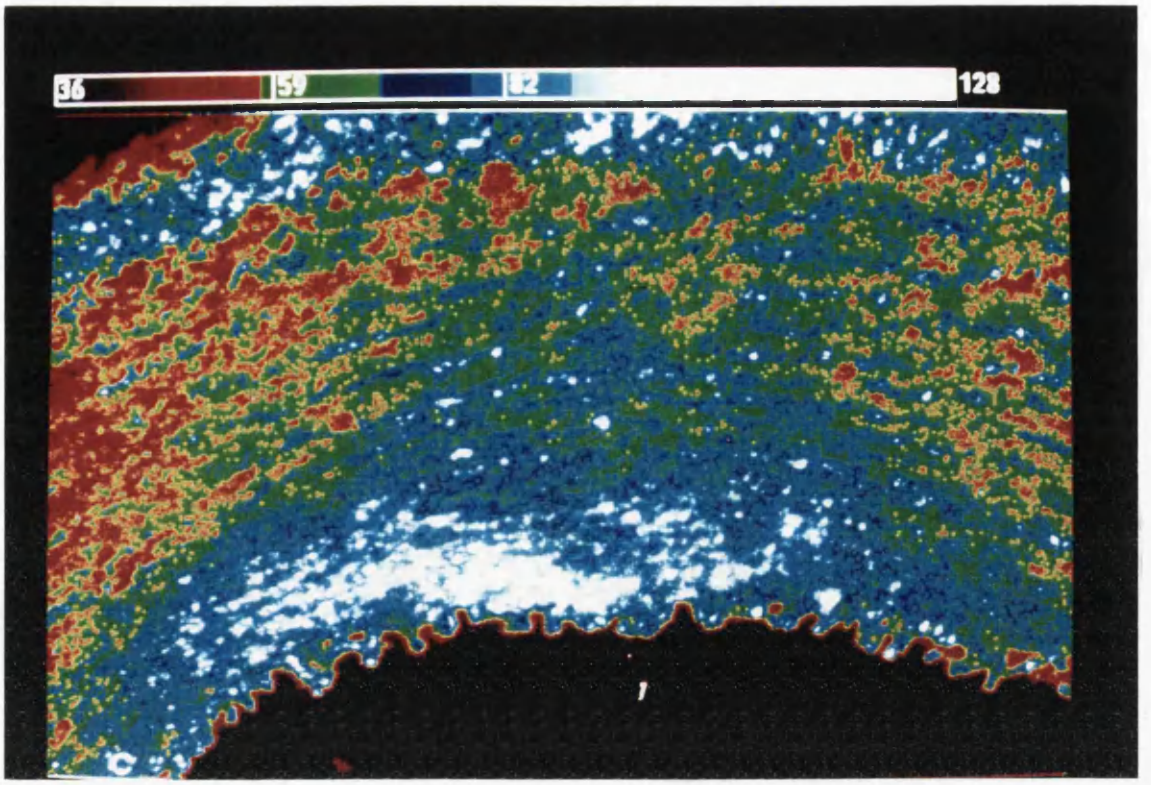


Figure 5.4 Transverse arterial sections showing (a) false colour fluorescence image (120 mg/Kg ALA at 1.5 hours) and (b) corresponding histological section (H&E).

(a)



(b)

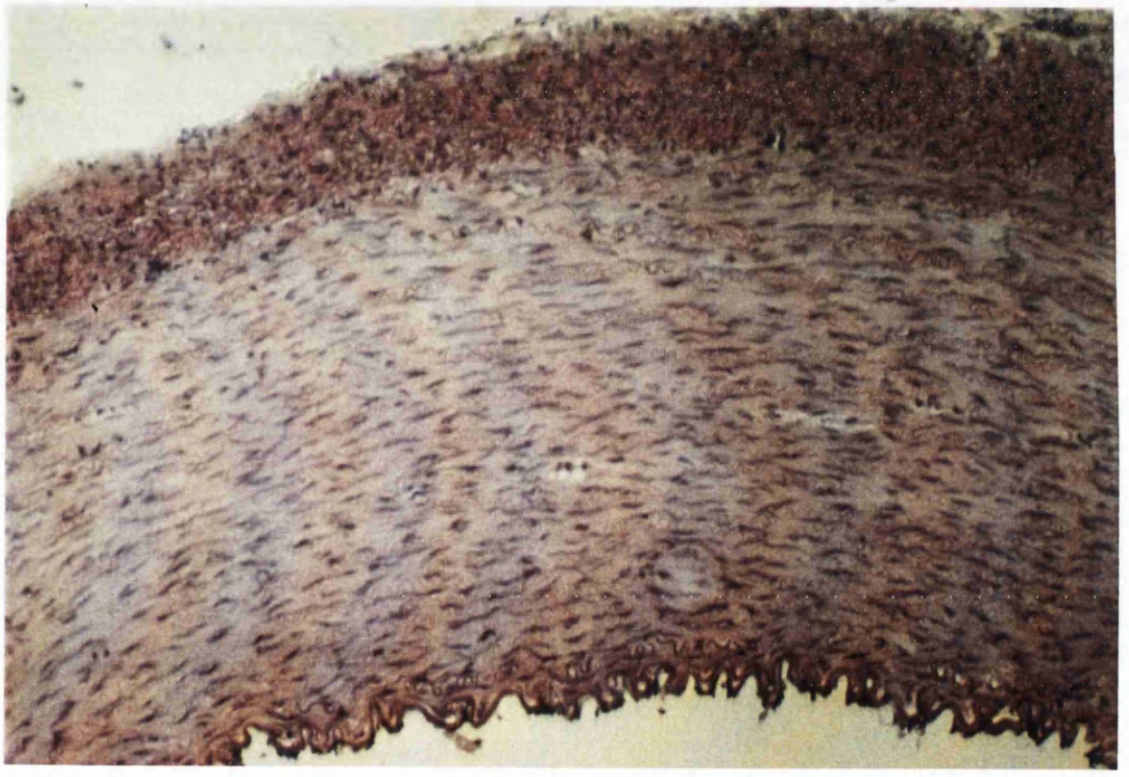
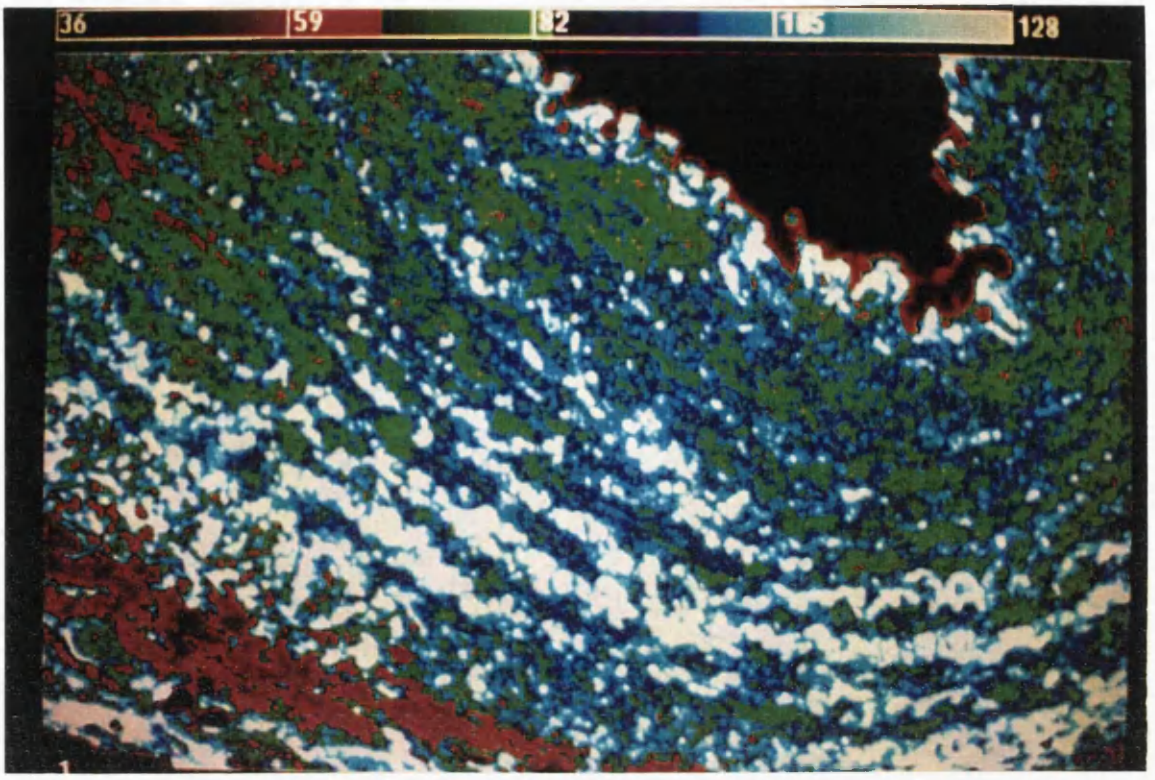


Figure 5.5 **Transverse arterial sections showing (a) false colour fluorescence image (120 mg/Kg ALA at 4 hours) and (b) corresponding histological section (H&E).**

(a)



(b)

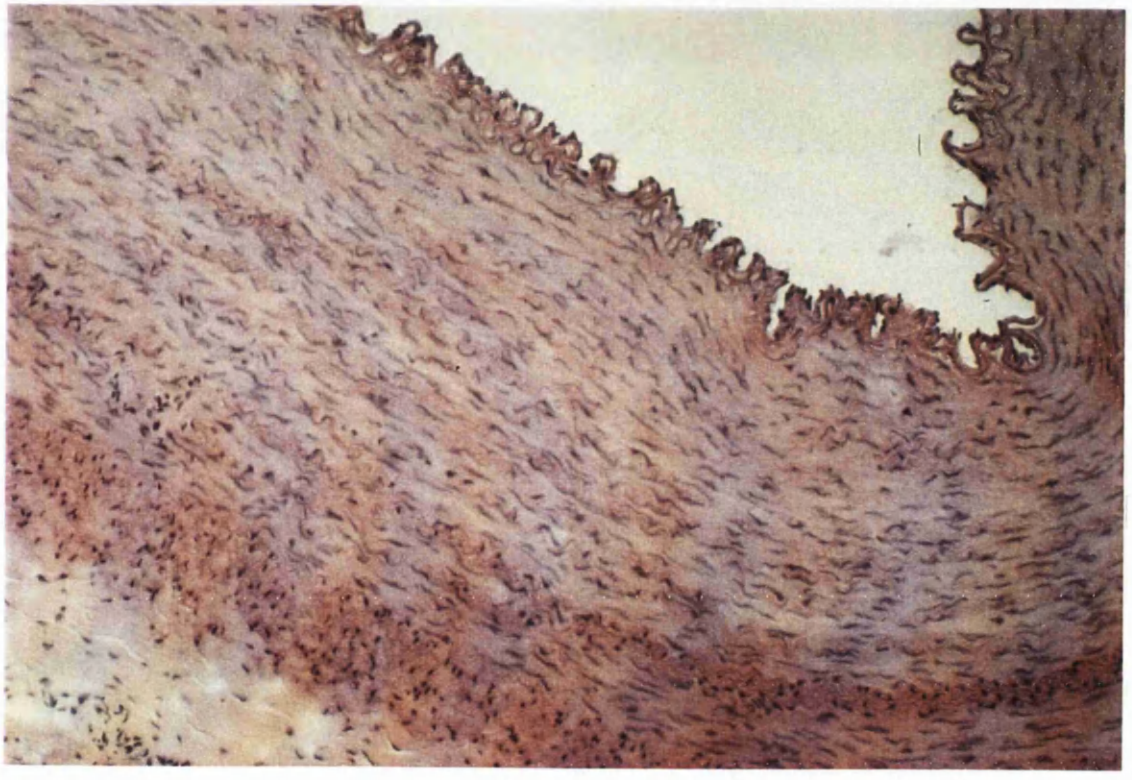


Figure 5.6 **Transverse arterial sections showing (a) false colour fluorescence image (120 mg/Kg ALA at 6 hours) and (b) corresponding histological section (H&E).**

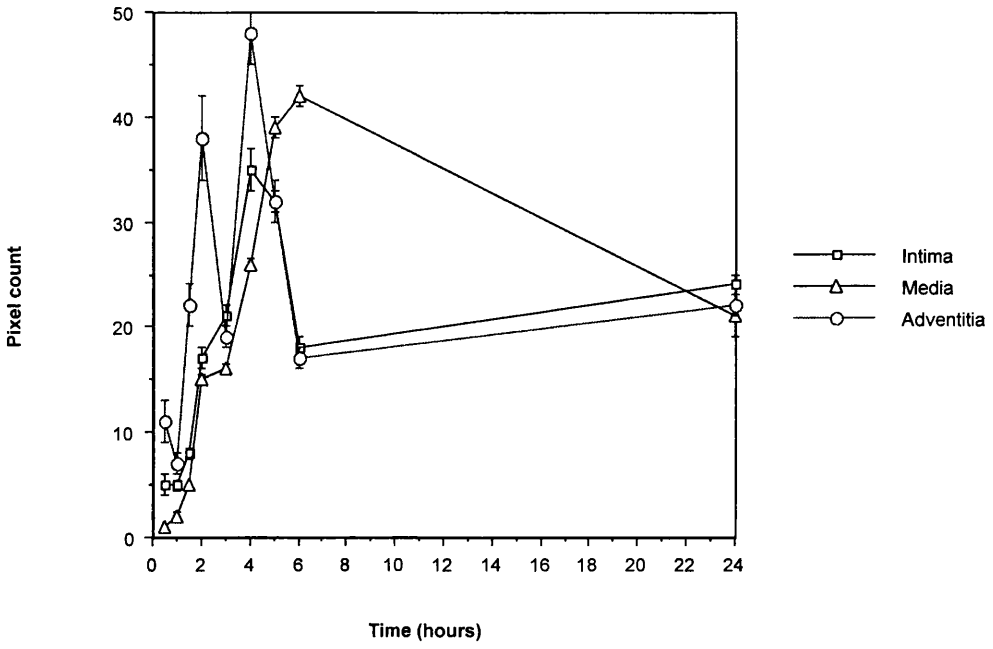


Figure 5.7 **Temporal profile of fluorescence following 60mg/Kg ALA. (Error bars represent standard errors)**

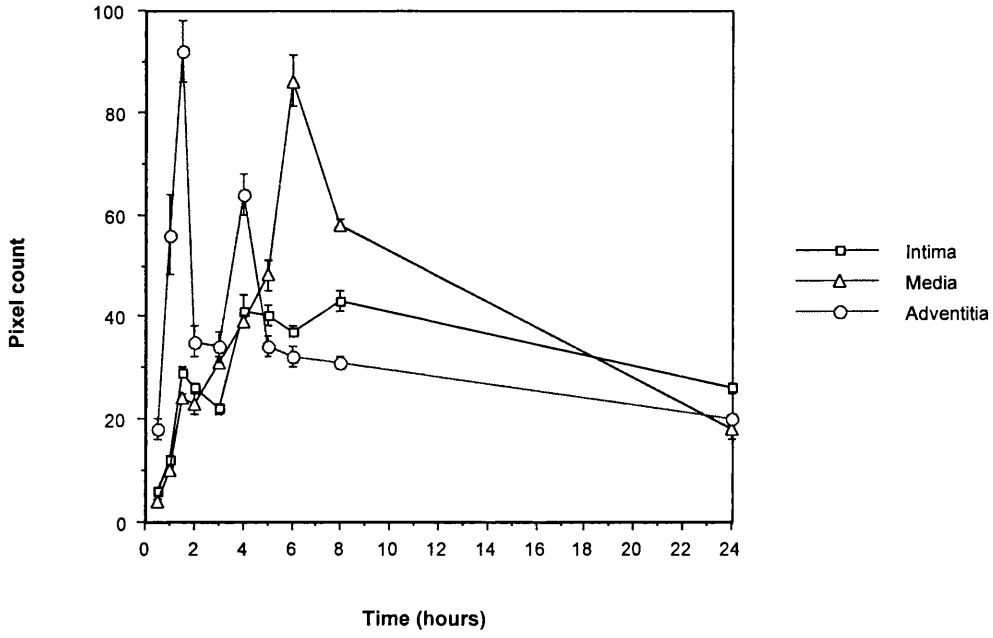


Figure 5.8 **Temporal profile of fluorescence following 120mg/Kg ALA. (Error bars represent standard errors)**

5.4 DISCUSSION

Unsensitised arteries demonstrated some degree of autofluorescence which was especially evident within the adventitia. ALA at 60 and 120mg/Kg produced a dose-dependent increase in PpIX fluorescence in all three arterial layers. The magnitude and temporal profile of the peak fluorescence varied between layers, but followed a similar pattern at both doses.

The unexpected finding from this work was the double peak seen in the adventitial layer, contrasting with the almost linear increase in fluorescence for other layers. This may be explained by the dual blood supply of the arterial wall which is particularly relevant to the adventitia. The adventitia is supplied both directly by a network of vasa vasora (which lies on the external arterial surface) and by diffusion of blood from the arterial lumen. Both the intima and media however, obtain the majority of their blood supply from the lumen by diffusion. The initial adventitial peak may therefore be as a result of the vasa vasorum supply and the second peak the result of luminal diffusion.

The time course of these results differs from that seen in the rat where previous work had found a peak at 1 hour (Nyamekye et al, 1995) and 3 hours (Grant et al, 1994) post sensitisation. Moreover the distribution of fluorescence in the rat differed from the pig results presented here. Nyamekye, using the rat carotid artery showed fluorescence levels in the media double those in the intima and adventitia in contrast to Grant (using the femoral artery) who found relative counts in the intima twice that of the media. The histological make up of the rat arterial wall differs markedly form that of the pig and man, especially with respect to the intima which is only one endothelial cell thick in the rat. This may explain the conflicting results obtained by Nyamekye and Grant where fluorescence measurement in the intima would have been difficult to measure accurately and may also account for differences between the rat studies and this

work. The essential contrast in terms of the adventitial fluorescence profile and the much later medial fluorescence peak could both be explained by the different inter-species arterial histology, notwithstanding the difference in animal size and metabolic rate.

The only unknown factor is the effect of vessel injury on PpIX pharmacokinetics. It could be argued that balloon injury, by disrupting the arterial wall, would alter the uptake and metabolism of ALA, thereby modifying the optimum drug-light interval. This is justified in part, but evidence from Hasan's laboratories (LaMuraglia et al, 1993) would suggest that greater photosensitisation is achieved in injured compared with normal arteries. Moreover, in the model to be described in Chapter 7, photosensitisation occurs prior to the proposed time of injury and light delivery is applied immediately after injury.

In summary, these pharmacokinetic studies have shown that the porcine arterial wall exhibits fluorescence in a dose-dependent manner following iv ALA administration. In the media, the fluorescence peak is seen between 4-7 hours at 60mg/Kg and 5-8 hours at 120mg/Kg with that of the intima occurring a little earlier and the adventitia showing a double peak. This information is essential for determining the optimum drug-light interval for ALA PDT.

CHAPTER 6 ENDOVASCULAR PDT OF NORMAL ARTERIES

6.1 INTRODUCTION

As outlined in Chapter 4, previous arterial PDT work has been disadvantaged either by the need for an external light source, or the use of a flawed intravascular one that did not totally exclude blood from the segment to be treated. To be of value for clinical use, light delivery would have to be endovascular and preferably be delivered via the same catheter as that used for angioplasty. This would avoid exchanging catheters during the procedure and ensure that the segment that was ballooned, also received light. I set out to show that endovascular light can be delivered via a balloon catheter and that this results in effective PDT in a sensitised animal.

6.2 METHODS

Animals used, photosensitiser administered and anaesthetic techniques were the same as outlined in Chapter 5. To maximise the chance of showing an effect and hence proof of concept, the larger of the two doses of ALA (120mg/Kg) was used.

6.2.1 Light Delivery

A pulsed (12kHz) copper vapour pumped dye laser (Oxford lasers, Oxford, UK) using a mixture of two Rhodamine dyes (Rh 590 and Rh 640) tuned to a wavelength of 635nm was used to generate light energy. This was conveyed intra-arterially by a laser fibre with a terminal cylindrical diffuser manufactured to my specification by Rare Earth Medical (West Yarmouth, USA). For coronary illumination, a 100μm fibre with a 2cm diffuser was used and for iliac illumination, this was increased to a 200μm fibre with a 4cm diffuser, each sized to fit the guidewire channel of the catheter and the length of the balloon

segment. The iliac balloon catheter (4mm x 4cm) was made to my requirements by Cordis (Roden, The Netherlands) as a modification of a standard catheter which was then made transparent. A suitable coronary catheter already existed in the form of the *Azuka*® manufactured by Schneider (Staines, UK) and this was used in the 2.5mm x 2cm size.

An initial energy dose of $50J/cm^2$ was selected, based on previous work in the rat model which showed that light doses of 50, 100, 200 and $250J/cm^2$ all produced absolute VSMC depletion (Nyamekye et al, 1995). Although in reality a threshold effect had not been demonstrated, $50J/cm^2$ was chosen as the lowest dose seen to be effective albeit from an external light source and in a small animal model. Fluence rates were adjusted in order that the power density was kept below the thermal threshold and thus avoid thermal effects which have been shown to generate NIH and lead to cross-sectional narrowing (Douek et al, 1992). The time required to deliver the above dose varied between 1210-1950 seconds for iliac segments and 485-538 seconds for coronary segments depending on the laser performance. During illumination, the laser source was switched off for 60 seconds after 20% of the dose had been administered as there is evidence from animal (Messmann et al, 1995) and clinical work (Messmann et al, 1997) that such fractionation of light delivery increases the efficacy of PDT.

