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PRECLINICAL INTERVENTIONAL RESEARCH

PhotoPoint Photodynamic Therapy Promotes Stabilization of Atherosclerotic Plaques and Inhibits Plaque Progression

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Objectives The purpose of this study was to determine how photodynamic therapy (PDT) promotes stabilization and reduction of regional atherosclerosis.

Background Photodynamic therapy, a combination of photosensitizer and targeted light to promote cell apoptosis, has been shown to reduce atherosclerotic plaque inflammation.

Methods Forty New Zealand White rabbits were fed with cholesterol. The iliac arteries were balloon denuded and randomized to receive either PhotoPoint PDT treatment (photosensitizer and light) (Miravant Medical Technologies, Santa Barbara, California), photosensitizer (MV0611) alone, or light alone and were then compared at 7 and 28 days. Arteries were examined for evidence of plaque volume, cell number, macrophage and smooth muscle cell (SMC) content, and plaque cell proliferation.

Results Compared with contralateral iliac artery controls at 7 days, plaque progression was reduced by approximately 35% ($p < 0.01$); plaque progression was further reduced to approximately 53% ($p < 0.01$) by 28 days, leading to an increase in lumen patency ($p < 0.05$). At 7 days after PDT, percent plaque area occupied by macrophages decreased by approximately 98% ($p < 0.001$) and SMCs by approximately 72% ($p < 0.05$). At 28 days after PDT, removal of macrophages was sustained (approximately 92% decrease, $p < 0.001$) and plaques were repopulated with non-proliferating SMCs (approximately 220% increase, $p < 0.001$). There was no evidence of negative or expansive arterial remodeling, thrombosis, or aneurysm formation.

Conclusions Photodynamic therapy simultaneously reduces plaque inflammation and promotes repopulation of plaques with a SMC-rich stable plaque cell phenotype while reducing disease progression. These early healing responses suggest that PDT is a promising therapy for the treatment of acute coronary syndromes. (J Am Coll Cardiol 2008; 52:1024–32) © 2008 by the American College of Cardiology Foundation

Acute coronary syndrome arising from plaque rupture is one of the leading causes of cardiovascular-related mortality (1–4). In sudden coronary death and acute myocardial infarction (AMI), lesions resembling plaque rupture (thin-cap fibroatheroma, vulnerable plaque) have been reported in other arterial sites remote from the culprit plaque (5,6). Chronic inflammation caused by macrophage infiltration, foam cell formation, size of necrotic core, fibrous cap, and degradation of collagen are intrinsic to the natural history of symptomatic and asymptomatic atherosclerotic disease. These vulnerable, unstable plaques, however, are considered "rupture-prone" because of the higher (10% to 15%) incidence of repeat AMI in patients previously presenting with AMI (4,7). This evidence suggests that the genesis and etiology of plaque disease are complex and diverse. Human

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plaques vary morphologically in length, volume, location, and are composed of heterogeneous cellular components. However, plaque inflammation, degree of stenosis (1,7) and smooth muscle cell (SMC) composition (8,9) independently or collectively play a critical role in promoting plaque instability. Therefore, a versatile therapeutic paradigm, which can be localized either to a region of the

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plaque or to a length of artery with significant plaque burden, to simultaneously reduce plaque inflammation and promote plaque healing and stabilization is required.

Photodynamic therapy (PDT) is being used as a treatment modality for proliferative neoplasms (10–12) and age-related macular degeneration (13). Recently endovascular PDT has emerged as a promising therapy for the treatment of restenosis after injury (14) and in short-term studies has shown efficacy in limiting atherosclerotic plaque inflammation in animal models (15–18).

PhotoPoint PDT is a combination endovascular therapy designed to systemically deliver a photosensitizer drug (gallium chloride mesoporphyrin dimethyl ester that belongs to the metallotetrapyrrollic group [MV0611]; Miravant Medical Technologies, Santa Barbara, California) (18) that is capable of being taken up by macrophages and other plaque inflammatory cells. A light delivery catheter connected to a laser light source is then guided to areas of plaque formation to provide localized activation of the photosensitizer, which in turn creates the formation of singlet oxygen that induces localized cell apoptosis. In an uninjured rat carotid artery model we characterized the photosensitizer (MV0611), optimal dose of the MV0611 (3 mg/kg), the wave length (542 nm) of the light for an adequate activation of the MV0611, and the dose of the light to detect optimal denucleation of the medial cells (17). We also tested the efficacy of MV0611 in stented and nonstented native porcine coronary arteries (18). We then tested the hypothesis that MV0611-PDT could significantly eliminate atherogenic cells in New Zealand White (NZW) rabbits with established lesions. The safe removal of cells that are essential to the development of atherosclerosis could provide a valuable treatment strategy for the unstable plaque.

Methods

Animal care and procedures were carried out in accordance with the guide for the care and use of laboratory animals. Forty adult male NZW rabbits weighing 3.5 to 4.0 kg (Robinson Services, Inc., Clemmons, North Carolina) were used for the study. Rabbits were fed an atherogenic diet (1% cholesterol; 6% peanut oil; Bio-Serve, Frenchtown, New Jersey) for 7 days followed by iliac artery endothelial denudation. Rabbits were maintained on an atherogenic diet for an additional 4 weeks after denudation to allow plaque development.

