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BRIEF REPORT

Comparison of the effects of pulsed and continuous wave light on axonal regeneration in a rat model of spinal cord injury

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Background

Light therapy (LT) has been investigated as a viable treatment for injuries and diseases of the central nervous system in both animal models and clinical trials. Based on in vivo studies, LT has beneficial effects on the treatment of spinal cord injury (SCI) [1–3], traumatic brain injury [4], stroke [5–9] and neurodegenerative diseases [10, 11]. Recent clinical trials on stroke have demonstrated that light is safe for the treatment of ischemic stroke in humans [12, 13].

The biomodulatory effects of LT have been investigated to a much greater extent with continuous wave (CW) mode than in pulsed mode. However, there is no clear consensus that one mode is superior to the other. A recent review of the published literature that compared the effects of light in CW or pulsed mode reported that some studies have shown pulsed light to be more effective than CW light, while other studies have shown no effect or a worsening effect with pulsed light compared to no treatment [14]. The studies in which pulsed light was more effective than CW light include LT on wound healing, pain attenuation, bone stimulation and ischemic stroke in animal models and human studies [14]. Lapchak et al. compared CW light with two different frequencies of pulsed light and found that transcranial treatment with both pulsing regimes significantly improved behavioral deficits compared to the CW light [6]. Why pulsed light in some cases improves the outcome of LT is currently unclear, and further investigation is needed.

Our previous studies have shown that LT promotes axonal regeneration and functional recovery in both hemisection and contusion models of SCI in rats with CW laser light [2, 3]. The purpose of this study was to compare the effects of CW and pulsed laser light on nerve regeneration in a hemisection model of SCI.

Materials and methods

This study was approved by the Institutional Animal Care and Use Committee, Uniformed Services University of Health Sciences. The study animals comprised 15 female Sprague-Dawley rats (200–250 g; Taconic Farms, Germantown, NY) which were randomized into the following three experimental groups: control, treatment with CW laser light, and treatment with pulsed laser light (PW). All rats underwent SCI by dorsal hemisection of the spinal cord at the T9 vertebral level as described previously [2, 3]. This dorsal hemisection model of SCI results in complete transection of the corticospinal tract, a major descending tract connecting the cerebral cortex with the motor neurons in the spinal cord that plays a dominant role in paralysis after SCI.

Within 15 minutes after SCI surgery, the CW and PW groups were transcutaneously irradiated with an 808-nm diode laser (provided by PhotoThera). Irradiation was delivered via a 3-mm diameter probe with direct contact to the skin above the lesion. Both treatment groups were treated at a power density of 0.5 W/cm^2 on the skin surface and an energy density of 1,500 J/cm^2. For the CW group, the output power was 35 mW. For the PW group, the

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parameters of the light were 175 mW peak power with 20% duty cycle, and a frequency of 100 Hz. Our previous study showed that irradiation with the same power density and energy did not induce significant heating at either the skin level (1.832±0.068ºC) or the spinal cord level (0.350±0.018ºC) [2]. Animals were irradiated for 50 min daily for 14 consecutive days. The total survival time for all rats was 3 weeks.

Ten days before the animals were killed, anterograde labeling was performed in all groups. A total of 12 μl 5% tetramethylrhodamine biotinylated dextran (mini-ruby; Molecular Probes, Carlsbad, CA) was injected into the primary motor cortex with six different coordinates (from bregma: −0.11 AP and ±1.60 ML, −1.33 AP and ±1.50 ML, −2.85 AP and±1.40 ML) [2]. This fluorescence dye labels the axons in the corticospinal tract located in the white matter [2, 3]. Axons were counted in 20-μm thick longitudinal sections of the spinal cord that extended from the lesion site to 20 mm caudal to the lesion using a TRITC filter (excitation 528–553 nm) and ×20 magnification using a Nikon Labophot fluorescence microscope (Tokyo, Japan). Total axon number and average axonal length were calculated as described previously [2].