6.2.2 Experimental Protocol

Pigs were commenced on a daily dose of 300mg aspirin the day prior to intervention and this was continued until culling. They were photosensitised with 120 mg/Kg ALA following which they were kept in subdued light until anaesthetised. Arterial access was gained via a 5cm longitudinal incision made in the left neck to expose the carotid artery which was controlled between slings. An arteriotomy was made and a 9FG sheath introduced over a guidewire using the Seldinger technique and 5000 IU of heparin given intra-arterially.

A catheter was advanced into the abdominal aorta and a peripheral angiogram was performed with 10 mls of Omnipaque (350 mgI/ml) and stored on an image intensifier (Siemens) to produce a 'road map'. Under screening, a 0.018inch guidewire was advanced into one common iliac artery. A 4mm/4cm transparent balloon catheter was advanced over the wire (Figure 6.1) and the balloon inflated to 4 atmospheres within the common iliac artery (to occlude, but not distend the artery) and a further angiogram performed to confirm occlusion of the artery. The guidewire was then removed and exchanged for a 200*u*m laser fibre with a 4 cm radial diffuser at its tip which was positioned in order that the diffuser exactly matched the balloon segment (Figure 6.2). During light administration the balloon was inflated, but was deflated to allow limb perfusion during the fractionation period.

Prior to coronary intervention, bretyllium (160mg), glyceryl trinitrate (50μg) and magnesium sulphate (4mmol) were given iv and glyceryl trinitrate (50μg) given directly into the coronaries via a guidecatheter. A 4FG Guidecath was used to perform a coronary angiogram which was then 'captured' on one screen of the image intensifier for reference. Individual coronary arteries were approached with a 0.014inch guidewire and a 2.5mm/2cm coronary balloon catheter advanced under screening. The guidewire was exchanged for a 100μm laser fibre with a 2cm diffuser which was advanced into the balloon segment of the catheter and the balloon inflated to 2-3 atmospheres. During light administration, the balloon was inflated in a cycle of 45 seconds inflation to 15 seconds deflation in order to prevent cardiac ischaemia as judged by the ST segments on the ECG monitor. The light was given continuously over this period as balloon deflation allowed blood perfusion of the segment, which effectively fractionated the dose received by the arterial wall.

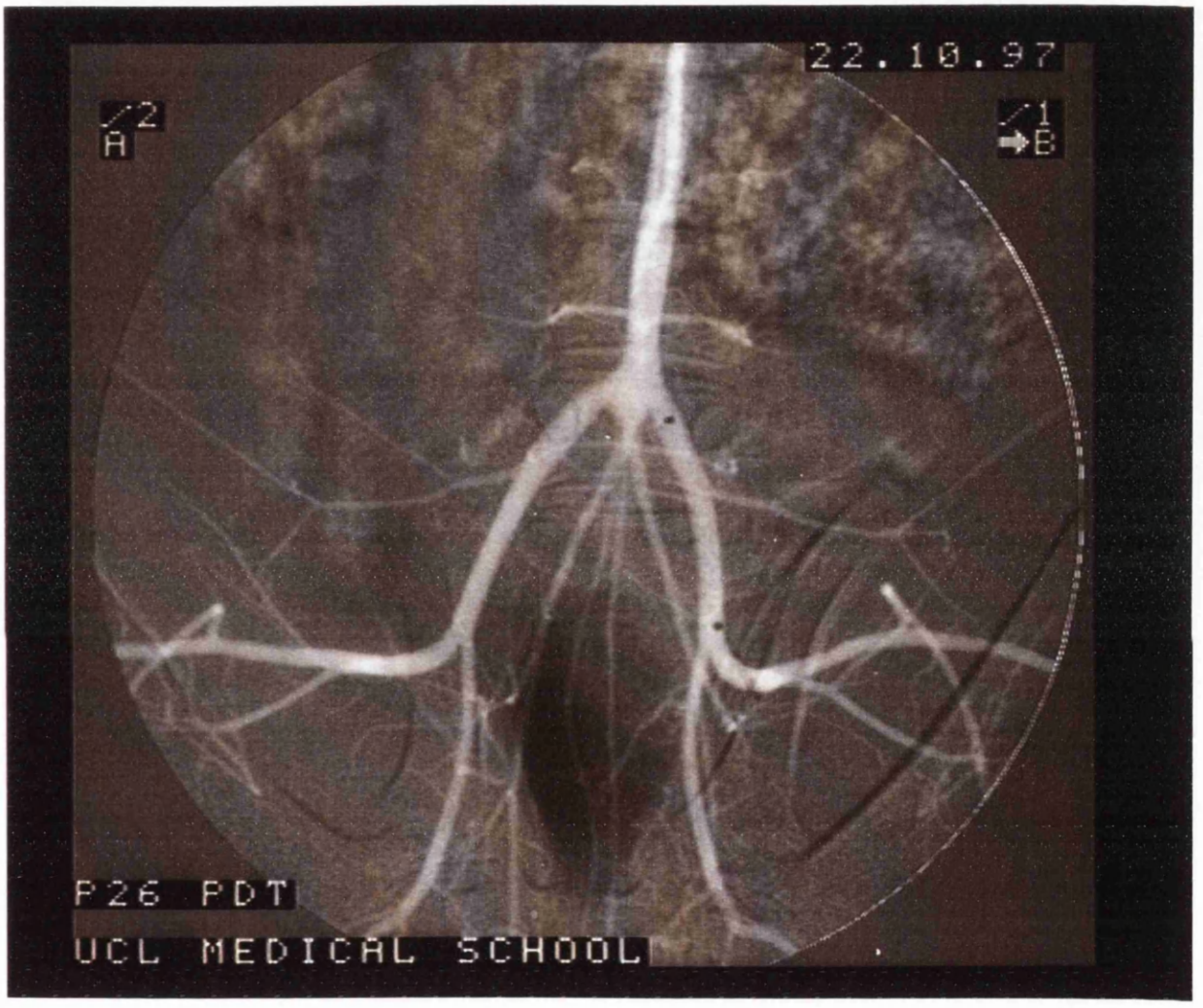


Figure 6.1 **Aortogram showing a 4cm balloon (radio-opaque markers) within the left common iliac artery prior to inflation.**

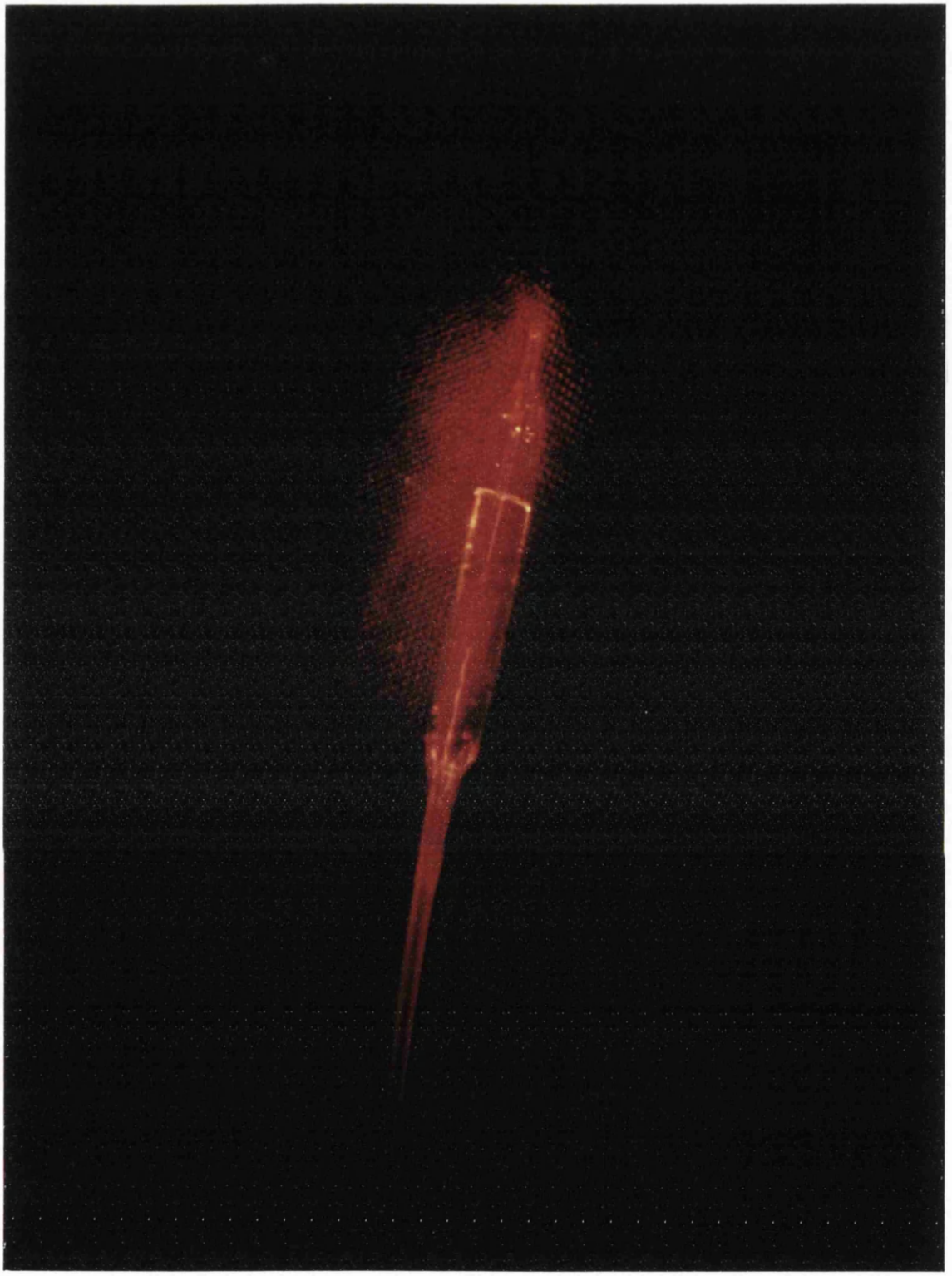


Figure 6.2 **Laser fibre with radial diffuser within the balloon segment of a transparent coronary catheter.**

Ten animals were photosensitised and both iliac segments treated in each animal. Six animals were culled at 3 days and four at 14 days post-procedure. Iliac segments received light at various drug-light intervals corresponding with the temporal pharmacokinetic profile of PpIX as demonstrated in Chapter 5. Of the animals culled on day 3, 4 iliac arteries were treated at 1.5 hours and 4 at 6-7 hours post sensitisation. From those culled on day 14, 2 arteries were treated at 1.5hours, 2 at 2.5 hours and 4 at 6-7 hours following sensitisation. Control procedures involved giving light alone to an unsensitised animal (2 arteries) and exposing an arterial segment to the balloon catheter without light in a sensitised animal (4 arteries), both of which were culled on day 3.

In six of the above pigs, the LAD and circumflex (Cx) coronary arteries were also treated with PDT at a drug light interval of 3.5-5 hours and were compared with sensitised control segments (right and Cx arteries) which did not receive light (6 arteries). Five treated arteries were obtained from 3 pigs culled at 3 days and 5 from 3 culled at 14 days.

Following the procedure, completion angiography was performed to check patency. The introducer sheath was then removed, the carotid artery ligated and the skin closed. Animals were recovered and kept in subdued light for over 24 hours.

6.2.3 Tissue Harvesting and Processing

Animals were culled by a lethal dose of iv pentobarbitone. The heart was removed via a median sternotomy and the coronary sinus cannulated. The coronary arteries were then pressure perfused with 4% formyl saline at 100mmHg and the whole heart stored in formyl saline for approximately 24 hours. Each coronary artery was then carefully dissected out and divided into 5mm transverse sections (8-10 per artery) and labelled from proximal to distal. By comparison with hard copies of the angiogram, the treated and control segments were documented.

A bilateral retroperitoneal dissection was made to expose the treated iliac segments which were controlled with slings and clamped proximally and distally. A 20 FG intra-venous catheter was inserted via a constant side branch and the segment pressure perfused in situ with a 4% solution of formyl saline at 100mmHg. The whole common iliac artery (≈4cm in length) was excised and divided into proximal, middle and distal treated segments and stored for 16 hours in 4% formyl saline fixative.

Each segment was embedded in paraffin wax from which two 4μm thick transverse sections were cut, mounted and stained with H&E. Each section was examined by light microscopy (Nikon Labophot-2) to assess the number of VSMCs per high power field (HPF). Microscopy images were transferred onto a 486 personal computer via a colour camera (JVC TK-1281) and morphometric analysis performed using a Lucia-M (Version 3.52a) programme. Each section was imaged and the number of VSMCs per HPF counted in 4 fields per section at 12, 3, 6 and 9 o'clock. Counts for PDT treated segments were averaged and compared with controls.

Sections from one block obtained at each treatment time point were stained with polyclonal Factor VIII (Dako, Denmark) using a Strep avidin horse radish peroxide technique as a specific marker for endothelial cells. These were examined in a qualitative manner by light microscopy.

6.2.4 Statistics

Means and standard deviations for each group were calculated. Statistical analysis between treatment groups and controls was by analysis of variance (ANOVA) using a Statview 4.5 computer programme (Abacus Concepts, California). Subgroups were compared using Fishers' PLSD post hoc test if the F-value was significant.

Inter- and intra-observer variability was assessed by reanalysis of a representitive sample of the histological sections. VSMCs were counted in 24 individual HPFs by the author at a time interval of 4 months and by a second independent observer. Results were compared between each observer and with the original counts and the variation calculated by comparing the standard deviation of the differences; the coefficient of variability being twice the standard deviation of the differences according to the method of Altman (Bland and Altman, 1986).

6.3 RESULTS

6.3.1 Iliac Arteries

All animals survived to culling and there was no evidence of thrombosis, rupture or aneurysm formation in any of the PDT treated or control segments. A transverse section of a treated and control iliac artery is seen in Figures 6.3 and 6.4 showing depletion of the VSMC population in the segment which received PDT. Mean ±SD VSMC counts per HPF were 115±17 for control sensitised arteries; 103±8 for light alone treated unsensitised arteries; and 27±29 [$p<0.0001$] for all arteries receiving PDT at all drug light intervals and harvested at both 3 and 14 days post-procedure.

Arterial segments treated at the same drug light interval, but harvested at either 3 or 14 days were analysed separately (Figure 6.5.) Mean ±SD VSMC/HPF from treatment groups harvested at 3 days (8±12) and 14 days (17±19) were significantly [$p<0.0001$] reduced compared with controls.

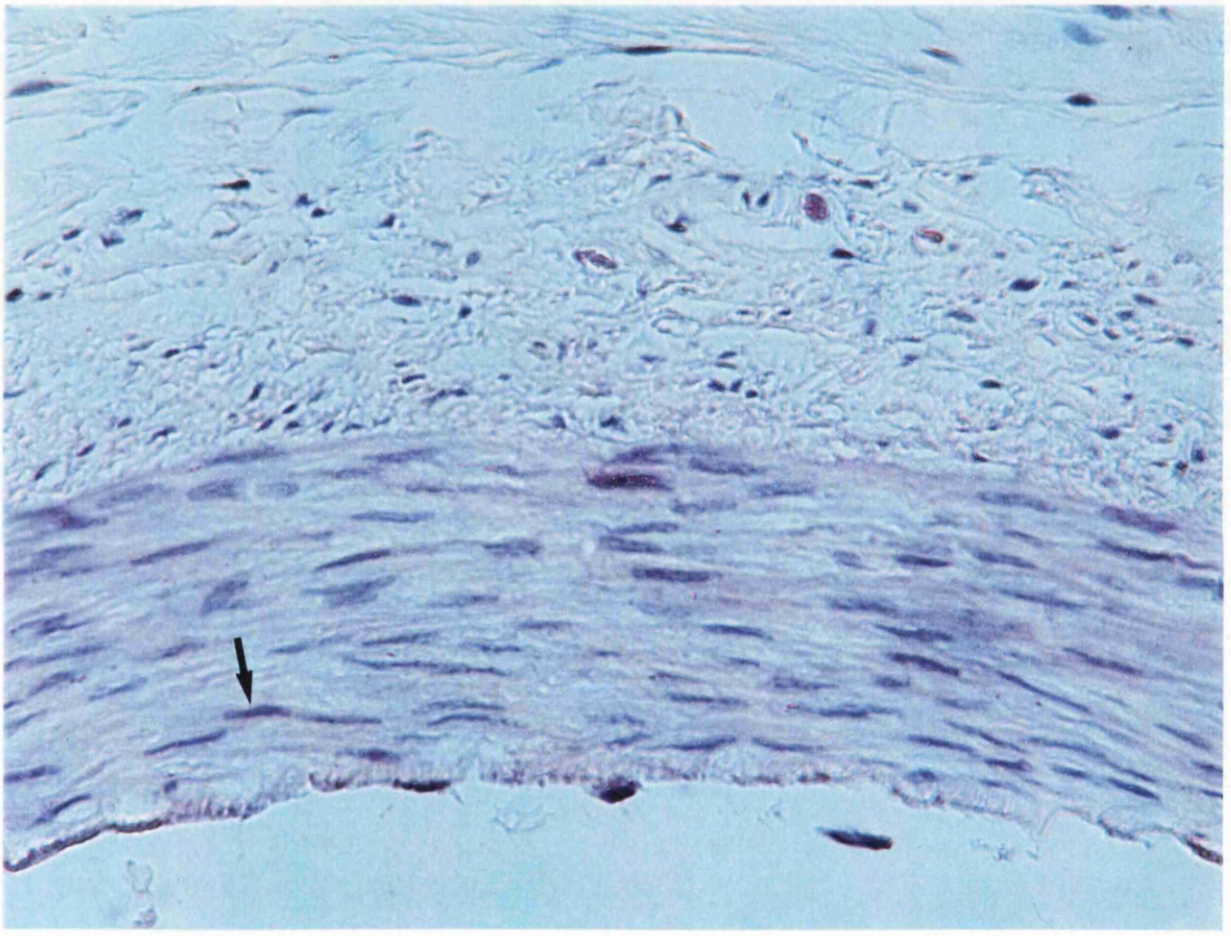


Figure 6.3 **Transverse arterial section (H&E) from control iliac vessel. (Arrow shows nucleus of VSMC).**

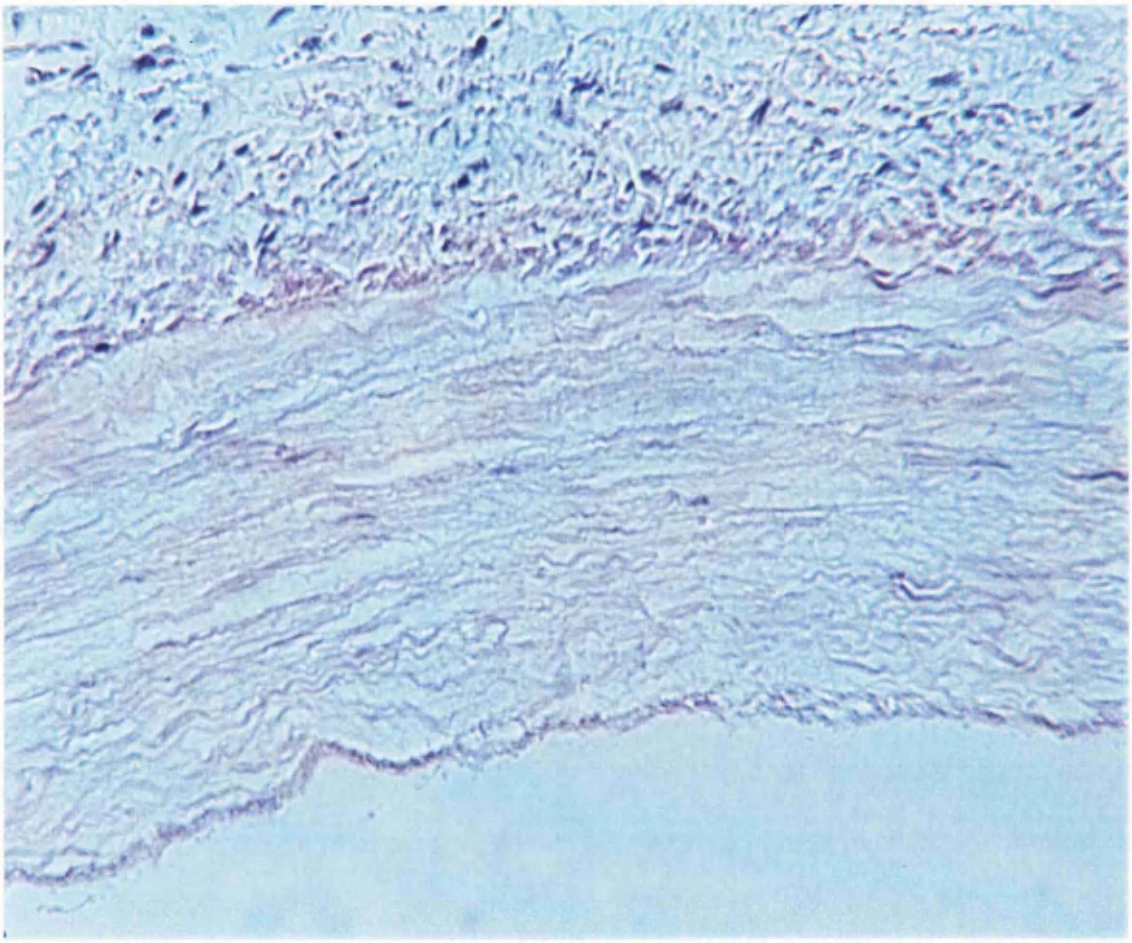


Figure 6.4 Transverse arterial section (H&E) from iliac vessel following PDT.