Surgical procedure. Rabbits were sedated with an intramuscular injection cocktail containing ketamine, xylazine, and acepromazine (7:1:0.5). Anesthesia was sustained throughout treatment procedures with 1% to 3% isoflurane delivered in oxygen (2.0 l/min). Animals were monitored throughout each procedure. A balloon dilation catheter (Swan-Ganz, Arrow International, Reading, Pennsylvania) was guided through the descending aorta into the right and left iliac arteries with guidewire assistance (Cordis, Miami Lakes, Florida) after arteriotomy of the left common carotid artery. The balloon catheter was placed with fluoroscopic guidance (diatrizoate meglumine sodium contrast. Mallinckrodt, Hazelwood, Missouri), inflated to 4 atmospheres (ATM) in right and left distal iliac arteries, and pulled proximally by 2 cm to induce 2-cm lesions. The catheter was then repositioned at the distal starting point, reinflated to 6 ATM, and pulled back a second time for effective removal of the endothelium and disruption of the subintima.

Abbreviations and Acronyms

AMI = acute myocardial infarction
ATM = atmospheres
EEL = external elastic lamina
IEL = internal elastic lamina
MV0611 = Miravant photosensitizer compound
NZW = New Zealand White
PDT = photodynamic therapy
SMC = smooth muscle cell

Preliminary drug and light dose optimization pilot study. Preliminary studies to characterize the nature of the photosensitizer (MV0611) and to find the optimal dose of the effective photosensitizer (3 mg/kg), the wave length (542 nm) of the light for an adequate activation of photosensitizer, and the dose of the light to detect optimal reduction of the plaques were described in earlier studies (17,18). However, these studies were conducted in the rat and the porcine models. To confirm that the drug uptake pattern in the atherosclerotic rabbit model is similar to that found in the pigs and rats (17,18), a preliminary pilot study was conducted. The photosensitizer MV0611 (3 mg/kg intravenous [IV] bolus) was administered to 6 cholesterol-fed rabbits with balloon-denuded iliac arteries who were then incubated for 4, 8, and 24 h (n = 2/group). After drug incubation, 1 rabbit from each group was killed; an extra rabbit (cholesterol-fed and balloon-denuded) without drug infusion served as an untreated control. The iliac arteries were snap-frozen in liquid nitrogen and cryosectioned for the fluorescent detection of MV0611 in plaques. After incubation, iliac arteries of the 3 remaining rabbits were light-treated (542 nm green light, MV0611-PDT), harvested at 3 days after treatment, embedded in paraffin, sectioned, and examined for evidence of cell depletion. Efficacy of treatment was established as plaque cell apoptosis (terminal deoxynucletidyl transferase-mediated dUTP nick end labeling [TUNEL]) and macrophage cell depletion (RAM-11) throughout continuous plaque lengths of treated arteries. Miravant photosensitizer compound was detected in cryosections with a microscope equipped with epifluorescence (Nikon, Melville, New York). Peak fluorescence was observed in macrophages between 8 and 24 h after injection and correlated with maximum cell depletion.

MV0611-PDT procedure. In all subsequent rabbits (n = 24), MV0611 (3 mg/kg IV bolus) was administered via the ear vein 8 h before light administration. Under anesthesia, a Miravant light diffusing catheter (3 cm) was advanced via the right carotid artery into iliac arteries with flexible

guidewire assistance. Angiography was used to place the catheter entirely over the 2-cm plaques. To cover the entire plaque area and also to avoid geographic miss, a 3-cm-long light source was chosen. The light diffuser, connected to a diode laser, delivered 18 J/cm^2 of light over a period of 90 s to activate MV0611 localized within the vessel wall. Contralateral iliac arteries served as controls, whereby the light catheter was placed in the arterial wall but not activated. Light alone in the absence of MV0611 was administered to a separate cohort of 6 rabbits (n = 12 arteries). The iliac artery was briefly occluded (90 s), before light activation or sham treatment, by low pressure balloon inflation to help remove blood and to provide a uniform treatment field. After catheter removal, an angiogram was obtained to confirm lumen patency. After surgery, rabbits were maintained under filtered light for 24 h. After PDT treatment, rabbits were maintained on a diet containing 0.05% cholesterol until scheduled follow-up of the first group at 7 days (n = 12 arteries MV0611 alone; n = 12 arteries MV0611 and light; and n = 6 arteries light alone) and the second group at 28 days (n = 12 arteries MV0611 alone; n = 12 arteries MV0611 and light; and n = 6 arteries light alone). This dietary strategy maintains circulating levels of cholesterol to sustain the inflammatory stimulus required for plaque progression.

Plasma cholesterol. Plasma samples were collected from whole blood at baseline (before atherogenic diet), at endothelial denudation, at PDT treatment time, and at death for the measurement of plasma cholesterol by colorimetric end point analysis (Antech, Lake Success, New York).

Tissue harvest. Rabbits were euthanized at either 7 or 28 days after PDT after anesthesia with an overdose of potassium chloride IV via the ear vein. The arterial tree was perfused and fixed at 100 mm Hg, and samples comprising the aortic bifurcation and iliac arteries were stored in 10% neutral buffered formalin for 24 h, then processed and embedded in paraffin. Arteries were measured and cut just distal to the internal iliac artery at the bifurcation at 3 intervals with 3 to 4 segments at each interval and labeled as proximal, mid-section, and distal.

