PHOTODYNAMIC THERAPY OF ARTERIES: PRESERVATION OF MECHANICAL INTEGRITY

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ABSTRACT

Photodynamic therapy (PDT) of tumours, as a primary treatment or as an adjunctive intra-operative therapy, may expose major vascular structures to injury. PDT has also been proposed to prevent neointimal hyperplasia following angioplasty of stenotic arteries. This study aimed to determine the effect of PDT on the normal rabbit carotid artery, and to determine whether this injury resulted in weakening of the vessel wall. PDT of the carotid arteries of NZW rabbits, using either disulphonated aluminium phthalocyanine, or 5-aminolaevulinic acid induced protoporphyrin IX, as photosensitisers, was performed using a light dose of 100J/cm2. Histological examination of the carotids treated with both drugs demonstrated full thickness loss of cellularity 3 days following photodynamic therapy. Treated vessels all remained patent and no inflammatory infiltrate was observed. Elastin van Gieson staining showed preservation of inner and medial elastic laminae and medial and adventitial collagen. Further rabbits were similarly treated with PDT to I cm segments of both common carotids and sacrificed at 3, 7, and 21 days. The carotids were exposed and control and treated segments subjected to intraluminal hydrostatic distension until the vessels ruptured. No reduction in the pressure re~guired to rupture the vessels was evident in treated vessels compared with controls. It is concluded that in spite of full thickness cell death, PDT treated arteries are not at risk of thrombotic occlusion or haemorrhage.

Keywords: photodynamic therapy, arteries, mechanical integrity, aminolaevulinic acid, phthalocyanine

2. INTRODUCTION

PDT tumour destruction has resulled in fatal carotid haemorrhage in two cases,lZ2 and in major blood loss from a Colorectal carcinoma3. As direct invasion of large vessels by tumour in these cases is quite likely to have occurred, it is necessary to confirm the safety of PDT when used on tumours adjacent to major arteries. Intra-operative adjunctive PDT may offer the prospect of decreased local recurrence due to the ablation of microscopic residual malignant disease495. Such applications may be of value for example in the treatment of the operative field following radical neck dissection for locoregional metastatic spread from upper aerodigestive tract malignancies. The normal carotid artery and its major branches would potentially be at risk in such procedures. Blood vessels have been shown to be susceptible to photodynamic injury. Star observed capillary haemorrhage in rat ear observation chambers following PDT using haematoporphyrin derivative6 and microvascular injury is accepted to be a major feature of PDT necrosis. In larger vessels, characteristic loss of endothelium and medial smoolh muscle cell death have been demonstrated7~9.

The possibility of weakening of larger vessel walls with loss of mechanical integrity and haemorrhage is a potential catastrophic outcome. The objective of this study was characterise the injury sustained by rabbit carotid arteries exposed to photodynamic injury, and to determine if this injury resulted in weakening of the vessel wall as detected by intraluminal hydrostatic distension to bursting poinL

3. MATERIALS AND METHODS:

New Zealand White rabbits were injected with either 200mg/kg aminolaevulinic acid (ALA) or lmg/kg disulphonated aluminium phthalocyanine (AlS2Pc). They were then anaesthetised, had their necks shaved and incision and dissection performed to expose the common carotid arteries in the paratracheal gutter on both sides. The carotids were gently

dissected and optically isolated from surrounding structures by placing a piece of foil-lined opaque card deep to them. A I cm length of each artery was then exposed, at one hour for the AlS2Pc group and three hours for the ALA group, to 100J/cm2 laser light at 630nm. We have previously demonstrated arterial injury in rat femoral arteries using both AlS2Pc and ALA induced PPIX9. In this study preliminary fluorescence quantification studies demonstrated peak sensitiser presence at one hour following intravenous administration of AlS2Pc and three hours following ALA.

