Transcranial Infrared Laser Therapy Improves Clinical Rating Scores After Embolic Strokes in Rabbits

Paul A. Lapchak, PhD; Jiandong Wei, MD; Justin A. Zivin, MD, PhD

Background and Purpose—Because photon energy delivered using a low-energy infrared laser may be useful to treat stroke, we determined whether transcranial laser therapy would improve behavioral deficits in a rabbit small clot embolic stroke model (RSCEM).

Methods—in this study, the behavioral and physiological effects of laser treatment were measured. The RSCEM was used to assess whether low-energy laser treatment (7.5 or 25 mW/cm²) altered clinical rating scores (behavior) when given to rabbits beginning 1 to 24 hours postembolization. Behavioral analysis was conducted from 24 hours to 21 days after embolization, allowing for the determination of the effective stroke dose (P50) or clot amount (mg) that produces neurological deficits in 50% of the rabbits. Using the RSCEM, a treatment is considered beneficial if it significantly increases the P50 compared with the control group.

Results—in the present study, the P50 value for controls were 0.97±0.19 mg to 1.10±0.17 mg; this was increased by 100% to 195% (P50=2.02±0.46 to 2.98±0.65 mg) if laser treatment was initiated up to 6 hours, but not 24 hours, postembolization (P50=1.23±0.15 mg). Laser treatment also produced a durable effect that was measurable 21 days after embolization. Laser treatment (25 mW/cm²) did not affect the physiological variables that were measured.

Conclusions—This study shows that laser treatment improved behavioral performance if initiated within 6 hours of an embolic stroke and the effect of laser treatment is durable. Therefore, transcranial laser therapy may be useful to treat human stroke patients and should be further developed. (Stroke. 2004;35:1985-1988.)

Key Words: laser ■ neuroprotection ■ embolism ■ stroke, acute ■ stroke, ischemic ■ clinical trials

Laser therapy has been shown to be effective in a variety of settings, including treating lymphoedema and muscular trauma, and it is now approved by the Food and Drug Administration for the treatment of carpal tunnel syndrome.1,2 Recent studies have shown that laser-generated infrared radiation (ie, photon or light energy) is able to penetrate various tissues, including the brain, and modify function. Laser-generated infrared radiation (ie, photon energy) can penetrate various tissues, including the brain,3–6 and can induce angiogenesis,5 modify growth factor (transforming growth factor-β) signaling pathways,6 and enhance protein synthesis.7 Of importance to the current study are recent reports showing that laser treatment could reduce lesion size in the rat heart after myocardial infarction.5,6,8 Because there are similarities between cardiac and cerebral ischemia, we investigated whether laser treatment reduces stroke-induced behavioral deficits. For these studies, we used the rabbit small clot embolic stroke model (RSCEM).9–12 Which is produced by injection of blood clots into the cerebral vasculature, resulting in ischemia-induced behavioral deficits that can be measured quantitatively with a dichotomous rating scale.9–12

Materials and Methods

Male New Zealand White rabbits (Irish Farms, Norco, Calif) were anesthetized and a catheter was inserted into the common carotid artery, through which microclots were injected, as described in detail previously.9–12 The procedures used in this study were approved by the Department of Veterans Affairs and the Veterans Administration San Diego Healthcare System (VASDHS).

Embolic Strokes

For the RSCEM, microclots were prepared from blood drawn from a donor rabbit and allowed to clot at 37°C, as described in detail previously.9–12 The microclots were resuspended in phosphate-buffered saline, then washed and allowed to settle, followed by aspiration of the supernatant and spiking of the particles with tracer quantities of 15-μm radiolabeled microspheres. The specific activity of the particles was determined by removing an aliquot, after which appropriate volumes of phosphate-buffered saline solution were added so that a predetermined weight of clot could be rapidly injected through the catheter. After the injection, the syringe and catheter were flushed with normal saline.

Quantal Dose–Response Analysis

To evaluate the quantitative relationship between clot dose and behavioral deficits, logistic (S-shaped) curves are fitted by computer to the quantal dose–response data as described in detail previous-
Figure 1. Laser-induced behavioral improvements. A. The control curve (dotted line) has a P50 value of 0.97 ± 0.19 mg (n=23). Laser treatment (7.5mW/cm², 2 minute duration; dark solid line) initiated 3 hours after the stroke increased the P50 value to 2.21 ± 0.54 mg (n=28, *P<0.05), but treatment 24 hours after a stroke (dashed line) was ineffective (P50 = 1.23 ± 0.15 mg, n=32). B, The control curve (dotted line) has a P50 value of 1.10 ± 0.17 mg (n=27). Laser treatment (25mW/cm², 10-minute duration; dark solid line) initiated 1 (dashed line) or 6 (dark solid line) hours after a stroke increased the P50 value to 2.02 ± 0.46 mg (n=18, *P<0.05) and 2.98 ± 0.65 mg (n=26, *P<0.05), respectively.