Histopathology and immunohistochemistry. Serial transverse arterial sections (5 μm) were stained with hematoxylin and eosin to establish plaque nuclei content. Demarcations of the internal elastic membrane (IEL), external elastic membrane (EEL), and lumen were determined with Movat's stain. Macrophages were detected after antigen retrieval (0.01% protease, 20 min) with a monoclonal antibody to RAM-11 (Dako, Carpinteria, California) diluted 1:300 and 1:200 in 1% bovine serum albumin. Smooth muscle cells were identified with a polyclonal antibody directed against α-actin (1:1,000, Sigma-Aldrich, St. Louis, Missouri). The cell proliferation marker Ki67 (1:250, Santa Cruz Biotechnology, Santa Cruz, California) was used to identify proliferating cells. The von Willebrand factor VIII (1:20, Sigma) was used to detect endothelium. All antigens were detected with standard peroxidase anti-peroxidase and/or immunoalkaline phosphatase techniques and used either diaminobenzidine or AEC (Dako) substrate chromogens.

TUNEL. For the preliminary optimization study, plaque cell apoptosis was observed in arteries by the TUNEL technique (TACS TdT Kit TA4627, R&D Systems, Minneapolis, Minnesota). Sections of rabbit spleen, stained in parallel, served as external positive controls. Internal controls comprised endonuclease pre-treatment of non–PDT-treated iliac arterial sections (data not shown).

Histomorphometry. A skilled computer operator blinded to the study performed all morphometric measurements. Measurements of plaque area, IEL, EEL, and plaque cell number/unit area were obtained in 8 consecutive transverse sections of proximal, midsection, and distal portions from each artery (n = 12 arteries; 96 total segments/group), with a computerized image analysis system (IP-Lab Spectrum image processing software, Signal Analytics Corporation, Vienna, Virginia). Cell counts were obtained automatically with a threshold value and binary processing of positively stained nuclei. Color digital images of a total of 32 sections/artery were assessed separately for quantification of areas occupied by RAM-11 (macrophages) and α-actin (Bioquant, Nashville, Tennessee).

Statistical analysis. Data are presented as means ± SD. Plasma cholesterol levels were analyzed by repeated measure 1-way analysis of variance with Fisher protected least significant difference. Because there were no statistical differences in any of the parameters measured between light-only and drug-only controls, comparisons were made only between drug-only control and PDT treatment groups. These data were analyzed by the unpaired Student *t* test. Significance was established by a value of $p \leq 0.05$.

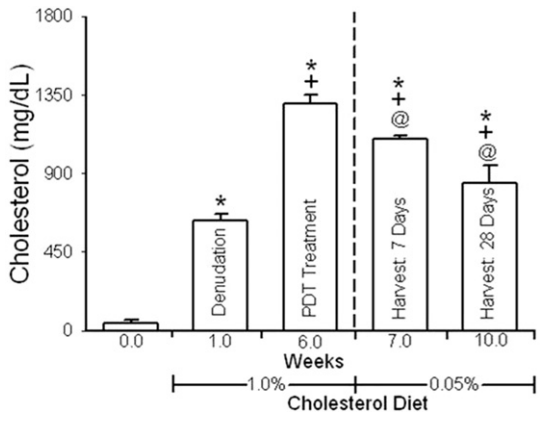
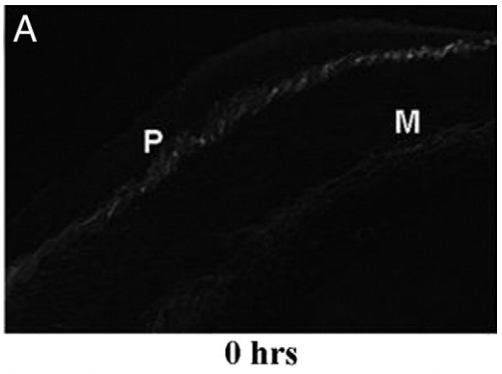
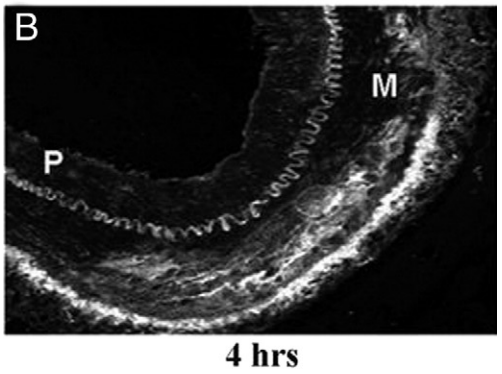
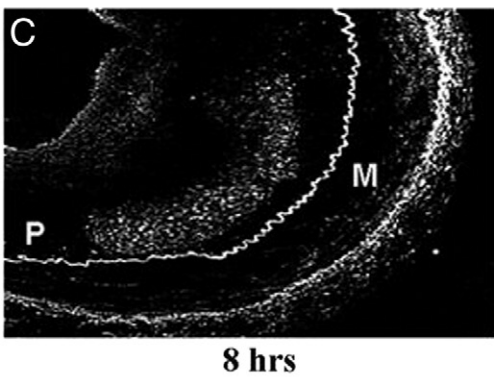


Figure 1 Plasma Cholesterol Levels

Plasma cholesterol levels were measured in the plasma collected from the rabbits before starting on the diet supplemented with cholesterol (0 week), at the time of denudation (1 week), at the time of photodynamic therapy (PDT) treatment (6 weeks), and 7 or 28 days after PDT treatment (7 or 10 weeks). Note that levels remain significantly elevated, despite a lowered maintenance diet of 0.05% dietary cholesterol at 28 days. *$p < 0.001$ compared with 0 weeks; +$p < 0.001$ compared with 1 week; @$p < 0.01$ compared with 6 weeks.







