

Photoremodeling of Arterial Wall Reduces Restenosis After Balloon Angioplasty in an Atherosclerotic Rabbit Model

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Objectives. This study evaluated the long-term impact of endoluminal low power red laser light (LPRL) on restenosis in an atherosclerotic rabbit model.

Background. Despite widespread application of balloon angioplasty for treatment of coronary artery disease, restenosis limits its clinical benefits. Restenosis is a complex process and may be partly attributed to the inability of the vascular endothelium to regenerate and cover the denuded area at the site of arterial injury. We previously demonstrated that LPRL stimulates endothelial cell proliferation in vitro and contributes to rapid endothelial regeneration after balloon injury in nonatherosclerotic rabbits.

Methods. Rabbit abdominal aortas (n = 12) were treated in separate zones with balloon dilation and balloon dilation plus laser illumination. Endoluminal laser therapy was performed using a laser-balloon catheter delivering a single dose of 10 mW for 3 min from a helium-neon laser (632 nm). Angiography was performed before and after treatment and was repeated 8 weeks before harvesting the aortas.

Results. Quantitative angiographic analysis demonstrated no differences in the minimal lumen diameter (MLD) between the two zones before treatment; an increase in the MLD in both zones after balloon angioplasty and a significant versus slight reduction of the MLD in the balloon treatment versus balloon plus laser zones at 8 weeks. Histologic examination showed a very high level of myointimal hyperplasia in the balloon treatment zones but a minimal level in the LPRL-treated zones. Morphometric analysis revealed a statistically significant difference in the lumen area, intimal area and intima/media ratio between the balloon versus balloon plus laser treatment sites.

Conclusions. Our experimental data indicate that endoluminal irradiation with LPRL prevents restenosis after balloon angioplasty in an atherosclerotic rabbit model.

(J Am Coll Cardiol 1998;31:1152-7)

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The introduction of percutaneous transluminal coronary angioplasty (PTCA) in late 1977 began a new era in the management of coronary artery disease, but the frequent occurrence of restenosis (30% to 50%) significantly limits the long-term clinical benefits (1,2).

Endothelial cell disruption/dysfunction after coronary artery intervention may mediate restenosis, suggesting that rapid regeneration of endothelial cells might impede this process (3-6). It has been postulated that the process of restenosis has histologic and pathophysiologic similarities to wound healing in other living tissues (7-9). Numerous reports indicate that noncytotoxic doses of low power red laser light (LPRL) stimulate in vitro cell proliferation and contribute to tissue

repair (10-12). We previously identified that LPRL does enhance endothelial growth as well as attachment in vitro at a dose that has no effect on smooth muscle cell (SMC) proliferation (13). In vivo studies in nonatherosclerotic rabbits treated with balloon angioplasty and endoluminal LPRL confirmed the presence of accelerated endothelial regeneration (13,14). The objective of the present study was to determine the long-term impact of endoluminal LPRL on restenosis in an atherosclerotic rabbit model.

Methods

Experimental animals. Fifteen adult New Zealand White rabbits weighing 2.5 to 3.5 kg were used in this study. Experimental atherosclerosis was induced by a cholesterol diet and balloon injury as previously described (15). After 2 weeks of atherogenic diet, the animals were anesthetized by a mixture of ketamine (10 mg/kg body weight) and xylazine (1 mg/kg) administered intravenously and then maintained with pentobarbital (12 mg/kg intravenously) when required. The femoral artery was exposed and ligated, and the abdominal aorta was deendothelialized by a 4F balloon embolectomy catheter passed via an arteriotomy retrograde into the abdominal aorta

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Manuscript received August 13, 1997; revised manuscript received December 17, 1997, accepted December 30, 1997.

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Abbreviations and Acronyms

LPRLL	= low power red laser light
MLD	= minimal lumen diameter
PTCA	= percutaneous transluminal coronary angioplasty
SMC	= smooth muscle cell

and withdrawn inflated three times. The animals recovered from anesthesia and were continued on the atherogenic diet for 4 weeks before balloon/laser treatment. Rabbits were fasted overnight before all surgical procedures.

