Percutaneous Delivery of Low-Level Laser Energy Reverses Histamine-Induced Spasm in Atherosclerotic Yucatan Microswine

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Background. Previous in vitro experiments performed in our laboratory have shown that low-level laser energy may produce prompt reduction in isometric tension of vascular smooth muscle. The present study was designed to extend these previous in vitro findings to an in vivo model and thereby investigate the hypothesis that laser light delivered percutaneously in vivo could successfully reverse arterial spasm.

Methods and Results. Spasm defined as >50% reversible reduction in luminal diameter persisting for ≥5 minutes was successfully provoked by injection of histamine (100–400 μg/kg) in 13 arteries among 10 atherosclerotic Yucatan microswine; the magnitude of histamine-induced vasoconstriction was then documented angiographically by repeated injections of contrast media for as long as 30 minutes (controls). After return of angiographic luminal diameter to baseline, spasm was reproduced with a second injection of histamine into the same artery. Representative wavelengths generated by ultraviolet (UV), visible, and infrared lasers were then delivered percutaneously via conventional fiberscopes to the site of spasm, and angiographic assessment was repeated for as long as 30 minutes (treatment trial). In three arteries treated with UV (351 nm) light from an excimer laser, angiographic luminal diameter narrowing decreased from 100% to 23.9%, 50.0% to 9.3%, and 76.0% to 42.3%, respectively. The magnitude of laser-induced increase in luminal diameter was 50.2±22.7%, which was significantly greater than the magnitude of relaxation observed spontaneously during the control trials (10.9±9.8%, p=0.02). Visible light from a helium-neon (632 nm) laser accomplished complete reversal of histamine-induced spasm in two of four arteries; in the remaining two arteries, luminal diameter narrowing percentages were reduced from 57.0% to 20.0% and from 76.5% to 30.8%, respectively. The magnitude of helium-neon laser-induced relaxation (55.8±17.9%) was again significantly greater than that observed during the control trials (0.9±1.9%, p=0.01). Finally, infrared irradiation from a diode-pumped neodymium:yttrium aluminum garnet (1,064 nm) laser decreased histamine-induced luminal diameter narrowing in three arteries from 100% to 21.4%, 56.0% to 8.7%, and 68.3% to 35.3%, respectively. The magnitude of infrared laser–induced improvement in luminal diameter narrowing was 53.0±23.3%, which was significantly greater than that observed during the control trials (12.9±10.7%, p=0.01). In three additional arteries, fiberoptic sham trials (without laser irradiation) failed to produce relaxation of histamine-induced spasm.

Conclusions. These findings document for the first time that light-induced relaxation of vascular smooth muscle, previously documented in vitro, may be reproduced in vivo. (Circulation 1992;86:756–768)

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dynamic arterial narrowing resulting from a transient increase in arterial tone, or spasm, is a recognized basis for a variety of clinical disorders. By definition, such an increase in tone resolves spontaneously after a variable time interval. When the time course is protracted, however, and the degree of narrowing is severe, spasm may have deleterious consequences. Experimental and clinical studies have established that some of these consequences may be successfully aborted by treatment with a variety of pharmacological agents.

It is less widely appreciated that increased vascular tone has proved reversible in response to certain physical stimuli administered in vitro. Experiments performed in the early part of this century with nonlaser sources demonstrated that the interaction of light and smooth muscle could result in a reduction of muscular tone.\(^1\)\(^-\)\(^4\) Furchgott et al\(^5\)\(^,\)\(^6\) subsequently showed that such photorelaxation could be achieved in vascular smooth muscle and did not require the use of exogenous photosensitizing agents. Experiments performed in our laboratory using low-level laser energy generated from a visible (488–514 nm) continuous wave laser produced prompt tension reduction in vascular rings prepared from normal and atherosclerotic New Zealand White rabbit aorta\(^8\) and from human coronary arteries.\(^9\) More recent in vitro experiments demonstrated that photorelaxation of vascular smooth muscle may be achieved using pulsed laser sources such as the excimer laser.\(^10\)

The present series of experiments was designed to extend these previous in vitro observations to an in vivo model and thereby investigate the hypothesis that laser light delivered percutaneously in vivo could successfully reverse arterial spasm. The experiments were carried out in atherosclerotic swine in which reversible spasm was provoked by intra-arterial administration of histamine.\(^11\) The results of these experiments indicate that the photorelaxant properties of laser light previously demonstrated in vitro may be reproduced in vivo.

**Methods**

**Preparation of Atherosclerotic Lesions**

Ten male Yucatan microswine (Charles River Laboratories, Wilmington, Mass.) 20.3±1.9 months old were used in the present study. Healthy and vaccinated animals from a selected and controlled breeding stock were housed and handled according to a protocol that was approved by the Department of Laboratory Animal Medicine, Tufts University School of Medicine.

Atherosclerotic lesions in all 10 animals were induced using a previously described protocol,\(^12\) according to which the animals were fed an atherogenic diet for 1.25±0.6 months before and 17.2±6.9 months after percutaneous balloon endothelial denu- dation of the iliac and femoral arteries.