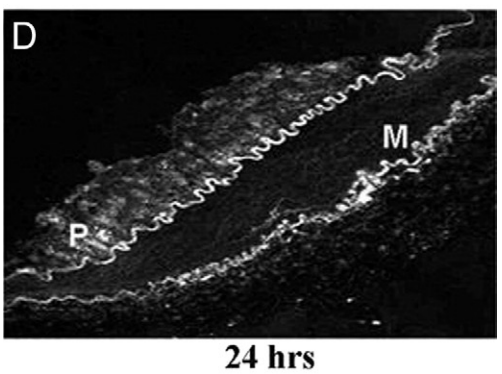


Figure 2 Tissue Localization of MV0611 Photosensitizer

Fluorescent localization of Miravant photosensitizer compound after incubation at 0 **(A)**, 4 **(B)**, 8 **(C)**, and 24 h **(D)**. Note at 4 h there is evidence of photosensitizer in media (M) and plaque (P). At 8 and 24 h after injection, photosensitizer is present within plaque regions containing macrophages.

Results

Plasma cholesterol. Baseline rabbit cholesterol values before receiving atherogenic diet were 44.5 ± 19 mg/dl, increasing after 1 week to 629 ± 38 mg/dl at endothelial denudation and after 5 weeks to 1,299 ± 56 mg/dl at PDT treatment time. At 7 days after PDT, the cholesterol values fell to 1,100 ± 21 mg/dl, then decreased further to 843 ± 101 mg/dl at 28 days after PDT and remained significantly elevated on the 0.05% maintenance cholesterol diet (Fig. 1).

PDT tolerance. Three rabbits died during induction of anesthesia. At autopsy, there was no evidence to suggest vessel toxicity that could relate the death to the photosensitizer. Electrocardiograms in all remaining rabbits were normal. After MV0611 administration, rabbits exhibited no physical or behavioral abnormalities, and histological analysis of arteries showed no evidence of thrombosis, inflammation, aneurysm formation, or adventitial fibrosis.

PDT optimization pilot study. Progression of MV0611 from adventitia to plaque was dependent on the duration of

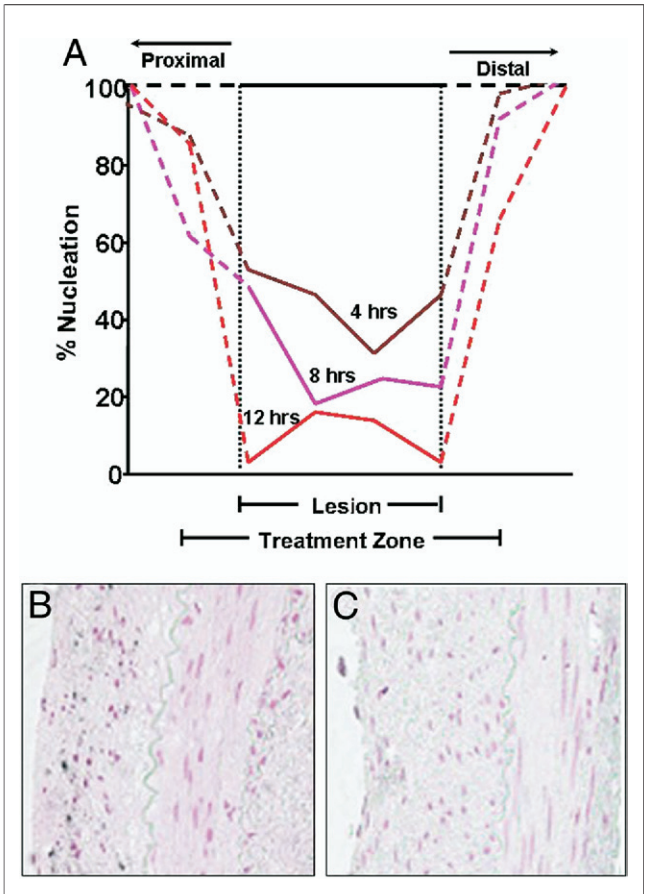


Figure 3 Effect of PDT on Cell Depletion

(A) The effects of Miravant photosensitizer compound incubation time on cell depletion throughout entire regional plaques. Note that maximum cell depletion is observed at 8 and 24 h after injection. Also note, in rabbits killed at 3 days after photodynamic therapy (PDT), evidence of significant plaque cell apoptosis by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling **(black)** staining **(B)** compared with contralateral untreated control **(C)**.

incubation. Maximum detection of in-plaque macrophages was observed between 8 and 24 h after injection. (Fig. 2) The temporal and spatial distribution of MV0611 suggests photosensitizer delivery via the adventitial vaso vasora and lumen. After treatment with PDT, plaque cell loss increased as a function of escalating drug incubation time throughout entire 2-cm lengths of concentric and eccentric plaques (Fig. 3). Maximum plaque cell depletion throughout entire lengths of plaque was observed with 24-h drug incubation (Fig. 3A). Plaque cell depletion with 8-h drug incubation varied from 55% to 72% (few proximal sections at the lower end and most of the distal sections at the higher end), and plaque cell depletion with 24-h drug incubation varied from 74% to 100% (both maximal and distal sections at the higher end and the middle sections at the lower end). Because variation between these 2 time points is not statistically significant and 8 h of incubation would be more clinically friendly, this time point for drug incubation was selected for the rest of the studies.