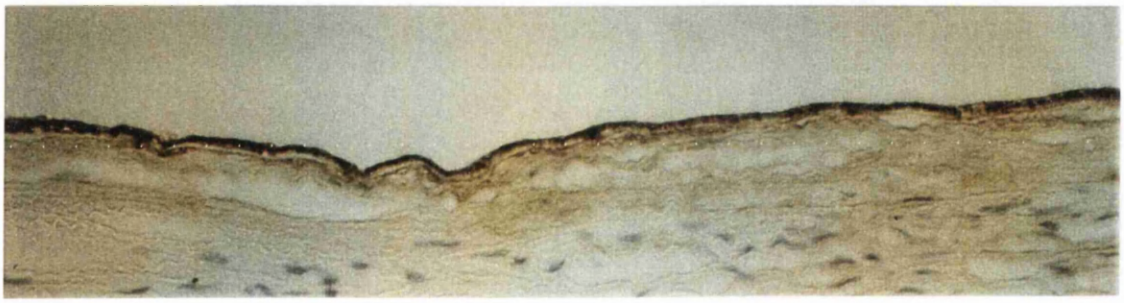


Figure 6.7 High power (40x) photomicrograph of transverse arterial section (polyclonal factor VIII) 14 days following PDT.

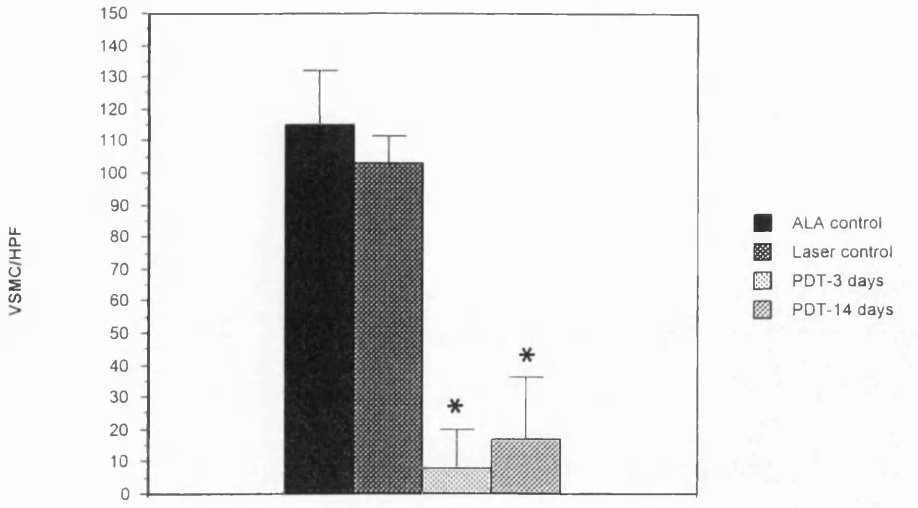


Figure 6.5 **Histogram of mean cell counts / HPF for iliac arteries harvested at 3 and 14 days. (Error bars represent standard deviations and * = p< 0.0001).**

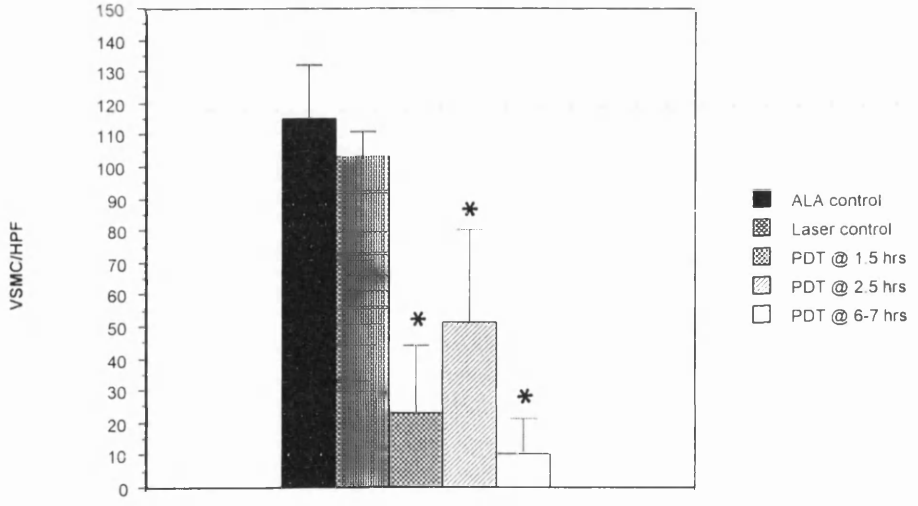


Figure 6.6 **Histogram of mean cell counts / HPF for iliac arteries exposed to PDT at 1.5, 2.5 and 6-7 hours post sensitisation. (Error bars represent standard deviations and * = p< 0.0001).**

Comparing PDT at different drug-light intervals showed that the maximal effect (as measured by VSMC depletion in the media) was seen at 1.5 and 6-7 hours post sensitisation, corresponding with peaks in the fluorescence profile of PpIX as seen in Figure 5.8. From tissue harvested at 14 days, mean ±SD VSMC/HPF in treatment groups was reduced at all time points compared with controls; 22±19 [$p<0.0001$] at 1.5 hours; 51±29 [$p<0.0001$] at 2.5 hours and 11±10 [$p<0.0001$] at 6-7 hours as seen in Figure 6.6. Depletion appeared to be most at times corresponding to the first adventitial fluorescence peak and the later medial fluorescence peak demonstrated in Figure 5.8.

The presence or absence of endothelial cells was assessed using polyclonal Factor VIII as a marker which showed complete absence of endothelial cells from sections harvested at 3 days, but repopulation by day 14 (Figure 6.7). Endothelial cell repopulation occurred early, and no appreciable neointimal hyperplasia occurred in treated sections.

6.3.2 Coronary Arteries

VSMC depletion was also evident in treated coronary arteries, but more variable than within iliac segments. Within control coronaries given ALA, but not exposed to light, the mean VSMC/HPF was 83±21 compared with 29±17 and 46±12 [$p<0.0001$] for animals culled at 3 and 14 days respectively following PDT (see Figure 6.8).

6.3.3 Inter- and Intra-observer Variation

The mean difference ±SD for VSMC counts between two observers was 4.0 ±4.2 and that between the same observer at different time points was 12.3 ±12.3. The coefficient of repeatability for inter-observer and intra-observer variations was therefore 8.4 and 24.6 respectively.

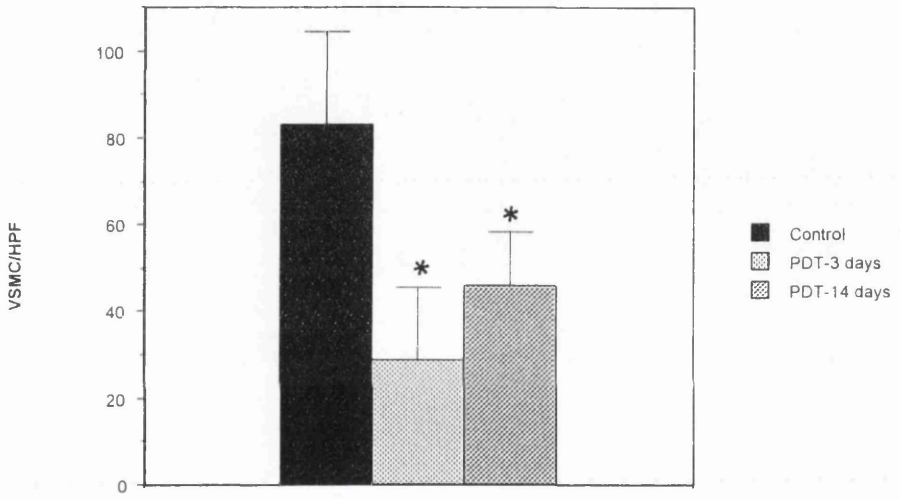


Figure 6.8 **Histogram of mean cell counts / HPF for control coronary arteries and those exposed to PDT and harvested at 3 and 14 days. (Error bars represent standard deviations and * = p< 0.0001).**

6.4 DISCUSSION

The above results establish the efficacy of endovascular light delivery for arterial PDT. As with studies using an external light source, PDT in the above model was seen to significantly reduce the VSMC population of the media around the whole arterial circumference. Moreover, there were no sensitiser-related or arterial complications. In particular, no evidence of thrombosis, arterial rupture or aneurysm formation was evident at either 3 or 14 days and as expected in a non-injury model, no appreciable NIH was seen in any sections. Specific staining for endothelial cells confirmed the findings of others (Grant et al, 1994; LaMuraglia et al, 1994; Nyamekye et al, 1995) by revealing a complete absence of endothelium at 3 days, but re-growth by day 14.

Subgroup analysis of iliac segments treated at various drug-light intervals revealed differences in the degree of VSMC depletion produced. With reference to the temporal fluorescence profile of PpIX, (Figure 5.8), maximal PDT effect on medial VSMCs would be expected at around 6 hours post-sensitisation when maximum fluorescence peaked in the media. This was borne out by the results presented above which show that maximum VSMC depletion occurred with PDT at a drug-light interval of 6-7 hours when counts were approximately 10% of control counts. Treatment at a drug-light interval of 2-2.5 hours (when fluorescence was low in all arterial layers) did result in VSMC depletion, but to only 50% of control values. The surprising finding was the magnitude of effect produced by treatment at a drug-light interval of 1.5 hours when VSMC depletion occurred almost equivalent to the maximum demonstrated at 6-7 hours. The fluorescence profile of PpIX shows a peak in adventitial fluorescence at this time, but fluorescence in the media and intima are still very low.

It is intriguing to consider that treating at this time localises the PDT effect to the adventitia, but that this exerts an influence on VSMCs within the media. The adventitia is now thought to play a more important role in restenosis than

once thought with evidence from animal work that it exerts an influence on both NIH (Barker et al, 1994) and remodelling (Shi et al, 1996). The adventitia has also been suggested as a target for local drug delivery to prevent restenosis (Huehns et al, 1996) and in the absence of any currently suitable drug, PDT at an early drug-light interval may be a promising alternative. Another potential explanation for this phenomenon is the presence of PpIX in the media which was not apparent on the CCD analysis, although this is unlikely to be of sufficient magnitude to explain the results. Finally, it could be argued that this represents a threshold effect of PpIX and treatment at any time after sensitisation would produce an equivalent result. However, if that were the case, treatment at a drug-light interval of 2.5 hours should produce equal depletion, which it does not. This raises the possibility that treatment of the adventitia exerts an effect throughout the arterial wall.

Analysis of data from animals culled at 3 and 14 days shows a trend to a slight increase in VSMC by day 14 in both iliac and coronary arteries, although this does not reach significance at the 5% level. Such an increase was also seen in the rat model (Nyamekye - personal communication), but after a longer time interval. The relevance is unclear, although it is unlikely to influence long term efficacy as repopulation by VSMCs over 2 weeks following an angioplasty means that the new cells have not been exposed to the insult of angioplasty and have therefore not been stimulated to proliferate.

The coronary PDT VSMC depletion results are not as marked as those from the iliac segments probably due to the logistics of the protocol resulting in a slightly sub-optimal drug-light interval and some degree of laser fibre/balloon mismatch. On a few occasions difficulty was experienced in inserting the 100μm laser fibre into the coronary catheter. This was especially evident in the distal few centimetres within the balloon segment, which resulted in the fibre being slightly proximal to the inflated balloon and therefore probably lead to a reduction in light reaching the arterial wall. A new design of catheter by

Schneider has resulted in a non-deformable balloon segment that should eliminate this problem.

These results have shown that catheter-based light delivery is feasible in both coronary and peripheral arteries and that PDT is effective as assessed by the end-point of VSMC depletion in normal arteries. The hypothesis that this will influence the response to injury, and hence angioplasty, will be investigated in Chapter 7.

CHAPTER 7 ENDOVASCULAR PDT IN AN INJURY MODEL

7.1 INTRODUCTION

It is now universally accepted that clinical restenosis occurs as a result of NIH and remodelling, although some studies persist in reporting NIH dimensions alone. Using an over-distension balloon injury in the pig model, I set out to investigate whether the endovascular PDT technique established in Chapter 6 will influence the arterial response to injury. Furthermore, I aimed to assess any effect produced both in terms of NIH development and overall arterial dimensions, thus addressing both the currently known components of restenosis.

7.2 METHODS

Animals used, photosensitiser administered and anaesthetic techniques were the same as described in Chapter 5.

7.2.1 Experimental Protocol

ALA was given at 120mg/Kg and the experimental procedure followed as set out in Chapter 6. Following vascular access and angiography, 20 iliac and 11 coronary arteries underwent balloon injury by an overdistension method using a balloon approximately 1.5x their diameter (Steele et al, 1985; Karas et al, 1992; Bonan et al, 1993; Humphrey et al, 1994). This equated to a 3mm/2cm coronary and 7mm/4cm iliac balloon which were deployed to 8 atmospheres to produce an injury as demonstrated in Figures 7.1 and 7.2 respectively. Of the vessels injured, 5 coronary (3 circumflex and 2 left anterior descending) and 10 iliac were also illuminated with light and were designated the 'treated' group. During coronary illumination, the balloon was inflated and deflated in a 45/15 second regimen and light delivered simultaneously to treatment vessels as

outlined in Chapter 6. Iliac arteries were initially injured by balloon distension to 8 atmospheres for 60 seconds followed by a reduction to 4 atmosph eres during the remainder of light or sham-light delivery. A completion angiogram was performed in all cases (Figure 7.3).

Light was delivered at a drug-light interval of 5-7 hours corresponding to the peak in medial PpIX fluorescence following ALA. Light (635nm) was again given at a fluence of approximately 50J/cm^2 which took between 503-1500 seconds for iliac and 200-365 seconds for coronary illumination depending on the power output of the laser. Fluence rates were kept below the threshold of 250mW/cm^2 for thermal effects, but did vary between 20-66mW/cm^2 and 114-211mW/cm^2 for iliac and coronary arteries respectively. Due to the difficulties in inserting the 100μm laser fibre for coronary illumination in the previous study, in this study, the fibre was fixed within the 3mm balloon and the two introduced as a single unit.

In 6 coronary and 10 iliac control arteries sham illumination was given (with the laser switched off) over the same time course as required to deliver the light dose to the treated arteries. Animals were recovered in the normal way and given aspirin until culling on day 28 post-operatively.

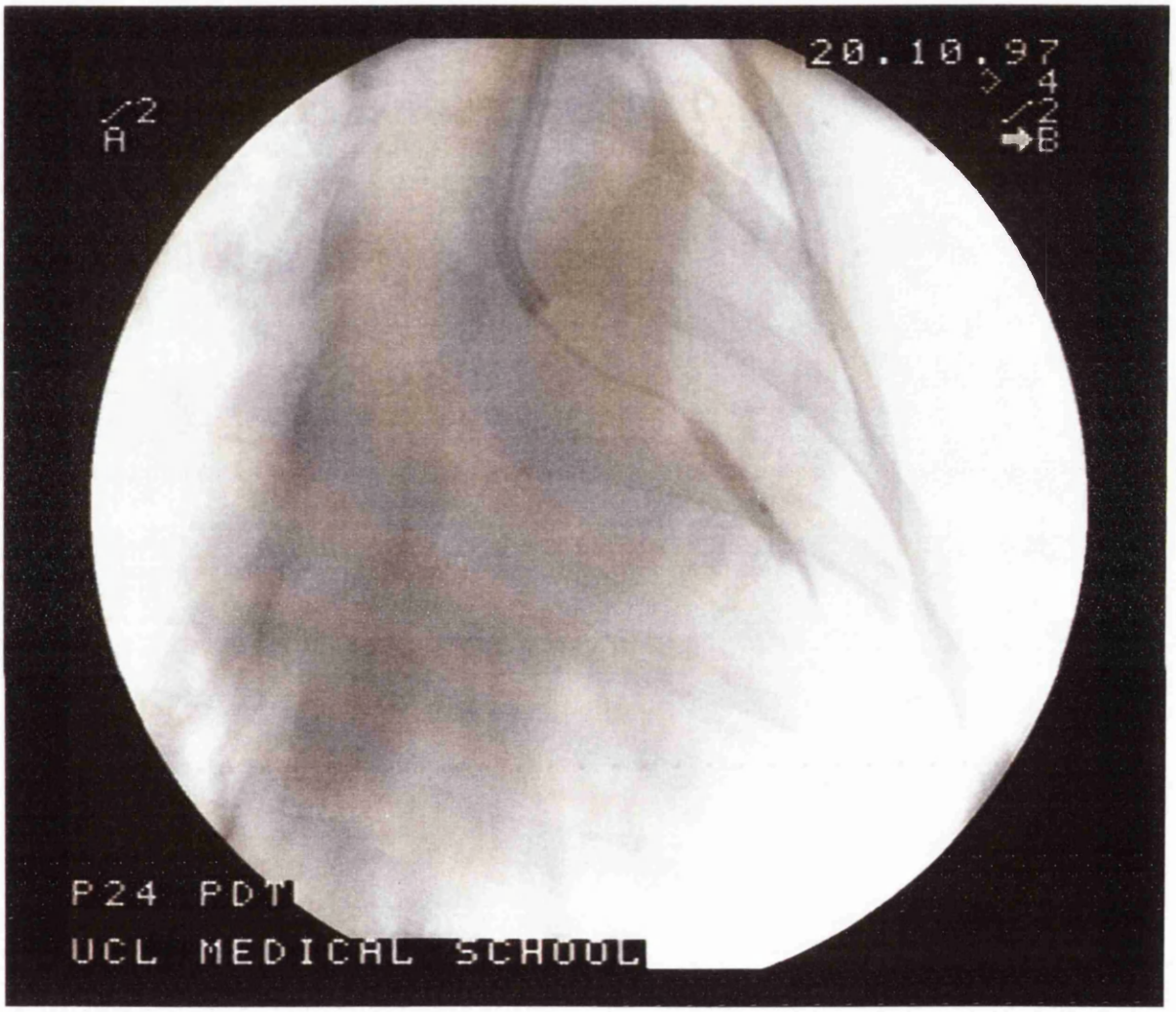


Figure 7.1 **Coronary balloon inflated within the circumflex artery with the guide catheter seen within the aortic sinus.**

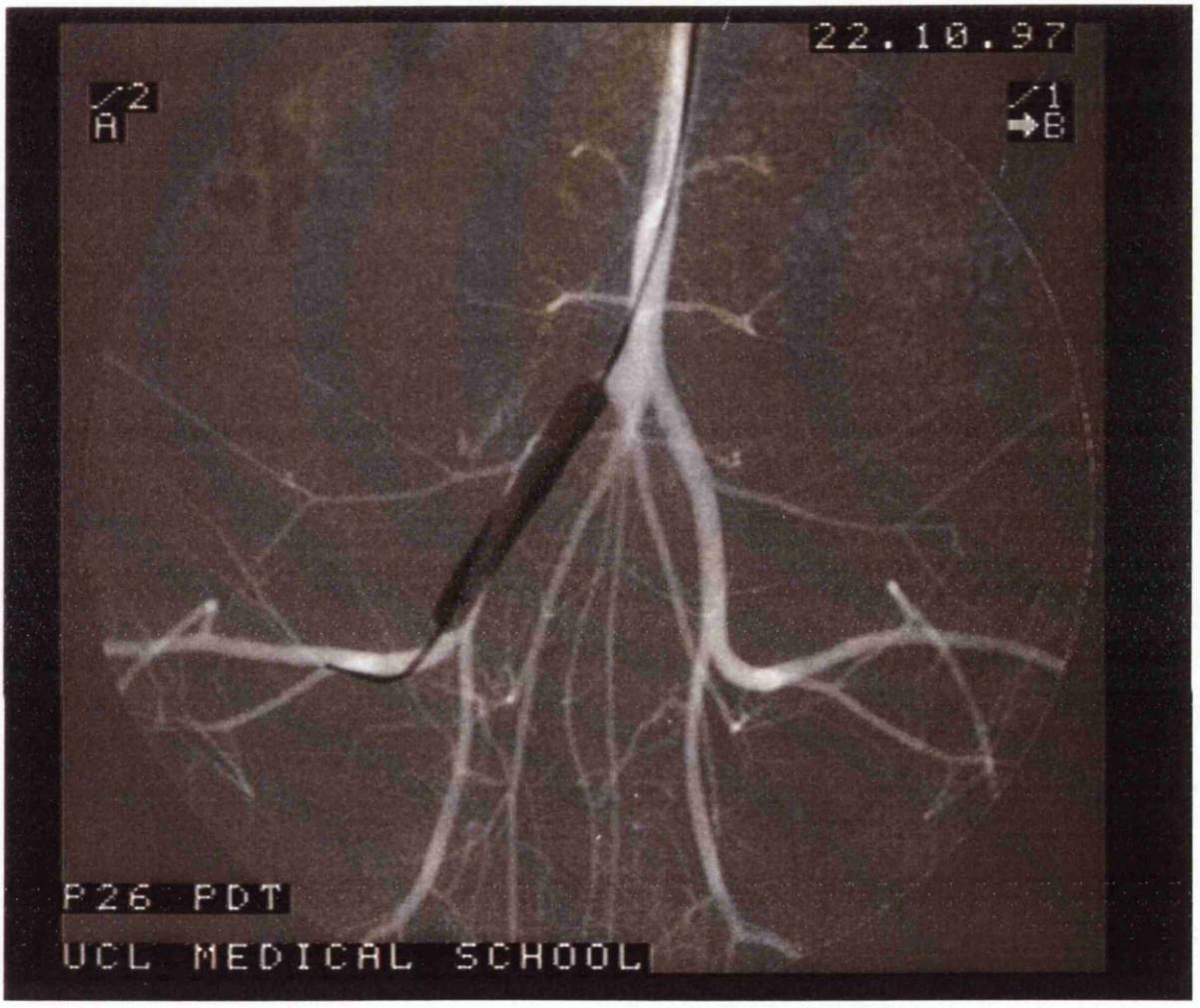


Figure 7.2 **Iliac balloon inflated within the right common iliac artery.**

7.2.2 Tissue Harvesting and Processing

Animals were culled and tissue harvested as outlined in Chapter 6. A view of the retroperitoneal dissection required to harvest iliac arteries post injury is seen in Figure 7.4. Additionally, histological material was stained with elastin van Gieson (EVG) and subjected to morphometry using a computerised image analysis system as described in Chapter 6. A 1x and 4x objective were used for iliac and coronary artery analysis respectively. The area of the lumen and that within the IEL and EEL was measured and recorded in mm^2 and from this, the area of the neointima and media derived.