Light was delivered by surface illumination, using a 400 micron fused silica optical fibre with a microlens attachment. Light doses of this order represent typical tumouricidal dosesl ~ll. A power density of 127mW/cm2~ below the threshold for thermal injury as confirmed in the control experiments described below was used, and each treatment lasted 785 seconds, the time required to deliver a total dose of 1001/cm2. The treated segments were marked by the placing of two 5:0 silk sutures in the adjacent fascia of the carotid sheath, care being taken not to damage the adventitia of the vessels. Following PDT the incisions were closed, the animals were recovered, and returned to their cages. No animal suffered any obvious adverse effect from the therapy.

To confirm that photodynamic therapy produced a vascular injury, four rabbits, two from each drug group, were sacrificed at three days, the carotids removed, fixed in buffered formaldehyde, and sectioned transversely for haematoxylin and eosin (HtE) and elastin van Gieson (EVG) staining. Sections were taken from treated segments and also from adjacent segments not exposed to light, to act as "drug + dissection but no light" controls. Two further rabbits had identical light irradiation but without prior photosensitisation to act as light only controls, and were also killed at three days.

A further eighteen rabbits underwent identical treatment, nine with AlS2Pc and nine with ALA. Three rabbits from each drug group were sacrificed on day 3, day 7 and day 21. The neck incisions were re-opened and the carotids re-exposed and the treated segments identified. A 4:0 silk ligature was tied at the midpoint of the treated segment, identified by the sutures placed at the time of treatment and by a slightly blanched appearance of the vessel. A second ligature was loosely placed just inside the marker for the end of the treatment segment. An incision was then made in the untreated artery wall about half a centimetre away from the treated segment, a blunted 18 gauge butterfly needle inserted to lie within the the treated zone, and the segment irrigated gently with normal saline. The loose ligature was then tied so that the needle tip was isolated in the half centimetre of treatment segmenL The fluid filled butterfly needle and tubing was connected to one "leg" of a three way tap. The remaining legs were connected to a pressure transducer capable of measuring pressures up to 10bar (7600mmHg) and to the output of a water filled pressurised vessel. The pressure transducer was connected a +IOV stabilised power supply and to a chart recorder and digital voltmeter. The input to the pressure vessel was connected to a compressed air cylinder through a manual pressure regulator capable of delivering up to 16bar (12000mmHg). When the butterfly needle was appropriately located and fixed in the prepared section of artery, the three way tap was rotated to the position shown and the gas supply opened. The pressure was manually increased until the isolated segment of vessel ruptured, usually after a period of several seconds. Rupture was indicated by a fluid leak and sudden dramatic fall in pressure which was noted and recorded on the flat bed recorder. The voltmeter output was monitored for cross-comparison with the reading from the Ratbed recorder. This procedure was repeated for the other half of the treated segment, and for proximal and distal normal segments of the same artery to act as controls. The whole procedure was repeated on the contralateral treated and non treated segments of carotid giving four treatment values and four controls per rabbit. Mean values for treated and control segments were calculated. Bursting pressure measurements from each treatment group were compared with controls and statistically analysed using Student's t-test.

4. RESULTS

All rabbits tolerated the treatment satisfactorily and showed no ill-effects in the post-operative period. On exposure of the treated and control arteries no evidence of dilatation, aneurysm formation or obvious diminished blood flow could be determined. Macroscopically the treated sections appeared a little paler than adjacent untreatred segments.

The treated arteries sampled at three days all demonstrated evidence of characteristic PDT injury. The same injury was observed in each drug group on HOE staining and comprised loss of endothelium and evidence of complete cell death throughout the media and adventitia. The adjacent segments of artery which had been sensitised and dissected but not exposed to laser light showed normal arterial features with an intact and cellular intima, media and adventitia. EVG staining for elastin and collagen demonstrated no discernible difference between treatment and control arteries. Both showed normal configuration of collagen in the media and adventitia as well as an intact inner elastic lamina and medial elastic laminae.

Control arterial segments (drug plus dissection but no light) were found to burst at a mean of 5200 mm Hg (+/- SD 990 mm Hg.) There was no significant difference in the hydrostatic pressure required to burst treatment or control groups at three days. At 7 and 21 days however, considerably greater pressure was required to burst the treated segments than the controls in both groups. The pressure required to burst all vessels was vastly supra-physiological, being in the order of 50 to 70 times that of arterial blood pressure. Both control and treated arteries tended to rupture in a linear split along the length of the isolated segment.

