Laser recanalization of occluded atherosclerotic arteries in vivo and in vitro

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ABSTRACT Controlled laser irradiation was used to recanalize atherosclerotic stenoses in vivo and in vitro. In 15 rabbits with atherosclerotic arteries a catheter was positioned in the distal aorta for angiographic examination and as a guide for a small silica optical fiber. Both Nd-YAG and argon lasers were used for recanalization with varying power and duration. As determined by angiographic studies, the severity of iliofemoral stenoses in eight of 15 arteries decreased from 78 ± 18% to 32 ± 11% (mean ± SD). In one additional artery the stenosis improved from 45% to 25%, but this was associated with perforation. The other six arteries were perforated (two after fiber manipulation, four after laser discharge) without obvious improvement in severity of stenosis. No angiographic loss of distal circulation was noted. To better define tissue-laser interactions in the live rabbits, lasing of totally occluded atherosclerotic rabbit arterial segments in vitro was done while the optical fiber was advanced or fixed. When the fiber was fixed, serial sections showed that the new lumen was flame shaped. The width and depth of the lumen increased with increasing laser energy. When the fiber was advanced, histologic examination showed a smooth cylindrical vascular channel with limited lateral tissue damage. This study demonstrated that lasers can recanalize atherosclerotic stenoses in a live animal preparation; however, arterial perforation remains a problem.


LASER RADIATION has been shown to recanalize atherosclerotic vascular occlusions in human postmortem vessels.1-3 The laser has the potential to be used intravascularly, since it can be delivered to the site of vascular obstruction by transmission through standard flexible optical fibers. The purpose of this study was to evaluate short-term effects of laser radiation applied in vivo to recanalize arterial obstructions in rabbits with atherosclerotic arteries. Results of laser recanalization were evaluated angiographically and histologically with light and electron microscopy. In addition, to provide further insight into the mechanism of the observations made in the preparation in vivo, experiments were performed in vitro to evaluate the effects of laser beam divergence and energy decay on the size of the recanalized channel.

Methods Induction of atherosclerotic stenoses. Twenty-one New Zealand White male rabbits weighing 5 to 7 pounds were fed an atherogenic diet of 2% cholesterol and 10% lard (ICN, Cleveland), which caused an elevation in serum cholesterol levels to between 800 and 1500 mg/dl after approximately 2 weeks. With animals under ketamine and xylazine anesthesia, a No. 4F Fogarty catheter was inserted via the right femoral artery and endothelial stripping of the aorta and right iliac artery was performed. The atherogenic diet was continued for an additional 2 to 3 months.4-6

Experiments in vivo Delivery of laser radiation. A 0.5 mm optical fiber measuring 80 to 100 cm in length with a 0.2 mm silica core (Medicor Optical Fiber MED200; Quartz Products, Plainfield, NJ) was used to deliver laser radiation. This optical fiber was polished smooth and was flat at both ends. One end was aligned with the focused laser beam and the other end delivered the laser energy intravascularly. A metal marker at the tip of the fiber allowed fluoroscopic visualization (figure 1).

Localization of arterial stenoses. A right carotid cut-down

Vol. 71, No. 2, February 1985
was performed in 15 rabbits under ketamine and xylazine anesthesia. A No. 4.5F or 5F angiographic guide catheter (Ducor, Cordis, Miami) equipped with a hemostasis valve was introduced and advanced to the abdominal aorta by means of 6 inch fluoroscopic image intensification. This catheter system allowed performance of angiographic studies with the optical fiber positioned inside the guide. Single-plane cineangiography was performed by hand injection of a mixture of 50% meglumine diatrizoate (Renografin 76) and physiologic saline at the level of the mid to distal abdominal aorta. When an area of severe stenosis was localized, the catheter was advanced toward the stenosis. Localization was assisted by the use of video recorder replay. An attempt was made in all 15 rabbits to advance the guide catheter across the stenosis, and in nine rabbits attempts were made to advance the optical fiber across the stenosis. After these attempts at mechanical recanalization, a second angiogram was obtained for comparison with angiograms obtained after the laser radiation procedure.

