Lasers in Medical Science
https://doi.org/10.1007/s10103-018-2447-3

ORIGINAL ARTICLE

Photobiomodulation improves motor response in patients with spinal cord injury submitted to electromyographic evaluation: randomized clinical trial

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Received: 8 September 2017 / Accepted: 12 January 2018
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Abstract
Photobiomodulation is a treatment that has been widely used in neurotrauma and neurodegenerative diseases. In the present study, low-level laser therapy was administered to patients with spinal cord injury. Twenty-five individuals were divided into two groups: placebo photobiomodulation plus physiotherapy and active photobiomodulation plus physiotherapy. Electromyographic evaluations were performed before and after 12 sessions of phototherapy as well as 30 days after the end of treatment. In the active phototherapy group, median frequency values of the brachial biceps and femoral quadriceps muscles were higher at rest and during isotonic contraction 30 days after photobiomodulation ($p = 0.0258$). No significant results were found regarding the rest and isotonic conditions in the pre-photobiomodulation period ($p = 0.950$) or immediately following photobiomodulation ($p = 0.262$). The data provide evidence that phototherapy improves motor responses in individuals with spinal cord injury, as demonstrated by differences in the EMG signal before and after treatment. Trial registration: NCT 03031223

Keywords Spinal injuries · Phototherapy · Electromyography

Introduction

Spinal cord injury (SCI) can lead to paraplegia or quadriplegia, exerting a significant impact on quality of life and life expectancy as well as economic burden in the form of the considerable costs associated with primary care and the loss of income [1].

The regenerative process after the occurrence of SCI is very complex and current therapeutic approaches fail to promote functional recovery. However, it is generally agreed that it is essential to modulate the initial inflammatory process and control secondary tissue loss related to SCI in order to offer a more appropriate environment for the stimulation of neuroplastic processes [2, 3].

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Published online: 13 February 2018

Although there are no fully restorative treatments for SCI, many rehabilitative, cellular, and molecular therapies have been tested using animal models. Among these treatments, the stimulatory effects of photobiomodulation on biological tissues have been highlighted [1, 2]. Photobiomodulation has been found to stimulate mitochondrial activity/function as well as increase the synthesis of ATP, the amount of reactive oxygen species, and the activity of cytochrome c oxidase [4]. As the mechanism of photobiomodulation is proposed to rest on the absorption of photons by cytochrome c oxidase (CCO), which is the terminal enzyme in the mitochondrial respiratory chain that catalyzes the reduction of oxygen to energy metabolism, an increase in CCO activity translates to greater oxygen uptake and energy metabolism is produced through mitochondrial oxidative phosphorylation. As CCO is an inducible enzyme, a longer metabolic effect is achieved by the concentration of CCO regulated by LLLT, which improves the capacity for the metabolism of cellular oxygen. As neurons are highly dependent on the metabolism of oxygen, this photon-bioenergetic effect results in metabolic and hemodynamic changes that facilitate neuronal functioning. A previous study involving neuron cultures demonstrated that the most effective wavelengths were near the infrared band, parallel to the absorption peaks of CCO, demonstrating that the effects of phototherapy on CCO depend on the wavelength of the laser used for stimulation [5, 6].

Using an experimental SCI model in rats, Song et al. [3] found that 810-nm laser altered the polarization state to an M2 tendency, reporting the potential for reducing inflammation, regulating macrophage/microglia polarization, and promoting neuronal survival. Also, using an experimental SCI model in rats, Wu et al. [7] found that low-level laser therapy (LLLT) (810 nm, 150 mV, and 1589 J/cm^2) increased axonal regeneration and improved functional recovery.

An important systematic review reports that all studies involving animal models demonstrate the positive results of phototherapy with regard to spinal tissue regeneration and functional improvement. The only study involving humans showed that the combination of cell transplantation and laserpuncture (irradiation at acupuncture points rather than at the injury site) was able to promote some voluntary activity below the level of the SCI. Different laser parameters, such as different wavelengths, produce similar results, but the variety of parameters shows the lack of standardization in studies, making it difficult for researchers and clinicians to know which parameters to employ [4].

To enable a better evaluation of the motor response in muscles, electromyography (EMG) has been employed as an important complementary method that captures information on the electrical activity of muscles of interest. EMG can assist in the determination of ducts, demonstrate results obtained through specific therapies, and offer information on different forms of muscular contraction [8, 9].

