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Application of the 1-μsec pulsed-dye laser to the treatment of experimental cerebral vasospasm

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✓ Laser energy of 480 nm was applied in 1-μsec pulses varying between 2.2 and 10 mJ to *in vitro* and *in vivo* models of cerebral vasospasm. First, the pulsed-dye laser was applied intravascularly via a 320-μm fiber to basilar artery segments from six dogs. The segments were mounted in a vessel-perfusion apparatus and constricted to, on average, 70% of resting diameter by superfusion with dog hemolysate. Immediate increase in basilar artery diameter occurred to a mean of 83% of control. In a second model, the basilar artery was exposed transclivally in the rabbit. In three normal animals, superfusion of the artery with rabbit hemolysate resulted in a reduction of mean vessel diameter to 81% of control. Following extravascular application of the laser, vessels returned to an average of 106% of the resting state. In six rabbits, the basilar artery was constricted by two intracisternal injections of autologous blood, 3 days apart. Two to 4 days after the second injection, the basilar artery was exposed. Extravascular laser treatment from a quartz fiber placed perpendicular to the vessel adventitia resulted in an immediate 53% average increase in caliber to an estimated 107% of control. No reconstriction was observed over a period of up to 5 hours. Morphologically, damage to the arterial wall was slight. This preliminary investigation suggests that the 1-μsec pulsed-dye laser may be of benefit in the treatment of cerebral vasospasm.

KEY WORDS • **pulsed-dye laser** • **basilar artery** • **subarachnoid hemorrhage** • **vasospasm** • **laser** • **dog** • **rabbit**

PULSED-DYE lasers have been utilized in various medical applications because their wavelength flexibility allows tuning to specific chromophores within target tissues. Furthermore, laser pulses in the microsecond range are generally short enough that thermal energy does not diffuse to surrounding structures.[1] This laser was first used clinically in the treatment of port-wine stains, because 577 nm light is absorbed preferentially by hemoglobin, resulting in minimal damage to surrounding tissue and, therefore, little postoperative scarring.[2,8] Recently, the pulsed-dye laser has been shown to have applications for the fragmentation of urinary calculi,[19] the ablation of atheromatous plaques,[14,15] and thrombolysis following experimental acute myocardial infarction.[11]

The vasodilatory effect of pulsed-dye lasers was first reported from this hospital by Gregory, *et al.*,[10] in 1988. During an investigation of the ability of lasers to cause vasoconstriction, it was noted that intravascular delivery of low pulse energies induced prolonged dilatation in rabbit femoral arteries which had been constricted acutely by serotonin. In this study, we examine the potential utility of dye lasers in the treatment of cerebral vasospasm.

Materials and Methods

Application to Dog Basilar Artery In Vitro

The basilar artery was excised from six adult mongrel dogs sacrificed by exsanguination under thiamylal sodium anesthesia. The vessel was cannulated at each end on blunt No. 19 needles, and side branches were ligated with 10-0 nylon suture. The artery was mounted in an *in vitro* vessel-perfusion apparatus and perfused luminally at a constant pressure of 120 mm Hg with modified Krebs-Henseleit physiological saline (PSS).[13] The PSS

contained (mM): NaCl 118; KCl 4.1; NaH_2PO_4 1.54; $NaHCO_3$ 24.9; $MgSO_4$ 1.19; $CaCl_2$ 2.54; and glucose 5. The perfusate was kept at 37°C in a water bath and was maintained at pH 7.4 by bubbling with 5% CO_2/20% O_2/balance N_2 gas. A fused silica quartz fiber 320 μm in diameter with a fire-polished tip was inserted into the lumen of the artery. Autologous erythrocytes were prepared from anticoagulated whole blood by centrifugation at 150 G for 8 to 10 minutes and washed three times in saline by similar centrifugation. Intact autologous erythrocytes were added to the luminal perfusate to obtain a hematocrit of 40%. The vessel was superfused simultaneously with PSS. After a 20- to 30-minute period of equilibration, the resting diameter was measured (see below).

Washed autologous erythrocytes were lysed by brief ultrasonication and centrifuged at 1600 G for 5 minutes to remove cell debris. Supernatant soluble hemolysate was diluted into PSS to a final hemoglobin concentration equivalent to 0.5 optical density units at 575 nm (approximately 10^{-5} M). When superfusate containing hemolysate was applied to the vessel, acute vasoconstriction resulted. After a 20- to 30-minute interval to allow stabilization, vessel caliber was again measured. Laser light (at a wavelength of 480 nm, a pulse duration of 1 μsec, pulse energies between 2.5 and 10 mJ, and at a repetition rate of 1 to 2 Hz) was applied coaxially within the lumen of the vessel through the quartz fiber from a pulsed-dye laser.* Pulse to pulse variation was less than 10%.

