Reduction in the response to coronary and iliac artery injury with photodynamic therapy using 5-aminolaevulinic acid

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Abstract

Objective: Photodynamic therapy (PDT) uses red light (non-thermal, non-ionising) to activate a previously administered photosensitising drug. This inhibits neointimal hyperplasia in injured arteries in small animals where it appears safe and well tolerated. Our aim was to develop a method for percutaneous application of PDT to iliac and coronary arteries in a large animal model and investigate its influence on the remodelling and intimal hyperplastic response to balloon injury. Methods: Studies were undertaken on 13 juvenile Large White-Landrace crossbred pigs (15–20 kg). After intravenous administration of the photosensitising agent 5-amino laevulinic acid (ALA), the arterial tree was accessed via the left common carotid artery and balloon injuries made by overdistension in both common iliacs (thirteen animals) and one or two main coronary arteries (eight animals). Half the injured sites were then illuminated with red laser light transmitted via the catheter. Animals were culled 28 days later and tissue harvested for histomorphometry. Results: Compared with control injured vessels, PDT treated, balloon injured coronary arteries had a larger lumen (1.4 vs. 0.8 mm², P<0.002), larger area within the external elastic lamina (2.8 vs. 2.2 mm², P<0.006) and smaller area of neointimal hyperplasia (0.4 vs. 0.7 mm², P=0.06), 28 days after intervention. Less neointimal hyperplasia and the absence of negative remodeling resulted in the lumen of PDT-treated, injured segments being the same as that of adjacent reference segments (1.5 vs. 1.6 mm²). Similar trends, but with smaller differences, were seen in the iliac vessels. Conclusions: Intra-arterial, trans-catheter PDT favourably influences the arterial response to balloon injury in both the coronary and peripheral circulations. This technique offers a promising new approach to restenosis after endovascular procedures. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Restenosis is the major limiting factor of percutaneous transluminal angioplasty (PTA) and other interventional endovascular techniques. Its incidence is dependent on the site and nature of intervention and the methods employed in post-operative assessment, but it probably follows 15–40% of angioplasties [1–3]. As a cause of such a significant clinical and economic burden it is not surprising that many therapies have been directed at its prevention. Stenting is the only intervention which has improved results in the medium term, especially after coronary angioplasty (PTCA), where it has been fundamental in the prevention and treatment of abrupt vessel closure [4], but restenosis due to neointimal hyperplasia (NIH) within stents is an increasing problem [5]. An effective therapy which can be applied at the time of PTA, with or without
stenting, that might prevent subsequent restenosis, is still awaited.

Several studies have looked at ways of reducing vascular smooth muscle cell (VSMC) proliferation leading to NIH after balloon injury in the normal rat carotid artery. Although some agents were effective in this model, none was successful in clinical trials, at least partly because normal arteries in small animals are histologically so distinct from their atheromatous counterparts in man. However, VSMC proliferation is now thought to be only one element of a multifactorial response to arterial injury so research that focuses just on ways of inhibiting this may be inappropriate in trying to prevent restenosis. Post et al. [6] demonstrated that the whole arterial dimension (and not just the lumen) undergoes remodeling following an insult such as PTA and that this contributes more to restenosis than NIH. The situation where restenosis is almost exclusively secondary to VSMC proliferation and matrix deposition is after stenting as this effectively fixes the eventual arterial diameter.

Recently, it has been shown in both animal [7] and clinical studies [8] that intra-coronary ionising irradiation (brachytherapy) decreases neointima formation and maintains larger lumen dimensions after PTA. Pilot clinical studies have shown coronary brachytherapy to be tolerably safe acutely [9], but the medium and long term outcome is unknown. Even though the doses of radiation used for brachytherapy are relatively low, the known long term risks of ionising radiation, including carcinogenesis and chronic, irreversible damage to blood vessels, make it an unattractive option for treating non-malignant disease if there are any alternatives, and it is not a treatment that can be readily repeated.

A new option is photodynamic therapy (PDT). This involves the interaction of non-ionising, non-thermal light (usually low power, red laser light) with a pre-administered photosensitising agent to produce reactive intermediates (mainly singlet oxygen) which are cytotoxic [10]. PDT can deplete the media of smooth muscle cells and reduce neointima formation following experimental injury to carotid, iliac and femoral arteries [11,12]. To date, PDT has not been evaluated in coronary arteries, nor has its effect on total arterial dimensions (rather than just the extent of the neointima) been studied in a large animal model.