The main question guiding the present study regards possible modifications in the behavior of muscles affected by spinal cord injury submitted to photobiomodulation with regard to electrical activity and muscle contraction, starting from the hypothesis that such modifications would be expected.

Methods

This study received approval from the Human Research Ethics Committee of the university under protocol CAAE: 56952716.2.0000.5511. All participants or their legal guardians received clarifications regarding the objectives and procedures of the study and those agreeing to participate signed a statement of informed consent.

Recruitment

Individuals were recruited from the physical therapy clinics of University Nove de Julho (São Paulo, Brazil) as well as spinal injury associations.

Inclusion criteria

Partial spinal injury, patients with quadriplegia or paraplegia, injury between C3 and L5 and up to 1 year elapsed since the injury and ASIA B, C, and D.

Exclusion criteria

Complete spinal cord injury, cognitive impairment, and ASIA A and E.

Randomization and blinding procedures

Individuals were allocated to different groups based on numbers randomly generated by a randomization site (randomization.com). Allocation was concealed with the use of sealed opaque envelopes. Each envelope was numbered and contained the name of a participant. The envelopes were randomly selected using the randomization site, with the formation of the control group (placebo photomodulation plus physiotherapy) or PBM group (active photobiomodulation plus physiotherapy). EMG evaluations were performed before and after the intervention as well as 30 days after the end of treatment.

Physiotherapy protocol

All individuals in both groups underwent physiotherapy at the Physiotherapy Clinics of Nove de Julho University prior to receiving photobiomodulation. Forty-minute sessions of conventional physiotherapy (stretching, strengthening, sensory training, and proprioceptive training) were held three times a week for 4 weeks.

Experimental protocol

Thirty-three individuals with spinal cord injury were recruited. Eight individuals were excluded: two patients had a diagnosis of complete spinal cord injury and the other six patients did not complete the phototherapy protocol. A larger number of patients were recruited than the minimum number determined by the sample calculation to compensate for possible losses of individuals during the study. The volunteers were randomly allocated to either the control group or treatment group. Evaluations were conducted before and after the intervention using electromyography to assess the motor response.

The phototherapy protocol was the same for both groups. The individuals were placed in lateral decubitus. The spinal injury was located through palpation of the transverse processes of the vertebrae. The operator placed the laser tip in contact with the skin at the previously marked spot. The device emits the same sound delineating the beginning and end of stimulation, giving the patient sound stimuli, but no irradiation was emitted in the placebo group (Fig. 1).

The treatment group received phototherapy using a protocol based on Byrnes et al. [10] and Holanda et al. [11] Irradiation was administered transdermally to the injury site at a wavelength of 808 nm using a Quantum diode laser (Ecco Fibras e Dispositivos, Brazil). Twelve sessions were held (three per week over 4 weeks) using the parameters described in Table 1. According to the literature, this dose is capable of enhancing functional recovery following an injury [6].

Electromyography

The EMG signals were captured using a four-channel acquisition system (EMG 432 C, EMG System do Brasil Ltda.) consisting of a signal conditioning module, bipolar active electrodes, analog band pass filter from 20 to 500 Hz, and common mode rejection ratio of 120 dB. The sampling frequency was 2 kHz, scanned using analog/digital (A/D) conversion board with 16 bits of resolution. The acquisition program was the EMG Lab (EMG System