It was felt that the contralateral iliac control vessel was unlikely to differ in size from the treated one, but there was scope for more variability between the treated and control coronaries. Therefore a reference segment was analysed: the most proximal histological section in the uninjured territory was examined from each coronary artery and the area within the EEL measured to compare the pre-injury arterial calibre in both PDT and control groups.

Similarly, to ensure that a comparable injury was produced in both groups, the degree of injury was objectively classified for every section. The injury score was calculated according to a modification of a method originally developed by Schwartz (Schwartz et al, 1990) in which points were attributed for an increasingly extensive injury following coronary wire coil implantation. The score was calculated thus:

Score 0 Endothelium denuded; IEL intact: media compressed.

Score 1 IEL lacerated: media compressed, but not lacerated.

Score 2 IEL lacerated; media lacerated; EEL intact.

Score 3 Media disrupted; EEL lacerated.

An example of a grade 2 and 3 coronary injury is seen in Figures 7.5 and 7.6.

A section from one block from each artery was stained with polyclonal Factor VIII (Dako, Denmark) using a Strep avidin horse radish peroxide technique as a specific marker for endothelial cells. These were examined in a qualitative manner by light microscopy.

7.2.3 Statistics

Descriptive statistics were calculated as mean ±SD for arterial areas in mm^2. Treated and control groups were compared using the unpaired Student's t-test and calculations were performed with a Statview 4.5 computer programme.

Inter- and intra- observer variation were assessed by reanalysis of 15 area calculations by the author and a blinded independent observer, according to the method used in Chapter 6.

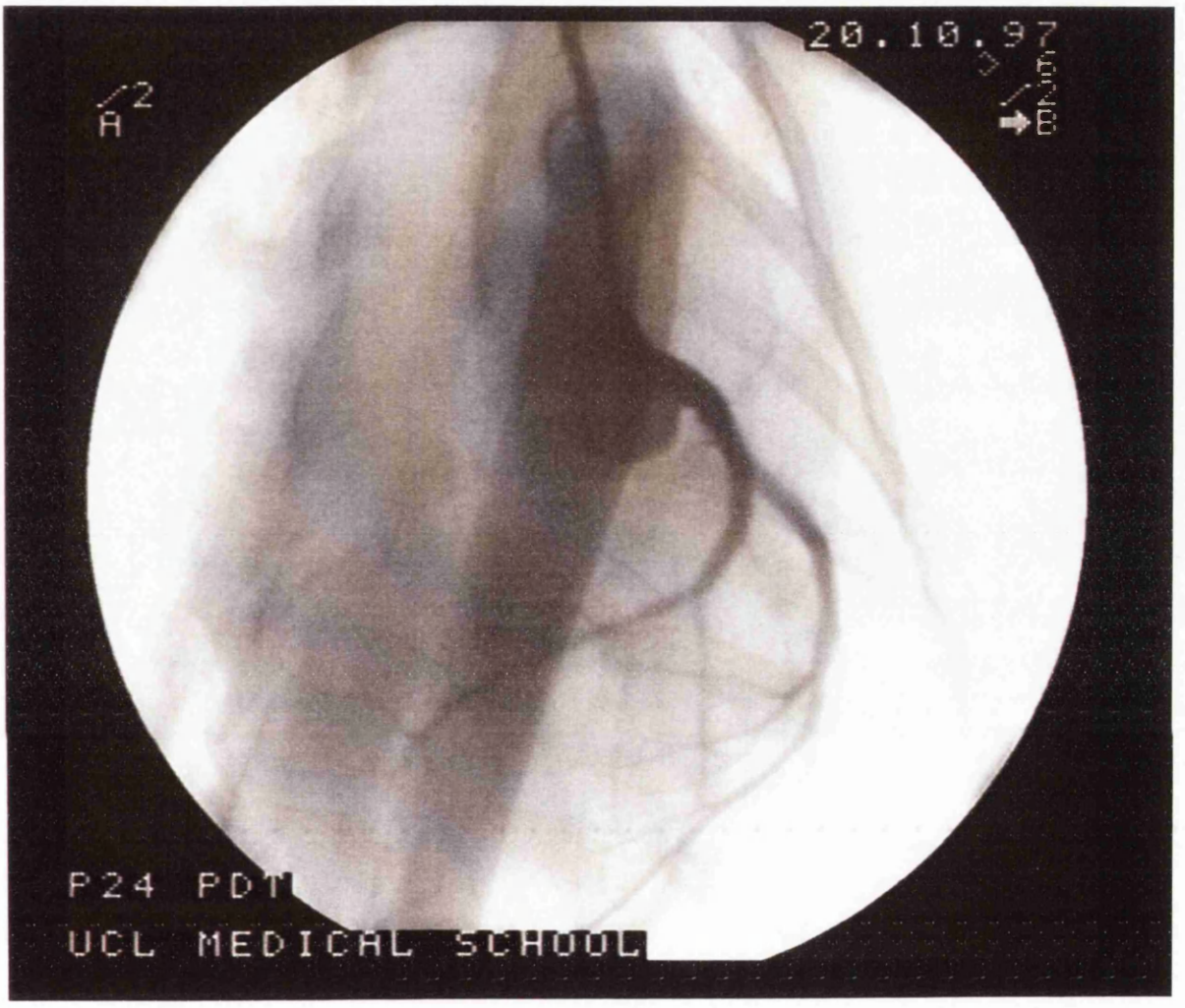


Figure 7.3 **Completion coronary angiogram following control injury to the circumflex coronary artery.**

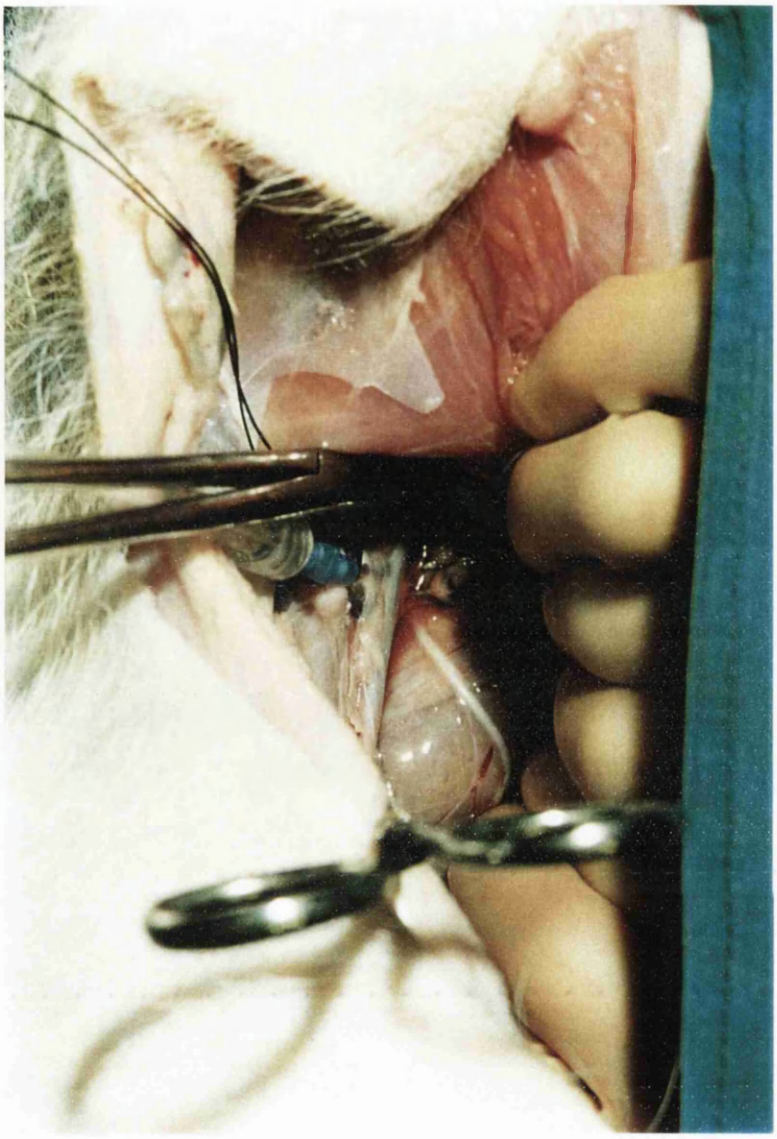


Figure 7.4 **Right common iliac artery exposed and clamped with irrigating catheter secured in side branch.**

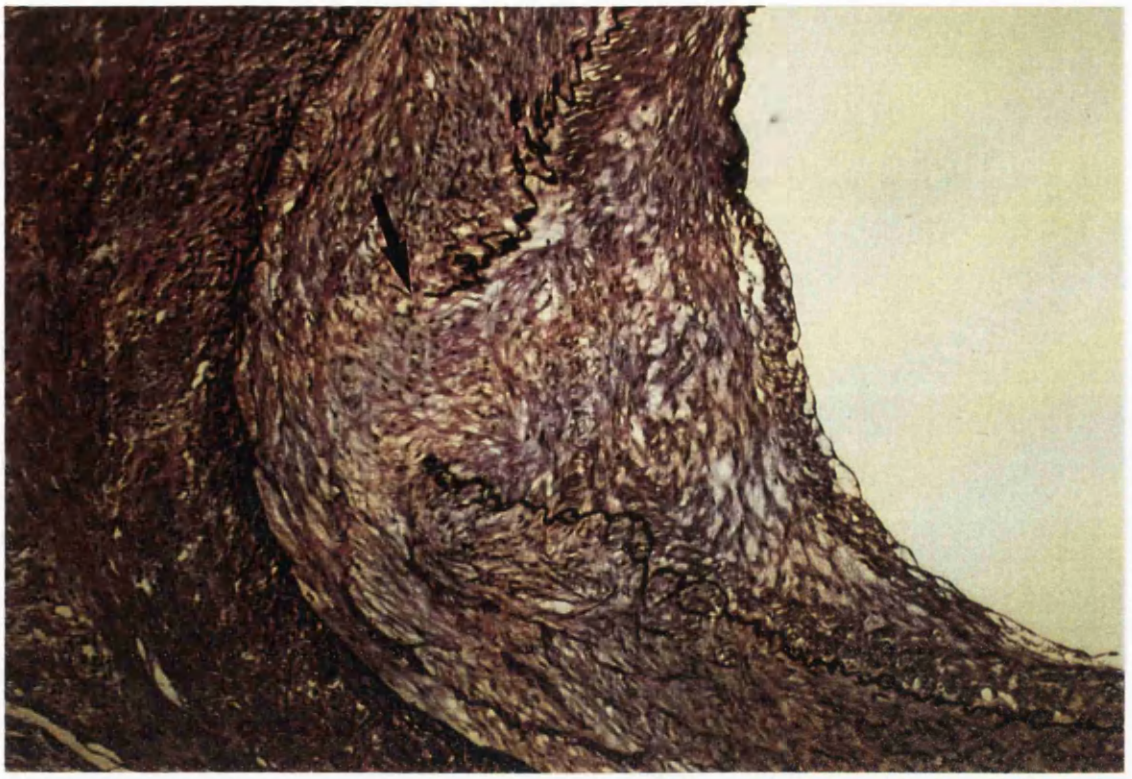


Figure 7.5 Photomicrograph (EVG staining) showing a grade 2 coronary injury with rupture of the IEL (arrow).

Figure 7.6 Photomicrograph (EVG staining) showing a grade 3 coronary injury with rupture of the EEL (arrow).

7.3 RESULTS

Vessels showed a consistent injury response, which was greater in coronary than the more elastic iliac arteries. There was no significant difference between the mean injury score for PDT and control arteries. In coronary arteries the mean (± SD) injury score for control and treated arteries was 2.3±0.8 and 2.1±0.8 respectively, compared with 0.4±0.5 and 0.6±0.6 for iliac control and treated arteries.

The area within the EEL of the reference coronary segment was no different for arteries that received PDT or acted as controls. The mean area within the EEL in control arteries and those treated with PDT was 2.91±1.1 and 2.94±0.49mm^2 respectively. The two groups were therefore considered comparable in terms of injury score and pre-operative calibre.

7.3.1 Coronary Arteries

Coronary arteries receiving PDT post injury were found to have a significantly larger lumen and EEL area compared with controls. An example of a control and treated coronary segment following injury is shown in Figure 7.7 and 7.8. Mean lumen area was 1.39±0.6mm^2 in treated arteries compared with 0.79±0.5mm^2 in controls (p=0.002) and mean EEL area was 2.84±0.7mm^2 following treatment and 2.15±0.8mm^2 in controls (p=0.006). The neointimal area in treated arteries was just over half that of controls: 0.39±0.2 mm^2 against 0.70±0.7mm^2 (p=0.06) as seen in Figure 7.9.

Figure 7.7 Photomicrograph of a control coronary injury (EVG) showing IEL rupture (arrow) and resultant NIH.

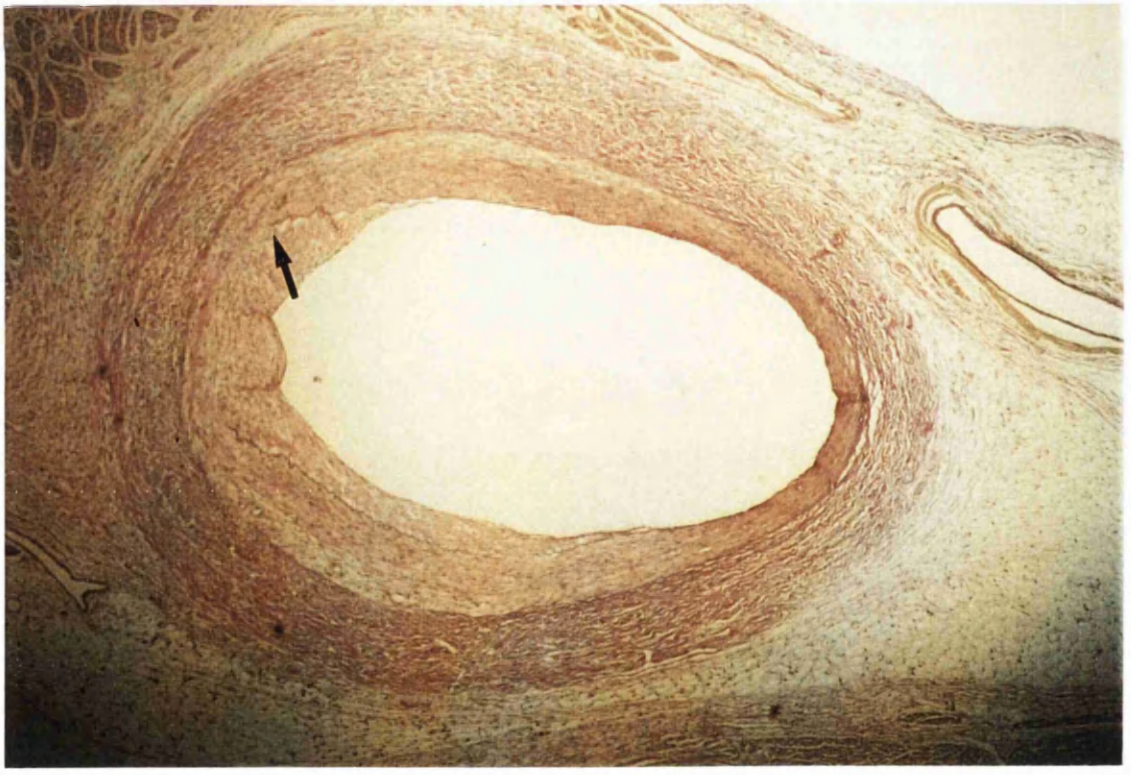


Figure 7.8 Photomicrograph of a coronary injury followed by PDT (EVG) showing IEL rupture (arrow) with minimal NIH.

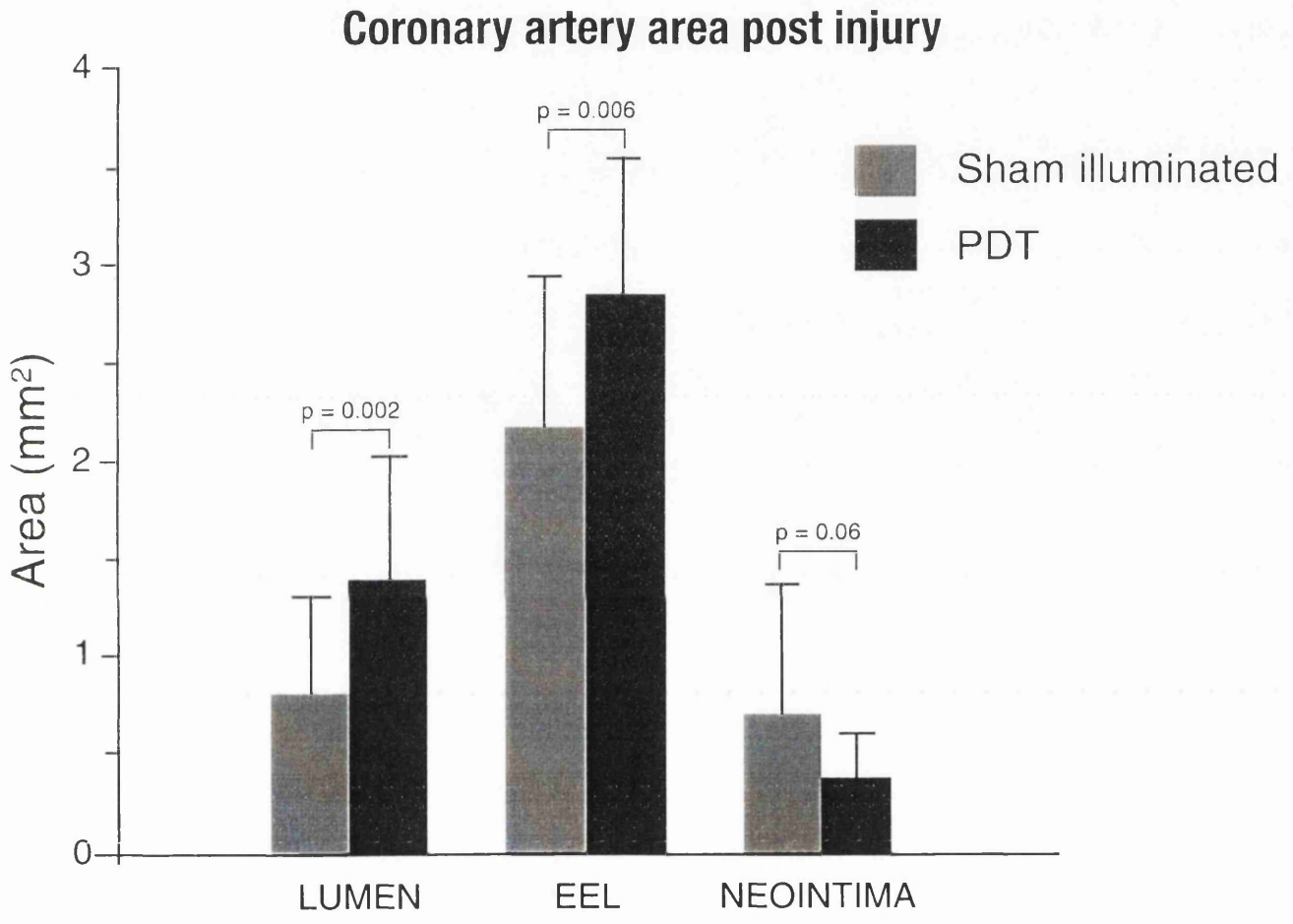


Figure 7.9 **Histogram showing morphometry of injured coronary arteries with and without PDT. Error bars represent standard deviations.**

7.3.2 Iliac Arteries

Iliac lumen, EEL and neointimal areas showed a similar trend for treated and controls, but the differences were not as marked which may reflect the lesser injury sustained. Mean lumen, EEL and neointimal areas in treated and control segments were 9.77±3.7 and 7.99±4.8 (lumen, p=0.1); 15.2±4.3 and 12.5±5.4 (EEL, p=0.03); and 0.76±0.5 and 0.95±0.8 mm^2 (neointima, p=0.2) respectively as demonstrated in Figure 7.10.

Light microscopy of sections stained with polyclonal factor VIII showed almost complete endothelial cover at 28 days.

Three pigs died before culling. One death was immediate due to an iliac rupture sustained during a control injury and two others occurred at 25 and 27 days. One of these had macroscopic and microscopic evidence of a myocardial infarction in the control-injured and PDT territories (Figure 7.11), but no obvious cause was apparent in the other although there was evidence of myocardial scarring. It is interesting to note that both these animals were given PDT following an overhaul of the laser and fluence rates during their treatment were much higher (211mW/cm^2) than in other animals. In both pigs post mortem thrombus prevented pressure perfusion at fixation and therefore the histological sections produced were not analysed morphometrically. Light microscopy revealed the lumen to be filled with thrombus with a thin rim of NIH no greater than that seen in other PDT treated segments. It seems plausible that the myocardial scarring observed may have been secondary to thermal effects at the time of treatment which may have lead to a conduction abnormality which could have precipitated a fatal arrythmia.