On the day of the second procedure, the marginal ear vein was cannulated with a 22-gauge angiocatheter and flushed with heparin to ensure patency. Anesthesia was then induced with a mixture of ketamine (10 mg/kg) and xylazine (1 mg/kg) administered as a bolus injection via the marginal ear vein, and then maintained with pentobarbital (12 mg/kg) given intravenously as a bolus injection to achieve effect when required. The abdomen and groin were shaved and the skin cleaned for the operation using betadine. The abdomen was opened and the abdominal aorta carefully exposed from the iliac bifurcation to an area adjacent to the renal arteries. Two treatment zones 2 mm in length averaging 2.7 to 3.5 mm in diameter were identified by placing a fine prolene stitch in the adventitia. In addition, the distance from each stitch to the iliac bifurcation was measured and recorded.

Animals used in this study received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 85-23, revised 1985).

Experimental protocol. Two treatment zones of 2.0 cm in length, each within the abdominal aorta, were identified with prolene stitches as described previously. All catheters were advanced using a 4F or 5F pediatric introducer sheath through a small incision in the femoral artery. The introducer catheter was continuously flushed (1 to 2 ml/min) with lactated Ringer's solution. Immediately before introducing the catheters, heparin (1,000 IU) was administered through the introducer catheter as a bolus injection. Each zone was subjected to one of the following treatments: 1) Balloon dilation alone, in a selected aortic segment with 1.1:1 balloon/vessel ratio using three cycles of inflation to 8 atm pressure for 30 s, followed by one cycle of inflation to 2 atm for 180 s using a laser-balloon catheter. 2) Balloon dilation plus laser illumination. Three cycles of inflation to 8 atm for 30 s each, followed by laser illumination. Endoluminal LPRLL therapy was performed using a laser-balloon catheter with a 200 μ m fiberoptic with a diffuse tip at the end to provide a uniform distribution of light in the radial and axial directions of the balloon (Fig. 1). In all animals a single dose of 10 mW was given for 180 s generated from a helium-neon laser (wavelength 632 nm). During illumination



Figure 1. Laser-balloon catheter. Note the homogenous diffuse character of laser irradiation.

the balloon was inflated with a mixture of nonionic contrast and saline to 2 atm.

The anatomic order of each treatment site was systematically rotated to avoid any inherent bias. Angiography was performed in all animals immediately before and after balloon-laser treatment and was then repeated 8 weeks before harvesting the aortas for tissue processing. After treatment, the catheter was removed and the site of the catheter introduction repaired with one stitch using a prolene suture. The skin was closed and the animals allowed to recover. Antibiotics were given as necessary. Twelve animals recovered from the second procedure and were kept on atherogenic diet for an additional 8 weeks.

Tissue processing. The animals were then reanesthetized and heparinized, the abdomen opened, and the abdominal aorta exposed to identify the marking tags.

The animals were euthanized to ensure blood was not delivered to the abdominal aorta. The aorta was then cannulated proximal to the treatment zones. The iliac arteries were severed and all side branches of the abdominal aorta ligated. The entire vessel was then flushed *in vivo* with \approx 100 ml phosphate-buffered saline to remove blood from the lumen of the vessel. This was followed with \approx 100 ml of 10% buffered formalin. The aorta was then fixed at hydrostatic pressure (80 mm Hg) for 30 min using 10% buffered formalin. Following fixation, the artery was dissected to reveal the two treatment zones. This procedure was designed 1) to minimize the generation of artifacts and to preserve the normal diameter and length of the vessel, and 2) to avoid a potentially bloody dissection during harvest by enabling the fixed vessel to be dissected out from a dead animal.

Tissue staining. Each vessel was serially sectioned in 2-mm increments to produce a minimum of nine 4- μ m cross-sections. Following embedding in paraffin and dehydration, the sections were stained for light microscopic analysis with the following agents: 1) hematoxylin and eosin, 2) Movatt's elastic tissue pentachrome, 3) smooth muscle cell actin, and 4) proliferating cell nuclear antigen.