A footprint test was used as a functional assessment for this study. Briefly, animals walked along a ramp with their hind paws inked. Their footprint pattern was recorded on a strip of paper and measured for angle of rotation (angle formed by the intersection of the line through the prints of the third digit and the line through the central pad parallel to the walking direction). Only angle of rotation was measured in this study because our previous research showed that other factors, such as stride length and base of support, are not affected in this model of SCI [2, 3]. This test was performed prior to injury (as baseline) and on days 7, 14 and 21 after injury.

All statistical tests were performed using the GraphPad Prism program (GraphPad Software, San Diego, CA). One-way ANOVA was used to compare groups and Student's *t*-test was used for comparison between individual groups. Significant difference was defined as a p value less than 0.05.

Results

There were significantly higher total axon numbers in both the CW group and the PW group (504.0±150.6 and 724.8±78.9, respectively) compared to the control group (111.3±47.4, $p<0.05$ and $p<0.01$, respectively; Fig. 1a). No significant difference was found between the CW group and the PW group. Distal to the lesion site, the CW group and the PW group had significantly greater average lengths of axonal regeneration (10.28±0.81 and 13.54±0.70 mm, respectively) compared to the control group (6.42±1.18 mm, $p<0.05$ and $p<0.001$, respectively; Fig. 1b). Also axonal length distal to the lesion site was significantly greater in the PW group than in the CW group ($p<0.05$).

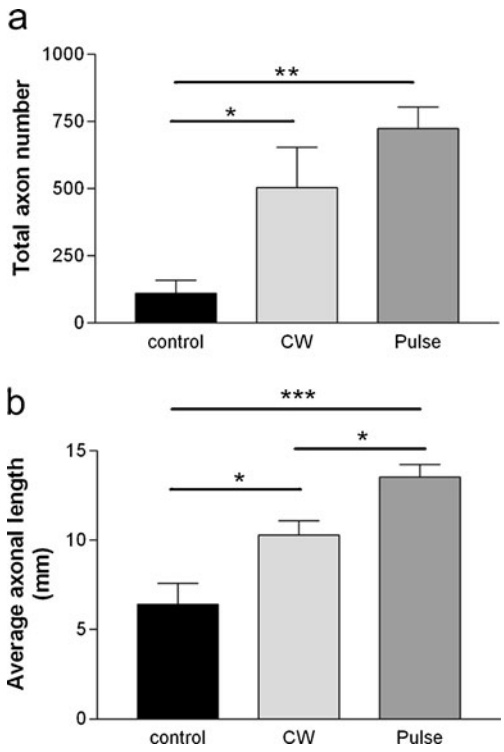

Fig. 1 Assessment of regrowing axons after SCI in control and light-treated groups: **a** total axon number; **b** average axonal length distal to the lesion site. *$p<0.05$, **$p<0.01$, ***$p<0.001$. *Error bars* represent means±SEM

Behavioral assessment showed a significant difference in angle of rotation in both the CW group and the PW group compared to the control group ($p<0.01$) when normalized to each group's baseline at 21 days after injury (Fig. 2). This reduction in angle of rotation indicated functional

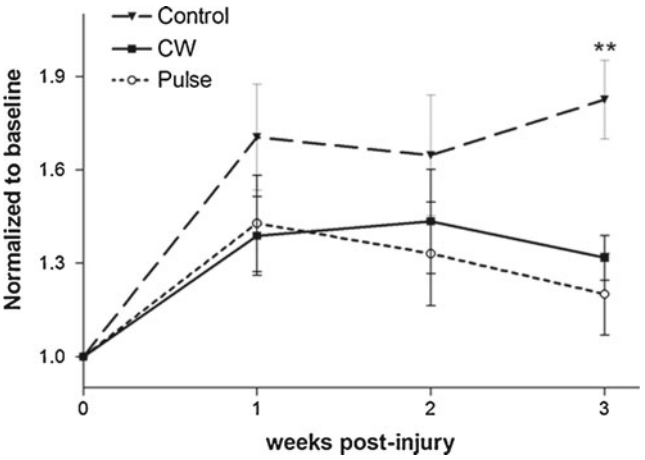

Fig. 2 Footprint test as functional analysis. **$p<0.01$. *Error bars* represent means±SEM

recovery after SCI. No significant difference was found between the CW group and the PW group.