The present study was undertaken to assess the effectiveness of PDT with the photosensitising agent 5-aminolaevulinic acid (ALA, Levulan) was provided by DUSA Pharmaceuticals (Valhalla, NY, USA) in powder form and dissolved in water for injection, buffered with 8.4% sodium bicarbonate to pH 4.8 for intravenous injection. Animals were sensitised with a dose of 120 mg/kg given as a bolus into an ear vein 4–6 h prior to the procedure, based on our previous pharmacokinetic studies [12].

2. Methods

2.1. Photosensitiser

5-Aminolaevulinic acid (ALA, Levulan) was provided by DUSA Pharmaceuticals (Valhalla, NY, USA) in powder form and dissolved in water for injection, buffered with 8.4% sodium bicarbonate to pH 4.8 for intravenous injection. Animals were sensitised with a dose of 120 mg/kg given as a bolus into an ear vein 4–6 h prior to the procedure, based on our previous pharmacokinetic studies [12].

2.2. Animals

Juvenile Large White–Landrace crossbred pigs (15–20 kg) were used for all experiments. All animal care was in accordance with the Animal (Scientific Procedures) Act 1986. Pigs were commenced on a daily dose of 300 mg of aspirin the day prior to intervention and this was continued until culling. Each animal was pre-medicated with 0.01–0.02 mg/kg intramuscular medetomidine hydrochloride and an endotracheal tube inserted. Anaesthesia was maintained with 1.5% halothane and a mixture of 0.5 l/min nitrous oxide and 4 l/min oxygen.

2.3. Arterial injury and PDT

On each animal, the left common carotid artery was exposed by a 5 cm cut down incision and a 9 French guage sheath inserted through which all subsequent instrumentation was performed. Prior to coronary intervention, bretyllium (160 mg), glyceryl trinitrate (50 µg) and magnesium sulphate (4 mmol) were given intravenously and glyceryl trinitrate (50 µg) given directly into the coronaries via a guide catheter. With guidance from X-ray screening, over distension balloon injuries (dilatation to approximately 1.5 times the natural arterial diameter) were attempted on both common iliacs and the circumflex and left anterior descending coronary arteries using transparent PTA balloons (4 cm×7 mm Cordis (Roden, Netherlands) for the iliacs and a 2 cm×3 mm Schneider Asuka® (Staines, UK) for the coronaries). Immediately after the balloon distension, with the balloon still in place, half the injured sites were illuminated with 50 J/cm² red light (635 nm) from a copper vapour pumped dye laser (Oxford Lasers, Oxford, UK). This was achieved by passing a thin laser fibre (Rare Earth Medical, West Yarmouth, USA) through the guidewire channel of the balloon catheter device. The 0.2-mm core diameter fibre with a 4-cm diffuser tip used in the iliac arteries was strong enough to be passed through the guide wire channel with the catheter in situ, but in some cases, the 0.1-mm core diameter fibre with a distal 2-cm radial diffuser tip used in the coronaries.
was more conveniently preloaded prior to catheter insertion.

In the coronaries, the balloon was inflated to 8 atmospheres and deflate in a cycle of 45 s inflation and 15 s deflation during illumination to allow for adequate coronary perfusion and avoid prolonged myocardial ischaemia. In the iliac segment, following arterial injury at a pressure of 8 atmospheres, the balloon pressure was reduced to below 4 atmospheres for trans-catheter illumination. During the period of iliac illumination, the laser beam was interrupted for 60 s after 20% of the light dose had been delivered as there is evidence that such fractionation of light delivery potentiates the effect of PDT [13]. Laser power was measured prior to the start of each period of illumination and the duration of illumination adjusted to keep the total light dose constant. The illumination time required to deliver the appropriate light dose varied between 500 and 1500 s, and 200 and 390 s for the iliacs and coronaries, respectively. The remaining half of the injured sites were used as controls and underwent the same protocol, but received only sham illumination. When the light deliveries and sham light deliveries were complete, angiography of all injured vessels was performed to confirm their patency. Control animals with balloon injury and light but no ALA were not included in this study as we had good evidence from our previous pig experiments [12] that light alone does not produce depletion of the medial smooth muscle cells in pig arteries (see Discussion).