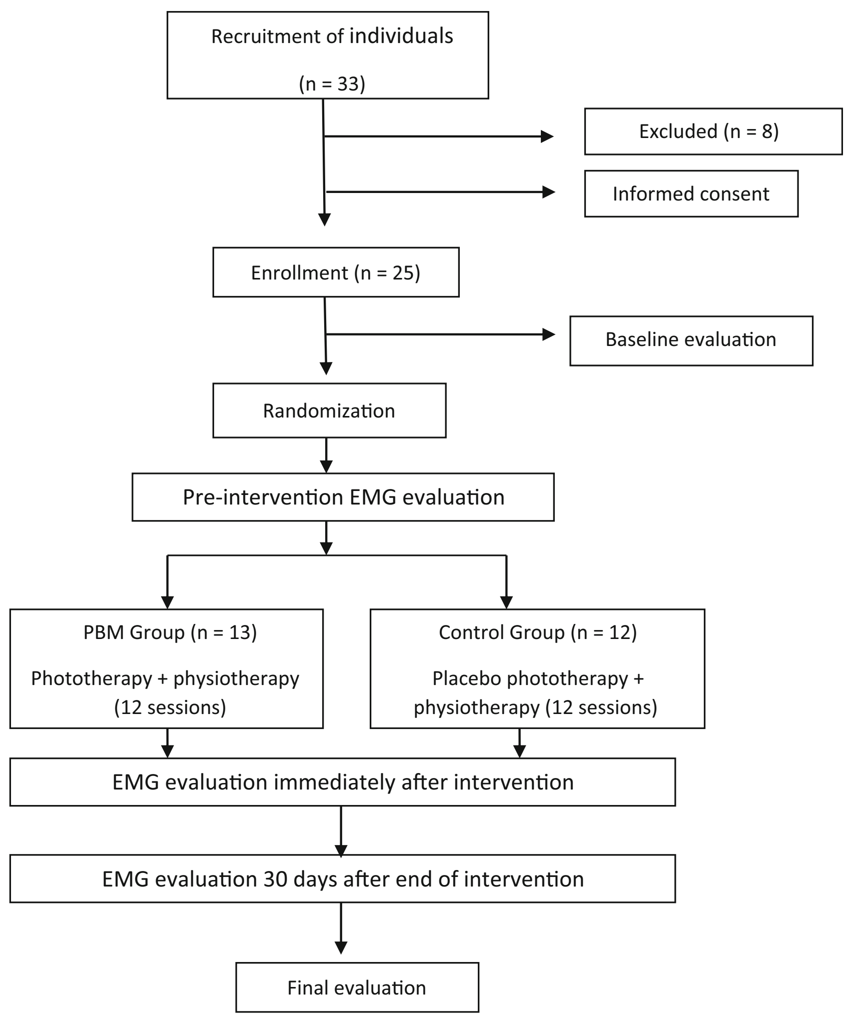

Fig. 1 Flowchart

Table 1 Laser parameters

Parameter	Infrared laser
Center wavelength [nm]	808
Spectral bandwidth [nm]	10
Operating mode	Continuous
Average radiant power [mW]	120
Polarization	Random
Aperture diameter [cm]	0.18
Irradiance at aperture [W/cm^2]	4.72
Beam spot size at target [cm^2]	0.0254
Irradiance at target [W/cm^2]	4.72
Exposure duration [s]	208 (per point)
Radiant exposure [J/cm^2]	983 (per point)
Energy density at aperture [J/cm^2]	983 (per point)
Radiant energy [J]	25 (per point)
Number of points irradiated	5
Area irradiated [cm^2]	0.0254
Application technique	Contact
Number and frequency of treatment sessions	Three per week over 4 weeks (12 sessions)

do Brasil Ltda.). Active bipolar electrodes with 20-fold preamplification were used. After cleaning the skin with 70% alcohol to decrease impedance, self-adhesive disposable Ag/AgCl surface electrodes (Noraxon) with a diameter of 10 mm were attached to the belly of the muscle in the region of greatest tonus. The inter-electrode distance was 20 mm center to center, as suggested by the European Society Recommendations for Surface Electromyography (SENIAM) (FRERIKS, 2000). The electrodes were placed on the right brachial biceps muscle in quadriplegic patients and the right femoral quadriceps muscle in paraplegic patients. As reference, an electrode was placed in the left hand of the individuals to prevent the effect of interference from external noise.

The EMG activity of the muscles was determined under three conditions: (i) rest, (ii) isometric contraction (maximum voluntary clenching—MVC), and (iii) isotonic contraction. The individuals were placed in the positions recommended by SENIAM to collect the EMG signals. The resting signal was collected first, followed by isometric contraction and finally isotonic contraction. All readings were extracted with a cut of 1 s before and 1 s after the center of the EMG signal. For the normalization of the EMG data, the mean of three MVC readings was used, employing the muscle function test suggested by Kendall (1993). Each reading lasted 10 s, always respecting a rest 2-minute interval between conditions to avoid the effects of fatigue (Fig. 2). The individuals were asked to perform isometric contraction for the purposes of normalizing the EMG signal. However, as some individuals did not exhibit visually perceptive contractions, it was not possible to use this evaluation. Thus, the electromyographic response was evaluated based on median frequency.

Statistical analysis

The level of significance for all statistical tests was $\alpha = 5\%$. The Anderson-Darling test was used to determine the normality of the data, which proved to be not normally distributed, requiring nonparametric tests. Thus, the Kruskal-Wallis was used to determine difference between groups and the Mann-Whitney test was used for the analysis of the variables within the same group (Table 2).