A wide range of lesion volumes is induced to generate normal and abnormal animals with various behavioral deficits. Using 3 or more different doses of microclots generated each quantal analysis curve. In the absence of treatment, we find the low end of the curve (small numbers of microclots cause no grossly apparent neurologic dysfunction) and the high end (large numbers of microclots invariably cause encephalopathy or death). Each animal is rated as either normal or abnormal (including dead animals), and interrater variability is very low (<5%). Behaviorally normal rabbits did not have any signs of impairment, whereas behaviorally abnormal rabbits had loss of balance, head leans, circling, seizure-type activity, or limb paralysis. With this simple rating system, the composite result for a group of animals is quite reproducible. Briefly, to evaluate the quantitative relationship between numbers of clots in the brain and neurologic dysfunction (coma or death), logistic (S-shaped) curves are fitted by computer to the quantal dose–response data. These parameters are measures of the amount of microclots (in mg) that produce neurologic dysfunction in 50% of a group of animals (P50). A separate curve is generated for each treatment condition and a statistically significant increase in the P50 value compared with control is indicative of a behavioral improvement. For these studies, rabbits were randomly allocated into treatment groups before embolization, with concealment of the randomization sequence until all behavioral and postmortem analyses were complete. The data were analyzed using the t test, which included the Bonferroni correction when appropriate.

**Laser Treatment**

Rabbits were placed in a Plexiglas restrainer for the duration of the treatment. The laser probe was placed in direct contact with the skin. An ACCULASER (PhotoThera, Inc) low-energy laser fitted with an OZ Optics Ltd fiber optic cable and laser probe measuring 2 cm in diameter was used (wavelength of 808 ± 5 nm). Instrument design studies showed that these specifications would allow for laser penetration of the rabbit skull and brain to a depth of 2.5 to 3 cm, and that the laser beam would encompass the majority of the brain if the laser probe was placed on the skin surface posterior to bregma on the midline. Although the surface skin temperature below the probe was elevated by up to 3°C, the focal brain temperature directly under the laser probe was increased by 0.8°C to 1.8°C during the 10-minute laser treatment using the 25 mW/cm² energy setting. Focal brain temperature returned to normal within 60 minutes of laser treatment.

**Physiological Measurements**

To determine if laser treatment alters physiological variables, 14 rabbits were randomly divided into 2 groups, a control group and a laser-treated group (25mW/cm² for 10 minutes). Blood glucose levels were measured for all embolized rabbits using a Bayer Elite XL 3901B Glucometer, and body temperature was measured using a Braun Thermoscan Type 6013 digital thermometer as described previously by Lapchak et al.14
Results

Transcranial Laser Treatment Improves Clinical Rating Scores

In this series of experiments, we used a laser with a power density setting of 7.5 mW/cm² and treatment duration of 2 minutes. Laser treatment initiated 3 hours after embolization significantly improved behavioral performance compared with controls measured 24 hours after treatment. The effect was durable and was measurable 3 weeks after embolization (Figure 1A). However, the same setting did not improve behavior if there was a long delay (24 hours) after embolization (Figure 2).

We also investigated whether a longer duration of laser treatment at a higher power density would have a beneficial effect on behavioral function. For this, we used a laser with energy settings of 25 mW/cm² and treatment duration of 10 minutes. When initiated 1 or 6 hours postembolization, this laser treatment also significantly increased behavioral performance ($P_{50}=2.02\pm0.46 \text{ mg; } n=18$; and $2.98\pm0.65 \text{ mg; } n=26$, respectively) compared with controls (Table 1; Figures 1B and 2).

Physiological Variables

Blood glucose levels and body temperature were measured to determine if laser treatment (25mW/cm² for 10 minutes) affected either measure after a small-clot embolic stroke. Figure 2. Composite figure showing the therapeutic window for laser-induced behavioral improvements after small-clot embolic strokes in rabbits. Results are shown as clinical rating score ($P_{50}$) given as mean±SEM for the number of rabbits per time point (number in brackets) for laser treatment initiated 1, 3, 6, or 24 hours after embolization as shown on the x-axis. The horizontal line represents the mean of the control $P_{50}$ values (*$P<0.05$).

