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

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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/cm^2) 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 (P_{50}) 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 P_{50} compared with the control group.

Results— In the present study, the P_{50} value for controls were 0.97±0.19 mg to 1.10±0.17 mg; this was increased by 100% to 195% (P_{50}=2.02±0.46 to 2.98±0.65 mg) if **laser** treatment was initiated up to 6 hours, but not 24 hours, postembolization (P_{50}=1.23±0.15 mg). **Laser** treatment also produced a durable effect that was measurable 21 days after embolization. **Laser** treatment (25 mW/cm^2) 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** treatment may be useful to treat human stroke patients and should be further developed.

Introduction

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,[3] modify growth factor (transforming growth factor-ß) signaling pathways,[4] 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 previously.[9–12] 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 neurological deficits (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 (P_{50}).[9–12] A separate curve is generated for each treatment condition and a statistically significant increase in the P_{50} value compared with control is indicative of a behavioral improvement. For these studies, rabbits were randomly allocated into treatment groups before **emboli**zation, 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

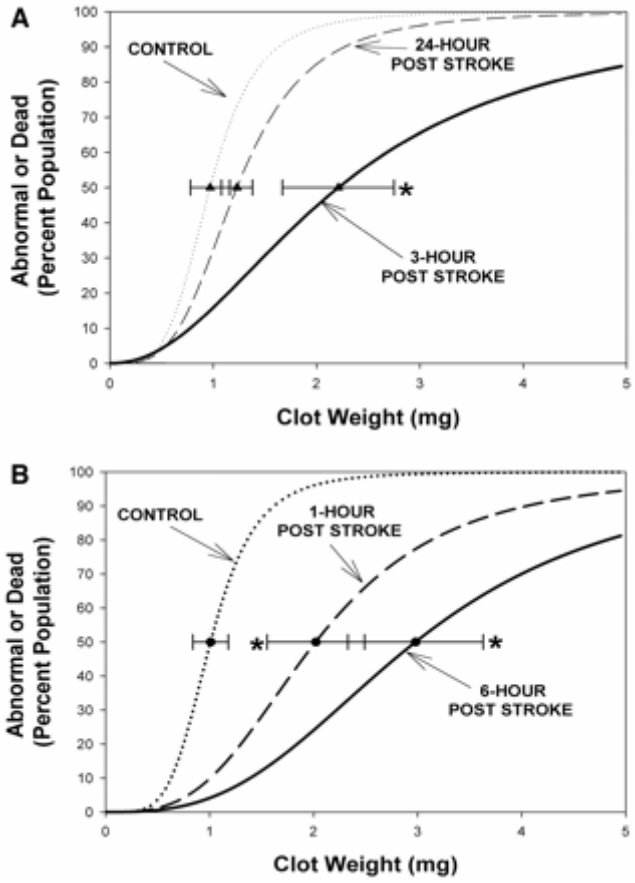
Rabbits were placed in a Plexiglas restrainer for the duration of the treatment. The **laser** probe was placed in direct contact with the skin. An ACCU**LASER** (PhotoThera, Inc) low-energy **laser** fitted with an OZ Optics Ltd fiber optic cable and **laser** probe measuring 2 cm in diameter was used (wavelength of 808±5 nm).[13] 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 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/cm^2 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/cm^2 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/cm^2 and treatment duration of 10 minutes. When initiated 1 or 6 hours post**embolization**, this **laser** treatment also significantly increased behavioral performance

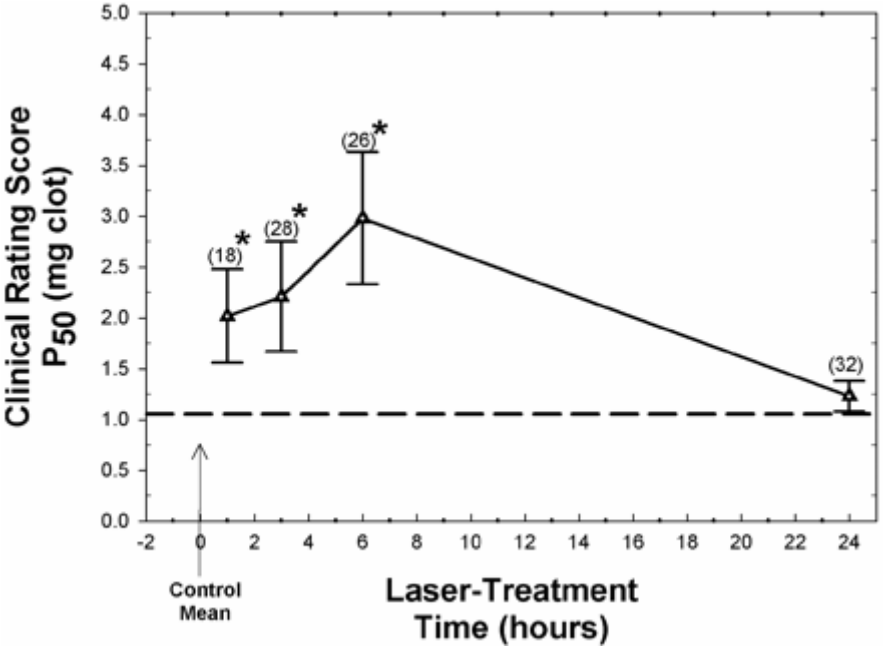
(P_{50}=2.02±0.46 mg; n=18; and 2.98±0.65 mg; n=26, respectively) compared with controls (Table 1; Figures 1B and 2).