Animals were recovered and kept in subdued lighting for 24 h post procedure to avoid any risk of skin photosensitivity to ambient light. Culling took place 28 days later by a lethal injection of intravenous pentobarbitone. The heart was removed and the coronaries pressure perfused with 4% formyl saline at 100 mmHg. The treated segments were identified prior to removal by reference to the angiograms taken immediately after PDT and by histological confirmation that the internal elastic lamina had been ruptured. The segment of uninjured artery immediately proximal to the treated segment was also harvested as a normal reference. The aorta and iliacs were approached by a retroperitoneal dissection. The treated segments extended from the aortic bifurcation to the iliac bifurcation, so were easy to identify. They were isolated between clamps and then similarly pressure perfused with fixative prior to their removal. As the limits of the injured segments in the iliac arteries were at vascular bifurcations, there were no suitable sections to take as references for the dimensions of uninjured arteries. Paraffin sections of all arterial sections were prepared and serial sections stained with either elasin van Gieson or haematoxylin and eosin.

Vessel morphometry was assessed using a light microscope (Nikon Labophot-2, Nikon, Tokyo, Japan) from which the image was transferred to a 486 computer by a colour camera (JVC TK-1281, Tokyo, Japan). Image analysis was performed using a LUCIA-M (Version 3.52a) computer programme (Laboratory Imaging, Nikon UK, Kingston, Surrey, UK). The integrity or rupture of the internal and external elastic laminae (IEL and EEL) of each section were documented and the area of the lumen and the area subtended by the EEL measured. For each injured coronary the vessel dimensions of sections from 4 to 8 blocks of the injured segment were measured for comparisons between treated and sham illuminated controls. The degree of injury was recorded for each section and assessed according to a modification of a method devised by Schwartz [14] (grade 0: endothelial denudation, grade 1: IEL disruption, grade 2: IEL and medial disruption, grade 3: EEL disruption). A section from the segment of coronary artery immediately proximal to the injured segment was analysed as a reference to document the dimensions of the untreated vessel for comparison with the treated segment and to ensure that PDT treated and control vessels were of comparable calibre. In addition to the morphometry, each section was examined to count the number of VSMCs per high power field (HPF). Counts were made in four fields, at 12, 3, 6 and 9 o’clock, on each section.

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

Statistical analysis was by the comparison of means from each group using the unpaired Student’s t-test. Inter- and intra-observer variability was assessed by reanalysis of a representative sample of the histological sections. Area calculations were made by the first author on two separate occasions and by a second independent observer on one occasion. Results were compared between each set of observations 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 [15].

3. Results

Experiments were undertaken on a total of thirteen pigs. Three pigs died before day 28, as described below. In the ten animals killed as planned at 28 days, a total of 20 common iliac and 11 coronary arteries received an over distension balloon injury. For technical reasons, balloon injuries could not be made on the other coronary arteries. Ten iliac and five coronary arteries were treated with red light and the others received sham illumination.

On histological examination, uninjured control segments appeared normal with an intact internal elastic lamina and no neointima. All balloon injured segments showed an injury response and this was greater in the coronary than in the more elastic iliac arteries. There was no significant difference between the mean injury scores for PDT and control arteries. The mean (±standard deviation) coronary
control and PDT artery injury scores were 2.3 (±0.8) and 2.1 (±0.8), respectively, compared with 0.4 (±0.5) and 0.6 (±0.6) for the iliac control and PDT arteries. Light microscopy of sections stained with polyclonal factor VIII showed almost complete endothelial cover at 28 days in all sections examined.

In the coronary artery studies, the area within the EEL and the lumen of the reference segments (adjacent to the treated segments) was no different for arteries that acted as controls or received PDT (area: 2.9±1.1 mm$^2$ for controls and 2.9±0.5 mm$^2$ for PDT treated arteries, lumen: 1.6±0.7 mm$^2$ for controls and 1.4±0.8 mm$^2$ for PDT treated). Thus the control and PDT groups were comparable in terms of injury score and calibre. The mean number of VSMC per high powered field within the media was less in PDT-treated than sham illuminated injured vessel sections (PDT treated 64±36 and sham 86±12), but this difference was not statistically significant. This suggests there had been repopulation of the medial vascular smooth muscle cells that we have previously demonstrated to be depleted in the media 3 days after treatment [12].

An example of a control and PDT treated coronary segment following injury is shown in Fig. 1. Coronary arteries receiving PDT after balloon injury were found to have a significantly larger lumen area (1.4±0.6 mm$^2$) and area within the EEL (2.8±0.7 mm$^2$) than injured controls (0.8±0.5 and 2.2±0.8 mm$^2$, $P=0.002$ and 0.006, respectively), but the area within the EEL of the PDT treated segments was almost exactly the same as that in the uninjured segment of the same vessels (2.9±0.5 mm$^2$). The neointimal area following PDT was 0.4±0.2 mm$^2$ compared with 0.7±0.7 mm$^2$ ($P=0.06$) in controls (Fig. 2a). This 42% reduction in neointimal area failed to reach statistical significance at the 5% level. Examination of the section from the segment that had the smallest lumen within the injury site showed that it had been maintained to the same size as its reference segment in the PDT-treated vessels (1.5 vs. 1.6 mm$^2$).