Table 2 presents the results of the blood glucose measurements. For these measurements, 7 rabbits were included in each group. They were embolized with small clot after establishing baseline body temperatures and blood glucose levels. Postmortem analysis showed that the control group was embolized using $5.68\pm0.41 \text{ mg of clots}$, whereas the laser-treated group received $5.52\pm0.52 \text{ mg of clots}$. In the laser-treated group, 6 of 7 rabbits survived 24 hours, whereas only 4 of 7 of the control rabbits survived 24 hours. Within 60 minutes of embolization, there was an increase in blood glucose levels in both groups that was maintained for the 2 hours postembolization observation time. Blood glucose levels returned to control levels by 24 hours, regardless of the extent of stroke-induced behavioral deficits. Laser treatment did not significantly affect glucose levels at any time. Neither embolization nor laser treatment significantly affected body temperature in either group of rabbits (Table 3).

Discussion

In the present study, we assessed the pharmacological effects of transcranial laser therapy in the embolic stroke model that was used in the preclinical development of tPA.15,16 The results in the RSCEM showed that laser treatment significantly improved behavioral rating scores after embolic strokes in rabbits and it is effective when initiated up to 6 hours after strokes; which is later than any other previously effective single therapy in the same preclinical stroke model.9–12 Moreover, the effect is durable and is measurable up to 21 days after embolization. Laser therapy is also effective at improving behavioral performance after strokes in rats (Dr Michael Chopp, Henry Ford Health Science Center, Detroit, MI, personal communication). The magnitudes of laser-induced improvement in rabbits are similar to previously tested thrombolytics (alteplase, tenecteplase, and microplasmin10,12) and neuroprotective compounds (NXY-0599), which are undergoing clinical development.

Although our studies indicate that laser treatment may attenuate stroke-induced behavioral deficits in rabbits, additional preclinical device development studies are required to evaluate the safety aspects of laser therapy. The use of lasers for the treatment of stroke should be approached with caution in light of findings that laser treatment can regulate a wide range of genes and induce translation of pre-existing mRNA species into their corresponding proteins.7 Because the mechanisms involved in laser-induced behavioral improvements remain unknown, studies are underway to determine if laser treatment increases neuronal survival after embolic strokes and to elucidate the cellular mechanism involved in the process.

Table 2. Effect of Laser Treatment on Blood Glucose Levels After Embolic Stroke

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Baseline</th>
<th>5 min</th>
<th>60 min</th>
<th>70 min</th>
<th>90 min</th>
<th>120 min</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>140.3±8.6</td>
<td>150.2±5.8</td>
<td>225.7±37.2</td>
<td>211.2±31.6</td>
<td>200.5±29.3</td>
<td>185.2±26.6</td>
<td>119.5±1.2</td>
</tr>
<tr>
<td>Laser-treated</td>
<td>140.0±5.3</td>
<td>155.5±3.6</td>
<td>189.4±23.8</td>
<td>189.3±24.2</td>
<td>193.5±22.8</td>
<td>192.5±29.9</td>
<td>138.1±6.7</td>
</tr>
</tbody>
</table>

Effects of embolism and laser treatment on blood glucose levels given as mg/dL. In the laser-treated group, 6/7 rabbits survived 24 hours, whereas only 4/7 of the control rabbits survived 24 hours. Embolization increased glucose levels within 60 minutes, an increase that was sustained for the 2-hour observation period and then returned to baseline levels by 24 hours. There were no statistically different effects of laser treatment on blood glucose levels measured at any time point.
Conclusion
We have shown that transcranial laser therapy effectively improves clinical rating scores if initiated within 6 hours of an embolic stroke in rabbits. Moreover, laser treatment improves behavior without affecting the 2 physiological variables (body temperature and blood glucose levels) that were measured in the study. Overall, our preclinical study indicates that transcranial laser therapy is a promising candidate for development as a treatment for acute stroke.

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References

### TABLE 3. Effect of Laser Treatment on Body Temperature After Embolic Stroke

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Baseline</th>
<th>5 min</th>
<th>60 min</th>
<th>70 min</th>
<th>90 min</th>
<th>120 min</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.3±0.5</td>
<td>100.2±0.4</td>
<td>101.2±0.8</td>
<td>101.5±0.9</td>
<td>101.8±0.3</td>
<td>101.6±0.4</td>
<td>100.5±0.7</td>
</tr>
<tr>
<td>Laser-treated</td>
<td>101.5±0.7</td>
<td>101.3±0.3</td>
<td>100.9±1.0</td>
<td>100.6±0.9</td>
<td>101.8±0.5</td>
<td>101.2±0.5</td>
<td>99.9±0.6</td>
</tr>
</tbody>
</table>

Effects of embolism and laser treatment on body temperature given in °F. There was no significant effect of either embolization or laser treatment on body temperature.
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