5. DISCUSSION

The histological effects of PDT using these sensitisers was as expected, with loss of endothelial cells from the intima and a complete loss of cellular structures in the media. Similar findings have been previously documented by ourselves and others7~9, in rat arteries. As well as confirming a similar response in the larger rabbit carotid, it was necessary to confirm that the timing of light exposure was appropriate for this model, and that the injury was circumferential and not just sustained by the surface of the vessel on which the light was directly incidenL All treated segments demonstrated that full circumferential injury was caused by the treatment parameters chosen. Control "drug + dissection only" groups, and "light only" groups demonstrated preservation of a normal cellular intima, media, and adventitia. The fact that bilateral common carotid PDT did not result in thrombotic occlusion is evidenced by the failure of any treated rabbit to show signs of stroke. Furthermore no histological evidence of mural thrombosis was found. The main purpose of this study was to determine and document any weakening of the vessel wall in the early weeks following PDT. This study demonstrates that in spite of full thickness cell death induced by PDT in the vessel wall no weakening or loss of mechanical integrity took place. It is not unreasonable to suppose that, in the presence of such extensive necrosis, an inflammatory response might be observed, and that the release of inflammatory mediators might contribute to weakening of the vessel wall. We have previously suggested that PDT in this situation may provoke a form of programmed cell death similar to apoptosis in which the inflammatory response is absent9. This would help explain the preservation of mechanical integrity of the vessels in this study. Furthermore it is apparent that PDT does not result in the degradation or denaturation of collagen and elastin12 and this is likely to be a contributing factor in functional preservation. Barr et al, in studies in PDT treated rat colon, and Smith et al, in studies in rat trachea have simlarly shown preservation of mural collagen and no reduction in the hydrostatic pressure required to rupture the treated organ13Xl4. A possible contributory factor is that PDT brings about cross-linking of collagen, through singlet oxygen reactions, which increases its resistance to deformation15~l7. The increase in pressure required to burst the vessels at 7 and 21 days might be explained further by the repopulation of the media, and to a lesser extent the intima, by proliferating smooth muscle cells, resulting in a minor degree of neointimal hyperplasia.

The photosensitising agenus used here offer advantages over currently used drugs such as Photofrin. Phthalocyanines absorb light at a longer wavelength than Photofrin, thus allowing greater penetration of tissues, and are less readily activated by sunlight thus diminishing cutaneous photosensitivity18. ALA induced protoporphyrin IX is cleared within approximately 24 hours of administration thus virtually eliminating the prolonged skin photosensitivity associated with Photofrin. Both ALA

and AlS2Pc have potential applications in tumour therapy. It is reassuring that major vessels, once photosensitised and exposed to light will not rupture or thrombose. This has significance for the use of PDT in the treatment of tumours occurring in proximity to major vessels, as may occur in the head and neck, and also in the intraoperative adjunctive

use of PDT to ablate microscopic residual disease. Such applications as the treatment of the neck field immediately following radical neclc dissection, or the illumination of the peritoneal cavity following resection of lower GI tumours, may reduce the incidence of recurrence following surgery.

A further potential application of PDT is in the treatment of vascular stenotic lesions, either on its own, or following angioplasty. Porphyrins have been shown to selectively locate in atherosclerotic plaquesl9~22, and PDT has been proposed to treat such stenoses. Similarly following angioplasty, myointimal proliferation is responsible for re-stenosis in up to 50gs of cases. PDT ablation of medial smooth muscle cells has been shown to prevent early restenosis in experiment models8w23f24. The preservation of mechanical integrity is obviously of vital importance in this situation.

6. CONCLUSION

This study demonstrates that in spite of full thickness vascular mural cell death following PDT using ALA or AIS2Pc, treated vessels do not undergo mechanical weakening of the wall. PDT is thus likely to be a safe treatment modality from the point of view of injury to major vessels in the treatment field.

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