Analysis of data. The areas of lumen, intimal hyperplasia and media were determined using a Tamaya Digital Planimeter (Lietz, Overland Park, KS) in all zones at 8 weeks after

Table 1. Quantitative Angiographic Analysis

	Balloon Angioplasty	Balloon Angioplasty Plus Endoluminal Laser
MLD before treatment (mm)	1.82 ± 0.09	1.85 ± 0.10*
MLD after balloon angioplasty (mm)	2.53 ± 0.43	2.46 ± 0.46*
MLD before harvest (mm)	1.62 ± 0.16	2.32 ± 0.06†
Acute gain (mm)	0.71 ± 0.06	0.61 ± 0.24*
Late loss (mm)	0.91 ± 0.05	0.14 ± 0.04†
Net gain (mm)	-0.2 ± 0.01	0.47 ± 0.12*
Loss index	1.28 ± 0.21	0.22 ± 0.02*

* $p > 0.05$, † $p < 0.05$ for balloon versus balloon plus laser. Data presented are mean value ± SD. MLD = minimal lumen diameter.

treatment. From this data the ratio of the total intimal area to the total medial area was derived.

In addition, the two treatment zones were analyzed histologically in a blinded fashion by two cardiac pathologists.

The angiographic data were analyzed for changes in luminal diameter for all treatment zones immediately before and after laser/balloon treatment and 8 weeks later before harvest of the vessels. Quantitative analysis of angiograms was performed on all vessels. Two observers, in blinded manner, recorded the diameter of each treatment zone by tracing the original image of the video recordings onto clear transparent sheets. This method is reproducible within 0.2 mm in our laboratory. For all treatment sites the minimal lumen diameter (MLD), the percent of stenosis in comparison with an inflated balloon as an internal standard was determined. From these data acute gain and late loss were derived.

Statistical analysis. Data (mean value ± SD) were analyzed for overall differences between treatment groups by Student *t* test, incorporating the Bonferroni correction for multiple comparisons. Comparison of the mean values with a *p* value of <0.05 was considered statistically significant. Correlation analysis was then performed between all measured variables using Pearson product moment analysis as well as Spearman rank order correlation analysis.

Results

Quantitative angiographic analysis. Quantitative angiographic analysis in 12 animals demonstrated that there were no differences in MLD between the balloon zone (1.82 ± 0.09 mm) and balloon plus laser zone (1.85 ± 0.10 mm) before treatment (Table 1). Balloon angioplasty resulted in an increase in MLD in both balloon alone and balloon plus laser treatment sites to 2.53 ± 0.43 mm and 2.46 ± 0.46 mm, respectively. An acute gain in lumen diameter (defined as the difference in predilation and postdilation MLD) was present in both the balloon group (0.71 ± 0.06 mm) and the balloon plus laser group (0.61 ± 0.24 mm).

Angiography was then repeated at 60 days immediately before vessel harvest and revealed a significant reduction of MLD at the balloon treatment sites (1.62 ± 0.16 mm). By