**Provocation of Spasm**

Each animal was parenterally premedicated with 2.5 mg/kg azaperone (Pitman Moore, Washington Crossing, N.J.) and 0.05 mg/kg atropine sulfate (Dexter, Chagrin Falls, Ohio). Anesthesia was induced and maintained with a mixture of isoflurane (1.75–2.0%) and nitrous oxide administered through an endotracheal tube; all animals were allowed to breathe spontaneously. A standard lead II ECG was monitored continuously. The left carotid artery was exposed via a ventral midline incision of the skin and cannulated with an 8F or 9F arterial sheath (Cordis, Miami, Fla.) through which an 8F or 9F guiding catheter (Schneider, Minneapolis, Minn.) was inserted. The catheter was advanced in antegrade fashion under fluoroscopic guidance into the distal abdominal aorta and then selectively into the iliac and superficial femoral arteries. Selective angiograms were obtained using meglumine diatrizoate to document baseline luminal diameter. Only arteries in which there was insignificant luminal narrowing by atherosclerotic plaque were selected for study of vascular reactivity; arteries with sites of extensive angiographic luminal narrowing, including arteries that were totally occluded, were excluded.

Reversible luminal diameter narrowing, or spasm, was provoked with a single intra-arterial injection of histamine of 100, 200, or 400 \(\mu\)g/kg in six, 15, and one artery, respectively (Tables 1 and 2). The evolution and resolution of spasm were systematically monitored by contrast angiography for as long as 30 minutes or until spasm appeared by visual inspection to have resolved. Angiograms were recorded on both 105-mm spot film (1 frame/sec) and 1-in. videotape (Grundig LDL 8740/10, Philips, Shelton, Conn.) using 10–15 ml of contrast media administered at 1-minute intervals for the first 5 minutes and then at 5-minute intervals for the subsequent 25 minutes.

**Hypothesis and Model**

The study was designed to test the hypothesis that laser light delivered percutaneously in vivo could successfully reverse arterial spasm. In vivo models of vascular spasm are limited to that described by Shimokawa et al.\(^11\) These investigators demonstrated that intra-arterial administration of histamine to swine with experimentally induced atherosclerosis produced transient vasoconstriction.

Although this animal model has been used subsequently to investigate mechanisms responsible for vascular spasm, there are to our knowledge no reports in which this model has been used to evaluate antispasmodic agents. Consequently, certain aspects of this model remain undefined, in particular, the reproducibility of spasm after serial administration of histamine to the same site in the same artery and the uninterrupted time course of histamine-induced vasoconstriction.

Accordingly, we adopted prospectively certain arbitrary definitions regarding histamine-induced
TABLE 1. Outcomes After Histamine and/or Laser Irradiation

<table>
<thead>
<tr>
<th>Case</th>
<th>Provocation</th>
<th>% DN</th>
<th>Duration</th>
<th>Provocation</th>
<th>% DN</th>
<th>Duration</th>
<th>Provocation</th>
<th>% DN</th>
<th>Duration</th>
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<tbody>
<tr>
<td>1</td>
<td>H (200 μg/kg)→</td>
<td>75.4</td>
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<td>76.0</td>
<td>5 min→</td>
<td>Laser (UV)→</td>
<td>42.3</td>
<td>4 min→</td>
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<td>2</td>
<td>H (200 μg/kg)→</td>
<td>67.0</td>
<td>30 min→</td>
<td>H (200 μg/kg)→</td>
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<td>5 min→</td>
<td>Laser (UV)→</td>
<td>23.9</td>
<td>10 min→</td>
</tr>
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<td>3</td>
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<td>50.0</td>
<td>5 min→</td>
<td>Laser (UV)→</td>
<td>9.3</td>
<td>4 min→</td>
</tr>
<tr>
<td>4</td>
<td>H (400 μg/kg)→</td>
<td>63.8</td>
<td>5 min→</td>
<td>Laser (VIS)→</td>
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<td>H (400 μg/kg)→</td>
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<td>5</td>
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<td>5 min→</td>
<td>Laser (VIS)→</td>
<td>20.0</td>
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<td>30 min</td>
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<td>Laser (VIS)→</td>
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<td>83.7</td>
<td>30 min→</td>
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<td>5 min→</td>
<td>Laser (IR)→</td>
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<td>56.0</td>
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<td>Laser (IR)→</td>
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<td>Laser (IR)→</td>
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<tr>
<td>13*</td>
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<td>57.6→</td>
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<td>16</td>
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<tr>
<td>17</td>
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<td>18</td>
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<td>32.2→</td>
<td>EXC</td>
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<td>EXC</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

DN, maximum percent luminal diameter narrowing; Duration, time during which ≥50% angiographic DN persisted; EXC, excluded; H, histamine; IR, infrared (1,064 nm); UV, ultraviolet (351 nm); VIS, visible (632 nm).

*In these three cases, %DN was judged by on-line visual inspection to be <50% at 5 min after administration of histamine; accordingly, no treatment trial was performed.

†So-called “treatment trial” (see text).

spasm in this model. The purpose of these definitions was to be confident that the temporal and spatial resolutions of spasm during laser irradiation could be attributed to the effect of laser light. Thus, requirements were adopted concerning a minimum reduction in luminal diameter narrowing (≥50%) and a minimum duration (≥5 min) for histamine-induced vasoconstriction. The former is similar to angiographic criteria adopted previously for this model, whereas the latter has remained undefined in previous experiments with this model.