Laser radiation procedure. Two different continuous-wave laser power sources were used. Laser radiation was delivered from either a Nd-YAG (neodymium–yttrium aluminum, garnet) laser (Model 510; Control Laser, Orlando) with a wavelength of 1064 nm or an argon laser (Model 554A; Laser Iomics, Orlando) with principal wavelengths of 488 and 514 nm. Power at the optical fiber tip was varied from 0.5 to 15 W and duration of exposure from 1 to 10 sec.1

The optical fiber was advanced beyond the guide catheter to the stenosis. Laser discharge was performed while slowly advancing the fiber with gentle pressure. Once the stenosis was crossed, laser delivery was discontinued and the fiber was withdrawn into the catheter. Angiographic studies were immediately repeated. In eight rabbits, within 10 min of the initial laser discharge the fiber was again advanced to the stenosis and a second laser discharge was performed; on most occasions (seven of eight) twice the power for a similar duration of exposure was used. The fiber was then withdrawn into the catheter and angiographic studies were immediately repeated. End points were either a large decrease in the percentage of arterial diameter stenosis or arterial perforation. Rabbits were then killed with an overdose of pentobarbital between 15 to 30 min after the last procedure. Since we previously demonstrated effective laser delivery in a system in vitro with argon and Nd-YAG lasers in a blood medium, the procedure was done in the circulating blood.1

Evaluation of laser recanalization. The effect of laser radiation was evaluated by angiographic and histologic studies. Angiograms were reviewed after the first and second applications of laser radiation. An Angiogram Viewer Analyser (Model 474; Berthcher General X-ray, El Monte, CA) was used to enlarge the images to improve accuracy of measurements. Percentage stenosis was measured in a single view as the ratio of the minimum arterial diameter with the “normal” prestenotic or poststenotic segment. Measurements were made by two independent observers. To ensure precise localization of the segments treated with laser radiation, the guide catheter was left 2 to 3 cm above the segment and used to identify the area after the animal was killed. Three successfully recanalized arteries were pressure perfused (80 mm Hg) and fixed in situ with 2.5% glutaraldehyde in 0.2M cacodylate buffer and examined by light and electron microscopy. The other 12 arteries were excised and fixed in 10% neutral buffered formalin and examined by light microscopy alone. Tissue sections for light microscopy were stained with hematoxylin-eosin, Masson’s trichrome, or Verhoeff’s elastic stain. Irradiated arterial segments fixed in glutaraldehyde were opened longitudinally. Five millimeter segments were subjected to critical-point drying in liquid CO₂, mounted on stubs, and gold coated in a sputter coater. The intimal surface was examined under a JEOL 35C scanning electron microscope. For transmission electron microscopy cross sections from irradiated and nonirradiated areas were cut in 2 mm pieces, embedded in Spurr resin, and thin sectioned with a diamond knife. Sections were stained with uranyl acetate and lead citrate and viewed in a Philips-300 electron microscope. Vessels were processed so as to obtain representative tissue sections above, at, and below the sites of laser delivery.

Experiments in vitro
Characteristics of energy (power × time) distribution in tissue. To evaluate laser beam divergence and decay, two types of experiments were performed. Fifteen 2 cm segments of totally occluded atherosclerotic femoral arteries from six other rabbits were exposed to laser radiation (argon) in air medium. Power was constant at 3 W. First, in eight segments the tip of the optical fiber was fixed at the end of the occluded artery, pointed in a coaxial direction, and used to create a new vascular lumen. Five segments were irradiated for 10 sec each, 2 for 20 sec, and 1 for 30 sec. Second, in seven segments, after the tip of the optical fiber was aligned in a coaxial fashion with the arterial wall, the fiber was slowly advanced as laser delivery was performed. All segments were then fixed in either 2.5% glutaraldehyde in cacodylate buffer or neutral buffered formalin and embedded in paraffin. In arteries irradiated from a fixed fiber, the entire tissue block was serially cross sectioned at 4 μm intervals. Staining was done with toluidine blue, O. The cross-sectional diameter of the new lumen created by the laser was measured in the vertical and horizontal axis with a Bausch and Lomb calibrated magnifying eyepiece. The largest diameter of the new channel was expressed as a percentage of the native internal lumen diameter of the artery. In arteries irradiated while the fiber was advanced, a longitudinal incision of the artery was done and scanning electron microscopy was performed as described earlier.

Statistical analysis. The amount of energy in laser discharges with and without arterial perforation was compared by an unpaired test, and the frequency of perforation with each power source was compared by Fisher’s exact test. A p value < .05 was considered statistically significant.