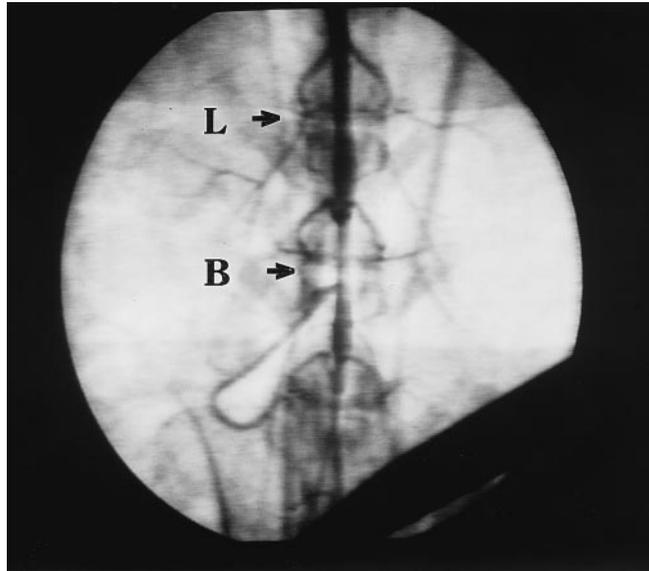


Figure 2. Angiogram of rabbit aorta at 8 weeks after treatment before harvest. Note that the distal portion of the abdominal aorta subjected to balloon angioplasty is restenosed. The midportion of the abdominal aorta subjected to balloon angioplasty and endoluminal LPRLI irradiation is widely open, without significant reduction of the lumen. B = balloon angioplasty; L = balloon angioplasty plus endoluminal laser.

contrast, the MLD of sites subjected to endoluminal laser therapy after balloon dilation was only slightly reduced (2.32 ± 0.06 mm) (Fig. 2).

Late loss (defined as the difference between MLD at the time of harvest and after dilation) ranged from 0.91 ± 0.05 mm in the balloon group to 0.14 ± 0.04 mm in the balloon plus laser group ($p < 0.05$ balloon vs. balloon plus laser). In the balloon group there was a significant correlation between acute gain and late loss ($r = 0.47$, $p < 0.05$); in the laser-treated arteries there was no correlation between acute gain and late loss ($r = 0.04$, $p > 0.05$).

Pathologic analysis. The angiographic findings also were reflected in the pathologic analysis based on histologic and morphometric criteria. Histologic examination of the extent of medial necrosis did not show any differences between treatment sites. There was a significant difference, however, in the level of myointimal hyperplasia. In the specimens obtained from balloon treatment locations we observed a very high level of myointimal hyperplasia. Interestingly, hyperplasia was concentric with a large number of SMCs. By contrast, the specimens subjected to endoluminal irradiation with LPRLI demonstrated minimal, eccentrically situated zones of myointimal hyperplasia (Fig. 3).

Morphometric analysis demonstrated that intimal area was five times greater in the areas subjected to balloon treatment alone (25.3 ± 1.9 mm²) than in the balloon plus laser treatment sites (4.2 ± 0.7 mm²) (Table 2). Also there was a statistically significant difference ($p < 0.05$) in the luminal area, intimal area and intima/media ratio between the two treatment sites.