Results

Median frequency values of the EMG signals were compared to determine differences between the control and laser groups under the conditions of rest and isotonic contraction. Figure 3 displays the results expressed in median and interquartile range (25 to 75%).

In the intra-group analysis of the laser group, median frequency values of the brachial biceps and femoral quadriceps muscles were higher at rest and during isotonic contraction 30 days after photobiomodulation ($p = 0.0258$; Mann-Whitney test). No significant results were found regarding the

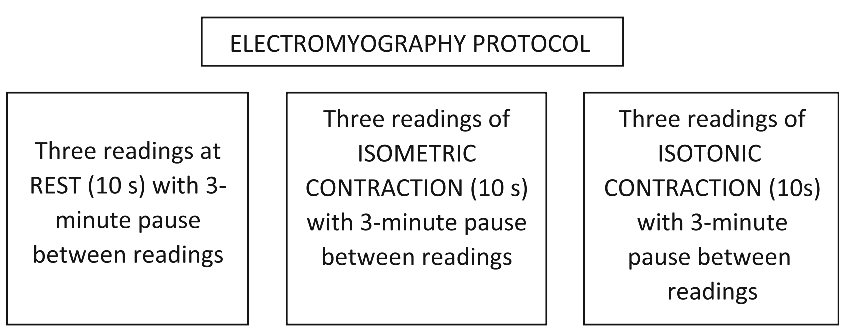

Fig. 2 Electromyography protocol

Table 2 Characterization of sample

Gender	Frequency	Percent
Male	22	88.0
Female	3	12.0
Level of Injury		
Paraplegic	16	64.0
Quadriplegic	9	36.0
Groups		
Control	12	48.0
Laser	13	52.0

rest and isotonic conditions in the pre-photobiomodulation period ($p = 0.950$) or immediately following photobiomodulation ($p = 0.262$).

The proportion between paraplegia and quadriplegia was compared between groups using the chi-square test corrected by Fisher's exact test, with the level of significance set at $\alpha = 5\%$. No significant difference was found between groups ($p = 0.0730$) (Fig. 4).

In the intra-group analysis of the control group, no statistically significant results were found regarding the rest and isotonic conditions in the pre-photobiomodulation period ($p = 0.850$), immediately after photobiomodulation ($p = 0.226$), or 30 days after photobiomodulation ($p = 0.184$).

In the intergroup analysis (Kruskal-Wallis test), no statistically significant differences were found at rest immediately after photobiomodulation ($p = 0.934$) or 30 days after photobiomodulation ($p = 0.900$). Moreover, no statistically significant differences were found during isotonic contraction immediately after photobiomodulation ($p = 0.542$) or 30 days after photobiomodulation ($p = 0.850$).

Discussion

Skeletal muscles are innervated by inferior motor neurons from the ventral horn of the spinal cord. The axions of these neurons form the ventral roots, which join with the dorsal roots (that transmit sensory information) and either form mixed spinal nerves or project through spaces between the vertebrae. The inferior motor neurons are classified as alpha and gamma and are responsible for the innervation of muscle fibers and the generation of force by muscles. A motor unit is formed by the motor neuron and the extrafusal muscle fibers that the neuron innervates; the number of muscle fibers that compose a motor unit varies depending on the muscle and its specialization in the composition of a given movement [8, 12].

When a motor unit is activated, the muscle contracts and action potentials are generated that can be captured and recorded with the use of electromyography. In this exam, the sum of the potentials are transformed into analog signals (graphs) that represent the discharge of all muscle fibers pertaining to the alpha motor unit [8, 9, 12].

In the investigation of the force or velocity of a muscle contraction, the amplitude of the EMG signal can be affected by factors that are not relevant to the production of force, such as the shape of the wave of the action potential of the motor unit. Moreover, the non-linear relationship between the response size and amplitude of the rectified EMG signal as well as changes in the amplitude of movement of the signal due to canceling limits the possibility of using the amplitude of the EMG signal as a variable to indicate the specific characteristics of the brachial biceps and femoral quadriceps muscles evaluated in the present study. Another disadvantage of analyses in the time domain is the need to record an additional EMG signal during the maximum volumetric period that can serve as a parameter for the normalization of the crude EMG