TABLE 1. Effect of Laser Treatment on Clinical Rating Scores of Rabbits

Control			Laser (6 h)		
Clot Dose (mg)	Normal	Abnormal	Clot Dose (mg)	Normal	Abnormal
0.031	1	0	0.06	1	0
0.07	1	0	0.12	1	0
0.08	1	0	0.15	1	0
0.18	1	0	0.18	1	0
0.20	1	0	0.23	2	0
0.25	1	0	0.52	1	0
0.44	1	0	0.62	1	0
0.45	1	0	0.97	1	0
0.68	1	0	1.29	1	0
0.78	1	0	1.30	0	1 (A)
0.86	0	1 (D)	1.35	1	0
0.93	1	0	1.61	1	0
0.98	0	1 (A)	1.70	1	0
1.46	1	0	1.79	1	0
1.64	0	1 (D)	1.94	1	0
1.65	0	1 (A)	2.30	1	0
1.71	0	1 (A)	2.52	0	1 (A)
2.72	0	1 (D)	2.62	1	0
2.10	0	1 (A)	2.68	0	1 (D)
2.27	0	1 (D)	2.73	1	0
2.65	0	1 (A)	3.03	1	0
2.91	0	1 (A)	3.24	1	0
2.94	0	1 (D)	3.47	0	1 (A)
3.13	0	1 (D)	3.51	0	1 (A)
3.76	0	1 (D)	3.92	0	1 (D)
5.49	0	1 (D)			
P_{50}= 1.10±0.17 mg (27)			P_{50}= 2.98 ±0.65 mg (26)		

Behavioral results are expressed as Normal or Abnormal rabbits for each clot dose shown in milligrams (mg).

n indicates the number of animals in each group; A, abnormal; D, dead.



Physiological Variables

Blood glucose levels and body temperature were measured to determine if **laser** treatment (25mW/cm^2 for 10 minutes) affected either measure **after** a small-clot **emboli**c stroke. Table 2 presents the results of the blood glucose measurements. For these measurements, 7 rabbits were included in each group. They were **emboli**zed with small clot **after** establishing baseline body temperatures and blood glucose levels. Postmortem analysis showed that the control group was **emboli**zed using 5.68±0.41 mg of clots, whereas the **laser**-treated group received 5.52±0.52 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 **emboli**zation, there was an increase in blood glucose levels in both groups that was maintained for the 2 hours post**emboli**zation 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 **emboli**zation nor **laser** treatment significantly affected body temperature in either group of rabbits (Table 3).

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

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

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.

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

Treatment Group	Baseline	5 min	60 min	70 min	90 min	120 min	24 h
Control	100.3±0.5	100.2±0.4	101.2±0.8	101.5±0.9	101.8±0.3	101.6±0.4	100.5.±0.7
Laser-treated	101.5±0.7	101.3±0.3	100.9±1.0	100.6±0.9	101.8±0.5	101.2±0.5	99.9±0.6

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.

Discussion

In the present study, we assessed the pharmacological effects of **transcranial laser therapy** in the **embolic** stroke model that was used in the pre**clinical** 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 pre**clinical** 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 microplasmin[10,12]) and neuroprotective compounds (NXY-059[9]), which are undergoing **clinical** development.

Although our studies indicate that **laser** treatment may attenuate stroke-induced behavioral deficits in rabbits, additional pre**clinical** device development studies are required to evaluate the safety aspects of **laser therapy**. The use of **laser**s 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.

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 pre**clinical** study indicates that **transcranial laser therapy** is a promising candidate for development as a treatment for acute stroke

Acknowledgments

This study was supported by funding from NS28121 (P.A.L./J.A.Z.), NS23814 (J.A.Z.), VA Merit Review (J.A.Z.), and the Christopher Reeve Paralysis Foundation (P.A.L.). We thank Donghuan Song for technical assistance.

Received February 4, 2004; revision received April 14, 2004; accepted April 22, 2004.

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