The figures for the iliac arteries showed similar trends but the differences were not as marked and did not reach statistical significance. The mean number of VSMC per high powered field within the media was less in PDT-treated than sham illuminated injured vessel sections (PDT treated 55±32 and sham 90±28, $P=0.07$). The mean lumen area in treated and control segments was 9.8±3.7 mm$^2$ and 7.9±4.8 mm$^2$ ($P=0.1$) and the mean area within the EEL was 15.2±4.3 mm$^2$ and 12.5±5.4 mm$^2$ in treated and control segments, respectively ($P=0.03$). The neointimal area was also less in treated arteries, 0.7±0.5 mm$^2$ compared with controls, 0.9±0.8 mm$^2$ ($P=0.2$) as demonstrated in Fig. 2b.

The mean difference±S.D. 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.

3.1. Procedural complications

Three animals died before the scheduled day for culling (28 days after the balloon injury). One death was immediate due to an iliac rupture sustained during a balloon injury. The other two occurred at 25 and 27 days and post mortem thrombus prevented pressure perfusion for fixation so these arteries were not analysed in terms of area calculation. Macroscopically, the segments receiving PDT showed evidence of thickening and induration extending 1–2 cm around the treated vessel, but without aneurysm formation or rupture. These changes were not seen around PDT treated segments in animals that survived until the scheduled date for culling. Microscopically, the whole lumen of all three main coronary arteries was filled with fresh thrombus with a thin rim of NIH at the injured site of the treated segment which was no greater than that seen in treated arteries of surviving pigs.

4. Discussion

This is the first report of PDT effects on balloon injured peripheral and coronary arteries in a large animal using light delivered intra-arterially via the guide wire channel of a balloon catheter. It is also the first study to look at the complete morphometry of PDT treated vessels. Earlier reports only described VSMC depletion and reduced neointimal proliferation or percentage stenosis [11,16]. We have shown that 28 days after intervention, PDT given at the time of balloon injury results in an increased lumen area compared with similarly injured sites not receiving PDT. This appears to be due to both a reduction in NIH and less negative remodeling.

Remodeling is now recognised as one of the key factors in the response of arteries to plaque formation and local injury. Although remodeling associated with plaque formation may be positive (increase in the area within the EEL) or negative (decrease in the area within the EEL) [17], the remodeling seen after balloon injury is predominantly negative [18]. The injury produced in pig coronary arteries by Andersen et al. [18] (circumferential in nine of eleven and through to the adventitia in all eleven animals) was more severe than in our animals, but the response was consistent with that seen in our injured arteries that did not receive PDT. In both series, the reduction in lumen 4 weeks after injury was considerably greater than could be accounted for by the area of NIH, confirming the important contribution of negative remodeling. In contrast, there was much less NIH in our PDT treated arteries (0.4 vs. 0.7 mm$^2$, $P=0.06$) and essentially no change in the area within the EEL (mean area within the EEL of 2.8 mm$^2$ in the PDT treated area and 2.9 mm$^2$ in the adjacent uninjured, untreated segment). Compared with injured, control segments, PDT treated segments had a significantly larger lumen diameter (1.4 vs. 0.8 mm$^2$, $P<0.002$) and...
Fig. 1. Photomicrograph (×10 magnification) of (a) injured control coronary artery and (b) injured coronary artery following PDT, both shown 28 days after injury. The ruptured IEL (arrow) is demonstrated in both cases with a larger neointimal response seen in the control, sham illuminated artery.
Fig. 2. Morphometry of (a) coronary and (b) iliac arteries following injury, expressed as mean area in mm² (with standard deviations). The EEL area represents the area within the external elastic lamina.

area within the EEL (2.8 vs. 2.2 mm², P<0.006). PDT reduced the area of NIH, but perhaps more importantly, appears to have dramatically reduced the negative remodeling seen in the control injured arteries, thus maintaining lumen area.

One could argue that removal of VSMCs would cause an artery to become an inert tube without contractile properties and that this could lead to an increase in arterial diameter. This has been demonstrated in normal rat femoral arteries (no arterial injury) when the luminal area 6 months after ALA-PDT was 0.09 mm² compared with 0.01 mm² in untreated controls, a very large difference [19]. In that study, there was complete depletion of smooth muscle cells in the media, even 6 months after treatment.