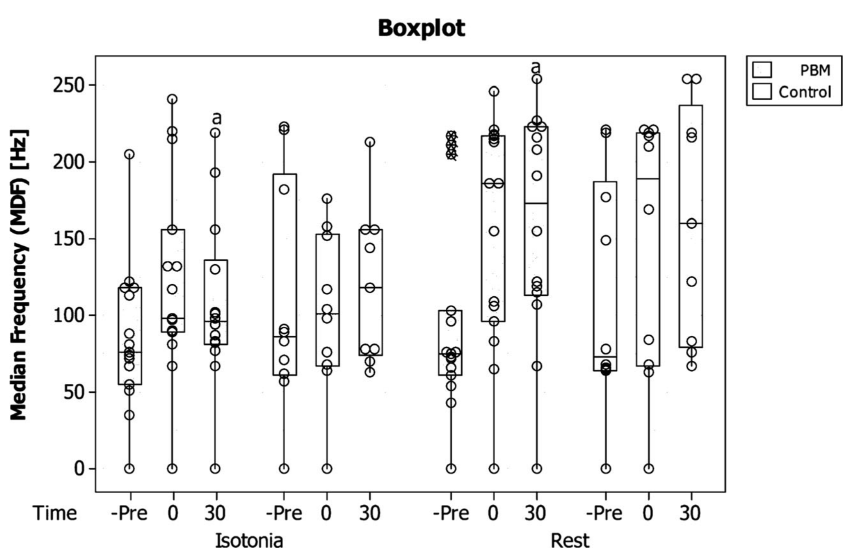

Fig. 3 Median (25%, 75% quartile) of median frequency recorded at rest and during isotonic contraction before (pre), immediately after treatment (time 0), and 30 days after treatment (time 30). Letter "a" indicates statistically significant difference ($p = 0.0258$)

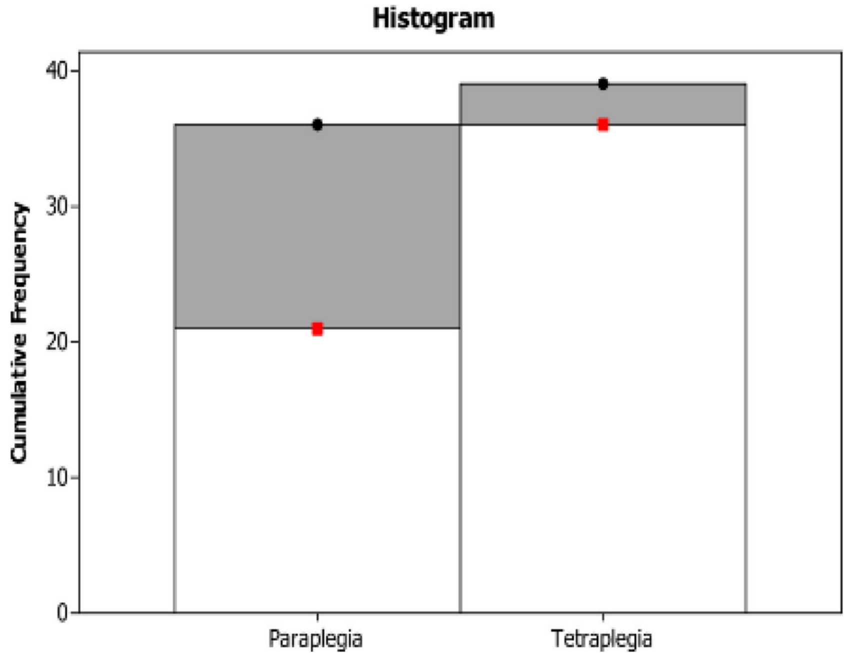

Fig. 4 Proportion between paraplegic and quadriplegic patients (histogram)

signal [9]. Many studies in the literature use the median frequency (MDF) to investigate muscle fatigue. This index can also be used to investigate other muscle phenomena. Due to the difficulty many patients have in performing voluntary muscle contractions, we used MDF as the parameter, since the root mean square (RMS) amplitude requires normalizing the signal by maximum voluntary contraction.

MDF and mean frequency are two parameters of the power density function (PDSF) that can be employed as useful measures of the EMG frequency spectrum. However, MDF has the advantage of being less affected by random noise, particularly in the case of noises located in the high frequency band of the EMG power spectrum. Moreover, MDF can be used as an index to identify recruitment strategies employed by muscles during different levels of force. In general terms, MDF is a global measure of PDSF restricted to a single spectrum frequency value and the analysis of frequency bands enables the acquisition of more detailed information regarding the contribution of these bands to the EMG signal [9].