The pulse energy delivered in early experiments was varied in order to optimize the laser parameters. If no dilatation was observed within 20 pulses, laser energy was increased. If, however, dilatation had occurred but was slight, additional pulses were administered at the same energy. In two experiments, laser energy was increased further after dilatation had occurred until perforation resulted. In the remainder, the vessel was observed for up to 5 hours after treatment. At the conclusion of the study the basilar artery was perfused *in situ* with buffered 1.5% glutaraldehyde and removed for morphological examination.

Application to Rabbit Basilar Artery In Vivo

New Zealand White rabbits of either sex, weighing 2.5 to 3.5 kg, were divided into two groups. Group I comprised three rabbits with no treatment prior to the acute study and Group II consisted of 13 rabbits subjected to a "two-hemorrhage" subarachnoid hemorrhage (SAH) protocol.[3] Anesthesia was induced with 2% to 4% halothane inhalation and intramuscular ketamine hydrochloride, 40 to 60 mg/kg. After endotracheal intubation, anesthesia was maintained with 1% to 2% halothane. The occipito-nuchal area was shaved, and the head acutely flexed. A No. 21 needle was inserted percutaneously into the cisterna magna, and 2 ml of autologous nonheparinized arterial blood was injected into the basal cistern. The needle was withdrawn immediately, and the animal was tilted 15° head-down for 20 minutes. Three days later a second SAH was induced in a manner similar to the first.

In both groups, the basilar artery was exposed transclivally using the operating microscope. Anesthesia was induced as before. Following endotracheal intubation, anesthesia was maintained with pancuronium bromide 0.25 to 0.5 mg/kg/hr, and a gas mixture of 1% halothane/70% NO_2/balance oxygen. Ventilation was maintained with a small-animal dual-phase respirator. End-tidal CO_2 was maintained between 38 and 42 mm Hg and arterial blood pressure was monitored continuously.† The animal was placed supine in a stereotactic frame. Through a ventral midline incision, the trachea and pharynx were displaced laterally. A clivectomy of approximately 8 × 15 mm was made using a dental drill cooled with saline. The dura was opened, and the vascular branch to the dura from the basilar artery was coagulated with bipolar cautery. In Group II animals, subarachnoid clot was removed by gentle suction with irrigation using warm Ringer's solution and the resting vessel diameter was measured. In Group I animals, the basilar artery was constricted by superfusion with lysed autologous red blood cells prepared in the same manner as for the canine studies. The three Group I rabbits (acute constriction) and eight animals from Group II (double SAH) received laser treatment. The remaining five SAH rabbits served as controls.

The 320-μm quartz laser fiber was mounted on a stereotactic manipulator, and was positioned perpendicularly to within 1 mm of the vessel wall. The field was filled with saline. Laser energy of 2.2 to 5 mJ/pulse was applied at a repetition rate of 1 to 2 Hz, in trains of 20 pulses until dilatation occurred. After laser treatment, or sham laser application, the vessel was observed for up to 5 hours, and measurements of vessel caliber were obtained repeatedly. The rabbits were sacrificed by exsanguination and were perfused through the ascending aorta with buffered 1.5% glutaraldehyde solution. The basilar artery was excised for morphological examination.

The vessel caliber in all experiments was measured from enlarged images captured by a 35-mm camera attached to the operating microscope. A mean was obtained of 20 measurements made by one experimenter and two observers blind to the procedure.

Results

Intravascular or extravascular application of the pulsed-dye laser resulted in vessel dilatation in all ex-

* Pulsed-dye laser manufactured by Dymed Spectrum, Series 3010, Dymed Corp., Marlborough, Massachusetts.

† Accucap CO_2 monitor manufactured by Datascope Corp., Paramus, New Jersey; blood pressure monitor, Model 150PC, manufactured by Micro Switch Division Honeywell Corp., Freeport, Illinois.