Results
Experiments in vivo. Tables 1 (Nd-YAG) and 2 (argon) summarize the effects of different lasers and
amounts of laser energy, measured in joules, on the percentage of arterial stenosis.

Angiographic observations after catheter and fiber manipulation. Attempts to cross and/or dilate the stenoses with the guide catheter were unsuccessful and did not change the angiographic appearance of any of the 15 stenoses or produce arterial perforation. By contrast, the fiber successfully crossed the stenosis in five of the nine rabbits (Nos. 6, 8, 10, 12, and 13) in which it was attempted. Angiography demonstrated improvement in lumen size in three of the five stenoses crossed (Nos. 8, 10, and 12) and an illustrative example is shown in figure 2. Stenoses that improved after mechanical revascularization had further improvement after laser radiation (see below). Stenoses in the other four rabbits (Nos. 5, 11, 14, and 15) could not be crossed. Fiber manipulation did not dilate the stenosis but did produce arterial perforation in two animals (Nos. 5 and 15).

Angiographic observations after initial laser discharge. After the first discharge of laser radiation, no change was noted in four rabbits (Nos. 1, 6, 7, and 14). An important decrease in stenosis severity was achieved in seven stenoses (Nos. 2, 3, 8, and 10 through 13). Illustrative examples are shown in figures 2 and 3. Angiographic improvement was seen with both lasers over a wide range of energies. Arterial perforation occurred in two rabbits (Nos. 4 and 9). Because of the magnitude of perforation, assessment of the effects of laser radiation on lesion severity could not be performed.

Angiographic observations after second laser discharge. In the four rabbits that showed no change after the initial procedure, laser radiation was repeated. This second procedure resulted in an improvement in the vascular lumen of one vessel (No. 6), an increase in lumen but perforation in another (No. 7), and no improvement but perforation in the other two (Nos. 1 and 14). Four of the rabbits (Nos. 2, 10, 12, and 13) that showed improvement after the first procedure underwent repeat laser radiation. Minor improvement was measured in animals 2 and 10, and no further important improvement was observed in the other two. After

TABLE 1
Angiographic results after laser radiation of atherosclerotic arteries in vivo with a Nd-YAG laser

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Control (% stenosis)</th>
<th>Fiber manipulation (% stenosis)</th>
<th>Energy (J)</th>
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<th>% Stenosis</th>
<th>Total energy (J)</th>
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TABLE 2
Angiographic results after laser radiation of atherosclerotic arteries in vivo with an argon laser

<table>
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<tr>
<th>Rabbit No.</th>
<th>Control (% stenosis)</th>
<th>Fiber manipulation (% stenosis)</th>
<th>Energy (J)</th>
<th>% Stenosis</th>
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FIGURE 2. Rabbit 12. Top panels, Low-power view of the lower abdominal aorta and both right and left femoroiiliac circulations. The right external iliac artery was severely stenosed (A, arrows). Bottom panels, Magnified views of the right internal and external iliac arteries showing the stenosed external iliac artery in more detail. Passage of the optical fiber across the stenotic segment resulted in mechanical recanalization with a small decrease in the percentage stenosis (B). After application of laser energy (15 J) further decrease in the percentage of stenosis occurred (C, arrows).

FIGURE 3. For legend see opposite page.
catheter and fiber manipulation and both laser discharges, no evidence of loss of distal circulation was noted by angiography in any of the rabbits.

**Further analysis of laser-induced arterial perforations.**
Five perforations occurred after laser radiation, two of six laser applications with the Nd-YAG laser and three of 15 with the argon laser (p = NS). In general, the total energy was higher in laser discharges resulting in perforation (49.5 ± 36 J with the Nd-YAG and 20 ± 13 J with the argon laser) than in those in which perforation did not occur (33.4 ± 15 J with the Nd-YAG, p = NS; 7.5 ± 5 J with the argon laser, p < .05).

**Histologic results.** Both light and electron microscopic examinations confirmed that there was severe atherosclerosis involving the abdominal aorta and iliac and femoral arteries. Furthermore, laser radiation in vivo consistently vaporized the intimal smooth muscle cell proliferation that characterized these atheromatous stenoses (figures 4 and 5).