The changes in the pig were not so dramatic although there is depletion of medial cells 3 days after PDT [12]. We demonstrate here that while medial cell number remains slightly less, that almost complete repopulation of coronary and iliac medial cells occurs in 28 days. However, loss of vessel tone due to early depletion of VSMCs may have balanced the reduction in lumen due to negative remodeling. The lumen size and overall arterial diameter after balloon injury and PDT are similar to those of the entirely untreated adjacent segments of artery. More complete VSMC depletion might lead to positive remodeling, because although the mechanism of remodeling is unknown it may be related to the muscle tone in the vessel wall.

Our over-distension method produced a consistent and equivalent degree of injury in control and treated segments (confirmed using the ‘injury score’), although the degree of injury was much greater in coronary arteries than the more elastic iliac arteries. This is the likely explanation for the milder responses to PDT seen in the iliac arteries compared with those seen in the coronary arteries.

The technique used here has the advantage that the PDT treated segment corresponded closely with the balloon injured segment. Most other arterial PDT studies have employed complete carotid or femoral endothelial denudation, but then only treated part of the denuded segment with PDT. We reported previously that laser light alone could inhibit NIH in balloon injured, rat carotid arteries [11]. However, this only occurred using a light dose that had been documented to cause VSMC depletion in the same arteries without a balloon injury. Further, the light dose used only halved the area of NIH whereas the same light dose after administration of 100 mg/kg ALA completely abolished the NIH response. In our recent studies on pigs [12], we treated uninjured, common iliac arteries with the same light dose as was used in the present study. In the light only controls, there was no VSMC depletion whereas in animals given 100 mg/kg ALA prior to illumination, the number of VSMC per high power field was reduced to as little as 10% of the numbers in each of the control groups (ALA alone or light alone). In view of these results, we felt it justified not to undertake further light only controls in injured pig arteries.
Using the same catheter to perform the injury (or angioplasty) and light delivery ensures accurate illumination of the injured and treated segments and reduces the number of catheter manipulations involved. Further, inflation of a balloon which conforms to the intimal surface of the artery ensures that blood is excluded from the segment to receive light therapy. Blood between the laser fibre and the surface to be treated would reduce the amount of light reaching that surface [20]. Our results indicate that the percutaneous, trans-luminal approach is feasible and effective.

Several photosensitisers are being studied for arterial PDT, but ALA has a number of advantages. Of practical importance for clinical use, the optimum drug–light interval for ALA is a matter of hours and systemic photosensitivity lasts for no more than 1–2 days, compared with much longer periods for other photosensitising agents [21–23]. Crucially, the PDT effect can be localised to the arterial wall as the depth of effect using ALA is limited to 1–2 mm, which makes it most unlikely that significant damage to extra-arterial tissues would occur [24]. This was confirmed in our experiments which showed no evidence of damage to adjacent myocardium or structures within the vicinity of the iliac arteries in the pigs that survived until the scheduled day for culling. Attempts are being made to deliver some photosensitising agents directly to the arterial wall to avoid systemic photosensitivity and increase local drug concentrations, but this is technically more difficult [25,26].

In the two pigs that died prematurely, there was evidence of myocardial scarring in the immediate vicinity of the vessels treated with PDT, not seen around PDT treated segments in animals that survived until the scheduled day for culling. Although the total light dose for all treatment sites was the same (50 J/cm²), these two pigs were treated with a light fluence rate of 210 mW/cm² (following servicing of the laser), compared with 65–130 mW/cm² for all other animals. Experiments in other organs have shown that the threshold for thermal effects is 150–200 mW/cm², so it is likely that there was some thermal damage which could have contributed to the effects seen. Both animals probably died of a cardiac arrhythmia, although we cannot exclude thrombosis of the treated segment. These two animals highlight the danger of delivering light at too high a fluence rate.

Our data would suggest that PDT is comparable to brachytherapy in the prevention of restenosis after angioplasty, but is more attractive in concept as it does not involve ionising irradiation and is much simpler to deliver. As there is no cumulative toxicity, the procedure can be repeated if necessary. The efficacy of PDT with ALA applied immediately after a balloon injury using a catheter-based light delivery system as demonstrated here justifies pilot clinical trials. We have recently reported such a study. Seven patients with eight femoral artery stenoses who had a symptomatic recurrence within 6 months of conventional balloon angioplasty had a repeat balloon angioplasty with adjuvant PDT using ALA. On follow up at 6 months, all were asymptomatic and none met the criteria for restenosis as measured by the peak systolic velocity ratio [27].

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References