To further distinguish spontaneous relaxation after histamine-induced vasoconstriction from relaxation induced by laser irradiation, we designed a protocol consisting of a preliminary control trial followed by a treatment trial. The control trial consisted of histamine administration only. To ensure that the control trial would constitute an appropriate experiment against which to judge the effects of laser irradiation, the two criteria required to be fulfilled were a minimum reduction in luminal diameter (≥50%) in response to histamine and a minimum duration (≥5 min) of histamine-induced vasoconstriction. Only if these minimum requirements were fulfilled during the control trial did we proceed to a treatment trial.

After return of angiographic luminal diameter to baseline dimensions, spasm was reproduced with a second injection of histamine delivered into the same artery (treatment trial). Furthermore, we presumed that the degree of vasoconstriction (≥50% luminal diameter narrowing) achieved during the control trial

TABLE 2. Outcomes After Histamine and/or Sham Treatment

<table>
<thead>
<tr>
<th>Case</th>
<th>Provocation</th>
<th>% DN</th>
<th>Duration</th>
<th>Provocation</th>
<th>% DN</th>
<th>Duration</th>
<th>Provocation</th>
<th>% DN</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H (100 μg/kg)→</td>
<td>66.0</td>
<td>30 min→</td>
<td>H (100 μg/kg)→</td>
<td>68.0</td>
<td>5 min→</td>
<td>Fiber/flush→</td>
<td>68.0</td>
<td>30 min*</td>
</tr>
<tr>
<td>2</td>
<td>H (200 μg/kg)→</td>
<td>70.0</td>
<td>30 min→</td>
<td>H (200 μg/kg)→</td>
<td>73.0</td>
<td>5 min→</td>
<td>Fiber/flush→</td>
<td>72.0</td>
<td>30 min*</td>
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<tr>
<td>3</td>
<td>H (200 μg/kg)→</td>
<td>72.0</td>
<td>5 min→</td>
<td>Fiber/flush→</td>
<td>68.0</td>
<td>30 min→</td>
<td>H (200 μg/kg)→</td>
<td>73.0</td>
<td>30 min</td>
</tr>
</tbody>
</table>

DN, maximum percent luminal diameter narrowing; Duration, time during which ≥50% angiographic DN persisted; EXC, excluded; H, histamine.

*Sham-equivalent so-called “treatment trial” in which the conditions of the treatment trial were repeated by using histamine to provoke spasm and then advancing the fiber into the spastic artery, instituting the saline flush, but not activating the laser. This sham treatment trial was preceded or followed by “control” trial during which spasm was provoked in the same arterial segment and monitored, in the absence of optical fiber and saline flush, for 30 min. When luminal diameter returned to the prehistamine baseline, histamine was readministered to the same artery.
TABLE 3. Laser Parameters and Angiographic Findings

<table>
<thead>
<tr>
<th>Laser parameters</th>
<th>Angiographic findings</th>
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<tbody>
<tr>
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<td>Control trial</td>
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<tr>
<td></td>
<td>Post-H (%) DN</td>
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<tr>
<td>Case</td>
<td>Wavelength (nm)</td>
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<tr>
<td>1</td>
<td>351</td>
</tr>
<tr>
<td>2</td>
<td>351</td>
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<td>3</td>
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<td>7</td>
<td>632</td>
</tr>
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<td>8</td>
<td>1,064</td>
</tr>
<tr>
<td>9</td>
<td>1,064</td>
</tr>
<tr>
<td>10</td>
<td>1,064</td>
</tr>
</tbody>
</table>

% Δ, Percent change; DN, diameter narrowing; EXP, exposure; H, histamine; Rep rate, repetition rate; Spon relax, spontaneous relaxation.

*Output was premeasured in vitro before intra-arterial delivery of optical fiber to site of spasm. For pulsed (ultraviolet or excimer) laser, premeasured fluence was 3.99 (case 1) and 2.38 (cases 2 and 3) J/cm², respectively.

must persist for a similar period (≥5 minutes) after administration of histamine during the treatment trial for interpretation of the response to laser irradiation to be considered valid. In two cases, the sequence of control trial–treatment trial was reversed to ensure that sequential administration of histamine did not bias the vasomotor response to laser irradiation. The sequential responses recorded among the 19 arteries in this investigation are listed individually in Table 1.

After spasm had been documented for 5 minutes, the arteries were irradiated with laser light. Hand injections of contrast media were repeated at 1-minute intervals during the first 5 minutes and then at 5-minute intervals until spasm appeared by visual inspection to have resolved. In certain cases, post-hoc quantitative analysis disclosed residual luminal narrowing in laser-irradiated arterial segments that was underestimated by on-line visual inspection at the point at which angiographic monitoring was interrupted (Table 1, cases 12–14 during control trial and cases 1 and 10 during treatment trial). In 17 of 19 arteries, the control provocation was performed first, and the provocation involving laser irradiation was performed second. In two cases (Table 1, cases 4 and 5), the sequence was reversed: Provocation followed by laser irradiation was performed first, and the control provocation was performed after angiographic luminal diameter had returned to baseline dimensions.