Arteries usually demonstrated centrally located channels without residual cellular debris, suggesting that the atheroma was vaporized. The intimal surface consistently showed a thin zone of charring (figure 4) with an underlying zone of thermal necrosis (figure 5). An eccentrically irradiated vascular channel, however, can lead to thermal necrosis of the entire thickness of the vessel wall and to perforation (figure 5). With both lasers the channel created had a cylindrical lumen that was similar in size and shape to the optical fiber (figures 4 and 6). Arterial segments that were pressure perfused during fixation looked similar to those fixed without perfusion fixation.

Scanning electron microscopic examinations of the irradiated arterial lumen revealed a flat smooth surface lining (figure 6). This surface was usually composed of fused cell membranes and denatured cell elements forming a smooth sheet. Occasional crenated red blood cells were seen adherent to the surface. These observations were supported by transmission electron microscopy, which showed the surface to be composed of a smooth layer of necrotic tissue without any recognizable cell type. In some areas there was an overlying amorphous surface that was dense and measured 5 to 10 µm thick. The necrotic layer below was less dense and also measured 5 to 10 µm. In this layer cellular structures could be identified but the cells appeared irreversibly damaged with clumping of chromatin material. Viable cells were usually seen at three to four cell layers below the irradiated amorphous surface.

**Experiments in vitro.** The fiber could not be forced through the occlusive atheroma in any experiment. With the fiber tip fixed, neither perforation nor complete recanalization of the 2 cm occluded segments occurred. Evaluation of approximately 3500 serial sections showed that the new channels were flame shaped. The initial point of laser impact created a new lumen that was similar in size to the optical fiber. In the midportion of the new channel the diameter was larger than the optical fiber, presumably because of laser beam divergence. Distally the new channel tapered over a longer area as the laser energy decayed. The depth of the new channel and the diameter of its mid and distal areas seemed related to the duration of exposure (figure 7). With the fiber tip advanced, no perforations occurred and recanalization was accomplished in all arteries within 5 to 10 sec. Multiple sections of these arteries showed a new cylindrical channel similar in diameter to the optical fiber along the entire length of the artery.

**Discussion**
This study demonstrates that laser radiation can be transmitted to arterial stenoses and can be used to en-

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**FIGURE 4.** Tissue section of an atherosclerotic femoral arterial stenosis recanalized by an argon laser. Angiographically, this vessel was severely stenosed before laser radiation. Intimal smooth muscle and foam cell proliferation occluded the vessel (arrows, IP), and laser radiation has cleanly vaporized a portion of this tissue. A thin darkened zone of charring was seen at the edge of the lumen. (Hematoxylin and eosin; original magnification ×68).

**FIGURE 3.** Rabbit 2. Left. Left iliac angiogram showing a stenosis of the external iliac branch before laser radiation (arrow). Right. After laser radiation of the stenosis with a total of 31.5 J (4.5 W, 7 sec) there is marked reduction in the percentage of stenosis (arrow).
large stenosed or occluded arterial lumens in live animals.

Both the Nd-YAG and argon power sources were equally effective in irradiating atherosclerotic stenoses in the rabbit. Additionally, arterial perforations occurred with both laser power sources. Theoretically, the deeper penetration into tissue by the Nd-YAG wavelength as compared with the argon wavelength might lead to a higher perforation rate, but the small number of studies we have performed does not presently substantiate this. It has been suggested that use of the carbon dioxide wavelength (10,600 nm), which has high tissue absorption, could lead to less deep tissue penetration and fewer perforations. A major limitation of the carbon dioxide wavelength, however, is its poor transmission in blood and saline media.

Lower energy levels were associated with less perforation; however, the overlap of energies resulting in recanalization or perforation led to further analysis of mechanisms leading to perforation. In our opinion, lack of a coaxial position of the fiber in the arterial lumen will be the major cause for laser-induced perforation regardless of energy used. Precise localization of the fiber tip with respect to the arterial lumen is essential. In the study in vivo, atherosclerotic lesions were localized by means of single-plane angiography. Other methods could be used to improve localization, such as a biplane angiographic system or direct visualization of the plaque with angioscopy before laser discharge. Experience of the operator and the tortuosity of the artery are likely to be important variables effecting perforation rate. Additionally, in the experiments in vitro, when laser delivery was done from a fixed optical fiber position, beam divergence from the fiber

FIGURE 5. Tissue cross section at the origin of the right common iliac artery showing an enlarged eccentric vascular lumen after radiation with an argon laser. There is considerable charring (C), thermal necrosis (N) with perforation (P), and extravasation of blood into the adventitia. (Hematoxylin and eosin; × 55).