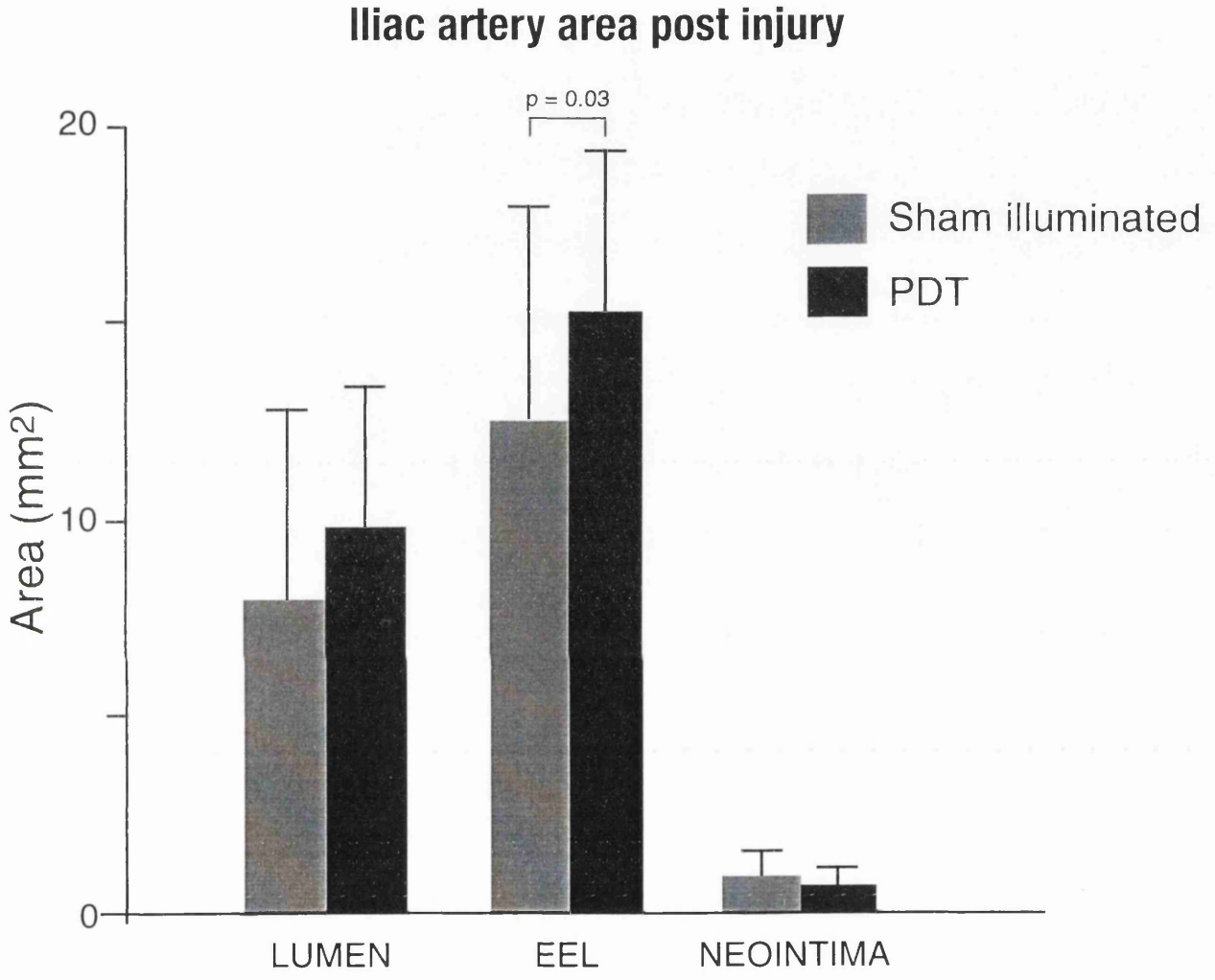


Figure 7.10 **Histogram showing morphometry of injured iliac arteries with and without PDT. Error bars represent standard deviations.**

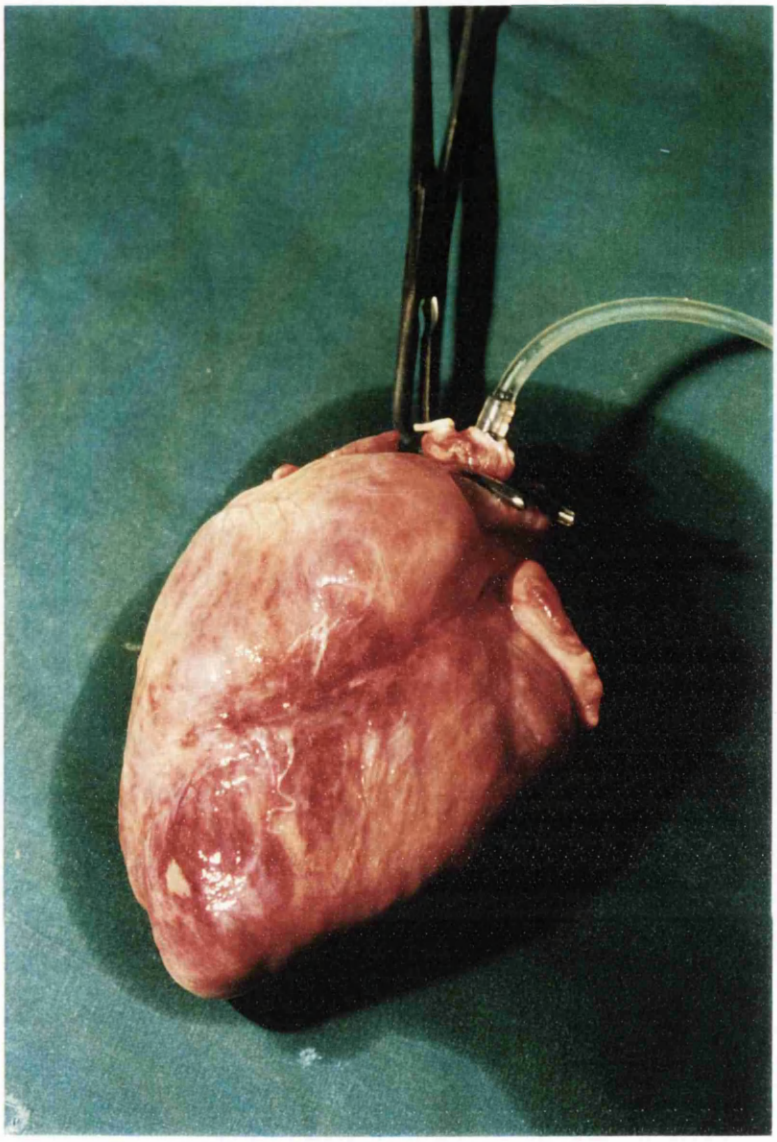


Figure 7.11 **Heart harvested from pig that died prior to culling. Gross scarring is seen adjacent to the LAD (PDT treated) with extensive infarct within the LAD and circumflex territories.**

7.3.3 Inter- and Intra-observer Variation

The mean difference ±SD for area calculations between two observers was 0.1 ±0.3 and that between the same observer at different time points was 0.1 ±0.3. The coefficient of repeatability for inter-observer and intra-observer variations was therefore 0.6 and 0.5 respectively.

7.4 DISCUSSION

These results demonstrate that when assessed at 28 days post intervention, PDT given at the time of injury results in an increased lumen area compared with controls. This seems to be secondary to both a reduced amount of NIH and an increase in overall arterial dimensions ie. positive geometric remodelling.

A consistent injury response was produced by the over-distension method and objective measurement of the injury score showed an equivalent degree of injury in control and treated segments. The degree of injury was much greater in coronary arteries than the more elastic iliac arteries which stretched without sustaining a deep injury right up to the point of complete rupture which was encountered in one case. This difference in injury response may explain the apparent difference in the magnitude of the PDT effect in iliac compared with coronary arteries. However, the validity of this injury method is borne out by its close resemblance to therapeutic angioplasty and is not disadvantaged by a long segment of endothelial denudation that occurs with other methods of arterial injury. All other arterial PDT studies except one (LaMuraglia et al, 1994) have relied on total carotid or femoral endothelial denudation, but then only treated a variable fraction of that segment with PDT. Studies using Fogarty balloon passage to produce endothelial injury and hence NIH have naturally focussed on the ability of PDT to influence neointimal area and have neglected to assess any influence on the remodelling aspect of the response to injury. Using a stent to produce an arterial injury would have been an alternative strategy in the very elastic pig iliac arteries, but this would have also produced an injury response

limited to NIH, as any remodelling would have been counteracted by the stent placement.

In this study, NIH development was not completely abolished, but the NIH area in segments that received PDT was less than that seen in controls. It has already been demonstrated in a similar porcine model that although the degree of injury correlates with NIH, NIH itself does not correlate with the most important parameter: lumen size (Anderson et al, 1996). In other words, final lumen size is influenced more by the remodelling response than by NIH as shown unequivocally by Anderson et al, who found a positive correlation between final arterial (EEL) size and NIH, but not the expected negative correlation between lumen size and NIH. Previous arterial PDT studies that reported NIH dimensions or percentage stenosis alone (Eton et al, 1992; Eton et al, 1995; Ortu et al, 1992; LaMuraglia et al, 1994; Nyamekye et al, 1995; Nyamekye et al, 1996a) have therefore been misleading in that their chosen end-point is not representative of the most important cause of restenosis.

The ability of PDT to reduce NIH and increase overall arterial dimensions in this model is impressive, but a number of questions remain to be answered. Firstly, the difference between the response in the coronary and iliac segments. Although I have suggested above that this may simply reflect the different degree of injury produced in both vessels (which itself is probably due to the different histological structure of muscular and elastic arteries), an alternative explanation may be the different protocol used for light delivery. During coronary light delivery, it was necessary to deflate the balloon every 45 seconds to allow myocardial re-perfusion, which also allowed arterial wall re-perfusion and hence oxygenation. In the iliac system however, the over-sized balloon remained inflated, albeit at a reduced inflation pressure compared with that used to produce an injury, (apart from during light fractionation) which may have reduced oxygenation to the arterial wall. The only comparable work to investigate this comes from the use of balloon-delivered light for PDT in a canine oesophageal model (Overholt et al, 1996). They showed that a large

balloon in a small dog could decrease the efficacy of PDT as measured by mucosal damage, presumably due to insufficient oxygenation. This could also be true for PDT in iliac arteries in this model.

The second unanswered question is the mechanism by which arterial PDT acts to influence lumen size. It has always been argued that NIH reduction was due to VSMC depletion after, or at the time of arterial injury, but this does not necessarily explain the change in arterial dimensions. One could argue that removal of VSMCs would cause the artery to become an inert tube without contractile properties and that this alone could explain the increase in arterial dimensions, but the true explanation is probably more complicated. Recent work has highlighted the role of TGF-β (Statius van Eps et al, 1997), activated neutrophils (Sluiter et al, 1996) and collagen cross-linking (Spikes, 1993) in the response to PDT, but the magnitude of their role in arterial PDT is at present unclear.

In summary, this work establishes the effectiveness of PDT in the porcine injury model. It is the first series reported where a completely percutaneous intra-arterial light source has been used both for iliac and coronary illumination and is the first to evaluate complete histomorphometric end points. It is validated by the objective assessment of injury score, analysis of inter- and intra-observer variation and measurement of a coronary reference segment in treatment and control groups.

CHAPTER 8 PREDICTION OF RESTENOSIS

8.1 INTRODUCTION

One of the dilemmas following the introduction of any new therapy is who should receive it. Should a method of limiting or abolishing restenosis be developed, a means of predicting those who would otherwise restenose would be desirable. The alternative would involve giving adjuvant treatment to all angioplasty patients which would over-treat approximately two thirds. Although this may be acceptable with a simple cheap method of prophylaxis, it would not be acceptable for a more sophisticated therapy and therefore finding a method of predicting the risk of restenosis is as important as finding a cure for it. Unfortunately the current literature relating to the prediction of restenosis is both confused and inconsistent.

8.1.1 Current Knowledge

Numerous patient-related and procedural factors have been reported as being associated with restenosis, but such factors are not always consistently implicated in all series. A recent editorial has reviewed the factors that have been associated with coronary restenosis as shown in Table 8.1 (Faxon, 1997). In the peripheral arterial tree the associations are almost identical, with the addition of a worse outcome for more distal PTAs in the lower limb. Although crural vessels are smaller in size than more proximal arteries, this alone does not account for the higher restenosis rates in these vessels, as in general, coronary arteries are even smaller, but respond much better to PTA. Although unproven, the difference must therefore be accounted for by the vastly different flow rates that occur in coronary compared with crural arteries.

With increased resolution from both peripheral duplex and IVUS, evidence is also emerging that plaque characterisation may also be important in determining the risk of restenosis. In a small series of 23 patients, hypo-

echogenic lesions within the femoro-popliteal segment have been shown to exhibit a significantly higher restenosis rate compared with fibroatheromatous ones (Baumgartner I et al, 1996b). This has recently been confirmed by Aly and colleagues who used a more objective classification of echogenicity where computer-assisted image analysis was used to determine a mean pixel value of the plaque. In an assessment of 42 lesions from both iliac and femoral segments, those containing plaque within the lowest echogenicity group were all found to have restenosed within the first 3-12 weeks post-procedure (Aly et al, 1998b).

Table 8.1 Factors associated with coronary artery restenosis.

Patient Factors	Sex, history of restenosis, diabetes, hyperlipidaemia, hypertension, unstable angina, renal impairment and smoking.
Anatomical Factors	Severity of stenosis, presence of calcium, eccentric nature, length of lesion, total occlusion, ostial and proximal lesions, reference vessel size, and location within the left anterior descending artery or saphenous vein graft.
Procedural Factors	Balloon-to-artery ratio, residual stenosis and gradient, and extent of dissection.

IVUS assessment of vessels following coronary angioplasty has resulted in contradictory information with respect to the risk factors for restenosis. Early series found dissection or plaque rupture lead to an increased restenosis rate (Tenaglia et al, 1992b), but a recent report involving 200 patients has found no association between outcome and the presence and extent of plaque rupture or arterial dissection (Peters et al, 1997). Unlike the peripheral arterial system, the

PICTURE (Post-Intracoronary Treatment Ultrasound Result Evaluation) study reported by Peters et al, found no association between restenosis and plaque morphology, although the assessment of plaque morphology in this study was crude and subjective. It would be counter-intuitive to predict that plaque morphology would not influence restenosis and based on the findings in the peripheral arterial tree, it may be that a more sophisticated assessment of plaque morphology in coronary arteries may yield different results. The only strongly positive association found in the PICTURE study was the immediate post-angioplasty lumen area (and to a lesser extent total vessel and plaque area) and minimal lumen diameter at follow up, leading to the "bigger is better" philosophy advocated by protagonists of atherectomy. The presence of residual stenosis following angioplasty is the one factor which is consistently implicated as a risk factor for restenosis. Rather than being thought of as a true risk factor however, it is better considered as a failure of adequate initial therapy and therefore a facilitator of a more rapid reduction to a lumen area which fulfils a definition of restenosis. This last statement underlines two important potential problems which underlines all such analysis. Firstly, restenosis is an ongoing dynamic process, but for the purpose of analysis is studied as categorical data once a value reaches the threshold for a given definition at a pre-determined time point. Secondly, it is unclear whether certain factors are true variables or merely confounding variables; for example, in the above case, it may be that the presence of a residual stenosis is unimportant but reflects a particular lesion characteristic that is both difficult to angioplasty and is prone to restenosis.

In addition to detailed studies of lesion and procedure variables, clinical data and mathematical models have also been assessed. However, analysis of a large number of clinical variables (Weintraub et al, 1993) and mathematical models (Hirshfeld et al, 1991) based on known associations with restenosis have added little to our knowledge and prediction of restenosis remains as elusive as it did a decade ago.

8.1.2 ACE

It has been known since the 1890s that extracts of renal tissue could produce hypertension in experimental animals. In the 1930s it was shown that inducing renal ischaemia by ligating one renal artery lead to hypertension and later that the proteolytic enzyme renin was responsible. Renin is secreted by the juxtaglomerular cells bordering the afferent arteriole as it enters the Malpighian corpuscle and is responsible for the cleavage of angiotensinogen to produce angiotensin I (Ang I). Ang I, which is essentially biologically inactive, is converted (primarily in the lung) to Ang II, one of the most potent pressor substances known. The renin angiotensin system (RAS) is one of the factors which controls blood pressure and via the interaction with aldosterone, fluid balance.

There is now evidence to suggest that all the components of the RAS described above also exist within the arterial wall (Campbell and Habener, 1986; Campbell, 1987). The evidence for the existence of local arterial ACE comes from both animal (Aiken and Vane, 1972) and human studies (Webb and Collier, 1986). Arterial injection of Ang I was found to produce vasoconstriction and as little, if any, Ang I to Ang II conversion occurs in plasma, this would suggest the existence of ACE within the arterial wall. It was initially thought that arterial ACE was confined to the endothelial layer, but against this, is data from experiments with endothelially-denuded rat aortic segments which contract in response to Ang I (Andre et al, 1990).

8.1.3 ACE and Restenosis

A potential therapeutic role for ACE inhibitors has been suggested by the observation that ACE inhibitors reduce the NIH response to rat carotid balloon injury by up to 80% (Powell et al, 1989). This response is thought to be mediated by a non-hypotensive mechanism as no such effect is seen with the hypotensive agents, minoxidil and verapamil (Powell et al, 1991). Laporte has shown that the response produced by ACE inhibitors is likely to be mediated

via Ang II inhibition by showing that Ang II antagonists are equally effective in reducing intimal thickness (Laporte and Escher, 1992). Moreover, non-hypotensive doses of an ACE inhibitor are also effective in reducing NIH (McEwan et al, 1992). Ang II itself has been shown to increase the area of NIH development after balloon injury and increase VSMC DNA synthesis (Daemen et al, 1991). The cellular mechanism by which Ang II acts, is unclear, but may involve induction of proto-oncogenes in VSMCs in a similar way to other growth factors.

Despite the experimental evidence which would suggest that ACE inhibition would be beneficial in reducing restenosis after angioplasty, trials in humans have demonstrated no such effect (MERCATOR Study group, 1992). It has been postulated that the lack of efficacy in clinical trials reflects the reduced dose levels used in humans (Rakugi et al, 1994), perhaps the timing of administration and the disease severity of the trial population. An alternative explanation is that clinical restenosis is a multi-factorial process involving other processes in addition to NIH.

8.1.4 ACE Polymorphism

The human ACE gene is found on chromosome 17 and contains a restriction fragment length consisting of the presence (Insertion, I) or absence (Deletion, D) of a 287 base pair sequence. Amongst the general population, D allele frequency varies between 0.57 and 0.59 (Rigat et al, 1992).

ACE polymorphism accounts for some of the variance in serum ACE concentrations. Significantly increased serum ACE concentrations were found in the homozygote deletion genotype (DD) compared with the heterozygote (I/D) which were in turn higher than the insertion homozygote (II) (Rigat et al, 1990). However, considerable overlap existed and although the ACE gene locus was thought to be the major determinant of serum ACE levels, polymorphism was found to account for only 47% of this variance.

Following the first study which revealed a link between the deletion variant of ACE and myocardial infarction (MI) (Cambien et al, 1992), a number of reports have both confirmed and refuted this (as reviewed by Singer et al, 1996). Many explanations have been put forward to explain the contradictory results of both prospective and case control studies. Selection bias, genetic background of the study population and possible genetic mistyping are three criticisms levelled at a number of both positive and negative studies. One of the main criticisms of the Cambien paper was that samples were taken many months after the diagnosis of MI and thus a preponderance of the DD allele may conversely represent improved survival amongst patients with that genotype. To test this hypothesis the same group categorised ACE polymorphism in a population of 213 cases of fatal MI from Belfast (Evans et al, 1994). When compared with controls from the same population the autopsy cases were found to have a significantly increased frequency of the D allele with an odds ratio of 2.2 for DD compared with II. This would suggest that their original observations were not confounded by a large number of early deaths being of the II genotype.

8.1.5 ACE Genotype and Restenosis

In addition to the genotype associations of ACE polymorphism with ischaemic heart disease, it has been suggested that the DD genotype may also be a predictor of restenosis following coronary PTA (Ohishi et al, 1993). In this study of 82 consecutive patients who underwent coronary PTA after MI, a significantly increased proportion of the restenosis group were of the DD genotype. The odds ratio for the development of restenosis was 4:1 for patients with the DD genotype compared with those of the I/D or II. However, these findings must be interpreted with caution as this study involved a small, selected group of patients, all of whom were Japanese, and the angiographic analysis was by visual means with an arbitrary definition of restenosis.

Three larger studies of elective coronary PTA (Hamon et al, 1995; Samani et al, 1995; van Bockxmeer et al, 1995) have since shown no evidence of a link between ACE genotype and restenosis and have thus concluded that genotyping for polymorphism would not be a useful predictor of restenosis risk following coronary PTA. Although the overall result of these trials was negative, there did seem to be a suggestion that ACE polymorphism may be linked to outcome for PTA in unstable angina (Samani et al, 1995) and evidence of an interaction with apoE genotypes with the combined carrier state having a higher restenosis rate (van Bockxmeer et al, 1995).

In a study which analysed the relationship between ACE genotype and tissue levels of ACE in atherosclerotic plaques (gained from atherectomy), the latter rather than the former was found to be related to restenosis (Haberbosch et al, 1997). The importance of this is uncertain, but may point to the fact that ACE influences the response to certain coronary interventions, but not all. Further evidence in support of this theory comes from a recent report which demonstrates a link between the DD genotype and an increased risk of restenosis after coronary stenting (Amant et al, 1997). In this study of 146 patients undergoing Palmaz-Schatz stent implantation, the relative risk of restenosis was found to be 2.0 per number of D alleles. The positive finding of a link between ACE polymorphism and restenosis after stenting, but not PTA, suggests that ACE may be related to the development of NIH, the major cause of restenosis following stenting, but a minority factor contributing to restenosis after PTA. This would be in line with experimental animal work showing that Ang II increased the NIH response to injury (Daemen et al, 1991) and be consistent with the proposed mechanism of action of ACE in inducing VSMC changes.

To date, ACE polymorphism has not been studied in relation to outcome following peripheral vascular intervention.

8.2 CLINICAL STUDY

8.2.1 Introduction

This study set out to investigate any relationship between ACE polymorphism and restenosis follwing PTA in the ilio-femoral segment. Restenosis in this segment is similar in aetiology to coronary restenosis and involves both NIH and remodelling. A further group of patients undergoing above knee synthetic femoro-popliteal bypass was also studied as it was felt that restenosis in this group may involve a greater contribution from NIH which in the coronary circulation seems to bear more relation to ACE polymorphism.

