Photodynamic Therapy for the Prevention of Intimal Hyperplasia in Balloon-Injured Rabbit Arteries

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This study was performed to demonstrate accumulation of the photosensitizer hematoporphyrin derivative (HPD) in atherosclerosis and to determine whether intimal hyperplasia, the main cause of restenosis after angioplasty, can be inhibited by photodynamic therapy (PDT). Forty Japanese White rabbits were subjected to balloon endothelial injury in the common iliac artery. Five groups of rabbits, ie, immediately after, or 3, 7, 14 or 28 days after the balloon injury, were injected with HPD. These rabbits were sacrificed 24 h after HPD administration, and HPD fluorescence was investigated in the injured arteries by fluorescence microscopy. Other groups of rabbits were injected with HPD 24 h before PDT, and they were then subjected to intravascular Hg-Xe flash-lamp irradiation immediately after (0D-PDT), or 3 days (3D-PDT), 7 days (7D-PDT), or 14 days (14D-PDT) after the balloon injury. All rabbits were sacrificed 28 days after the balloon injury, and histological sections of PDT-treated arteries were examined by light microscopy. Slight, uniform HPD accumulation was observed in the injured media immediately after the balloon injury, and throughout the entire media and the neointima on day 7. On day 14, HPD accumulation had diminished in the media and increased in the intima, and on day 28 no HPD remained in the media. In the 0D- or 3D-PDT groups, no inhibition of intimal hyperplasia was observed. In contrast, there was significant inhibition of intimal hyperplasia in the 7D- and 14D-PDT groups, and the most effective inhibition was in the 7D-PDT group. This study demonstrated that PDT with HPD inhibits smooth muscle cell growth and decreases the intimal hyperplasia response in rabbits. (Jpn Circ J 1999; 63: 387–393)

Key Words: Angioplasty; Intimal hyperplasia; Photodynamic therapy

Recent studies indicate that restenosis occurs in approximately 30–50% of cases after coronary interventional procedures such as balloon angioplasty, directional atherectomy and excimer laser angioplasty, but despite numerous attempts at prevention no effective means of reducing the incidence of restenosis has been found. The only treatment options for restenosis are repeat interventional therapy or by-pass surgery.

Restenosis is a complex mechanism that includes plaque collapse, vasoconstriction, thrombosis and remodeling, and proliferation of smooth muscle cells (SMCs) is an important component of the fibrocellular intimal hyperplasia process. A partial cause of the restenosis lesions has been identified as an increased mass of vascular SMCs and surrounding extracellular matrix, resulting from markedly accelerated migration, proliferation and protein synthesis by these cells. This appears to be a relatively non-specific response of the artery to many different types of vascular injury and suggests that reducing or inhibiting SMC migration and proliferation would be an effective treatment for an atherosclerotic lesion wound.

Photodynamic therapy (PDT) is an innovative treatment involving a combination of light and photosensitizers to selectively identify and destroy diseased cells. When photosensitizers are administered, they accumulate and are retained to a greater degree in hyperproliferating cells, such as those found in malignant tumor and atherosclerotic lesions, than in normal cells. The photosensitizer remains dormant until it is activated by light of a very specific wavelength. The light that activates the compound is produced by a light source and is often transmitted through specially modified fiber optics. The resulting photochemical reaction destroys diseased cells without affecting surrounding normal tissue.

Because the proliferative activity involved in atherosclerosis and restenosis is similar to that of various cancers, research was carried out to test the feasibility of using photosensitizers to treat cardiovascular disease. Our previous study investigated a novel approach using PDT with hematoporphyrin derivative (HPD), a photosensitizer, to treat intimal hyperplasia. The present study was performed to demonstrate that PDT is preferentially localized and retained by intimal hyperplasia. We also used a light-diffusing catheter designed to irradiate both radially and axially over the length of atherosclerotic lesions to investigate whether HPD-sensitized PDT can selectively inhibit intimal hyperplasia.