TABLE 1

*Pulsed-dye laser parameters inducing dilatation in dog and rabbit basilar artery**

Animal No.	Pulse Energy (mJ)	No. of Pulses	Outcome
dog basilar artery *in vitro*			
1	2.5	22	57% dilatation
2	10.0	25	19% dilatation
	15.0	16	perforation
3	5.0	10	29% dilatation
4	7.0	20	5% dilatation
5	10.0	20	14% dilatation
6	7.5	20	7% dilatation (focal)
	10.0	20	perforation
rabbit Group I			
1	2.2	80	30% dilatation
2	2.5	100	13% dilatation
3 proximal	5.0	20	focal dilatation
distal	5.0	20	52% dilatation
rabbit Group II			
1	2.5	60	45% dilatation
2 proximal	5.0	20	focal dilatation
distal	5.0	20	85% dilatation
3	5.0	20	84% dilatation
4	5.0	20	29% dilatation
5	5.0	20	27% dilatation
6	5.0	60	44% dilatation

* Experiments were conducted *in vitro* on dog basilar arteries and *in vivo* on rabbit basilar arteries. The 1-μsec pulsed-dye laser was used at a 480-nm wavelength.

periments. There was good agreement between vessel diameter measurements made by the three observers.

Dog Basilar Artery In Vitro

The mean resting vessel diameter after equilibration, at a constant perfusion pressure of 120 mm Hg, was 1.1 mm (range 1.01 to 1.26 mm). Superfusion with dilute hemolysate resulted in vasoconstriction to 70% of the control diameter (range 55% to 84%). Intraluminal laser energy of 2.5 to 10 mJ/pulse resulted in a mean diameter increase to 83% of control (range 75% to 88%). Between 10 and 25 pulses were required to induce dilatation. The parameters used in each experiment are shown in Table 1. With each pulse, dilatation was observed to progress away from the ball tip bidirectionally, in a stepwise fashion, until the entire segment was enlarged (Fig. 1). No reconstriction of the vessel was seen for up to 5 hours after treatment. In two experiments, vasodilatation was observed using energies of 7.5 to 10 mJ, but was not uniform throughout the whole vessel segment. The position of the quartz fiber was left unchanged, but laser energy was increased to 10 and 15 mJ, respectively. In both cases, the higher energy caused perforation.

Rabbit Basilar Artery In Vivo

Group I. Application of hemolysate to normal basilar arteries resulted in a mean constriction to 81% of the control diameter (range 75% to 90%). Extraluminal application of 20 to 100 pulses at an energy of 2.2 to 5 mJ/pulse resulted in a mean increase of vessel diameter to 106% of control (range 96% to 125%). Dilatation persisted throughout the 5-hour observation period after treatment.

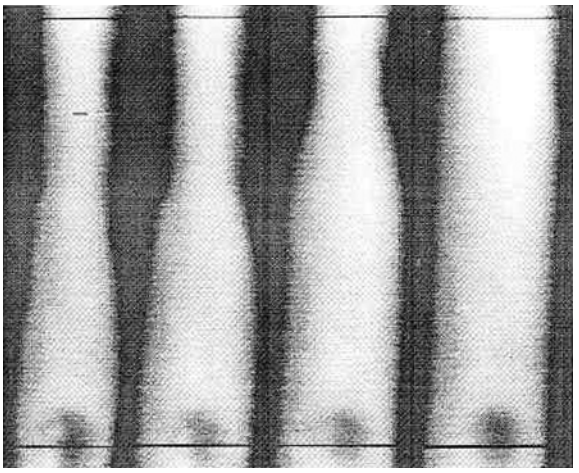

FIG. 1. Computer reconstruction of a videotape image showing the effect of consecutive intravascular laser pulses on a dog basilar artery mounted in a vessel-perfusion apparatus, and constricted with hemolysate. With each 1-μsec pulse, dilatation proceeds in stepwise fashion away from the ball tip.

Group II. All basilar arteries subjected to "two-hemorrhage" SAH developed vasospasm. Although the control basilar artery diameter was not measured in these animals, extrapolation from control measurements in Group I animals, together with previous studies from our laboratory using this model,[3] suggests that caliber was reduced to approximately 70% of baseline. Eight animals received laser application consisting of 20 pulses at an energy of 2.5 or 5/mJ pulse. In two, the fiber was placed less than 0.5 mm from the artery and, during treatment, the expanding vessel became impaled on the fiber resulting in perforation. In the remaining six, a mean increase of 53% in basilar artery diameter was observed (range 29% to 85%; 107% of control diameter). In four of the six animals, the entire length of basilar artery in the surgical field dilated uniformly after laser application at a single focus (Fig. 2). In the remaining two, dilatation was localized to within a few millimeters of the site of delivery. When laser treatment was repeated at a second point, uniform enlargement of the exposed basilar artery segment was obtained. No recurrence of spasm was observed in any animal for up to 5 hours after treatment (Fig. 3). Laser parameters required to induce dilatation are given in Table 1. In the control group, vasodilatation was not observed during the 5-hour observation period following evacuation of the subarachnoid clot alone and sham laser treatment.