Effect of PDT on plaque area. In 7- and 28-day controls, 2-cm lengths of iliac artery contained single or multiple eccentric and concentric luminal plaques. Despite lowering the cholesterol diet to 0.05%, there was continued plaque growth in control arteries with some changes in cellular composition (0.05%). In any of parameters determined, the values between light-only and drug-only controls were not statistically different (data not presented). Contralateral control plaques that received no light increased from 0.48 ± 0.13 mm^2 at 7 days to 0.79 ± 0.03 mm^2 at 28 days and occupied up to 45% of the lumen area. Compared with drug-only controls, plaque area in PDT animals was significantly smaller both at 7 days (0.31 ± 0.08 mm^2 in PDT vs. 0.48 ± 0.13 mm^2 in MV0611 control, $p < 0.01$) and 28 days (0.37 ± 0.05 mm^2 in PDT vs. 0.79 ± 0.03 mm^2 in MV0611 control, $p < 0.01$). At 28 days after treatment with PDT, plaque percent stenosis was significantly less than that in 28-day controls (34.2 ± 2.8% PDT vs. 45 ± 2.1% MV0611 control and 42 ± 2.9% light control, $p < 0.01$ for both) (Fig. 4A). In PDT animals with a decrease in plaque area and plaque percent stenosis, lumen area increased (0.77 ± 0.08 mm^2 in 7-day PDT treated vs. 1.02 ± 0.03 mm^2 in 28-day PDT treated, $p < 0.05$) (Fig. 4C). There was no significant effect of PDT on the EEL at either 7 or 28 days and, therefore, no evidence of negative or expansive arterial remodeling (Fig. 4D).

Effects of PDT on plaque histology and nuclei number. In control arteries harvested at 7 and 28 days, treatment with MV0611 alone or light alone had no effect on plaque nuclei number (Fig. 5A). After PDT treatment at 7 days, there was a transient decrease in plaque nuclei number/mm^2 of plaque compared with controls (756 ± 320 PDT vs. 3,011 ± 712 MV0611 controls and 2,945 ± 650 light controls, $p < 0.001$ for both) (Figs. 5A, 5B, and 5D). By 28 days after PDT, plaque nuclei number was not different from controls and cell counts in medial segments outside of the 3-cm light treatment zone showed no evidence of injury or cell proliferation due to catheter placement (Figs. 5A and 5C).

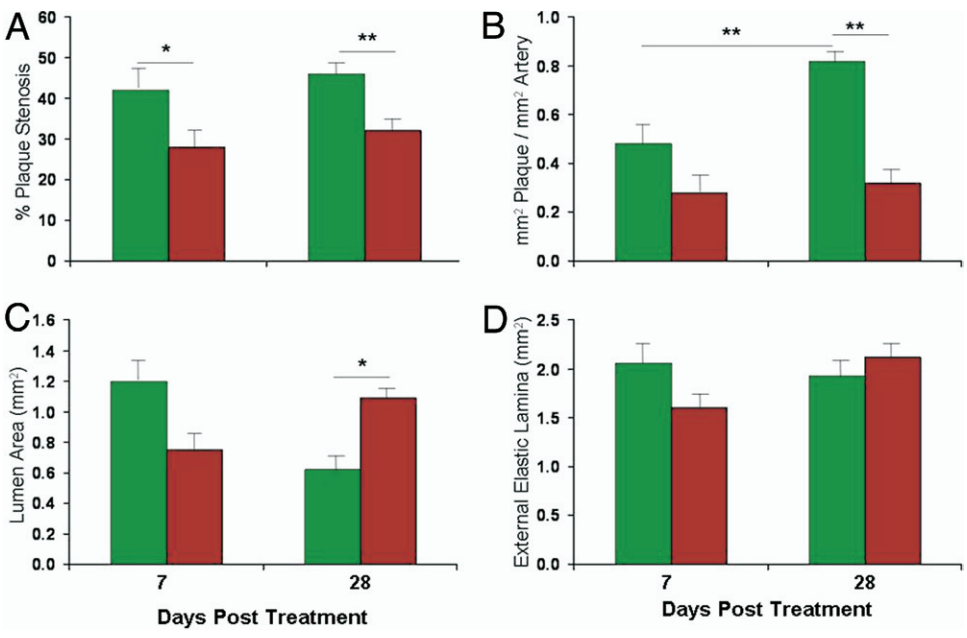


Figure 4 Effect of PDT on Vessel Parameters

Green bars are drug-only controls, whereas **brown bars** represent drug and light combination. **Graphs A to D** show the effects of photodynamic therapy (PDT) on plaque stenosis, plaque area, lumen area, and external elastic membrane area (n = 12 arteries/group). Note that by 28 days after treatment, PDT significantly reduced plaque percent stenosis **(A)**, reduced plaque area **(B)**, and increased lumen area **(C)** with no evidence of negative or expansive arterial remodeling **(D)**. *$p < 0.05$; **$p < 0.01$.