**Laser Irradiation**

Laser irradiation was generated from laser sources representing the ultraviolet (UV), visible, and infrared portions of the electromagnetic spectrum. UV light was generated from an excimer laser (model EM-52 MSC, Lambda Physik, Acton, Mass.) with a pulse duration of 12 nsec, using a mixture of XeF and helium gas to achieve an output at 351 nm; repetition rate was 25 Hz, and pulse energy varied from 3 to 5 mJ/pulse. Visible light (632 nm) was generated from a continuous wave helium neon (HeNe) laser (model 127-25, Spectra Physics, Mountain View, Calif.); output power was 17–20 mW. Infrared light (1,064 nm) was generated from a diode-pumped neodymium: yttrium aluminum garnet (Nd:YAG) diode laser (model ALC 1064-150, Amoco, Naperville, Ill.); output power was 90 mW.

In each case, the laser output beam was coupled to a fused silica optical fiber with a core diameter of 200 (Polymicro, Phoenix, Ariz.) or 400 μm (Ensign Bickford, Avon, Conn.). The optical fiber was advanced within a 5F catheter; the proximal end of the optical fiber was secured by a Touhy-Borst Y-connector so that the distal tip of the fiber protruded <1 mm beyond the tip of the catheter. The catheter-fiberoptic assembly was advanced via the guiding catheter through which injections of contrast media were delivered.

Laser parameters for each of the wavelengths used are summarized in Table 3. During laser irradiation, the laser catheter was moved into the artery with a gentle to-and-fro motion, and the distal tip of the optical fiber was hand- or pump-flushed with saline (5 ml/min). Laser irradiation was applied to sites of arterial spasm until there was angiographic evidence of improved luminal caliber as described above.

To exclude the possibility of mechanically induced relaxation resulting from the catheter-fiberoptic assembly and flush solution, sham trials were performed in three additional arteries. The sham trials reproduced each of the conditions of the treatment trial, except that laser irradiation was not used. Specifically, in each of these three arteries, histamine was administered to provoke spasm, after which the optical fiber was advanced into the spastic artery, saline flush was initiated, but the laser was not activated. In two of three arteries (Table 2, cases 1 and 2), a control provocation was performed first; after angiographic luminal diameter...
returned to baseline dimensions, the sham trial was performed. In the remaining one of the three arteries, this sequence was reversed: The sham trial was performed first, and after return of angiographic luminal diameter to baseline dimensions, the control provocation was performed.

**Quantitative Angiographic Analysis**

Angiographic assessment of luminal diameter narrowing observed in response to histamine, relaxation observed spontaneously, and relaxation observed after laser irradiation were quantified using 105-mm spot films digitized on a laser film digitizer (Laser-Scan, ImageComm, Santa Clara, Calif.). Digitizing resolution was 512×512 pixels and 8 bits per pixel over the 100-mm-square area of the film. Image files were stored on a local area network file server (Norvell, Provo, Utah), accessed by an imaging workstation (SciView, ImageComm), and then analyzed at the workstation using custom software (Quantum 2000 I, QCS, Ann Arbor, Mich.). The analyzed software provides automatic tracking of the internal aspect of the vessel wall with a relative density technique; measurement accuracy of this program has been verified. Percent luminal diameter narrowing was calculated as the quotient of [(LD1−LD2)/LD1]×100, where LD1 is baseline luminal diameter, and LD2 is luminal diameter after a given provocation.

**Histological Examination**

At the completion of each experiment, the animals were killed with a mixture of phenobarbital and phenytoin (Butanesthesia-D, Schering, Kenilworth, N.J.). The distal abdominal aorta and branch arteries were dissected free, removed en bloc, and preserved in 10% buffered formalin. The anatomic sites at which spasm had been documented in vivo were localized at necropsy by comparing the recorded angiograms with the harvested arteries using measured segment lengths in reference to various branch points.

Harvested arteries were then serially sectioned at 5-mm intervals. Each 5-mm segment was examined grossly for the degree of cross-sectional area narrowing by atherosclerotic plaque. Representative segments were cleared with xylene, impregnated with and embedded in paraffin, and cut in 4-μm intervals. Sections stained with hematoxylin and eosin and with Richardson’s elastic tissue-trichrome stain were then examined by light microscopy. Representative sections were examined morphometrically to quantify the percent of cross-sectional area narrowing by atherosclerotic plaque, using commercial software (Sigma Scan Jandel, Corte Madera, Calif.) as described previously.

**Statistical Analysis**

Data are presented as mean±SEM values. One-tailed Student’s paired t test was used to compare the angiographic outcomes observed during the control and treatment trials. A value of p=0.05 was considered to distinguish statistically significant from statistically insignificant results.

**Results**

**Control Trials**

Spasm (≥50% luminal diameter narrowing) was successfully provoked in 14 of 19 arteries (73.7%) during the control trial of histamine administration (i.e., histamine administration followed by uninterrupted evolution and resolution of angiographically documented vasoconstriction). Spasm was documented as early as 1 minute after injection of histamine. The maximum percent of angiographic luminal diameter narrowing produced among these 14 arteries was 71.3±3.0%. In three of the 14 arteries (21.4%) (Table 1, cases 12–14), percent luminal diameter narrowing elicited during the control trials was judged by on-line visual inspection to be <50% at 5 minutes after histamine administration. In one additional artery (Table 1, case 11), although spasm had been successfully provoked during the control trial, spasm could not be reelicited before intended laser irradiation. These latter four arteries as well as the five arteries in which histamine failed to provoke spasm (Table 1, cases 15–19) were therefore not subjected to laser irradiation.