8.2.2 Methods

8.2.2.1 Study Population

Consecutive patients (70 following ilio-femoral PTA and 30 following femoro-popliteal bypass) were recruited from clinic at follow up in a prospective manner. Each patient was interviewed, examined and duplex studies and/or angiograms reviewed. Any patient where outcome was unclear and whose treated segment had not been visualised post operatively underwent duplex scanning. Details of the patient's pre-intervention clinical state, risk factors and outcome were recorded as shown in the protocol sheet in the appendix. Patients in whom technical difficulties were encountered or where no discernible benefit was noted were excluded. Restenosis was defined by recurrence of a significant stenotic lesion (PSVR>2.0) on duplex or >50% stenosis on angiography within 6 months of intervention.

Venous blood was taken and 5mls of EDTA blood stored at -20°C prior to genotyping for ACE polymorphism.

8.2.2.2 ACE Genotyping

a) DNA Extraction

5ml EDTA blood samples stored at -20°C were used. To lyse blood cells, one litre of sucrose lysis buffer (SLB) was made and stored at 4°C. 100ml Nuclei Lysis Buffer (NLB) was made with distilled water, autoclaved and stored in a sealed bottle.

Twenty-four samples were processed on ice at any one time. Samples were defrosted, emptied into 30ml polypropylene tubes with 20 mls cold SLB, inverted repeatedly to mix, and left for 10 mins. Samples were centrifuged (10000 rpm for 15 minutes at 4°C), supernatant decanted, 3ml of SLB added to the nuclear pellet, and the pellet resuspended in 22ml SLB by vortex. Washing was repeated twice. 3ml of NLB was then added, the pellet vortexed and 1ml 5M sodium perchlorate added. The samples were agitated (room temperature for 15 min) before incubation in a waterbath (60°C for 90 min). 1ml cold (-20°C) chloroform was now added, and the samples then agitated for 15 minutes and centrifuged (3000rpm for 3 min) discarding the supernatant.

The DNA was finally precipitated by gentle agitation with an excess of iced (-4°C) 100% ethanol, wrapped around a drawn glass pipette tip then deposited with the broken tip in a 3ml Eppendorf tube containing 0.5ml of buffer (pH 7.2). The DNA was allowed to dissolve, and the samples stored in a refrigerator at 4°C.

b) Polymerase chain reaction amplification

The I/D polymorphism was identified by polymerase chain reaction amplification (PCR) and subsequent electrophoretic separation of fragments. A 3-primer PCR system was used with primers as described by Evans (Evans et al, 1994), but with a slightly modified protocol to reduce D mistyping. Reactions were overlaid with 20μl mineral oil. All 96 wells were always filled with reagents (mix or dummy reagents) to ensure constant thermal mass on the

block. Amplification products were visualised using electropheresis on 7.5% polyacrylamide gels. The accuracy of this method was confirmed by the fact that PCRs set up using only the primer pair ACE1 and ACE3, both at 8pmol per 20µl PCR reaction, always confirmed the presence of the D allele.

DNA fragments were separated using electropheresis on an 8.4% polyacrylamide gel. Fragments were identified by the incorporation of ethidium bromide into the gels, and viewing under ultraviolet light.

Statistics

Differences between genotype groups were compared by χ^2 contingency tables.

8.2.3 Results

The mean age of patients undergoing angioplasty was 73.5 years (67.7 years in those who restenosed) each having a mean number of 1.8 risk factors (1.7 for the restenosis group). There was no difference in mean age or risk factors between individual genotypes. 9 PTAs were performed for critical ischaemia and 61 for claudication. 23 iliac and 47 femoral PTAs were analysed with restenosis rates at 6 months of 39.1% and 44.7% respectively. The median follow up period for patent vessels was >2 years.

The mean age of patients undergoing femoro-popliteal bypass was 72.5 years (72.8 years in those who restenosed) each with a mean number of 1.8 risk factors (2.2 for the restenosis group). There was no difference in mean age or risk factors between individual genotypes. 12 bypasses were performed for critical ischaemia and 18 for disabling claudication. The restenosis rate at 6 months was 36.7% and the median follow up period for patent conduits was 5.5 years. 7 genotypes failed to be identified. The overall genotype distribution for this population is shown in the table below:

Table 8.2 Genotypes of study population.

Genotype	Number	Percentage
DD	19	20
ID	38	41
II	36	39

Of the PTA patients, there was a trend towards those with the DD genotype having a higher restenosis rate (60%) although this was not significant (p=0.48, χ^2). Of those patients undergoing bypass, it would appear that there was an inverse trend with the II genotype having the highest restenosis rate. In reality however, this involves only 9 patients and must therefore be viewed with caution.

Table 8.3 Proportion of each genotype in which restenosis occurred.

Genotype	Bypasses*			PTAs‡		
	No.Restenosed	Total	%	No.Restenosed	Total	%
DD	3	9	33	6	10	60
ID	3	12	25	10	26	38
II	5	9	56	11	27	41

*p= 0.34 (χ^2) ‡p= 0.48 (χ^2)

8.2.4 Discussion

Amongst this population with peripheral vascular disease, ACE genotyping is of no predictive value for estimating the risk of restenosis following PTA or graft failure after bypass surgery. The distribution of genotypes in this series differs from that reported by other groups reporting results from patients with ischaemic heart disease (Samani et al, 1995; Hamon et al, 1995; Amant et al

1995). In these three series the ID genotype was found in between 50.2-52.0% of patients with the DD genotype occurring in the majority of the remainder.

The higher percentage of II genotypes amongst our population might explain the finding of a larger than expected number of restenoses occurring amongst bypass patients with this genotype. An alternative explanation would be that the outcome in the relatively small number involved (30 patients) was determined more by technical considerations than any genetically determined predisposition. Against this however, is the fact that all bypasses were performed by the same surgical unit and any failures within the first week were excluded. Although this study group was chosen to represent NIH rather than the complexity of the restenotic process following angioplasty, it is likely that the small number (11) of graft restenoses is insufficient for meaningful genotype analysis.

There was a trend towards a higher percentage of patients with the DD genotype developing restenosis following PTA, but this was not significant. A larger population may reveal a stronger relationship, but from the data available, it is unlikely that any relationship would have a predictive value sufficient to be clinically useful in identifying patients at risk of restenosis.

To date, it is clear that other than a previous history of restenosis, the risk factor common to the majority of studies is an inadequate primary intervention which is more likely to restenose compared with a procedure which results in complete restoration of the lumen. This however, simply reflects the greater ability of a large lumen to delay being compromised by NIH and remodelling and therefore does not truly represent a reduction in restenosis risk. In terms of specific lesion prediction, exciting data from plaque characterisation using IVUS and Duplex linked to a computer generated grey scale representing density shows the most promise in being able to predict whether a given atherosclerotic lesion may restenose or not.

In summary, accurate prediction of restenosis remains impossible at present. As with prevention, prediction is handicapped by our limited understanding of the restenosis process. If as expected, restenosis is multi-factorial, it would be unreasonable for a single risk factor to predict all cases. It is currently not known if a restenosis risk is harboured by the patient, by the lesion to be dilated or even by the method of intervention. Further advancement in the understanding of the complex interaction of these three factors is awaited.

CHAPTER 9 ARTERIAL PDT IN THE MANAGEMENT OF ANGIOPLASTY RESTENOSIS

9.1 INTRODUCTION

Evidence from in vitro work and small and large animal studies would suggest that PDT applied to an artery following PTA would be efficacious in reducing the incidence of restenosis (see Chapters 3,6 and 7). Data from trials using PDT for other purposes suggests that a predictable response can be obtained by the correct combination of photosensitiser, light dose and drug light interval. The work described below is the first clinical series using arterial PDT to prevent angioplasty restenosis.

9.1.1 Justification of Methodology

9.1.1.1 PDT

ALA induced PpIX sensitisation was deemed to be the optimum method of photosensitisation for arterial PDT in a clinical study for a number of reasons. Firstly, the depth of tissue effect with red light activating PpIX limits extra-arterial tissue damage, which is a significant benefit compared with other photosensitisers. Secondly, the limited skin photosensitivity period of PpIX allied to the small number of reported side effects (Webber et al, 1997a) makes it an ideal choice for PDT for non-malignant conditions requiring a short hospital stay. This would allow photosensitisation on the day of admission, angioplasty later that day and discharge the following day, much in line with the normal practice for patients undergoing PTA alone.

An oral dose of 60mg/Kg was selected as this is the highest dose with an established safety profile (Webber et al, 1997a; Webber et al, 1997b). This was given in 3 divided doses to give a longer plateau phase and avoid a post-bolus peak (Regula et al, 1995). There is scant information on the optimum drug-

light interval for ALA-PDT in humans, but previous studies would suggest a plasma PpIX half life of 8 hours (Webber et al, 1997b) with maximum tissue concentrations reached in a variety of tissues 7-10 hours after oral administration (Webber et al, 1997a). Other studies on patients undergoing operations for gastrointestinal and colorectal malignancies show that peak fluorescence activity is reached 6 hours after oral ALA given as a bolus (Webber et al, 1997c) or in divided doses (Regula et al, 1995). For this study a drug-light interval of 5-7 hours was aimed for.

9.1.1.2 Patient selection

Full local ethical committee approval was obtained and all patients were consented following a full explanation of the trial backed up with written information. Angioplasty limited to the SFA segment was selected due to the relatively poor current outcome for PTA in this segment, and the ease by which bypass surgery could be carried out in the event of unexpected complications arising. In this pilot study, patients were offered entry into the trial if they had previously undergone femoral PTA that had resulted in symptomatic restenosis between 1 week and 6 months post intervention. All patients who were suitable were also offered bypass surgery as an alternative.

The reason for targeting patients who had already experienced restenosis was their perceived poor outcome from repeat PTA. There are few studies reporting results from repeat PTA, but most would agree that the results with restenotic arteries are worse. In a study of 100 patients undergoing PTCA, Bresee showed that previous restenosis (even in a different artery) was an independent risk factor for subsequent restenosis (Bresee et al, 1991), although this has since been disputed by a larger study (Berger et al, 1992). With femoro-popliteal disease, a study investigating plaque morphology indirectly reported a higher rate of restenosis within a restenotic cohort compared with a primary atherosclerotic group, concluding that hypoechogenic lesions (thought to

represent NIH as seen with duplex) impart a higher restenotic risk (Baumgartner I et al, 1996b).

9.2 METHODS

9.2.1 Patients

Patients were recruited prospectively. They were excluded if they had pre-existing liver disease (as ALA can cause elevation of liver enzymes) or complete femoral occlusions greater than 15cm in length. Long occlusions were excluded from this pilot study because the technical problems anticipated in achieving primary patency may lead to an unfair assessment of the influence of PDT on restenosis. All patients were symptomatic and all lesions were confined to the SFA and restenotic following previous successful PTA.

9.2.2 Protocol

All patients were interviewed by myself. Symptoms and findings on examination were documented and baseline blood tests performed. ABPI at rest and following a standard exercise protocol (4 minutes at 2 km/hr with a 10° upward slope) was measured. An arterial duplex scan was performed and the lesion which had previously been angioplastied visualised and confirmed to have restenosed. The distance from the centre of the stenosis to the medial femoral condyle was measured and recorded for each patient. The PSVR was calculated by the ratio of the peak systolic velocity (PSV) at the stenotic site to the PSV at a normal pre-stenotic site. A PSVR > 2.0 across the lesion was taken as the definition of restenosis (Jäger et al, 1996).

Patients were then admitted for a repeat PTA with adjuvant PDT. ALA was given at 60mg/Kg in 3 divided doses dissolved in 50mls of orange juice and patients kept in subdued lighting thereafter. Between 5-7 hours after the first dose, patients underwent angiography via an ipsilateral femoral puncture and the stenosis was identified by comparison with previous films. Oxygen

saturation was monitored using pulse oximetry and the probe rotated from finger to finger to avoid a skin burn in a photosensitised patient (Farber et al, 1996). A standard PTA using an appropriately sized (4-6mm) transparent 4cm PTA balloon (Cordis) was performed and angiography repeated to confirm a satisfactory result. The guidewire was then exchanged for a 200μm laser fibre with a 4cm radial diffuser (Rare Earth Medical) and up to 50J/cm^2 light at 635nm (power < 120mW/ cm^2) given with a fractionation break of 60 seconds after 20% of the dose.

Completion angiography was performed and the run off vessels identified. Patients were kept in subdued lighting overnight and underwent duplex assessment the following day. They were then re-assessed at 1, 3 and 6 months and underwent an ivDSA at 6 months. Angiograms were reviewed blindly by an independent Consultant Radiologist and the degree of narrowing at the treatment site compared with the initial post-angioplasty arteriogram. All angiograms were performed in the same radiological suite using the same magnification and individual angiograms were checked by measuring the distance between the medial and lateral femoral cortex. Restenosis was assessed by measuring the percentage lumen loss which was estimated by comparing the lumen width at 6 months (as measured with callipers in mm) with that immediately post-angioplasty.

Data was expressed as medians and interquartile range (IQR) and compared using a Wilcoxon signed rank test with significance taken at the 5% level. Liver function was assessed by venous blood analysis at day 1 and at 1 month. All patients were commenced on aspirin at a dose of 75mg per day for life.

9.3 RESULTS

Seven patients (2 females and 5 males) with a median age of 70 (range 59-86) underwent 8 angioplasties with adjuvant PDT. Risk factors for peripheral vascular disease included hypertension (3), diabetes mellitus (2) and smoking (3). Three patients had occluded arteries (<5cm) at the commencement of the study and the other 5 lesions in 4 patients were tight (>75%) stenoses <10cm in length. Following previous successful PTA, 3 patients had re-presented with angiographically proven restenosis at 2 months; 1 at 3 months; 2 at 5 months and 1 at 6 months post intervention. In this study, all lesions were again successfully dilated under radiological imaging with satisfactory immediate results.

9.3.1 Surveillance

At follow up, all patients were found to be asymptomatic at 1 month and thereafter remained asymptomatic (with walking distances not limited by claudication) throughout the study period. In 2 patients, a fall in ABPI from the immediate post-procedure value was noted, but in others the ABPI remained stable or continued to improve during the 6 month study period (Figure 9.1). Using the more specific PSVR measurement derived from duplex analysis, none of the above angioplasty segments reached the pre-determined definition of restenosis (a PSVR of >2) as seen in Figure 9.2. The initial PSVR prior to angioplasty shows that all arterial lesions were significant stenoses (PSVR>>2) or occlusions (no flow and hence a PSVR =0). Median (IQR) PSVR across stenotic segments was 4.7(2.0) (excluding occlusions) pre-angioplasty; 1.1(0.4) at 24 hours and 1.4(0.8) at 6 months post-intervention ($p=0.04$ compared with pre-op). Including the occluded segments (with a PSVR of zero) in the statistical analysis lowers the pre-op median PSVR from 4.7(2.0) to 4.1(5.1) and reduces the significance ($p=0.09$).

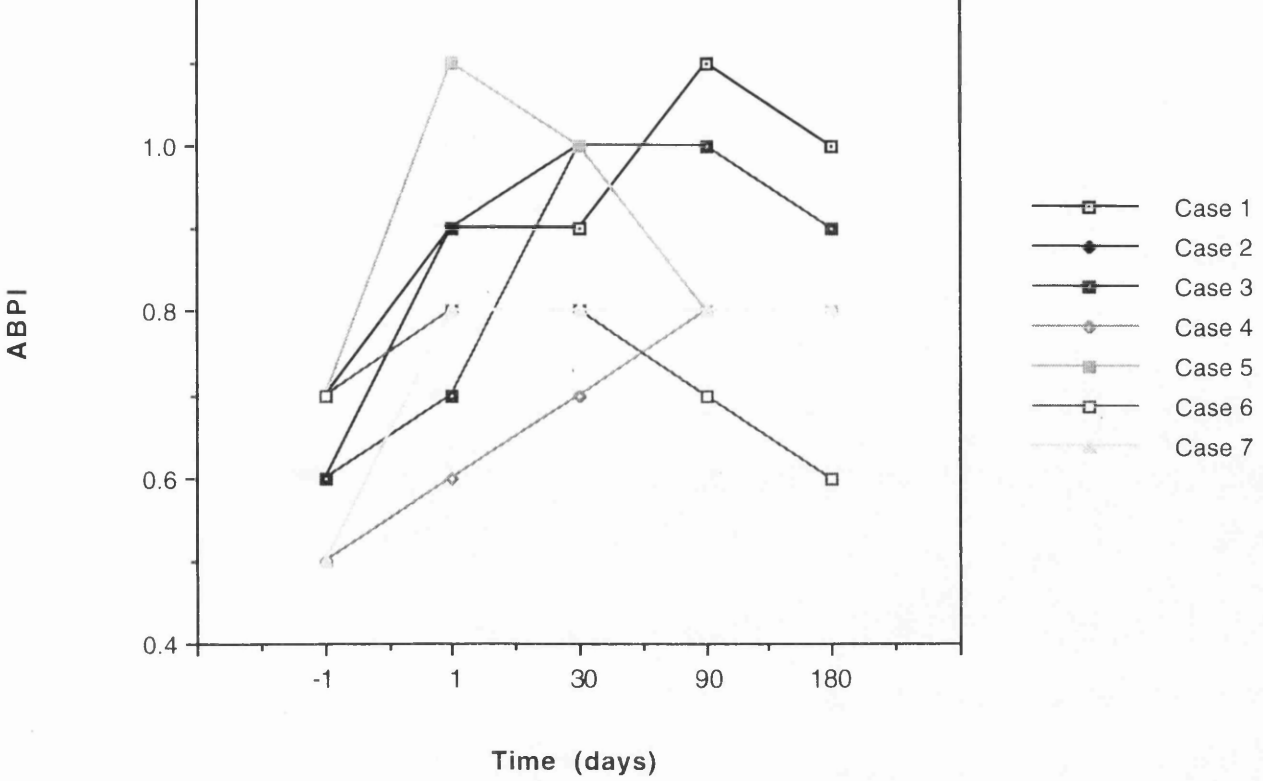


Figure 9.1 **Individual case data showing ABPI throughout the study period.**

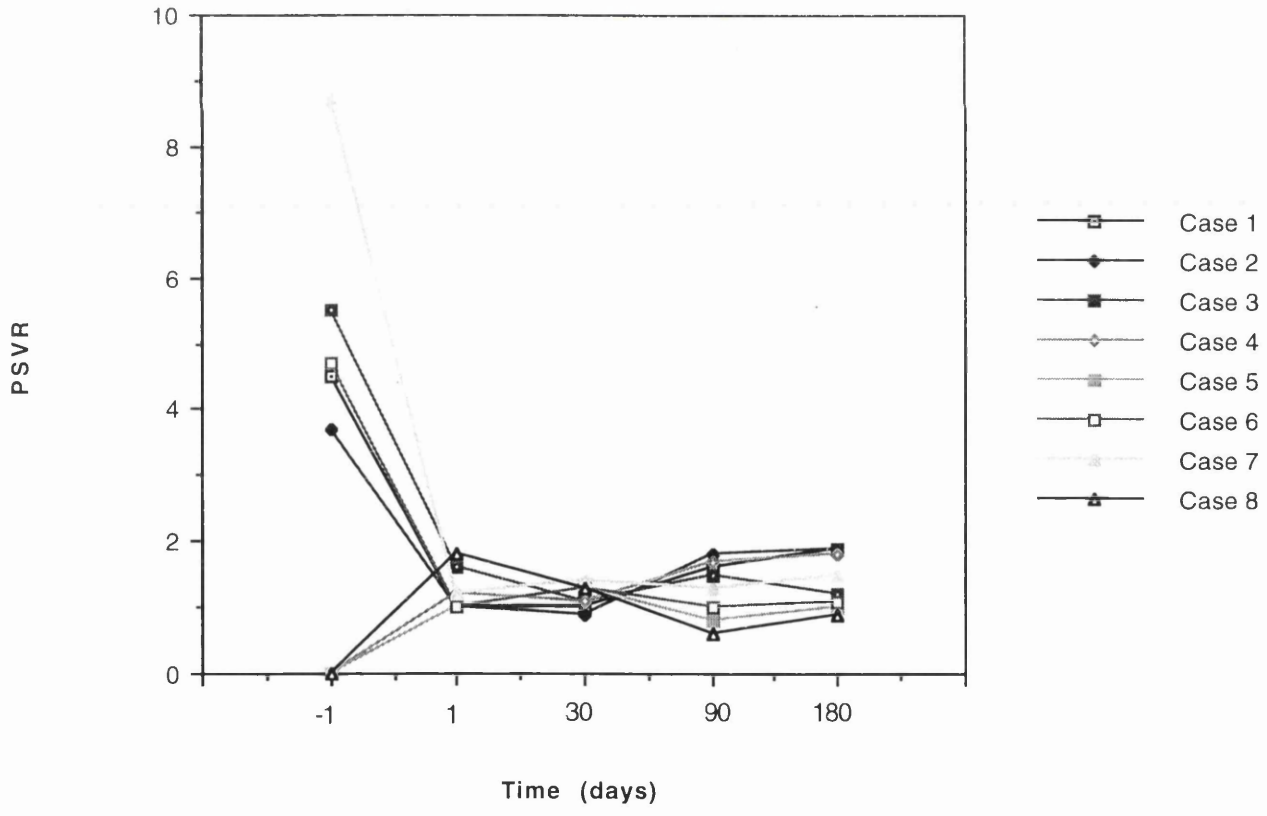


Figure 9.2 **Individual case data showing PSVR throughout the study period.**

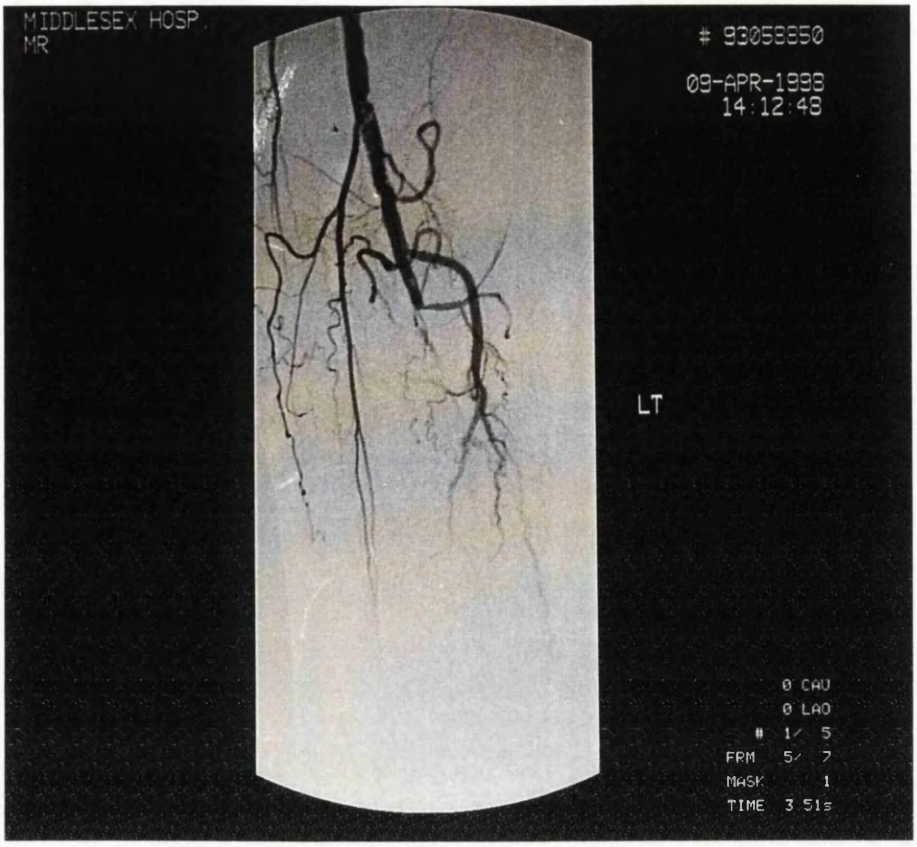
At 6 months, all patients underwent ivDSA which confirmed patency in all cases. Examples of pre-intervention, immediate post-intervention and 6 month angiograms are seen in Figures 9.3 and 9.4. Percentage stenosis as measured on angiography is shown for all cases in the table below.