Materials and Methods

Animal Preparations

Forty Japanese White rabbits weighing 3–4 kg were anesthetized intravenously with 30 mg/kg pentobarbital sodium. After the right femoral artery was exposed and incised under direct vision, a 4.0 French Fogarty balloon catheter was inserted and advanced in retrograde fashion toward the right common iliac artery. The catheter was then inflated, and the endothelium was stripped from a 10-mm length of the right common iliac artery. This procedure was
repeated 3 times. Thirty rabbits from this group were used for the PDT study and were subjected to the same balloon injury in the left common iliac artery. The remaining 10 rabbits were used for the HPD accumulation study and, therefore, the balloon injury was not carried out in the left common iliac artery of these rabbits. After these operations, the catheter was removed and the right femoral artery was surgically repaired. The rabbits were subsequently maintained on a 0.2% cholesterol diet with 10% peanut oil until they were killed.

**Photosensitization With HPD**

In the group of rabbits used for the HPD accumulation study, HPD (Pharmacy Department, Queen Elizabeth Hospital, Woodville, Australia) at a dose of 5 mg/kg was intravenously injected into the rabbits 24 h before being killed (n=10). In the other group of rabbits used for the PDT study, HPD at the same dose was injected into the rabbits 24 h before PDT (n=30).

**Arterial HPD Accumulation After Balloon Injury**

Five groups of 10 rabbits were sensitized with HPD immediately after (n=2), or 3 days (n=2), 7 days (n=2), 14 days (n=2) or 28 days (n=2) after the balloon injury. Twenty-four hours after the administration of HPD, the rabbits were sacrificed with an overdose of intravenous pentobarbital sodium. Both the injured and uninjured common iliac arteries were resected and removed en bloc. The uninjured left common iliac arteries of each rabbit were used as controls (n=10). These arteries were embedded in OCT medium (Tissue Tek 11 embedding compound, BDH) and immediately frozen in liquid nitrogen. Serial 5- to 10-μm sections were prepared on a cryostat (AO Cryocut) at −28°C. Frozen sections of artery specimen

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*Fig 1. Fluorescence photomicrographs of representative cross sections of the common iliac arteries without balloon injury (A) and with balloon injury (B–F). A, normal vessel; B, immediately after balloon injury; C, 3 days after balloon injury; D, 7 days after balloon injury; E, 14 days after balloon injury; F, 28 days after balloon injury. After HPD administration, normal vessels showed no accumulation in either endothelium or media. Slight HPD accumulation was observed in the media immediately after balloon injury, and focally in the media on day 3. On day 7, accumulation was observed throughout the entire media and the neointima. On day 14, it had diminished in the media and increased in the intima, and none remained in the media on day 28 although a large amount persisted in the neointima.*
were examined by fluorescence microscopy (AH-2, Olympus, Tokyo, Japan) to determine the HPD level within each specimen. Planimetric measurements were made directly from the slide in a blinded manner using an interactive computerized imaging analysis system (Colourmorph, Perceptive Instruments). The ratio of the area of HPD fluorescence in the media to the total area of the media on the slide was calculated and expressed as a percentage (%HPD). Other segments of the injured and uninjured arteries were fixed in formalin, embedded in paraffin, stained with hematoxylin and eosin (HE), and immunostained for cyclin/proliferating cell nuclear antigen (PCNA) (American Biotec, USA) for light microscopic examination. The number of cells that stained positive for PCNA were counted in the intima and in the media. PCNA-positive cells were expressed as a percentage of the total number of cells in the intima and media in each specimen (%PCNA-positive cells).

**Intraluminal Photoradiation Delivery Systems**

Intraluminal photoradiation was performed with a Hg-Xe flash-lamp. The light was carried by a 800-μm-core quartz fiber with the diffusing tip in the 4.0 French catheter. This catheter was designed to provide uniform light distribution both radially and axially over the length of atherosclerotic lesions. The total length of the diffusing tip was 1.0 cm.