**Treatment Trials**

The 10 arteries in which histamine provoked spasm persisting for ≥5 minutes during the control trial were reprovoked with histamine in preparation for laser irradiation. Histamine successfully reprovoked spasm in each of these 10 arteries. The maximum percent of angiographic luminal diameter narrowing produced in these 10 arteries in response to histamine during the treatment trial was 74.1±7.4%; this did not differ significantly from that observed in these same 10 arteries during the control trial of histamine administration (72.4±17.2%, p=0.38).

In each of these 10 arteries, percutaneous intraarterial laser irradiation produced partial or complete reversal of spasm (phoretolaxation) (Table 1 and Figure 1). Photorelaxation was typically observed first in the most proximal segment of the artery (i.e., the arterial segment immediately in contact with the distal tip of the optical fiber) (Figure 2b) and then progressed to involve the entire length of the spastic arterial segment (Figure 2c). The magnitude of improvement in percent angiographic luminal diameter for the group of 10 arteries as a whole was 53.5±18.7%. The time course of photorelaxation for these 10 arteries was such that minimum residual luminal narrowing was realized 19.5±10.9 minutes after the onset of laser irradiation. For each of the 10 arteries, the line pairs describing the magnitude and time course of spontaneous relaxation observed during the control trial versus laser-induced relaxation observed during the treatment trial are shown in Figure 1.
ULTRAVIOLET (351 nm)

Case No. 1

Control

Laser

Time (min)

Luminal diameter narrowing (%)

Case No. 2

Control

Laser

Time (min)

Luminal diameter narrowing (%)

Case No. 3

Control

Laser

Time (min)

Luminal diameter narrowing (%)

VISIBLE (632 nm)

Case No. 4

Control

Laser

Time (min)

Luminal diameter narrowing (%)

Case No. 5

Control

Laser

Time (min)

Luminal diameter narrowing (%)

Case No. 6

Control

Laser

Time (min)

Luminal diameter narrowing (%)

Case No. 7

Control

Laser

Time (min)

Luminal diameter narrowing (%)

INFRARED (1064 nm)

Case No. 8

Control

Laser

Time (min)

Luminal diameter narrowing (%)

Case No. 9

Control

Laser

Time (min)

Luminal diameter narrowing (%)

Case No. 10

Control

Laser

Time (min)

Luminal diameter narrowing (%)

FIGURE 1. Magnitude and time courses of spontaneous relaxation observed during control trial versus laser-induced relaxation observed during treatment trial for each of the 10 arteries in the present study. Percent angiographic luminal diameter narrowing is shown in each case on the ordinate; time course of relaxation is shown on the abscissa. In each case, repeated angiographic measurements were recorded systematically up to 30 minutes or until spasm appeared by visual inspection to have resolved. Post-hoc quantitative angiographic analysis disclosed varying degrees of residual luminal narrowing in laser-irradiated arteries that were not appreciated or underestimated by on-line visual inspection at the point at which angiographic monitoring was interrupted.
responses are also listed numerically in Tables 1 and 2. The results of laser irradiation are discussed as a function of the wavelength of laser light.

Treatment Trials

*UV laser irradiation.* Among the 10 arteries in which percutaneous laser-induced photorelaxation was attempted, the source of laser irradiation involved UV light in three (Figure 1, cases 1–3; Figures 2 and 3). Maximum percent luminal narrowing induced by histamine before laser irradiation in the treatment trials (75.3±25.0%) was similar to that observed during control trials (72.8±8.8%, p=0.45) for these three arteries. Laser irradiation reduced the percent angiographic luminal diameter narrowing from 76.0% to 42.3%, 100% to 23.9%, and 50.0% to 9.3%, respectively. The magnitude of improvement in percent angiographic luminal diameter was 50.2±22.7%, significantly greater than the magnitude of relaxation observed spontaneously in these three arteries during the control trials (10.9±9.8%, p=0.02). The onset of angiographically apparent increase in luminal diameter (i.e., photorelaxation) in response to laser irradiation was observed 1.3±0.3 minutes after the onset of laser irradiation.

*Visible laser irradiation.* Visible (632 nm) laser irradiation was used to treat four arteries (Figure 1, cases 4–7; Figure 4). Maximum percent luminal diameter narrowing documented in response to histamine in the control trials (75.3±4.1) was similar to that observed before laser irradiation in the treatment trials (68.5±4.9%, p=0.15) for these four arteries. In two of the four arteries narrowed by 63.8% and 76.8% in luminal diameter, continuous wave visible laser irradiation accomplished complete reversal of histamine-induced spasm. In the remaining two arteries, visible laser light reduced the percent angiographic luminal diameter narrowing from 57.0% to 20.0% and from 76.5% to 30.8%, respectively. The magnitude of improvement in percent angiographic luminal diameter was 55.8±17.9%, which was significantly greater than the magnitude of relaxation observed spontaneously during the control trials (0.9±1.9%, p=0.01). The onset of angiographically apparent increase in luminal diameter in response to laser irradiation was observed 7.7±3.0 minutes after the start of laser irradiation.