FIGURE 6. A. A low-power scanning electron micrograph showing a new vascular channel created by laser radiation in a totally occluded artery. The walls of the channel were smooth. The arrows point to two areas magnified below (B and C). Bar = 100 μm. B. High-power view showing a smooth surface composed of amorphous, fused cell elements. Several distinct but crenated red blood cells adherent to the surface were seen in all high power views. Bar = 10 μm. C. High-power view showing a mesh of burned, fused cell elements. Bar = 10 μm.
FIGURE 6. For legend see opposite page.
FIGURE 7. Graph summarizing experiments in vitro in totally occluded arteries with the optical fiber tip fixed. Increasing duration of exposure at constant power (3 W) led to a larger internal lumen diameter and deeper penetration into the atheroma. The size of the new channel created by laser radiation was expressed as the percent change in lumen diameter as compared with the size of the original occluding atheroma (vertical axis), and the depth of penetration is shown on the horizontal axis. Average changes in lumen diameter at selected depths of penetration are shown for exposures of 30, 20, and 10 sec durations. The new laser-created channels were flame shaped.

A wider area of damage. On the other hand, laser delivery as the fiber was advanced reduced duration of exposure at any site and resulted in a smooth cylindrical channel. Shorter exposure times to a certain site can be achieved by advancing the fiber or by short duration of exposure from a fixed location. Laser discharge in different media, such as blood or saline, also affects conduction of the thermal energy to the atheroma and may affect perforation rate. Blood enhances transmission of laser energy as compared with saline. 1

The histopathologic features of laser-irradiated human atherosclerotic plaques have been described in coronary arteries obtained at autopsy. 1,2,3 Features noted in the studies in vitro were similar to those noted after the intravascular irradiation of rabbit atherosclerotic plaques in vivo. In all studies laser radiation vaporized the smooth muscle of the atherosclerotic intima, leaving a thin zone of charring along the edge of the newly created channel. Adjacent to this zone was an area of variable thickness in which thermal necrosis of tissue was evident. Tissue vaporization was nearly complete even in the presence of blood; thrombosis was not seen either angiographically, on gross inspection, or microscopically. Ultrastructural studies showed the irradiated channel had a smooth wall composed of fused cell membranes and denatured cell elements. Since no acute platelet adhesion or thrombus formation was noted, the irradiated surface may be resistant to acute platelet aggregation and subsequent thrombus formation.

At present it is not certain how laser-irradiated arterial stenoses will heal and whether thrombus formation will occur after longer periods of observation. For example, depth of laser penetration into the atherosclerotic plaque with release of various cell elements may influence reclosure either via thrombosis or smooth muscle proliferation. Preliminary reports on the healing of laser-induced arterial injury in atherosclerotic swine and in normal dogs suggest that rapid healing and reendothelialization occur with minimal change to surrounding tissue. 10,11 In both preparations a fibrin platelet plug formed early after irradiation, with reendothelialization of the crater site by 2 to 4 weeks.

The rabbit preparation of atherosclerosis was one of the first developed. Although the rabbit develops atherosclerosis rapidly, this preparation posed several problems. First, the rabbit is a small animal and technically difficult to catheterize. Second, the arteries of the rabbit are fragile and easily torn by catheter and optical fiber manipulations without laser discharge. Third, even with endothelial stripping to intensify the severity of the atherosclerotic process, the degree of lumen stenosis is not always severe. 4,5 Fourth, the nature of the atheromas in this rabbit preparation differs from human atherosclerotic disease. 4,6

Since atherosclerotic human arteries are thicker than those in the rabbit, the frequency of perforation in the rabbit preparation may be higher than that in humans. By a combination of improved control of the optical fiber tip and understanding of laser-tissue interactions, the technique of laser recanalization may become an alternative method of treating occlusive arterial disease in patients. 12

We thank Wendie Smith for the laboratory assistance, Faye Nemeth for preparation of the transmission electron micrographs, the staff of the Cardiac Catheterization Laboratory, and Nancy Sajzuk for the typing of the manuscript. We also thank Cordis Research Corp., Miami; Trimecyn Inc., Santa Ana; and Webster Laboratories Inc., Altdena, CA for their support.

References

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