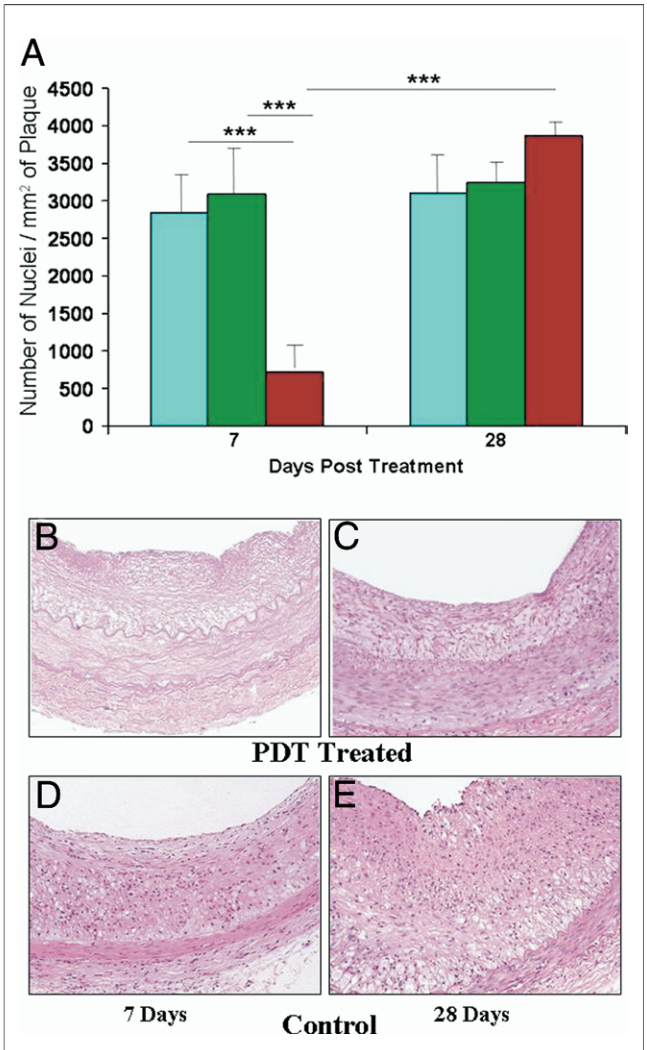


Figure 5 **Changes in Plaque Nuclei Number/mm² of Plaque in Response to Light, MV0611, and PDT**

Blue bars represent light-only controls, **green bars** are drug-only controls, and **brown bars** represent drug and light combination. **Graph A** shows initial (7 days) transient reduction in all atherogenic cells/mm² of plaque followed by plaque cell repopulation by 28 days (n = 12 arteries/group). Photomicrographs show hematoxylin and eosin staining of iliac arteries at 7 and 28 days after photodynamic therapy (PDT) **(B and C)** versus controls **(D and E)**. Note the loss of "foam" cells at 28 days in **panel C** compared with controls. Magnification ×200. ***p < 0.001.

Plaque macrophage and SMC content. Because there is no difference in the plaque nuclei number between light-only and drug-only controls, plaque macrophage and plaque SMC content was determined in the drug-only controls. After PDT treatment at 7 days, the percentage plaque area occupied by macrophages was significantly decreased compared with controls (0.23 ± 0.01 PDT treated vs. 14 ± 2.2 controls, p < 0.001) (Fig. 6A). The decrease in macrophages was sustained at 28 days after PDT (0.74 ± 0.02 PDT treated vs. 9.1 ± 2.4 controls, p < 0.001) (Figs. 6A, 7A, and 7F). At 7 days after PDT there is also a decrease in α-actin positive cells (6.1 ± 1.8 PDT treated vs. 22 ± 3.6 controls, p < 0.01) (Fig. 6B); however, at 28 days after PDT there is a significant increase in α-actin positive cells (42 ± 7 PDT treated vs. 13 ± 3.4 controls, p < 0.001) (Figs. 6B, 7B, and 7G).

Plaque healing and repair. At 28 days after PDT, plaques comprised densely packed quiescent SMCs (Figs. 7B and 7G). The Ki67 proliferation index (percent positive cells of total plaque cell number) of repopulated PDT treated plaques was less than controls (1.22 ± 0.42 PDT treated vs. 2.58 ± 1.4 controls, p < 0.01) (Figs. 7C and 7H). Staining of random arterial sections with Factor VIII revealed the presence of intact endothelium in both PDT-treated and control arteries at 28 days (Figs. 6D and 6J).