**PDT of the Artery After Balloon Injury**

Thirty Japanese White rabbits were sensitized with HPD immediately after, or 3 days, 7 days, or 14 days after the de-endothelialization procedure. These rabbits were intravenously anesthetized with 30 mg/kg pentobarbital sodium 24 h after administration of HPD, and a right femoral artery cutdown was performed. A 4.0 French light-diffusing catheter was then inserted and advanced in a retrograde fashion toward the injured artery segment. PDT was performed using a Hg-Xe flash-lamp (Medical Science, Tokyo) to illuminate the injured segment intraluminally at a total fluence of 27 mJ/cm² for 10 min and with a repetition rate of 2 Hz (1200 flashes). The 30 rabbits were divided into 5 groups: PDT of the right common iliac arteries was performed immediately after (0D-PDT, n=7), or 3 days (3D-PDT, n=7), 7 days (7D-PDT, n=8), or 14 days (14D-PDT, n=8) after the de-endothelialization procedure. Injured left common iliac arteries that were not subjected to PDT were used as controls (n=30). All rabbits were killed with an overdose of intravenous pentobarbital sodium 28
days after the balloon injury, and histological sections of the treated arteries were examined by light microscopy (BH-2, Olympus, Tokyo). Both left and right common iliac arteries subjected to and not subjected to PDT were resected and removed en bloc. These arteries were fixed in formalin, embedded in paraffin and stained with HE for light microscopic examination. Planimetric measurements were made from the light micrographs scanned by a Coolscanner (Nikon, Tokyo), a personal computer, and Photoshop (Adobe) using NIH Image, and the ratio of the thickness of the intima at the thinnest point to the thickness of the media at the same point was calculated (I/M ratio). The ratio of the total area of the intima to the total area of the media in the entire circumference of the vessel on the slide was calculated (IA/MA ratio).

**Statistics**

Results are expressed as means ± SEM. The Mann-Whitney U-test was used to compare differences in ratios between groups. A value of p ≤ 0.05 was considered to be significant.

**Results**

**Fluorescence Analysis**

Histologically, the formation of neointima was observed to be much more apparent on day 7 and day 14 after balloon injury to the vessel than immediately after or on day 3 after the damage. The endothelium damaged by a balloon had regenerated by day 14 after the injury.

Fluorescence microscopy showed a red fluorescence, indicating the presence of HPD in the artery. After HPD administration, normal, uninjured vessels showed almost no accumulation of HPD in either endothelium or media (Fig 1A). Immediately after the balloon injury, slight, uniform accumulation of HPD was observed in the media (Fig 1B), and focal and marked accumulation were seen in the media on day 7 (Fig 1C) and day 14 (Fig 1D) after the balloon injury.

**Table 1** Comparison of I/M and IA/MA Ratios in the 0D-, 3D-, 7D-, 14D-PDT and the Control Groups

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<th>I/M</th>
<th>IA/MA</th>
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<tr>
<td>Control</td>
<td>2.278±0.523</td>
<td>2.517±0.750</td>
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<tr>
<td>0D-PDT</td>
<td>2.130±0.151</td>
<td>2.517±0.359</td>
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<tr>
<td>3D-PDT</td>
<td>1.295±0.790*</td>
<td>2.209±0.552</td>
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<tr>
<td>7D-PDT</td>
<td>0.336±0.469**</td>
<td>1.252±0.530*</td>
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<tr>
<td>14D-PDT</td>
<td>0.727±0.427**</td>
<td>1.509±0.469*</td>
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*p<0.05, **p<0.01.
Vascular PDT to Inhibit Intimal Hyperplasia


the media on day 3 (Fig 1C). On day 7, a large amount of HPD fluorescence was observed throughout the entire media and neointima (Fig 1D). On day 14, HPD fluorescence had diminished in the media but increased in the intima, especially in migrating and proliferating SMCs immediately beneath the endothelium and on the internal elastic lamina (Fig 1E). Almost no accumulation remained in the media on day 28, but a large amount persisted in the neointima. The intensity of the fluorescence in the neointima progressively diminished towards the media (Fig 1F).

Light Microscopic Observation After PCNA Immunohistochemical Staining

The peak number of PCNA-positive cells in the media was observed on day 3, and the number decreased thereafter (Fig 2). By contrast, the number of positive cells in the intima was highest on day 14. On day 7, the number of PCNA-positive cells was almost the same in the neointima as in the media (photomicrographs not shown).