*Infrared laser irradiation.* Infrared (1,064 nm) laser irradiation was used to treat three arteries (Figure 1, cases 8–10; Figure 5). Maximum percent luminal diameter narrowing documented in response to histamine in the control trials (74.8±22.7%) was similar to that observed before laser irradiation in the treatment trials (72.5±9.7%, p=0.40) for these three arteries. Infrared laser light reduced percent angiographic luminal diameter narrowing from 100% to 21.4%, 56.0% to 8.7%, and 68.3% to 35.3%, respectively. The magnitude of improvement in percent angiographic luminal diameter was 53.0±23.3%, significantly greater than the magnitude of relaxation observed spontaneously during the control trials (12.9±10.7%, p=0.01). The onset of angiographically apparent increase in luminal diameter in response to

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**Figure 2.** Angiograms of excimer-laser induced photorelaxation (case 3). Serial angiograms were recorded (panel a) before laser irradiation, showing extent of histamine-induced vasospasm, and (panel b) after 1 minute and (panel c) 4 minutes of excimer (ultraviolet, 351 nm, 3 ml/pulse, and 25 Hz) laser irradiation.
laser irradiation was observed 5.3±4.5 minutes after the beginning of laser irradiation.

Temporal Analysis of Response to UV, Visible, and Infrared Laser Irradiation

Figure 6 illustrates the mean values for the vaso-motor responses illustrated individually in Figure 1. Laser-induced reduction of histamine-induced spasm was achieved most expeditiously with UV light.

Sham Trials

In three additional arteries, the effect of 30 minutes of sham treatment on spasm induced by administration of histamine was compared with the effect of 30 minutes of no treatment of histamine-induced spasm (standard control trial). In each of these three paired trials, sham treatment consisted of advancing the fiberoptic into the spastic artery, instituting the saline flush, but not activating the laser. In each of these three cases, sham treatment failed to produce relaxation of the spastic segment (Figure 7 and Table 2). Luminal diameter of the spastic segment of artery was 72% versus 73%, 68% versus 68%, and 73% versus 68% (30 minutes of sham treatment versus a 30-minute control).

Histological Examination

Histological examination of arterial segments in which spasm had been provoked confirmed the presence of cross-sectional area narrowing by atherosclerotic plaque in representative 5-mm sections examined from each of the arterial segments. The composition of plaque in these segments was typical of that described previously for this model.12 There was no demonstrable correlation between the magnitude of histological cross-sectional area narrowing and either the magnitude of spasm induced by histamine or the ability of laser light to induce relaxation. No arterial segments were totally occluded, and none contained antemortem thrombus. Finally, no histological section disclosed signs of laser-induced pathological alterations.

Discussion

Previous experiments performed in our laboratory have demonstrated that when laser light is used to irradiate isolated rings of vascular smooth muscle in vitro, the observed vaso-motor response represents the net effect of heat and light on vascular smooth muscle tone. Both visible7 and near-infrared14 continuous wave laser irradiation, for example, when applied at power levels in excess of 1.0 W, consistently produces an increase in ring tension, or vasoconstriction. Measurements of tissue temperature recorded simultaneously with recorded tension have consistently demonstrated that such augmented tension is associated with significant tissue heating. In contrast, the observed vaso-motor response to continuous wave laser irradiation applied in vitro at powers of less than 0.1 W is consistently limited to a reduction in ring tension, or relaxation. This outcome was initially documented in vitro for laser wavelengths in the visible portion (454–514 nm) of the spectrum.7 Subsequently, an identical response was achieved using longer wavelengths (632 and 1,064 nm) from lasers typically considered to be low level with regard to power, namely, HeNe15 and diode-pumped Nd:YAG lasers (see “Appendix”). In all such cases, simultaneous temperature recording documented that the laser-induced reduction in vaso-motor tone was unaccompanied by a significant increase in tissue temperature. These findings were interpreted to reflect the preeminent effect of light unopposed by heat on vascular smooth muscle.

Figure 3. Angiograms of excimer-laser induced photorelaxation (case 2). Panel a: Prelaser irradiation shows histamine-induced occlusive spasm (arrow). Panel b: After 3 minutes of excimer (ultraviolet, 351 nm, 3 mJ/pulse, and 25 Hz) laser irradiation, luminal patency has been restored; further improvement in luminal caliber is observed after 10 minutes of cumulative laser irradiation (panel c).
Laser-induced vasorelaxation has also been achieved in vitro with higher laser outputs by delivering energy from the laser in a pulsed mode\textsuperscript{10}; at a suitable frequency (typically <100 Hz), the pulse interval between each laser exposure is sufficient to prevent heat generated by laser–tissue interaction from exceeding that tissue’s thermal relaxation time.\textsuperscript{16} The pulsed laser source used initially for such experiments was the excimer laser, which generates ultraviolet radiation; both 308- and 351-nm wavelengths yielded reproducible relaxation of isolated rings of rabbit thoracic aorta over ranges of 0.8–5.5 J/cm\textsuperscript{2}, 10–50 Hz, and cumulative exposures of 10–120 seconds. Tissue temperature profiles recorded simultaneously during these experiments documented that tissue temperature increase did not exceed 5°C during these experiments. The magnitude of relaxation increased with increases in both repetition rate and pulse energy, up to 50 Hz and 5 mJ per pulse, respectively. Although most of these in vitro experiments were performed using isolated rings of rabbit aorta, identical vasomotor responses have been observed in segments of human coronary artery freshly harvested from explanted hearts at the time of cardiac transplantation.\textsuperscript{9}