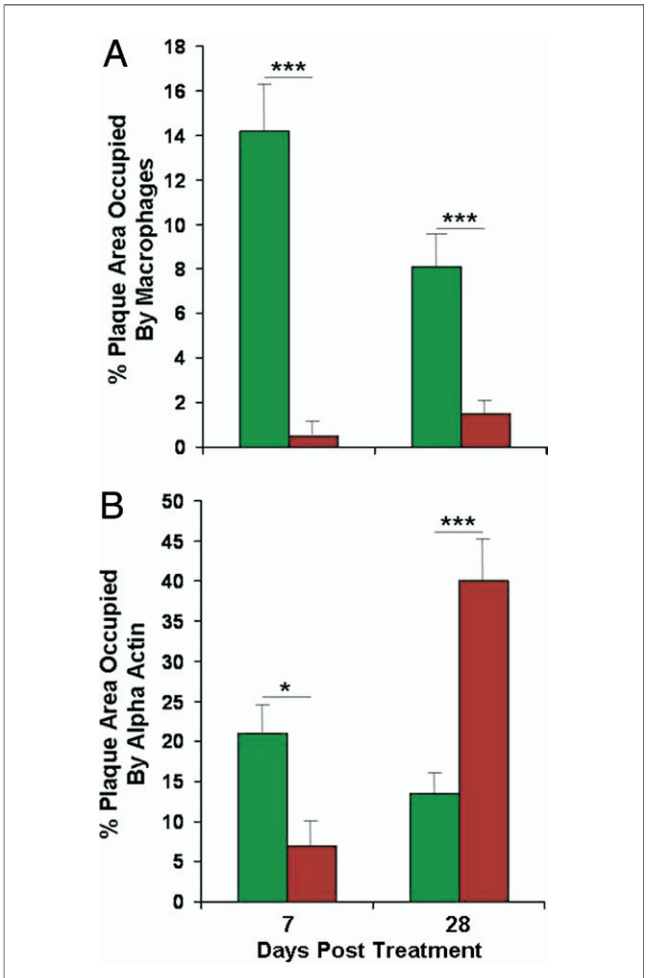


Figure 6 **The Effects of PDT on Plaque Cell Composition**

Green bars are drug-only controls, whereas **brown bars** represent drug and light combination. **Panel A** shows that the area of plaque (mm²) occupied by macrophages is reduced by photodynamic therapy (PDT) at 7 days after treatment and that macrophage removal is sustained at 28 days after PDT regardless of elevated plasma cholesterol, an acute inflammatory stimulus (n = 12 arteries/group). **Panel B** shows transient loss of plaque smooth muscle cells/mm² of plaque at 7 days after PDT, followed by an increase in α-actin area by 28 days, indicative of plaque repopulation with a stable plaque cell phenotype (n = 12 arteries/group). *p < 0.05; ***p < 0.001.

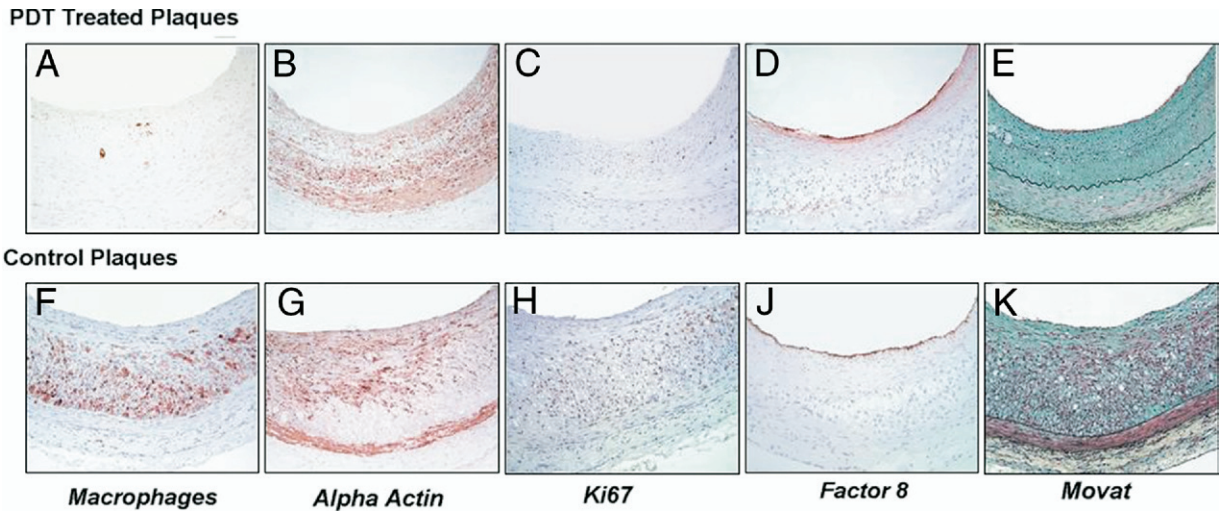


Figure 7 **Serial Sections Showing a Rabbit Atherosclerotic Plaque at 28 Days After PDT Treatment**

Photodynamic therapy (PDT) simultaneously reduced macrophages **(A and F)** and replaced existing plaque matrix with quiescent smooth muscle cells **(B and G)** in the non-G2 or S phases **(C and H)** as indicated by the Ki67 cell proliferation marker. After endothelial denudation of all rabbit arteries, staining with Factor VIII revealed the presence of endothelium in PDT-treated arteries and controls **(D and J)** indicative of arterial repair by 28 days. **Panels E and K** show the presence of the internal elastic membrane and external elastic membrane with Movat's stain. Note that plaque area is significantly less than controls at 28 days after PDT. Magnification ×200.

Discussion

Our findings in a cholesterol-fed balloon-denuded rabbit iliac artery model suggest that PhotoPoint PDT attenuates plaque progression with simultaneous plaque stabilization, vessel healing, and repair. At 7 days after treatment, PDT caused a decrease in plaque macrophage cell content throughout the entire 2-cm balloon-injured and PDT-treated vessel segment. This effect was sustained at 28 days after treatment. By 28 days after PDT, plaque matrix was almost entirely repopulated by α-actin positive SMCs, predictive of plaque stabilization and healing (19,20). Cell proliferation analysis by Ki67 showed that only 1% of the SMCs were in G2 or S phases of the cell cycle, suggesting that at 28 days after PDT, the majority of plaque cells are nonproliferating with limited potential for plaque growth or restenosis. Moreover, the endothelium, which was previously denuded, appeared intact at 28 days after PDT. Collectively, the cellular changes induced by MV0611-PDT led to significantly reduced neointimal growth with complete vascular healing.