Histological Findings. Vessels from all experiments were subjected to light and electron microscopic examination. Light microscopy of paraffin-embedded

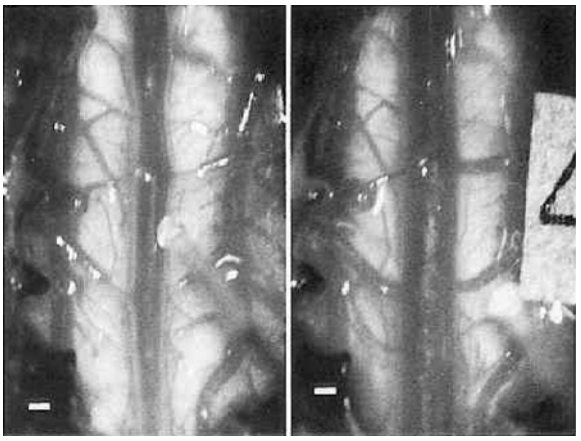

FIG. 2. Transclival exposures of a Group II rabbit basilar artery constricted by two intracisternal injections of autologous blood, 3 days apart. *Left:* Constricted vessel after removal of subarachnoid clot. The laser fiber has been positioned over the midpoint of the artery. *Right:* Immediately after delivery of 20 pulses at 5 mJ/pulse. Scale bar = 0.5 mm.

transverse sections stained with hematoxylin and eosin did not show morphological damage to nonperforated laser-treated vessels, as compared with controls. Likewise, scanning electron microscopy revealed no loss of endothelial cells (Fig. 3). By transmission electron microscopy, endothelial and smooth-muscle cells remained intact, although the former showed some irregularity of the luminal surface (Fig. 4), which was not present in control vessels.

Discussion

Analysis of Results

Results from the *in vitro* and *in vivo* studies indicate that 1-μsec pulses at energies of 2.5 to 10 mJ/pulse from a 480-nm pulsed-dye laser are able to dilate cerebral arteries constricted either acutely by the application of hemolysate or chronically by experimental SAH. Delivery of either intra- or extravascular laser energy fiberoptically can cause vasodilatation, although the effect is more focal with extravascular application. The degree of dilatation appears to be related to the pulse energy, the number of pulses delivered, and the distance from the fiber tip to the arterial wall.

The pharmacological responsiveness of a small number of vessels was studied *in vitro* after laser treatment. In the vessels examined, the response to serotonin and substance P was preserved. Histologically, only minimal damage to the arterial wall resulted from pulsed-dye laser treatment at low energies. Signs of thermal injury, such as pyknosis of endothelial nuclei or focal stripping of endothelial cells with adhesion of altered erythrocyte masses,[9] were not observed in our specimens. In the two vessels that perforated after intravascular delivery of energies of 10 mJ or greater, disruption of the endothelial and medial layers was accompanied by intramural hemorrhage, vacuolation, and scattered carbonized tissue debris.

It remains uncertain whether the perforations resulting from extravascular laser treatment were the result of mechanical injury by the quartz fiber or due to a laser-blood effect. However, the lack of thermal damage is more suggestive of the former. Furthermore, because a more distant placement of fiber from the artery provided good dilatation without perforation, there appears to be no need for very close apposition between the fiber and the vessel.

Potential Hazards

In this preliminary study, the difference between the energy required to cause dilatation and that which

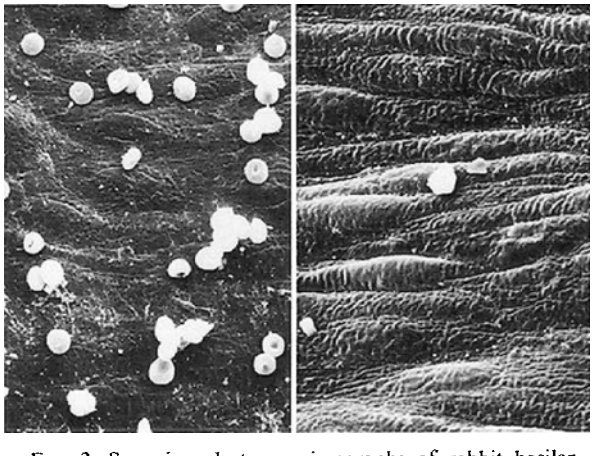

FIG. 3. Scanning electron micrographs of rabbit basilar artery segments. × 1500. *Left:* Normal basilar artery. *Right:* Basilar artery after delivery *in vitro* of 25 intravascular pulses at 10 mJ/pulse. No loss of endothelial cells is seen.