Phototherapy seems to exert positive effects on SCI through the reduction in the inflammatory process, reductions in some cytokines and chemokines, an improvement in the distribution of fibrocartilage/elastin, greater number and sprouting of axons and consequently less cavitation at the injury site, better somatosensory potential, and better functional recovery [4, 13, 14].

Few studies have addressed the use of phototherapy in cases of SCI. Paula et al. [15] found faster motor evolution in rats with spinal contusion submitted to phototherapy, with the maintenance of the effectiveness of the urinary system, the preservation of nerve tissue in injured areas, inflammation control, and an increased number of nerve cells and connections. Using a spinal cord crush injury model in rats, Song et al. [3] found that phototherapy altered the polarization state to a tendency toward M2 and increased levels of interleukin 4 and interleukin 3, demonstrating the potential to reduce inflammation, regulate macrophage/microglia polarization, and promote neuronal survival.

Only one study involving human subjects was conducted to evaluate the capacity of cell transplantation (olfactory ensheathing glia [OEG]) combined with Laserpuncture® with regard to restoring voluntary muscle activity below the level of the injury [16]. Three clinical cases (two men and three women aged 25 to 37 years) of SCI between T4 and T9 classified as paraplegics were analyzed. Several months after being submitted to OEG transplantation, the patients underwent 40 to 57 sessions of laserpuncture involving infrared laser applied to eight points following the paths of the acupuncture meridians (kidneys, stomach, *Ren Mai*, and *Dai Mai* in the anterior region of the body and *Chong Mai*, bladder, and *Daí Mai* in the posterior region of the body as well as over dermatomes that are not part of classic acupuncture). Twenty-minute sessions were held twice a day (morning and afternoon), 5 days a week with an interval of 8 weeks between each two sets of sessions (each ten sessions). The Laserpuncture® properties and parameters were not divulged due to the proprietary nature of the technique. For the evaluation of muscle activity, adhesive electrodes were attached to the skin 15 cm above the patella on the belly of the right and left quadriceps muscles. The results suggest some degree of voluntary muscle contraction below the level of the injury in the three individuals.

This is the first randomized controlled study in which the role of phototherapy was evaluated in patients with spinal cord injury. The results demonstrate that phototherapy stimulated the injured tissue, achieving an improved motor response. The EMG data demonstrate a difference in comparison to the pre-intervention evaluation, with higher MDF values at rest and during isotonic contraction 30 days after the end of treatment. MDF is related to the firing and synchronism of motor units. An increase in MDF indicates either an improvement in the firing synchronism of the motor units or an increase in the firing frequency of the motor units. Thus, LLLT may contribute to an improvement in motor recruitment.

The activity of a motor neuron is controlled by three pathways that modulate different aspects of its activity. The first pathway is formed by ganglion cells of the dorsal root that inform the length of the muscle innervated by the alpha neuron. The second pathway is important to the initiation of the control of voluntary movement that stems from motor neurons of the brainstem and motor cortex. The third pathway is composed of interneurons of the spinal cord and is responsible for spinal motor programs [8, 12]. One may suggest that phototherapy in the present study promoted the firing of motor neurons through the early activation of the third pathway of inferior motor neurons, as demonstrated by the increase in the median frequency of the EMG signal. In a scan of the literature, we found no studies that employed median frequency analysis in patients with SCI.

The findings of this initial investigation demonstrate that phototherapy exerted positive effects on the behavior of motor function, especially at rest and during isotonic contraction of the muscles stimulated. It therefore appears that long-term phototherapy could result in improvements in the motor function of affected limbs. As this is the first randomized clinical trial involving patients with SCI submitted to photobiomodulation, further studies should be developed to furnish more information and evaluate the effects of phototherapy on neuroplasticity following a spinal cord injury.

Conclusion

In the present study, phototherapy was effective at promoting a motor response in individuals with spinal cord injuries, as demonstrated by the electromyographic analysis. Studies investigating the effects of different laser parameters on spinal cord recovery are important to determining the effectiveness and safety of laser irradiation as well as the treatment parameters necessary for stimulation.

Compliance with ethical standards This study received approval from the Human Research Ethics Committee of the university under protocol CAAE: 56952716.2.0000.5511. All participants or their legal guardians received clarifications regarding the objectives and procedures of the study and those agreeing to participate signed a statement of informed consent.

Ancillary and post-trial care At the end of the study, all volunteers allocated to the control group received phototherapy with the same protocol administered to the treatment group to avoid any inequality regarding treatment among the individuals.

Competing interests The authors declare that they have no conflict of interest.

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