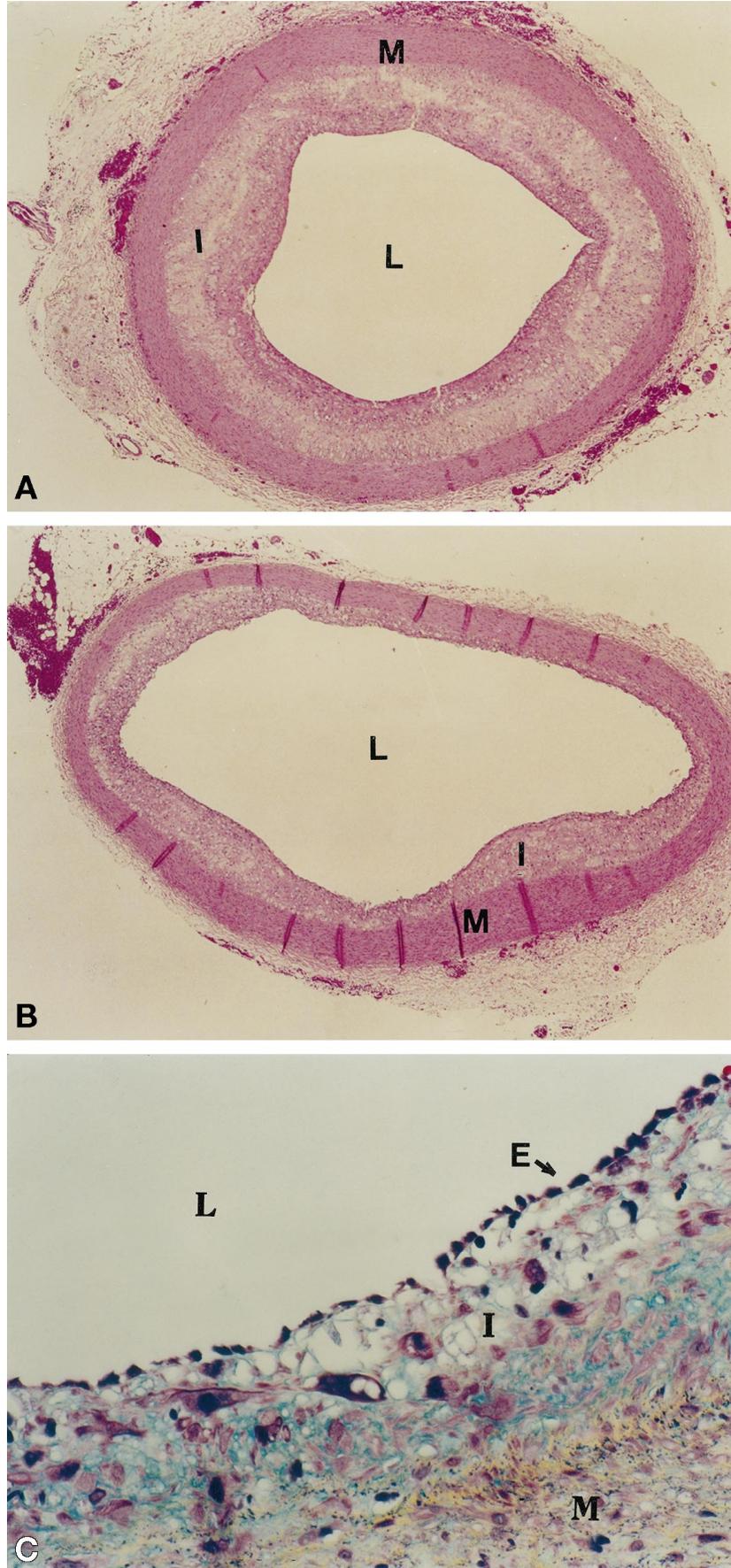


Figure 3. Impact of endoluminal LPRLL on restenosis at 8 weeks after treatment. **A**, Low magnification image of histologic cross section of rabbit abdominal aorta subjected to balloon angioplasty. Note the significant reduction of lumen area with marked intimal hyperplasia. Hematoxylin-eosin stain. **B**, Low magnification image of histologic cross section of rabbit abdominal aorta subjected to balloon angioplasty and endoluminal laser irradiation. Note the minor intimal growth with near normal lumen area. Hematoxylin-eosin light stain. **C**, Higher magnification of histologic cross section of rabbit aorta subjected to balloon angioplasty and endoluminal laser irradiation shows endothelial cell regeneration and mild SMC proliferation. Movatt's stain. E = endothelium; I = intima; L = lumen, M = media.

Table 2. Planimetric Analysis of the Harvested Arteries

	Balloon Angioplasty	Balloon Angioplasty Plus Endoluminal Laser
Lumen area (mm ²)	7.6 ± 0.9	33.9 ± 2.1*
Intimal area (mm ²)	25.3 ± 1.9	4.2 ± 0.7*
Intima/media ratio	2.1 ± 0.29	0.3 ± 0.02*

*p < 0.05 for balloon versus balloon plus laser. Data presented are mean value ± SD at 60 days.

Discussion

We demonstrated both angiographically and histologically that endoluminal laser irradiation of postangioplasty zones is capable of reducing the degree of myointimal hyperplasia at 60 days following balloon dilation in an atherosclerotic rabbit model.