Photodynamic Effects on the Artery After Balloon Injury

No hemorrhage, thrombosis, aneurysm formation, or thermal degradation such as carbonization were seen in any of the treated segments. In the control vessels of rabbits fed on a 0.2% cholesterol diet for 4 weeks after balloon injury and that were untreated by PDT, marked fibrocellular neointimal hyperplasia was seen around the entire circumference of the lumen (Fig 3). Compared with the 7D- and 14D-PDT groups, the vessels in the 0D- and 3D-PDT groups showed less medial cell necrosis; the 7D-PDT group showed the most extensive necrosis in the media. Intimal cell necrosis was found in the 7D- and 14D-PDT groups (Fig 4A–D).

The arteries in the 0D- and 3D-PDT groups showed no reduction in the degree of development of intimal hyperplasia compared with controls. A prominent inhibitory effect on intimal thickness was found over necrotic media (photomicrograph not shown).

Discussion

HPD is a photosensitizer that, after injection, selectively accumulates in certain tissues and renders them susceptible to photodynamic destruction. Proliferating cells preferentially accumulate photosensitizers, and PDT by specific photoradiation causes the death of these cells. The principle of this phenomenon is the cytotoxic reaction of singlet oxygen induced by the energy transfer of excited photosensitizers. Thus, accumulation of a photosensitizer in the tissue is the most important factor associated with the PDT effect.

HPD Accumulation

The mechanism of photosensitizer accumulation, including HPD, has not been sufficiently clarified, and many aspects of it remain unknown. However, it is generally considered that photosensitizers are taken up by proliferating cells, in which DNA synthesis is accelerated, such as cancer cells and migrating SMCs. Accumulation of HPD was confirmed to coincide with PCNA-positive cells in the intima and media of the injured arteries in the present study. However, as shown by the fact that peak accumulation of HPD in the media occurred 1 week after the vascular injury whereas the peak number of PCNA-positive cells in the media was observed on day 3 after the vascular injury, there was no steady correlation between the amount of HPD accumulated and the number of proliferating SMCs (Fig 2). In a similar manner, during the time when hardly any PCNA-positive cells were detected in the arteries immediately after the balloon injury, HPD accumulation, although only slightly, was observed in the media. These histological findings appear to support direct penetration into the blood vessel, based on the increased vascular permeability that occurs in association with endothelial injury or the existence of a passive uptake pattern by activated macrophages, as the mechanism of accumulation of HPD in blood vessels, in addition to incorporation into proliferating SMCs.

Comparison of the degree of HPD accumulation between the various time points after vascular injury showed that at 1 week after the injury, when the number of PCNA-positive cells was almost the same in the neointima as in the media, HPD accumulation in the media was at its highest level (Fig 2). At 2 weeks after the injury, there was more HPD accumulation in the neointima than in the media, and its accumulation in the media was consistent with the small number of PCNA-positive cells remaining there. Hardly any PCNA-positive cells were seen in the media at this time, however they were at their maximal level in the neointima. The hypothesized mechanism of vascular permeability for HPD accumulation, observed in the acute phase after vessel injury, would not be applicable to the situation 2 weeks after injury because of regeneration of the endothelium. The relationship between the amount of HPD accumulation in the intima and in the media and the frequency of the PCNA-positive cells suggested that uptake by proliferating cells appeared to be the principal mechanism of HPD accumulation at 2 weeks after injury.

Other photosensitizers that have been used to prevent intimal hyperplasia, such as 5-aminolevulinic acid (5-ALA) and chloroaluminium sulfonated phthalocyanine (CASPC), have been reported to accumulate more in the media than in the intima or adventitia of blood vessels in the acute phase after balloon injury. The physical properties responsible for the differences in the accumulation patterns of 5-ALA, CASPC and HPD in blood vessels are not known, but the differences in their patterns of accumulation may have a considerable impact on the effect of PDT in the vessels.

PDT Studies

HPD Photofrin, CASPC and 5-ALA have been used as photosensitizers, and the usefulness of PDT by extravascular irradiation has been reported in experimental arteriosclerosis. Reports have also been published on intravascular irradiation with intravascular probes as a new device for catheter intervention, based on the clinical application of PDT treatment of arteriosclerosis. According to these reports, the short-term efficacy of PDT in experimental models was demonstrated by the inhibition of the intimal hyperplasia that occurs soon after balloon injury of blood vessels, and by the reduction in the intimal thickening that is found in arteriosclerotic foci.