Table 9.3

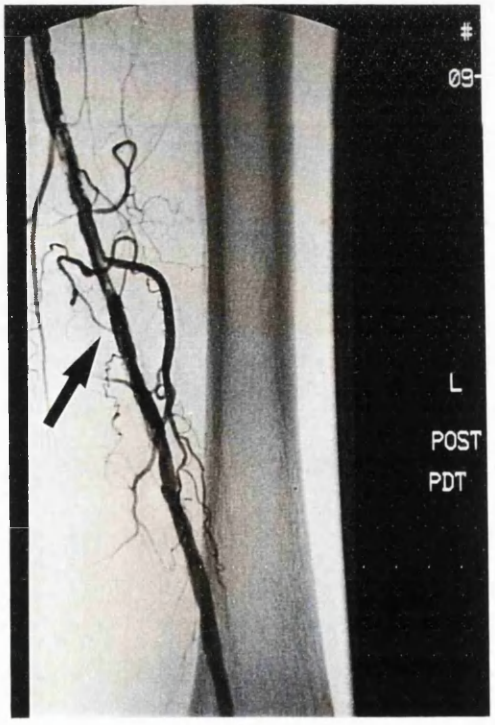
Patient	% Stenosis
TJ	33
NOB	33
	0
EM	50
KD	0
WE	50
MD	25
JA	0

No arterial or angioplasty complication occurred in any patient. One patient experienced a transient rise in liver enzymes and another mild nausea and temporary facial erythema. All of the above side effects have been previously described using ALA-photosensitisation (Webber et al, 1997a) and in this trial resolved spontaneously.

a).



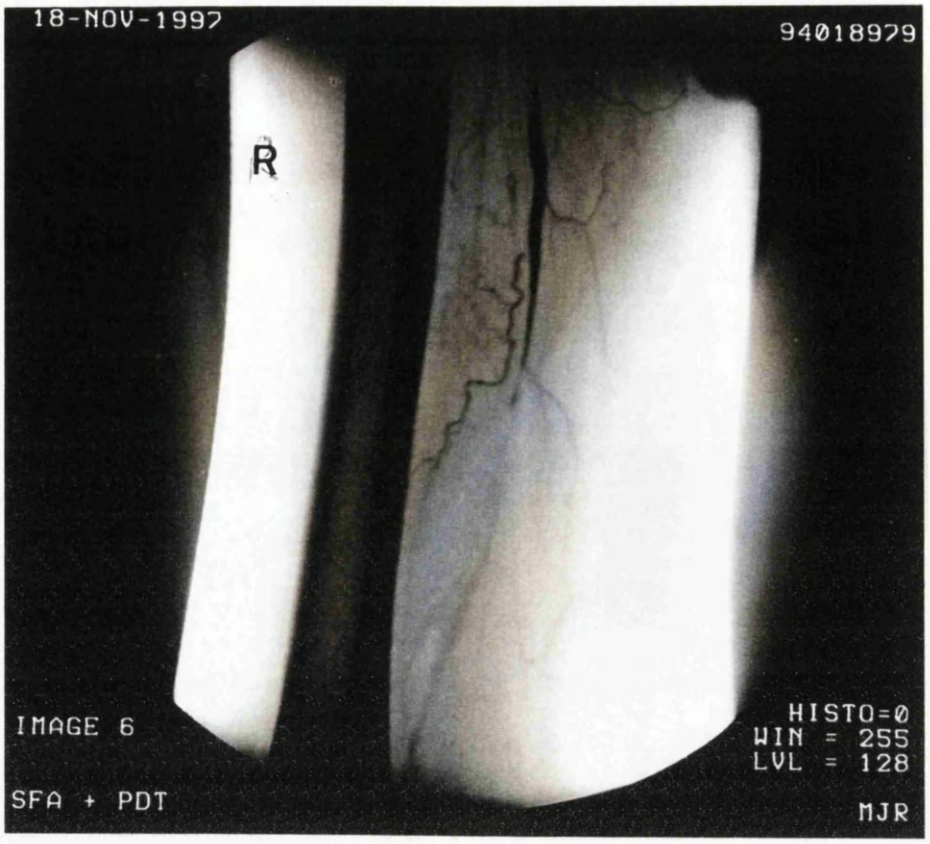
b).



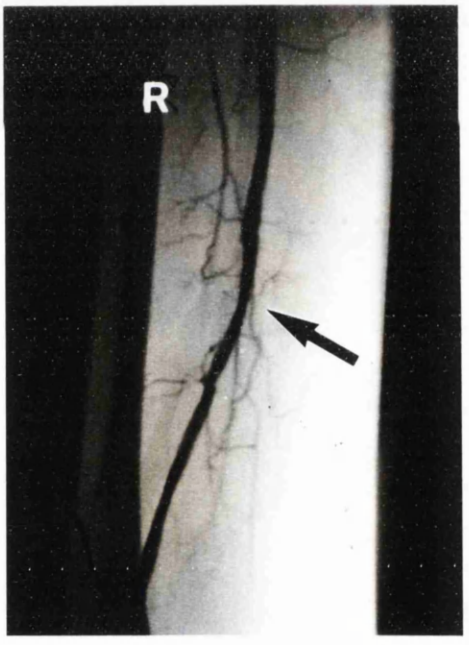
c).

Figure 9.3 **Example of angiograms a) pre-intervention, b) immediately post-intervention and c) at 6 months follow up (ivDSA). Arrow demonstrates the angioplasty site.**

a).



b).



c).

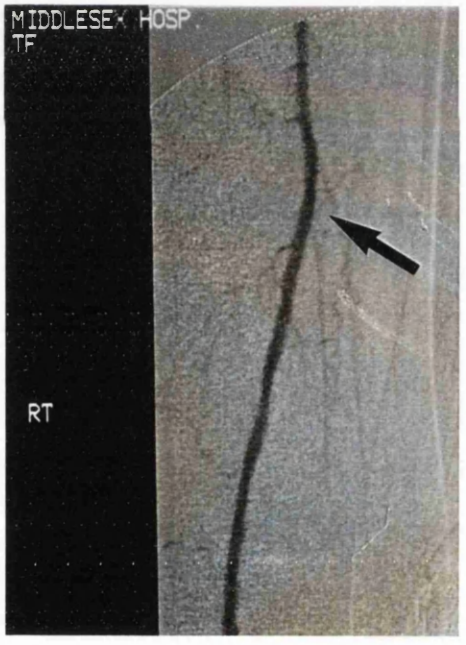


Figure 9.4 **Further example of angiograms a) pre-intervention, b) immediately post-intervention and c) at 6 months follow up (ivDSA). Arrow demonstrates the angioplasty site.**

9.4 DISCUSSION

This is the first clinical trial of arterial PDT as an adjuvant therapy to prevent restenosis following angioplasty. No major complications were noted at the time of the procedure and follow up to 6 months has revealed no evidence of clinical restenosis.

A pre-requisite for a therapy aimed at prevention of restenosis is that it is compatible with angioplasty. The minimally invasive, percutaneous nature of angioplasty has revolutionised the treatment of cardiovascular disease and any adjuvant treatment must be as safe and acceptable to physicians and patients alike. A long period of skin photosensitivity for example would be unacceptable in this context as the purpose of such treatment is aimed at prevention of a problem that is common, but by no means universal.

ALA was thought to be the ideal photosensitiser for targeting the vascular system as data from the pig model had revealed excellent PpIX fluorescence of the arterial wall and limited extra-vascular involvement. Moreover, the skin photosensitivity period of 24-48 hours and drug-light interval of the order of 5 hours make ALA-PDT a feasible adjuvant to angioplasty necessitating only one night in hospital. An oral dose of 60mg/Kg was selected as this is the highest dose with an established safety profile (Webber et al, 1997a; Webber et al, 1997b). Light delivery at the time of angioplasty was easily accomplished in the vascular suite and the use of the same balloon for angioplasty and as a vehicle for light transmission meant minimal delay and catheter manipulation during the procedure.

In this small cohort of patients judged to be at high risk of restenosis, little evidence of restenosis was seen within the study period. Review of the ivDSA films at 6 months revealed that in 2 patients, a degree of renarrowing occurred, but this did not encroach on greater than 50% of the lumen. Both these patients remained asymptomatic and the haemodynamic significance of the above

radiological findings must be questioned. As stated in Chapter 2, angiography gives a uniplanar anatomical view that may be of little relevance to the functional situation and is known to be inaccurate in the face of eccentric lesions. Duplex on the other hand is able to give anatomical information in more than one plane and can therefore identify eccentric plaques in addition to relating the disturbance in flow caused by a given stenosis. No lesions attained the pre-determined duplex definition of restenosis by the end of the surveillance period.

It is impossible to accurately predict the expected restenosis rate in such a small group of patients, but the evidence from other series would suggest that it should be much higher. Three patients in this series had occluded vessels and the reported success rates for first time recanalisation in an occluded femoral segment range from 22-44% after 1 year (Gray et al, 1997; Vroegindeweij et al, 1997). Even with stenotic disease, patency rates at 1 year are less than 50% (Matsi et al, 1994; Stanley et al, 1996) and as outlined above, results with restenotic lesions are probably worse.

Although the results of this pilot study would suggest that PDT is effective in reducing restenosis following angioplasty, the numbers are small and it would be premature to claim that ALA-PDT abolishes restenosis. However, the results are sufficiently promising to justify a randomised controlled trial of angioplasty with adjuvant PDT versus angioplasty alone.

CHAPTER 10 CONCLUSIONS

10.1 SUMMARY

Restenosis is a complex process that involves a combination of elastic recoil, NIH and negative geometric remodelling. Review of the current literature has lead me to conclude that restenosis should be thought of as a diverse process with the importance of each component varying depending on the primary vascular "insult" which leads to restenosis developing. Two decades of research and development has failed to find a solution to the increasingly important clinical and economic problem of restenosis. At the time of writing, it seems that radiotherapy via brachytherapy has achieved the vital threshold of efficacy coupled with global capability and acceptability in order that a single therapy to combat restenosis is investigated in a multicentre, multiculture domain. The long-term efficacy and safety consequences are awaited with interest.

The main aim of this thesis was to develop a safe and practical system of delivering PDT following PTA, to test it in a large animal model and then apply it in clinical practice within the confines of a pilot study. Fundamental to this was the choice of an appropriate photosensitiser that both encompassed the optimum characteristics to produce a limited depth of PDT effect and was suitable for clinical application to patients in the context of an overnight stay to undergo PTA.

In summary, the large animal work in this thesis has shown that:

1. Systemic ALA achieves arterial sensitisation with a drug-light interval four to five times that expected from small animal studies. This was the first study to investigate the pharmacokinetics of ALA in the pig model.

2. Differential temporal sensitisation between arterial layers was seen offering the possibility of manipulating the drug-light interval to target PDT to a particular vessel wall layer.

3. Endovascular light delivery was achieved via a standard (but, transparent) balloon catheter, and as judged by VSMC depletion, PDT was found to be effective following such a method of light delivery. Although a few other workers have used an intra-arterial light source, this has been limited to a diffuser housing a laser fibre, rather than an expandable balloon capable of conforming to the irregularities of an arterial lesion and therefore excluding blood from the segment to be treated. The other main advantage offered by this system is that the angioplasty balloon serves both as the vehicle for dilatation and phototherapy. This is the first study to report the results of such a system.

4. Following a balloon injury, it was seen that PDT results in a significantly larger lumen area compared with control vessels. This seems to be due to a combination of reduced NIH and less negative remodelling and was seen both in iliac and coronary arteries. No other workers have shown such an effect in the coronary circulation.

The clinical pilot study was designed to investigate the safety and feasability of arterial PDT as an adjuvant treatment to angioplasty. It is the first such study to be undertaken in the world. Over the surveillance period of 6 months, no major complications were encountered and in the population treated, the extent of restenosis was much less than expected. The ACE genotyping study did not show any meaningful association between the DD genotype and a predilection to restenosis after femoral PTA or arterial bypass. This is in keeping with the findings from coronary angioplasty studies, although a stronger association is seen with restenosis following coronary stenting. This is the first study to investigate any association between ACE genotype and outcome following PTA and bypass surgery for treatment of peripheral vascular disease.

10.2 STUDY LIMITATIONS

10.2.1 Animal Model

The most obvious disadvantage of any model of restenosis is that it does not exactly mimick the clinical situation. Regardless of how close the normal arterial histology approximates to man, it is impossible to control for the fact that atherosclerosis in man takes many decades to develop and the response of an atherosclerotic vessel to any intervention is not fully predictable from any animal model. In defence of the model chosen here, it is meant as a model of *restenosis* rather than *atherosclerosis* and as such is as good a representation of the result of balloon angioplasty as possible notwithstanding the above caveat.

The use of an expensive large animal model meant that it was impossible to perform a range of dose finding experiments and therefore optimum drug and light doses were estimated from the available data from both small animal and clinical trials using systemic ALA, mainly in the oral cavity. The inherent difficulty involved in light delivery within coronary arteries meant that the protocol in the iliac and coronary segments differed, making direct comparison difficult. In addition to variations in protocol, comparison difficulties are also compounded by vessel size differences and inherent differences between muscular and elastic arteries. The injury method used was probably insufficient to induce a large enough response in the elastic iliac segment and stent deployment would offer a more reliable iliac injury in future work. This underlines the shortcomings of using a model based on a single arterial segment and extrapolating results to represent the whole arterial tree.

10.2.2 Clinical Trial

The major limitation of the clinical PDT trial as reported is the lack of a control group. However, before testing arterial PDT in the context of a randomised control trial, a pilot study was needed establish safety and efficacy. The study described was meant as a pilot prior to a randomised study.

For the purpose of follow up assessment, with the advent of IVUS it must be accepted that the combination of duplex and ivDSA is a compromise. However, in the last few years, duplex has been validated beyond doubt as a tool for providing accurate diagnostic and surveillance information. A balance also has to be struck between the extra information gained from IVUS and the not inconsiderable cost and increased invasiveness involved.

10.3 FUTURE WORK

It would be desirable to increase the number of animals in the treatment group and then follow up a subgroup of these to 3 or 6 months. The rapid expansion of the use of therapeutic stenting in both coronary and peripheral arterial percutaneous treatment makes the investigation of how PDT would influence the response to stenting mandatory. This would solve the aforementioned problem of the limited iliac injury and lessen the concern that PDT alone may compromise the long-term integrity of the arterial wall perhaps leading to aneurysm formation. However, in combination with stent placement, PDT would only influence NIH as any remodelling effect would be negated by the stent.

Since these studies were commenced, an intravenous preparation of ALA suitable for clinical use has been developed. This would allow closer matching of the doses used in animal and clinical studies. The present maximum oral dose (60mg/Kg) used in clinical practice is limited by hepatotoxicity seen in doses above this. An intravenous preparation would potentially allow higher doses to be used safely by removing the first pass metabolism of the oral route. The intravenous ALA preparation is currently being tested in a group of patients undergoing treatment for oral cavity malignancy.

Following on from the clinical PDT trial, a randomised controlled trial would be essential to establish the role of PDT in reducing the incidence of post angioplasty restenosis. It would be relatively simple to randomly allocate

groups matched for severity of disease and risk factors to standard PTA and PTA with adjuvant endovascular PDT as described in the pilot study. Funding is currently being sought for such a trial. With the advent of a new 635 nm diode laser (Diomed) with sufficient power to achieve adequate fluence rates with the laser fibres used in the above studies, future experiments and trials promise to be technically much less challenging.

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APPENDICES

DATA TABLES

Table 5.1 Mean PpIX fluorescence intensity for ALA at 60mg/Kg.

Time (hours)	Counts per pixel ± SD		
	Intima	**Media**	**Adventitia**
0.5	5±6	1±1	11±11
1	5±3	2±2	7±7
1.5	8±2	5±2	22±12
2	17±5	15±3	38±25
3	21±4	16±2	19±7
4	35±10	26±5	48±20
5	32±6	39±6	32±14
6	18±4	42±5	17±6
24	24±5	21±6	22±5

Table 5.2 Mean PpIX fluorescence intensity for ALA at 120 mg/Kg.

Time (hours)	Counts per pixel ± SD		
	Intima	**Media**	**Adventitia**
0.5	6±3	4±2	18±10
1	12±6	10±2	56±47
1.5	29±8	24±6	92±35
2	26±6	23±11	35±20
3	22±4	31±3	34±13
4	41±11	39±4	64±13
5	40±8	48±13	34±8
6	37±4	86±18	32±7
8	43±7	58±4	31±5
24	26±6	18±10	20±4

Table 5.3 Mean PpIX fluorescence intensity for coronary arteries at 6 hours post ALA dose.

ALA Dose	Coronary Counts per pixel at 6 hours		
	Intima	Media	Adventitia
60 mg/Kg	26	28	16
120 mg/Kg	34	39	18

Table 6.1 Circumferential VSMC/HPF for control and treated iliac arteries harvested at 3 and 14 days.

	VSMC/HPF			
Intervention	**0°**	**90°**	**180°**	**360°**
ALA control	92	86	101	123
	105	109	113	121
	123	132	145	125
PDT - 3 days	3	0	11	12
	0	0	10	14
	34	40	41	5
	0	5	3	19
	0	1	1	2
	0	2	9	0
	0	0	3	2
PDT - 14 days	0	0	7	0
	2	0	0	35
	8	17	26	8
	38	0	3	57
	47	53	46	44

Table 6.2 Circumferential VSMC/HPF for control and treated coronary arteries harvested at 3 and 14 days.

	VSMC/HPF			
Intervention	**0°**	**90°**	**180°**	**360°**
ALA control	95	72	90	83
	41	37	83	78
	115	70	126	119
	76	85	93	71
	55	60	93	59
	76	87	75	87
	106	100	93	99
PDT - 3 days	8	0	0	6
	11	0	32	27
	35	17	33	10
	24	3	30	28
	52	52	36	38
	39	31	29	46
	51	45	53	44
	42	37	46	29
	43	39	41	50
	44	44	56	29
PDT - 14 days	27	13	19	23
	51	87	76	50
	68	47	66	48
	75	48	65	43
	40	36	58	66
	52	49	57	56
	53	41	52	54
	46	32	46	38
	26	42	56	44
	53	45	54	36

PDT - 14 days (cont)	0°	90°	180°	360°
	43	45	51	47
	62	46	54	50
	49	45	42	53
	51	38	51	44
	46	40	53	59
	29	38	36	32
	40	40	36	33
	51	33	39	38
	56	34	32	39
	54	48	33	35
	44	39	42	49

Table 6.3 Mean VSMC/HPF for control and treated animals harvested at 3 and 14 days.

Intervention	Mean VSMC/HPF ± SD	
	Iliac	Coronary
ALA control	115 ± 17	83 ± 21
PDT - 3 days	8 ± 12	29 ± 17
PDT -14 days	17 ± 19	46 ± 12

Table 6.4 Iliac VSMC/HPF at various drug-light intervals.

	VSMC/HPF			
Intervention	**0°**	**90°**	**180°**	**360°**
ALA control	92	86	101	123
	105	109	113	121
	123	132	145	125
Laser control	105	110	108	110
	100	88	89	102
	105	97	114	111
PDT @ 1.5 hrs	0	5	3	19
	8	17	26	8
	38	0	3	57
	47	53	46	44
PDT @ 2.5 hrs	66	64	0	0
	75	58	69	57
	63	50	58	62
	16	0	0	3
	79	66	74	61
	85	69	66	76
PDT @ 6-7 hrs	0	0	7	0
	2	0	0	35
	3	0	11	12
	0	0	10	14
	34	40	36	5

Table 6.5 Mean VSMC/HPF at various drug-light intervals.