A pivotal component of PDT in promoting plaque stabilization is sustained macrophage removal. Cytokines released by macrophages promote further atherogenesis (21,22) and continuous macrophage infiltration, which over time results in the accumulation of lipoproteins and aggregation of free cholesterol that contribute to necrotic lipid core formation (23) leading to plaque instability (24). Moreover, release of macrophage metalloproteinases and other proteolytic enzymes is considered, in part, to weaken the fibrous cap and promote plaque rupture (25,26). The finding that macrophage reinfiltration was prevented by MV0611-PDT treatment at 28 days is surprising, given that plasma cholesterol levels, a potent stimulus for macrophage activation, remained elevated until the time of sacrifice. These data suggest that factors other than cholesterol contribute to plaque macrophage infiltration and that stabilizing effects of PDT on plaque matrix might selectively inhibit macrophage migration. In vitro collagen gel studies, for the purpose of understanding PDT mechanisms of cell migration in restenosis, have shown that the effects of PDT at the molecular level are complex. On the one hand, PDT might cross-link collagen (27), thus providing a temporary barrier to invading SMCs (28). Yet at the same time, PDT has been shown to promote accelerated endothelialization (29). Although it is possible that the reconstituted endothelium might be functionally altered to delay macrophage adhesion and migration, the precise mechanisms of action that prevent the influx of macrophages into the intimal layer are unclear.

A critical component of plaque healing and repair is that after treatment with MV0611-PDT, the intimal matrix was repopulated by SMCs by 28 days, with an absence of macrophages. Although similar changes in plaque cell composition (favorable to plaque stabilization) have been reported in rabbits maintained on a 16-month hypolipidemic diet (30), the rapid change in plaque composition induced by PDT is striking. Moreover, although statins have shown long-term clinical efficacy for reducing atherosclerosis in humans (31), they are unsuitable for acute management of plaques that are rupture-prone (32). In contrast, the acute plaque stabilizing effects of MV0611-PDT might prove to be of

therapeutic benefit, because a complete change in plaque cell composition was rapidly achieved by 28 days.

At 28 days after treatment, MV0611-PDT–treated arteries were significantly less occluded than untreated contralateral control arteries. The MV0611-PDT achieved this by reducing neointimal growth, with a simultaneous change in the plaque composition toward a stable plaque. Given that these effects were observed throughout the entire 2-cm treated length of artery, these data suggest that PDT might prove effective for the treatment of regional atherosclerosis in humans. Moreover, systemic photosensitizer administration might allow for multiple vessel segment treatments within a single intervention and might prove more cost-effective than the deployment of multiple stents. Also, from a safety perspective, catheter removal after PDT treatment might avoid some of the pathological phenomena that are typically associated with stents, namely hypersensitivity, late stage thrombosis, delayed healing, malapposition, restenosis, adventitial fibrosis, and impaired re-endothelialization (33,34). Moreover, a well-tolerated biological response with appropriate vessel healing might compare favorably with drug-eluting stents that could be challenged in their capacity to locally deliver the combination of drugs required to reduce inflammation yet promote healing and repair mechanisms.

Initially, photosensitizers were designed specifically for the treatment of solid tumors and were designed to have large absorptions in the 620- to 740-nm range so as to photo-activate the drug that will penetrate to the greatest depths possible in all tissue types. In particular these photosensitizers were designed to absorb outside of the blood absorption spectrum profile, thus ensuring efficient photoactivation in most tissue types. When red light PDT was used in cardiovascular disease, a high energy was used to overcome the absorption in the blood that resulted in photochemically induced damage to normal myocardial tissue surrounding the artery (18). This adverse effect could be the result of nonselective photosensitizer uptake and long depths of red light penetration, which activates the photosensitizer in the myocardial tissue. The use of a lower wavelength of light (<600 nm) has a significant advantage, because such wavelengths have penetration characteristics that deliver the PDT effect to shallower tissue (plaque, media, and adventitia) and not to deep myocardial tissue (18).

Clinical use of PDT therapy has been limited to nonrandomized, nonplacebo-controlled trials, with red light activation of 5-aminolaevulinic acid at 630 nm or motexafin lutetium at 730 nm (35–38). Although motexafin lutetium is well tolerated at lower doses, at higher doses a significant number of patients had paraesthesia (38). The use of deep penetrating red light might be better tolerated in peripheral vessels than in coronary vessels, where PDT with 5-aminolaevulinic acid has shown evidence of myocardial scarring in porcine coronary arteries (39). Although the green light used in the current study requires a blood free field to avoid absorption by circulating red blood cells, the light exposure time using green light (90 s) is significantly less than the time used for red light (12 min) in prior studies.

Study limitations. The current study was carried out in cholesterol-fed balloon-denuded iliac arteries where the pathology of the plaque is not similar to that of human plaques. Moreover, this is a short-term study; thus, the long-term effect of MV0611-PDT treatment is not known.

Conclusions

Our findings that removal of plaque inflammatory cells and replacement with SMCs promotes plaque stabilization as early as 28 days suggest a timely, effective, and appropriate vascular healing and repair response after PDT treatment. PhotoPoint PDT could be an interesting intravascular therapy for the treatment of acute coronary syndromes and regional atherosclerosis.

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Key Words: atherosclerosis ■ macrophages ■ photodynamic therapy ■ plaque stabilization.