However, this study showed that 0D-PDT in the artery was not effective in preventing intimal hyperplasia, whereas PDT using 5-ALA or CASPC caused effective...
inhibition of the hyperplasia by inducing necrosis of all SMCs present in the media. This seems to be attributable to the fact that the concentration of the photosensitizer in the target tissue is an important factor governing the efficacy of PDT, and the tissue concentration of HPD, especially in the media, was inadequate.

In contrast, in the 7D- and 14D-PDT groups, both the I/M ratio and the IA/MA ratio were significantly lower than in the control. Two mechanisms of action may explain these results. One mechanism is that when PDT is performed extravascularly in conjunction with CASPe or 5-ALA there is cell damage in the media, the principal site of action. The other mechanism is that when PDT is performed intraluminally with HPD or Photofrin that have been found to have a greater affinity for the intima than for the media there is cell damage in the intima, which seems to become the principal site of action. Histologically, accumulation of HPD on day 7 and day 14 after the vascular injury was observed in both the intima and the media. Of particular interest is that the accumulation of HPD on day 7 after the vascular injury, besides being observed throughout almost the full thickness of the intima, was found to have reached its maximum level during the entire period in the media as well (Fig 2). According to these findings, in the 7D-PDT group, in addition to suppressing migration of proliferating SMCs into the intima from the media, direct changes occur in the intima, for example tissue sloughing and thinning after cell necrosis, and it appears that intimal thickening may be prevented as a result of both. In contrast to this, accumulation of HPD on day 14 after the vascular injury had decreased to the point of being scattered in the media. Intimal thickening had increased, and HPD accumulation was observed extending throughout almost the entire thickness of the intima. It was speculated that this increase in intimal thickness diminished light penetration into the media, and that this, coupled with the decrease in accumulation in the media, might be linked to the decreased tissue damage in the media. Furthermore, there was clearly greater HPD accumulation in the media on day 3 after vascular injury than on day 14. On the basis of this finding, even when the absence of neointima is taken into consideration, PDT conditions, such as the level of HPD accumulation in the media and depth of penetration of the excitation light into the media, were clearly more advantageous in the 3D-PDT than in the 14D-PDT group. However, more intense neointimal thickening was observed in the 3D-PDT than in the 14D-PDT group. The above results suggest that the principal effect of 14D-PDT in the vessel is on the intima itself and that this may be based on direct regression of the arteriosclerosis, such as by sloughing and thinning of intimal tissue, than on suppression of SMC migration. Based on the above, PDT to prevent neointimal formation would seem to be optimal when performed on day 7 after vascular injury, when efficacy can be expected in both the media and the intima.

Because the power output of the Hg-Xe flash-lamp used as the excitation source in this study is less than that of laser light and it irradiated the entire circumference of the vessel wall through the blood, it may not have been an adequate light source to excite the photosensitizing agent. If a light source with superior power output, depth of tissue penetration, and absorption by the photosensitizing agent were to be used, PDT immediately after and on day 3 after the vascular injury, when there is little HPD accumulation, may be capable of more intense inhibition of intimal thickening.

**Limitations**

The fact that thickened intima is already present in the coronary artery in humans must be taken into consideration when performing PDT to treat arteriosclerotic foci with HPD or Photofrin. According to the results of this study, it is suspected that HPD or Photofrin may not accumulate in the media of human coronary arteries unless there is considerable vessel wall injury, such as arterial dissection. Moreover, when irradiated with light from inside the vessel, presumably the excitation light is attenuated before it reaches the media by the presence of the intima, by the interposition of blood, by the diffusion of light to the entire circumference of the vessel, etc. If it were a matter of preventing intimal hyperplasia by using PDT to target SMCs in the media, PDT with CASPe or 5-ALA may be more effective. However, it will be necessary to make improvements and modifications to the wavelength of the light source and method of irradiation such that the excitation light will have adequate power and be capable of reaching the media.

**Conclusion**

HPD is a useful indicator of the SMCs whose DNA synthesis is accelerated in the tissue repair of injured vessels. PDT inhibits neointimal thickening after balloon injury. A regimen of 7D-PDT is the most effective treatment. PDT by intraluminal irradiation may offer a new approach to prevent restenosis after balloon angioplasty in humans.

**References**

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