Intervention	Mean Iliac VSMC/HPF ± SD
ALA control	115 ± 17
Laser control	103 ± 8
PDT @ 1.5 hrs	23 ± 21
PDT @ 2.5 hrs	51 ± 29
PDT @ 6-7 hrs	10 ± 11

Table 7.1 Coronary artery areas for control vessels post injury (EEL = external elastic lamina; NIH = neointimal hyperplasia; IS = Injury score).

Intervention	Cross sectional area (mm^2)			IS
	Lumen	**EEL**	**NIH**	
Control	1.42	2.95	0.30	2
	1.03	2.27	0.52	3
	0.79	2.49	0.88	3
	0.34	0.95	0.05	1
	0.48	1.37	0.34	2
	0.51	1.42	0.20	3
	0.14	2.31	1.55	3
	0.26	0.80	0.10	2
	0.82	1.78	0.23	2
	0.80	1.68	0.15	3
	0.21	0.57	0.03	1
	0.50	2.33	0.82	3
	0.16	3.02	1.71	3
	0.30	3.51	2.69	3
	0.40	1.97	0.74	3
	1.35	2.64	0.65	2
	1.19	2.86	0.71	2
	0.83	2.76	1.18	3
	1.12	2.01	0.35	2
	1.88	2.73	0.36	2
	1.71	2.70	0.34	1
	1.21	1.93	0.20	1

Table 7.2 Coronary artery areas for treated vessels post injury (EEL = external elastic lamina; NIH = neointimal hyperplasia; IS = Injury score).

Intervention	Cross sectional area (mm^2)			IS
	Lumen	**EEL**	**NIH**	
PDT	1.47	2.62	0.31	3
	1.24	2.16	0.20	2
	1.25	2.21	0.21	2
	1.62	3.38	0.56	3
	1.65	3.80	0.52	3
	1.45	3.23	0.56	2
	1.50	3.23	0.41	1
	0.94	3.13	1.16	2
	0.90	3.34	1.29	3
	0.57	2.36	0.95	2
	0.95	1.90	0.45	1
	2.49	2.94	0.08	1
	3.29	4.36	0.37	2
	1.05	2.33	0.75	2
	1.02	2.05	0.29	1
	1.60	3.42	0.37	1
	1.23	2.45	0.17	3
	0.28	2.19	0.39	3

Table 7.3 Mean coronary artery areas for control and treated vessels post injury (EEL = external elastic lamina).

Intervention	Cross sectional area (mm^2) ± SD		
	Lumen	**EEL**	**Neointima**
Control	0.79 ± 0.51	2.15 ± 0.78	0.70 ± 0.66
PDT	1.39 ± 0.64	2.84 ± 0.69	0.39 ± 0.22

Table 7.4 Iliac artery areas for control vessels post injury (EEL = external elastic lamina; NIH = neointimal hyperplasia; IS = Injury score).

Intervention	Cross sectional area (mm^2)			IS
	Lumen	**EEL**	**NIH**	
Control	2.36	8.85	2.20	0
	1.55	7.35	2.51	1
	4.05	7.68	0.65	1
	2.13	5.65	0.95	0
	2.88	5.95	0.77	0
	3.85	6.88	0.51	0
	5.90	9.60	0.30	0
	9.60	13.6	0.30	0
	10.4	17.2	0.70	1
	14.2	18.3	0.30	0
	13.4	16.9	0.50	1
	15.4	19.4	0.40	0
	5.20	8.30	0.10	0
	5.10	7.90	0.20	0
	5.30	8.30	0.10	0
	3.29	7.64	0.90	1
	3.89	8.06	0.76	1
	4.05	8.52	0.80	0
	14.2	19.2	1.45	0
	7.86	12.2	0.90	1
	8.88	12.3	0.72	0
	5.35	9.96	0.61	0
	6.52	10.7	0.54	0
	7.09	11.0	0.38	0

Intervention	Cross sectional area (mm^2)			IS
	Lumen	**EEL**	**NIH**	
	17.7	24.6	1.50	0
	17.4	25.1	2.24	0
	13.9	18.1	1.00	0
	11.7	18.1	2.63	1
	10.2	17.0	2.12	1
	6.51	13.1	1.86	1

Table 7.5 Iliac artery areas for treated vessels post injury (EEL = external elastic lamina; NIH = neointimal hyperplasia; IS = Injury score).

Intervention	Cross sectional area (mm^2)			IS
	Lumen	**EEL**	**NIH**	
PDT	3.95	8.86	1.32	0
	2.71	7.29	0.99	0
	3.40	7.70	1.10	0
	3.70	8.48	2.15	1
	6.04	9.91	1.54	1
	9.81	12.4	0.99	0
	10.5	17.2	0.70	1
	11.9	17.2	0.40	1
	15.5	20.8	0.50	0
	14.6	18.8	1.00	1
	12.2	16.7	0.40	1
	15.5	20.7	0.70	0
	12.6	17.5	0.80	0
	12.2	16.3	0.50	2
	9.20	13.3	0.50	0
	9.77	13.9	0.36	1
	5.41	9.71	0.41	0
	5.72	10.2	0.30	0
	9.83	18.5	1.20	1
	11.9	20.0	1.25	1
	12.6	19.6	0.56	0
	12.7	20.2	3.20	1
	11.2	15.9	0.65	0
	13.2	16.6	0.61	1

Intervention	Cross sectional area (mm^2)			IS
	Lumen	EEL	NIH	
	11.1	18.3	2.04	1
	13.1	21.3	1.34	1
	10.0	16.4	0.33	0
	7.82	13.7	0.16	1
	6.75	14.6	1.49	1
	8.05	13.8	0.54	1

Table 7.6 Mean iliac artery areas for control and treated vessels post injury (EEL = external elastic lamina).

Intervention	Cross sectional area (mm^2) ± SD		
	Lumen	EEL	Neointima
Control	7.99 ± 4.79	12.49 ± 5.44	0.95 ± 0.76
PDT	9.77 ± 3.66	15.19 ± 4.25	0.76 ± 0.48

Table 9.1 ABPI (Ankle brachial pressure index) before and after angioplasty with adjuvant PDT.

Patient	ABPI				
	Pre-op	Day 1	Day 30	Day 60	Day 90
TJ	0.7	0.9	0.9	1.1	1.0
NOB	0.6	0.9	1.0	1.0	0.9
EM	0.6	0.7	1.0	1.0	0.9
KD	0.5	0.6	0.7	0.8	0.9
WE	0.7	1.1	1.0	0.8	0.8
MD	0.7	0.8	0.8	0.7	0.6
JA	0.5	0.8	0.8	0.8	0.8

Table 9.2 PSVR (Peak systolic velocity ratio) before and after angioplasty with adjuvant PDT.

Patient	PSVR				
	Pre-op	Day 1	Day 30	Day 60	Day 90
TJ	4.5	1.0	1.0	1.6	1.9
NOB	3.7	1.0	0.9	1.8	1.9
	5.5	1.6	1.1	1.5	1.2
EM	Occluded	1.2	1.1	1.7	1.8
KD	Occluded	1.0	1.3	0.8	1.0
WE	4.7	1.0	1.3	1.0	1.1
MD	8.7	1.2	1.4	1.3	1.5
JA	Occluded	1.8	1.3	0.6	0.9

ACE GENOTYPE STUDY

Study No.

Name **Hospital No.**

Address **Age**

Tel.

Clinical Assessment: **Claudication**

Critical ischaemia

Risk factors	**IHD**	**Hypercholesterolaemia**
	Hypertension	**DM**
	CVA/TIA	**Renal failure**
	Smoker	**Family history**

Procedure	**Site**	**Side**
Date		
Benefit Y/N		**Duration**
Claudication dist. pre.		**Claudication dist. post.**
Assessment	**Duplex**	
	Angiogram	
Need for:	**Repeat PTA**	
	By-pass	
	Amputation	
ACE Genotype	**DD**	
	DI	
	II	

CLINICAL PDT TRIAL

NAME: **TRIAL NUMBER:**

PATIENT DETAILS

SURNAME: **SEX: M / F**

FORENAME(S): **DOB: / /**

ADDRESS: **HOSPITAL No:**

POST CODE:

TELEPHONE No:

GP:

GP ADDRESS:

GP TELEPHONE No:

CLINICAL PDT TRIAL

NAME: **TRIAL NUMBER:**

HISTORY

PRESENTING COMPLAINT:

CLAUDICATION Y / N REST PAIN Y / N TISSUE LOSS Y / N

BILATERAL / R / L

R L

DURATION:

DISTANCE:

WORSENING:

RISK FACTORS:

SMOKER	**Y / N**	**X - SMOKER**	**Y / N**
DIABETES	**Y / N**		
HYPERTENSION	**Y / N**		
FAMILY HISTORY	**Y / N**		
HYPERCHOLESTEROLAEMIA	**Y / N**		

RELEVANT PMH:

CVA / TIA	**Y / N**	
MI / ANGINA		**Y / N**
RENAL FAILURE	**Y / N**	
ARTERIAL SURGERY	**Y / N**	

CLINICAL PDT TRIAL

NAME: **TRIAL NUMBER:**

HISTORY CONT.

	R	L
PREVIOUS PTA &DATE	**CIA / SFA**	**CIA / SFA**
BENEFICIAL ?	**Y / N**	**Y / N**
DURATION:		

EXAMINATION

DATE: / /

	R	L
SKIN CHANGES:	**Y / N**	**Y / N**
ULCERATION / TISSUE LOSS:	**Y / N**	**Y / N**
PULSES: (++ / + / -)	**R**	**L**
FEMORAL:		
POPLITEAL:		
DORSALIS PEDIS:		
POSTERIOR TIBIAL:		

DOPPLERS:	R	L
BRACHIAL PRESSURE		
ANKLE DOPPLER PRESSURE		
DOPPLER INDEX		

CLINICAL PDT TRIAL

NAME: **TRIAL NUMBER:**

PRE-ANGIOPLASTY DUPLEX

DATE: / /

STENOTIC / OCCLUDED SEGMENT:

SIDE: R / L **LENGTH (cm):**

SITE:

DISTANCE FROM MEDIAL FEMORAL CONDYLE (cm):

OTHER LESIONS SAME SIDE? **RUN OFF: 1 2 3**

LUMINAL DIAMETER (mm):	**ANGLE 1 (TRANSVERSE)**	**ANGLE 2 (LONGITUDINAL)**
LESION 1		
LESION 2		
ADJACENY ARTERY - PROXIMAL		
DISTAL		

VELOCITIES (m/s):	
PROXIMAL	
LESION	
DISTAL	

EXERCISE PRESSURES:

DOPPLER INDEX AT REST:

DOPPLER INDEX AFTER EXERCISE:

TREADMILL TIME (2km/h):

CLINICAL PDT TRIAL

NAME: **TRIAL NUMBER:**

ANGIOPLASTY

DATE: / /

PHOTOSENSITIZER

PATIENT WEIGHT **Kg**

ALA DOSE **mg/Kg** **ORAL / IV**

mg

TIME FIRST DOSE:

TIME LAST DOSE:

LESION

SIDE: **R** **L**

SITE:

DISTANCE FROM MED. FEM. CONDYLE:

PROCEDURE

ACCESS SITE:

BALLOON SIZE:

TIME:

LASER ENERGY (J/cm2): **DURATION:**

IMMEDIATE RESULT:

CLINICAL PDT TRIAL

NAME: **TRIAL NUMBER:**

24 HOUR DUPLEX

LUMINAL DIAMETER (mm):	**ANGLE 1 (TRANSVERSE)**	**ANGLE 2 (LONGITUDINAL)**
LESION 1		
LESION 2		
ADJACENT ARTERY - PROXIMAL		
DISTAL		

VELOCITIES (m/s):	
PROXIMAL	
LESION	
DISTAL	

EXERCISE PRESSURES:

DOPPLER INDEX AT REST:

DOPPLER INDEX AFTER EXERCISE:

CLINICAL PDT TRIAL

NAME: **TRIAL NUMBER:**

1 MONTH DUPLEX

LUMINAL DIAMETER (mm): **ANGLE 1 (TRANSVERSE)** **ANGLE 2 (LONGITUDINAL)**

LESION 1

LESION 2

ADJACENT ARTERY - PROXIMAL

DISTAL

VELOCITIES (m/s): **PROXIMAL**

LESION

DISTAL

EXERCISE PRESSURES:

DOPPLER INDEX AT REST:

DOPPLER INDEX AFTER EXERCISE:

CLINICAL:

CLAUDICATION / Y / N **REST PAIN Y / N** **TISSUE LOSS Y / N**

BILATERAL / R / L **R** **L**

DISTANCE:

CLINICAL PDT TRIAL

NAME: **TRIAL NUMBER:**

3 MONTH DUPLEX

LUMINAL DIAMETER (mm): **ANGLE 1 (TRANSVERSE)** **ANGLE 2 (LONGITUDINAL)**

LESION 1

LESION 2

ADJACENT ARTERY - **PROXIMAL**

DISTAL

VELOCITIES (m/s): **PROXIMAL**

LESION

DISTAL

EXERCISE PRESSURES:

DOPPLER INDEX AT REST:

DOPPLER INDEX AFTER EXERCISE:

CLINICAL:

CLAUDICATION / Y / N **REST PAIN Y / N** **TISSUE LOSS Y / N**

BILATERAL / R / L

R **L**

DISTANCE:

CLINICAL PDT TRIAL

NAME: **TRIAL NUMBER:**

6 MONTH DUPLEX

LUMINAL DIAMETER (mm): **ANGLE 1 (TRANSVERSE)** **ANGLE 2 (LONGITUDINAL)**

LESION 1

LESION 2

ADJACENT ARTERY - PROXIMAL

DISTAL

VELOCITIES (m/s): **PROXIMAL**

LESION

DISTAL

EXERCISE PRESSURES:

DOPPLER INDEX AT REST:

DOPPLER INDEX AFTER EXERCISE:

CLINICAL:

CLAUDICATION / Y / N **REST PAIN Y / N** **TISSUE LOSS Y /N**

BILATERAL / R / L

R **L**

DISTANCE:

CLINICAL PDT TRIAL

NAME: **TRIAL NUMBER:**

6 MONTH IV DSA

DATE: / /

PATENT ? Y / N

LESION VISIBLE Y / N

% STENOSIS:

(% STENOSIS ON ORIGINAL ANGIOGRAM):

INFORMATION SHEET

PHOTODYNAMIC THERAPY (PDT) IN THE MANAGEMENT OF ANGIOPLASTY RESTENOSIS.

PROF SG BOWN, MR MP JENKINS, DR M RAPHAEL, MR CCR BISHOP

Symptoms arising from a blockage in the leg arteries may need an operation, but can often be treated by a simpler procedure using a balloon to stretch the artery open (angioplasty). Although angioplasty can be very effective in reopening the blockage, the problem can recur in about a third of patients. If you have already had a recurrence, or your blockage is a long one, then you are at increased risk of this complication.

Photodynamic therapy (PDT) is a relatively new treatment which is already being used in other branches of medicine and is known to be safe. Our preliminary studies show that PDT is also likely to be of value in preventing the leg arteries re-blocking after a balloon stretch. We would like to use this new treatment to see if we can prevent the blockage in your artery from returning once you have had the angioplasty.

The treatment involves taking a drink (or injection) containing a substance known as ALA (aminolaevulinic acid). ALA occurs naturally in the body, but by giving you more, we can make your arteries sensitive to light. During your angioplasty, a low power laser beam will be shone down the artery which will activate the ALA and this will suppress the cells responsible for the artery reblocking. The angioplasty will be normal and the new treatment is only a way of trying to reduce the likelihood of recurrence. The treatment should be painless and will add only 30 minutes or so to your angioplasty time. If any discomfort is experienced then appropriate pain killers will be given, but this is most unlikely to be necessary.

The main side effect is that the skin also becomes sensitive to light for about 24 hours after taking the drug and exposure to bright light during that time could result in an unpleasant sunburn-like reaction. You will therefore be kept out of strong light overnight. Providing these guidelines are followed we do not expect any side effects from the treatment.

You do not have to take part in this study if you do not want to. If you do decide to take part you may withdraw at any time without having to give a reason. Your decision as to whether to take part or not will not affect your care or management in any way. If you do agree to take part, we will ask you to sign a consent form. You are encouraged to ask for further information and to discuss it with anyone you wish before making your decision. The whole procedure will be explained to you in detail by Mr Jenkins and there will be ample opportunity to raise any queries you might have before signing the consent form.

All proposals for research using human subjects are reviewed by an ethics committee before they can proceed. This proposal was reviewed by the joint UCL/UCLH Committees on the Ethics of Human Research.

PATIENT CONSENT

PHOTODYNAMIC THERAPY (PDT) IN THE MANAGEMENT OF ANGIOPLASTY RESTENOSIS

PROF SG BOWN, MR MP JENKINS, DR M RAPHAEL, MR CCR BISHOP

Have you read the information sheet about this study?	YES/NO
Have you had the opportunity to ask questions and discuss this study?	YES/NO
Have you received satisfactory answers to all your questions?	YES/NO
Have you received enough information about this study?	YES/NO

My questions have been answered by

.....

I understand that I may withdraw from this study at any time without giving a reason and without it affecting my subsequent continuing care. I agree to take part in this study.

Signed ..... Print name .....

Witness ..... Print name .....

Investigator ..... Date :

PUBLICATIONS ARISING FROM THIS DISSERTATION

Abstracts and Presentations to Learned Societies

Jenkins MP, McEwan J, MacRobert AJ, Bishop CCR, Bown SG. Photosensitisation with 5-aminolaevulinic acid allows selective targeting of the arterial wall using photodynamic therapy. Presented at the *British Medical Laser Association* , Jersey - June 1997 and published in *Lasers in Medical Science,* 1997; **12**: 290.

Jenkins MP, Buonaccorsi GA, Bishop CCR, Bown SG, McEwan J. Photodynamic therapy (PDT) depletes the vascular smooth muscle cell population of arteries in a pig model and is therefore a potential therapy for restenosis. Presented at the *British Medical Laser Association* , Jersey - June 1997 and published in *Lasers in Medical Science,* 1997; **12**: 295.

Jenkins MP, Buonaccorsi GA, MacRobert AJ, Bishop CCR, Bown SG, McEwan J. Photodynamic therapy - a potential therapy for restenosis. *20 years of PTCA - Back to the cradle* , Zurich - September 1997.

Jenkins MP, Buonaccorsi GA, Bown SG, Bishop CCR, McEwan J. Endovascular photodynamic therapy reduces the response to balloon injury in coronary and iliac arteries in a swine model. *International Endovascular Symposium,* Sydney - December 1997.

Jenkins MP, Buonaccorsi GA, MacRobert AJ, Bishop CCR, Bown SG, McEwan J. The pharmacokinetics of 5-aminolaevulinic acid for endovascular photodynamic to reduce restenosis following angioplasty. Presented at the *International Society for Optical Engineering,* San Jose, January 1998

Jenkins MP, Buonaccorsi G, MacRobert A, Bishop CCR, Bown SG, McEwan JR. Intra-arterial photodynamic therapy to prevent restenosis following angioplasty. Presented at *Royal Society of Medicine,* Section of Surgery (MIA First prize) - February 1998 and published in *Ann R Coll Surg Engl,* 1998; **80**: 297.

Jenkins MP, Buonaccorsi GA, MacRobert AJ, Bishop CCR, Bown SG, McEwan JR. Photodynamic thrapy - a potential therapy for restenosis. *Second International Workshop on ALA Photodynamic Therapy and Photodetection,* Leeds - April 1998.

Jenkins MP, Buonaccorsi GA, Raphael M, Bown SG, Nyamekye I, Bishop CCR. Intra-arterial PDT - The first clinical experience. *Second International Workshop on ALA Photodynamic Therapy and Photodetection,* Leeds - April 1998.

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Jenkins M, Raphael M, Buonaccorsi G, Bown S, Nyamekye I, Bishop C. Preliminary results of a clinical pilot study using adjuvant photodynamic therapy following femoral angioplasty. *7th Biennial Congress of the International Photodynamic Association,* Nantes - July 1998.

Jenkins MP, McEwan JR, Buonaccorsi GA, Bown SG, Bishop CCR. Endovascular photodynamic therapy reduces the response to balloon injury in a swine model. Presented at *Surgical Research Society (Patey Prize section),* Dublin - July 1998 and published in *British Journal of Surgery,* 1998; **85**: 1565.

Jenkins MP, Buonaccorsi GA, Bishop CCR, Bown SG, McEwan JR. Intra-arterial coronary photodynamic therapy in an animal model of angioplasty restenosis. Presented at *American Society of Photobiology,* Utah - July 1998 and published in *Photochemistry and Photobiology,* 1998; **67**: 79S.

Jenkins MP, Buonaccorsi GA, Raphael M, Nyamekye I, Bown SG, Bishop CCR. A clinical pilot study of adjuvant photodynamic therapy to reduce restenosis following femoral angioplasty. Presented at *Association of Surgeons,* Brighton - May 1999 and published in *British Journal of Surgery*, 1999; **86**: 65.

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Jenkins MP, Buonaccorsi GA, MacRobert AJ, Bishop CCR, Bown SG, McEwan J. 1998 Pharmacokinetics and efficacy of 5-aminolaevulinic acid for endovascular photodynamic therapy in a swine model. *Proceedings of the International Society for Optical Engineering*; **3245**: 20-27.

Jenkins MP, Buonaccorsi GA, MacRobert AJ, Bishop CCR, Bown SG, McEwan JR. 1998 Intra-arterial photodynamic therapy using 5-ALA in a swine model. *European Journal of Vascular and Endovascular Surgery*; **16**: 284-291.

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