The effect of LPRLL was previously studied in vivo using the balloon injury model in normal rabbit iliac arteries (13). Quantitative analysis identified significant enhancement of re-endothelialization following intravascular LPRLL irradiation. Ultrastructural analysis of vascular segments using scanning electron microscopy confirmed these results. Endothelialization originated from the proximal and distal ends of the injured segments in control arteries. By contrast, re-endothelialization was homogenous throughout the vessel in the laser-treated arteries. Moreover, there was less platelet adhesion and less macrophage deposition in the laser-treated vessels.

The mechanisms by which laser light enhances endothelial repair were further evaluated in vitro by studying the adhesive properties of endothelial cells and their rate of regeneration (16). We show that stimulation or inhibition (or both) of cell proliferation in vitro can each be obtained with LPRLL by varying the energy level. These data correlate with other reports. It is recognized that LPRLL is capable of influencing cellular behavior (cell growth, differentiation, motility, migration and phagocytosis) in the absence of significant thermal effect (10,16-21). In our experiments endothelial cells were more sensitive to photobiomodulation with LPRLL than were SMCs. SMCs were more resistant to LPRLL, however, although a higher dose of LPRLL was cytotoxic for both endothelial cells and SMCs. We also observed that low doses of light significantly increased the attachment efficacy of endothelial cells. Similar results were reported by Karu et al. (11). They demonstrated that LPRLL stimulates adhesion of He-La cells in vitro and speculated that visible light alters the intensity of ion fluxes through the cell membrane, which may enhance cell sedimentation and adhesion. Functional behavior of tumor cells, however, is not as dependent on attachment and adhesion, whereas endothelial cells require attachment before proliferation (22).

Integrins are major receptors that mediate adhesive interactions in living tissue. They are intimately involved in the regulation of many cellular functions, including programmed cellular death, hemostasis-thrombosis, leukocyte activation

and the response of cells to mechanical injury (23). Integrins can trigger several signal transduction pathways and regulate changes in gene expression critical for proliferative response and affinity of membrane to growth factors. Experimental balloon angioplasty rarely results in complete denudation of endothelium. Based on in vitro (enhancement of cell adhesion) and in vivo (homogenous endothelialization of entire segment) findings, we believe that intravascular laser therapy may trigger integrin-dependent pathways of preserved endothelium that signal increased DNA synthesis. This results in endothelial cell proliferation. We investigated this hypothesis by determining the rate of (H³) thymidine uptake in confluent endothelial cell cultures (unpublished data). Low doses of red light increased DNA synthesis by 45.5% ± 6.8%. At higher doses, DNA synthesis diminished, consistent with a cytotoxic effect of higher dose therapy.

In summary, we found that endoluminal LPRLL after balloon angioplasty stimulates endothelial regeneration, thereby enhancing vascular healing before a hemodynamically important reduction in arterial lumen area can take place. This results in a decreased rate of restenosis.

Although the mechanisms by which this might occur have not yet been established, stimulation of endothelial repair with LPRLL may be mediated by integrin-dependent pathways that in turn prevent migration of SMCs into media as well as reduction of platelet activation and vascular reactivity (24-26).

Study limitations. Our data support the idea of beneficial effects of endoluminal LPRLL on restenosis; however, certain limitations of the present study must be acknowledged. First, is the small animal model, with the principal disadvantages of the atherosclerotic rabbit model, including the morphologic differences from human lesions; second, and perhaps more importantly, is that the biologic mechanism of action of LPRLL on the vascular wall needs further exploration. Furthermore, additional studies are required to access function and biologic activity of regenerated endothelium.

Conclusions. Our experimental data indicate that endoluminal irradiation with LPRLL prevents restenosis after balloon angioplasty in an atherosclerotic rabbit model. Endoluminal LPRLL therapy may have a significant role in interventional cardiology, not only to prevent restenosis after coronary interventions but also to stabilize vascular endothelium so that progression or redevelopment of the disease is inhibited. Randomized studies are required to establish this as an effective clinical tool in humans.

We are grateful to John R. Petersen, MD for review of the manuscript.

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