The present series of experiments was initiated to determine if these previous demonstrations of laser-induced relaxation, accomplished in vitro, could be reproduced in vivo. There were at least two principal reasons to question whether this could be accomplished. First, how did the magnitude of relaxation observed in vitro under conditions of optimized length–active tension translate into angiographically appreciable vasodilatation? The second involved successful transmission of relatively low levels of laser energy through an undiluted flowing blood field without constant fiber–tissue contact or a guaranteed angle of incidence between laser radiation and the target tissue.

With regard to the first issue, we were encouraged by previous investigations performed in our laboratory in which vasoconstriction produced in vitro after continuous wave laser irradiation was reproduced in vivo in two species of normal and atherosclerotic animals\textsuperscript{17}; these findings suggested that the magnitude of vasoconstriction observed in vitro was meaningful in terms of vasoconstriction that could be appreciated angiographically in vivo. Furthermore, Yamamoto et al\textsuperscript{18} described direct evidence for an angiographic in vivo correlate to vasoconstriction measured in vitro in atherosclerotic swine in which the angiographic response to histamine was evaluated in vivo, by angiography, and then after death, in vitro, by recording tension in vascular ring isolated from the same arterial segment.

With regard to the issue of wavelength transmission in a blood field, we were similarly encouraged by previous studies from our laboratory that had demonstrated that even the highly absorbed UV wavelengths transmitted from the excimer laser could penetrate an undiluted blood column up to 3 mm deep.\textsuperscript{19}

The findings in the present series of experiments confirmed that the previous in vitro observations could be reproduced in vivo. Not only did laser light lead to complete and/or partial resolution of histamine-induced spasm, but this was accomplished percutaneously in an intact circulation of undiluted blood. Furthermore, relaxation was achieved with laser wavelengths from the infrared and visible as well as UV portions of the spectrum. In a series of elegant experiments designed to evaluate the “action spectrum” of light on vascular smooth muscle, Furchgott et al\textsuperscript{5,6} demonstrated that UV and visible

**FIGURE 4.** Angiograms recorded before and after HeNe laser irradiation in case 6. Panel a: Prelaser angiogram discloses mild, focal histamine-induced spasm at origin of left internal iliac artery and more severe vasospasm distally. Panel b: After HeNe (visible, 632 nm) laser irradiation (20 mW), spasm virtually resolved.
light could produce relaxation of pharmacologically precontracted vascular strips. An initial peak for light-induced relaxation was observed at 250 nm, followed by a trough at 280 nm, a second peak at 310 nm, and a progressive decline in relaxation through 450 nm; at the remaining visible wavelengths, relaxation was minimal.

In contrast, as indicated in the previously reported in vitro experiments\(^\text{15}\) and the present in vivo trials, relaxation was achieved with visible and infrared wavelengths as well. The basis for these differing outcomes is probably the light source used. Furchgott et al performed their experiments with a xenon arc lamp and used a grating monochromator to select out individual wavelengths. Because such a broad-band light source produces illumination that is relatively weak and because the intensity is further diminished by filtering through the monochromator, the resultant intensities achieved were calculated to range from \(1.0 \times 10^{-5}\) to only \(3.5 \times 10^{-6}\) W/cm\(^2\). The fact that energy and power available from lasers used in the present series of experiments was substantially in excess of that generated by a xenon arc lamp probably explains the observation that relaxation could be accomplished with wavelengths of 632 and 1,064 nm.

It is perhaps noteworthy that laser-induced reduction of histamine-induced spasm was achieved most expeditiously with UV light. Previous in vitro experiments\(^\text{10,14,15}\) have consistently also demonstrated this finding. This observation may relate in part to the mechanisms underlying photorelaxation of vascular smooth muscle. Furchgott et al\(^\text{10}\) proposed that photorelaxation resulted from activation of "some endogenous photosensitive material" present in the aortic strip and compared the relaxing effect of light on vascular smooth muscle with "relaxation produced by the chemical relaxing agent NaNO\(_2\)." Subsequent investigations of this issue with broad-band light by others\(^\text{20–22}\) and with laser light in our laboratory,\(^\text{7,10}\) however, have

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**Figure 5.** Angiograms recorded before, during, and after laser (Nd:YAG diode) irradiation in case 9. Prelaser angiogram (panel a) shows occlusive spasm (arrow) of the left external iliac artery induced by intra-arterial administration of histamine. Panel b: Angiogram recorded 10 minutes after infrared (1,064 nm, 90 mW) laser irradiation shows improved luminal patency in proximal segment of artery with persistent subtotal occlusion distally. Panel c: Angiogram recorded after an additional 5 minutes of laser irradiation (same parameters) shows further improvement in luminal caliber.