Discussion

A number of recent reports on the comparison of the effects of pulsed versus CW light indicate that pulsed light may be more effective for LT applications than CW light. This potential advantage has stimulated interest and research resulting in a number of recent reports on the effects of pulsed light in LT.

CW light irradiation results in axonal regeneration in both the peripheral [15–17] and central injured nervous systems [2, 3]. As for LT using pulsed light for peripheral nerve injury, a few studies have shown either no effect or a worse outcome compared to no treatment [18, 19]. This study is the first to assess the efficacy of pulsed light in LT for SCI. The data showed that pulsed laser light significantly improved axonal regeneration in SCI compared to untreated controls and led to a significant increase in the length of regenerated axons distal to the lesion compared to the group treated with CW laser light ($p<0.05$). Recent studies on neurological diseases have provided some insight into the possible mechanism involved in the beneficial effects of CW and pulsed light treatment. Transcranial laser treatment using CW light in rabbits following embolic stroke resulted in an increase in cortical ATP of 41% and pulsed light with 5 times and 35 times more energy delivery resulted in increases in cortical ATP of 157% and 221%, respectively [9]. Higher ATP content and improved mitochondrial function have also been found in a transgenic mouse model of Alzheimer's disease following transcranial irradiation using pulsed light [11]. Increased ATP content and enhanced mitochondrial function could be an acute effect which ultimately leads to normalization of the tissue function and neurological improvement.

To date, there is no established optimal range of parameters for pulsed light in LT. Simply following the effective parameters established for CW light irradiation does not ensure an efficacious treatment. Optimization of parameters for pulsed light needs to include frequency, duration time and duty cycle, besides the other parameters typically used in CW light irradiation, such as wavelength, output power, and power and energy density. In a rabbit embolic stroke model, laser treatment with pulsed laser light at two different frequencies (100 Hz and 1000 Hz) initiated 6 h after stroke significantly improved behavioral function. However, CW laser light with the same power density (7.5 mW/cm^2) and treatment time (2 min) did not result in a statistically significant improvement [6]. In a rat stroke model produced by permanent middle cerebral artery occlusion, CW light irradiation resulted in significant functional improvement when applied 24 hours after stroke, while pulsed light irradiation at a frequency of 70 Hz resulted in a nonsignificant difference [7]. Although these two studies used different animal models of stroke, the difference in frequency of the pulsed light could be a main reason for the differences in the results. In this study, the parameters for the pulsed laser light were not optimized. The pulse parameters chosen were based on the same power density as used for CW laser light irradiation. The results of this study indicate a greater efficacy of pulsed laser light for repair of SCI. A follow-up study is planned to optimize the parameters of the pulsed light for clinical translation as a treatment for SCI.

For LT of a cutaneous or subcutaneous target, penetration depth is not a major issue. However, to treat diseased or injured tissues located deep inside the body, penetration depth is a critical factor. Depth of penetration depends upon the wavelength of the light used due to scattering and absorption of the light by tissue [20]. Therefore, if the same wavelength is used, CW and pulse modes of light will have the same penetration depth. However, the effective depth of penetration is determined by the depth where an adequate amount of energy is present to cause a photobiological reaction. Ilic et al. found that pulsed light could deliver 100 times the optimal power density without neurological damage at the cortical surface, while CW mode caused neurological deficits, including subdural necrosis with loss of neuronal tissue, at such a high power density [21]. The higher safe range of output power in pulsed light allows a greater effective penetration depth than CW mode. Hence, pulsed light can be used to treat not only a deeper target, such as nerves and muscles, but also the same target in larger organisms, which would allow the effective translation of LT from animal models to use in the clinic.

In conclusion, CW and pulsed laser light support axonal regeneration and functional recovery after SCI. Pulsed laser light has the potential to support axonal regrowth to spinal cord segments located farther from the lesion site. Therefore, the use of pulsed light is a promising non-invasive therapy for SCI.

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