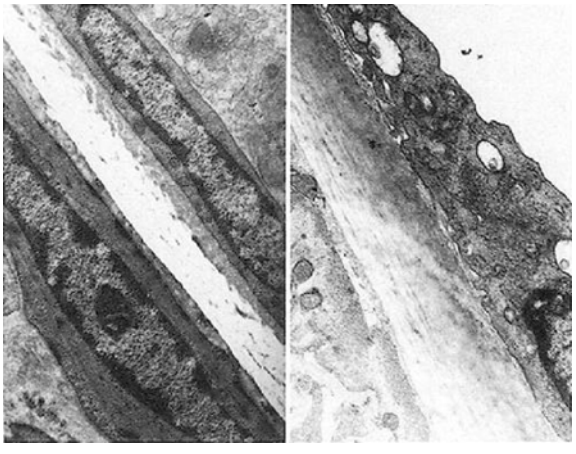

FIG. 4. Transmission electron micrographs of rabbit basilar artery segments. × 2000. *Left:* Normal basilar artery. *Right:* Results of extravascular laser treatment in a vessel subjected to double subarachnoid hemorrhage. Although intact, there is irregularity of the surface of endothelial cells.

caused perforation was relatively small. Yet the ability of the laser to restore the basilar artery to its prespasm caliber in most experiments, while causing only slight damage to the vessel wall (equivalent to or less than that caused by balloon angioplasty), indicates that a potential benefit for laser treatment of cerebral vasospasm may exist. An understanding of the mechanism or mechanisms of dilatation may enable laser parameters to be optimized in order to reduce the risk of vessel damage. In this regard, the distance from the laser fiber to the vessel wall is probably of particular importance. The laser pulse duration and pulse width used in this series are less than the thermal relaxation time of arterial tissue, and this may explain the absence of thermal injury to the vessels studied.

Although not observed in this study, a second potential hazard following laser therapy is that of arterial thrombosis. This may result from a combination of direct injury to the arterial wall, and the activation of clotting cascades.[1,12] Interestingly, however, the same laser is being employed currently at an identical energy for the removal of arterial thrombi.[11]

Mechanism of Dilatation

The mechanism of dilatation is at present unclear. Light-induced relaxation of vascular smooth muscle was first observed 35 years age by Furchgott, *et al.*,[5,6] and is related both to the duration and wavelength of irradiation. More recently, pulsed ultraviolet irradiation of high intensities from an Excimer laser has been shown to produce endothelium-independent relaxation of vascular smooth muscle in rabbit aorta.[17] Dilatation in this phenomenon is also wavelength- and time-dependent.[6,16] Possible mechanisms include an alteration in membrane permeability of smooth muscle resulting in an inability to maintain tone,[4] an alteration to the disulfide-sulfhydryl configuration of the cell membrane,[18] or a direct conformational alteration in contractile proteins induced by absorption of photons.[17]

It is extremely unlikely that an action of light on vascular smooth muscle is responsible for the dilatation observed in our study. Unlike the experience of Steg, *et al.*,[17] with the ultraviolet Excimer laser, dilatation with the pulsed-dye laser occurred immediately, required only 1-μsec exposures, and persisted for at least 5 hours after irradiation. Nor was it necessary to align the fiber perpendicular to the endothelium in order to obtain a response. The most likely mechanisms of action include a direct mechanical effect on the vessel wall caused by the rapid expansion and/or subsequent collapse of a cavitation bubble produced by rapid energy deposition or erythrocyte microvaporization[7] or by the generation of stray acoustic waves.

Clinical Applicability

These results suggest that 1-μsec laser pulses may be of benefit in the treatment of cerebral vasospasm. Because laser energy can be delivered by solitary silica quartz fibers, the potential exists for the dilatation of even very small arteries. Studies are currently in progress to establish the duration of dilatation, the mechanisms of action, parameters for optimum delivery of laser energy to vessels, and possible chronic effects on the arterial wall.

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