**Figure 6.** Plot of analysis of temporal response of histamine-induced spasm to laser irradiation. Numbers plotted represent mean values of responses illustrated individually in Figure 1. Laser-induced reduction of histamine-induced spasm was achieved most expeditiously with ultraviolet light.
FIGURE 7. Representative angiographic results of sham treatment trial. Baseline angiogram (panel a) shows luminal caliber of right internal iliac artery before histamine administration. Panel b: Magnitude of histamine-induced spasm. Panel c: The histamine-induced spasm in right internal iliac artery persists despite 5 minutes of sham treatment (fiberoptic advanced into the artery, saline flush initiated, but laser not activated). Panel d: At 30 minutes, histamine-induced spasm persists, despite sham treatment with fiberoptic and saline flush. Open white arrow indicates the 8F guiding catheter in the proximal right internal iliac artery. Closed white arrow denotes the radiopaque marker tip of the SF catheter containing the optical fiber.

disclosed no evidence that photorelaxation results from activation of known endothelium-generated relaxing factors; anatomically and functionally confirmed purposeful endothelial denudation failed to alter the vector or magnitude of light (including laser)-induced relaxation.

There is preliminary evidence, however, that at least one mechanism for light-induced relaxation of vascular smooth muscle may involve activation of an intracellular "endogenous photosensitive material." Preliminary studies of isolated vascular strips have demonstrated that UV irradiation from nonlaser sources produces a transient rise in tissue levels of cyclic GMP (cGMP) that is associated with increased activity of guanylate cyclase. This finding is particularly intriguing in view of the facts that guanylate cyclase contains stoichiometric amounts of heme, the wavelengths of light with which increased activity of guanylate cyclase has been reported parallel the absorption spectrum of heme, and photorelaxation of vascular smooth muscle appears to be optimized at wavelengths in the region of peak absorption described for both guanylate cyclase and heme.

These findings taken in toto suggest that the heme moiety of guanylate cyclase may serve as a chromophore to promote absorption of laser light, thereby activating guanylate cyclase and resulting in the production of cGMP and relaxation of vascular smooth muscle. Alterations in levels of intracellular cGMP and/or activity of guanylate cyclase after irra-
diation with the visible (632 nm) and infrared (1,064 nm) wavelengths used in the present in vivo study have not been previously reported. Accordingly, it remains to be determined whether this hypothesis satisfactorily explains photorelaxation of vascular smooth muscle achieved using these wavelengths at the higher fluences typical of laser irradiation.

Finally, the demonstration that laser-induced reversal of arterial spasm can be successfully accomplished in vivo may have potential implications for clinical therapy. At least three different excimer laser systems are currently undergoing clinical trials as ablative therapy for coronary and peripheral atherosclerotic stenoses; occasional cases of spasm have been observed with all three systems.22–26 The etiology of spasm in such cases is unclear, but it may be due to mechanical trauma from the catheter delivery system or excessive cumulative exposure to suprablation threshold energy levels that ultimately overwhelm the thermal relaxation time of the irradiated arterial segment.30 Whatever the cause, use of a pulsed laser source creates the theoretical option to treat the spastic segment directly with subablation energy levels, particularly when such spasm is resistant to pharmacological therapy. We have used this strategy successfully to reverse severe focal spasm (refractory to intra-arterial nitroglycerin) that developed during percutaneous, in vivo excimer laser angioplasty in an atherosclerotic swine model by switching from supra-ablation outputs to subablation parameters.15 Because lasers that emit invisible wavelengths are typically constructed with an adjunctive aiming beam of visible light, there exists the possibility that coirradiation with appropriate output levels of such visible light might provide a “background cover” of relaxation that could serve to optimize luminal cross-sectional area during laser-based interventions. Because vascular spasm has also been observed to complicate a variety of percutaneous interventions including balloon angioplasty,31 fiber-based low-level laser irradiation may constitute a potential alternative therapy for selected cases in which spasm is clinically significant and pharmacologically resistant.

Appendix

Previous research reported in abstract form by Chokshi et al15 described corroboration of laser-induced reduction of vasomotor tone (relaxation) using a longer (632 nm) visible wavelength than that used in our initial in vitro experiments (488–514 nm).7 The presentation of this abstract at the Annual Scientific Sessions of the American Heart Association (New Orleans, La., November 13, 1989) included description of similar in vitro experiments performed using infrared (1,064 nm) wavelengths as well. Because these experiments were performed subsequent to submission of the abstract, however, they were not included in the published text15 and have not yet been published elsewhere. Because these in vitro experiments represent the basis for the use of the infrared wavelengths used in the present study, we include here a representative recording from these experiments; shown in Figure 8 is an example of infrared laser–induced relaxation.

It is noteworthy that this was accomplished using 1,064 nm generated from a Nd: YAG pumped diode laser. At the power used to induce vasomotor relaxation, no significant increase in tissue temperature was observed. In contrast, the failure of previous attempts to induce vasomotor relaxation with infrared laser wavelengths may have resulted from the fact that it was not possible to accurately reduce the output of these generators below outputs associated with a significant increase in tissue temperature. Nevertheless, the output of this relatively low-energy diode laser source still exceeded by two orders of magnitude the light source used by Furchgott et al.6

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References


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