LOW-INTENSITY ELECTRICAL STIMULATION AND STEM CELLS IN A DOG WITH ACUTE SPINAL CORD INJURY


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ABSTRACT

Spinal cord injury (SCI) is classified as a neurological disorder that affects motor and sensory functions below the injury level [1] which causes functional loss to the axons of the spine and their downstream targets [2]. Since 1982 the low-intensity electrical stimulation (LIES) has been tested as an adjuvant to peripheral nerve regeneration [3] and it has been shown that it could effectively improve the speed and accuracy of nerve regeneration after peripheral nerve injury [4]. Early studies demonstrated that brief electrical stimulation applied to transected peripheral nerves accelerates the reinnervation of distal target tissues, such as muscles [5].

Other interesting finding of regenerative medicine is the applicability of stem cells (SC) in different human and animal tissues. Stem cells are defined as cells that have capacity of self-renewal as well the differentiation capacity in several lineage of cells [6]. SC therapy for patients with SCI can result in the replacement of injured neurons after trauma [7]. In addition, the application of stem cells on axons may result in remyelination and reconnection of neural pathway [8].

The electromechanical delay (EMD) is defined as time elapsed between the onset of muscle electrical activation and onset of force production. A dog diagnosed with incomplete thoracolumbar SCI followed by disc hernia between the vertebral segments L1 and L2, was evaluated by electromyography and mechanomyography after surgical decompression procedure, one SC transplantation and LIES. We observed motor response and decrease in the EMD from 8.25ms to 5.75ms after the procedures. The wavelet decomposition of EMG signals showed reestablishment of vastus lateralis muscle activity 30 days after the procedure. In addition, the EMGRMS increased from 3.3 µVrms to 157 µVrms. The preliminary results of this case report indicate positive effects of the hybrid therapy involving stem cells and low-intensity electrical stimulation after surgical decompression.

KEY WORDS

Electrotherapy, Neural Rehabilitation, Spinal Cord Injury, Stem Cells.
development rate and longer EMD (Δt +59%; p<0.001) when compared to people without the disorder [15].

The aim of this study was to evaluate the vastus lateralis muscle response in acute SCI dog by electromyography (temporal and wavelet domains) and electromechanical delay after surgical decompression procedure and stem cell transplantation followed by low-intensity electrical stimulation.

2. Materials and methods

2.1 Case Report

This work was approved by Animal Ethics Committee of Universidade Tecnológica Federal do Paraná according to the protocol number 2015 – 016. The study participant was of the breed Lhasa Apso, age: six years, sex: male and mass: 8kg, with thoracolumbar spinal cord injury degree 4 (incomplete spinal cord injury) [rating from 1 (intact) to 5 (cauda equina syndrome)] [16] followed by left herniation disc in the spinal segment between L1-L2.

2.2 Diagnosis

After the neurological examination and localization of injury between T3-L3, the mean differential diagnosis were: hernia disk disease, myelitis, neoplasia, disco spondylitis and traumatic. We performed computed tomography and diagnosed the hernia disk disease at level L1-L2. For more information we performed ultrasound and blood tests such as blood count and biochemical evidence of urea, creatinine, albumin, alanine aminotransferase and alkaline phosphatase, as well as urinalysis. Test results were significant for realization of pre-surgical anesthetic preparation.

2.3 Electromyography and Mechanomyography

The electromyography [17] technique captures the neuromuscular electric signal during contraction, while mechanomyography (MMG) records the vibration caused by the muscle during the contraction [18]. The gains of EMG and MMG were 2000x and 1x, respectively. The equipment used for signal acquisition consisted of four channel EMG System (BrasilLida®, 430C). The MMG sensor was a three axial accelerometer where only the perpendicular axis (Z) was used.

The pair of EMG electrodes was of acupuncture needle type (disposable) inserted subcutaneously in the vastus lateralis muscle. The reference electrode was positioned in the region of the spinous process of the fourth lumbar vertebrae. The MMG sensor was positioned on surface of the vastus lateralis muscle belly.

The evaluations were performed before and after treatment (post30 days). The dog underwent specific tests of residual functional capacity, as weight bearing maintenance on paws or capability to march.

2.4 Wavelet transform

The dog underwent specific tests of residual functional capacity, as weight bearing maintenance on paws or even march. The EMG acquisition rate was 1kHz. The signal processing was performed by a customized routine in MatLab® (MathWorks, Inc, version 2013). The EMG signals were filtered using a 3rd order Butterworth band pass filter (20-450 Hz) with notch filters on power line harmonics (60, 120, 180, 240, 300, 360 and 420 Hz). The root mean square (EMG_RMS) was extracted. The EMG signals were processed using the Cauchy wavelet transform (CaW) [19] with scale factor modified to 0.9. Thus, eighteen frequency bands were selected (20, 30, 42, 56, 72, 90, 110, 131, 155, 180, 207, 237, 268, 300, 335, 372, 410 and 450 Hz).

2.5 Electromechanical delay

The EMG and MMG signal acquisition rate was 4kHz. A stimulator developed in prototyping platform Arduino® configured with a peak voltage of 50 V and calibrated with a Tektronix® TDS 1002B two channels oscilloscope with a load of 2.2 kΩ, compliant with the protocol created by Morales [20]. Pulses with durations of 0.2 and 0.3 ms were used to generate biopotentials on the femoral nerve. An electrode 5 x 5 cm was used as the anode (positive pole) on gluteal region and a pen-type electrode was used as the cathode on femoral nerve.

The intervals between the electrical stimulus (ES) single twitch and MMG was calculated (Δt_ES-MMG). The signal processing was performed by a customized routine in MatLab® (MathWorks, Inc version 2013). The MMG signal was rectified. In order to avoid spurious signals, 20% above the baseline of MMG was selected as threshold, where inferior values were set to zero.

2.6 Treatment plan

The treatment plan included the surgical procedure of decompression associated with stem cell transplantation in epidural space, followed by LIES application.
2.7 Anesthetic and surgical procedure

The surgery procedure was accomplished in Veterinary Hospital CLINIVET, Curitiba - Brazil. As premedication, the patient administered 40 micrograms of fentanyl (5 mg/kg) intravenously and 8 micrograms of ketamine (1 mg/kg) also intravenously, then 40 micrograms of propofol bolus (5 mg/kg) intravenously. Maintenance of anesthesia was with isoflurane inhalation and 80 micrograms of fentanyl (10 mg/kg) per hour continuous intravenous infusion. Wide trichotomy was performed in the dorsal region, followed by antisepsis with polyvinylpyrrolidone, incisional adhesive film and placement of the surgical field.

With the patient positioned in sternal recumbency, the Hemilaminectomy surgery was performed, according to the technique described by Dewey [21].

2.8 Postoperative care

The patient remained under observation for 72 hours in the semi-intensive care unit of the veterinary hospital CLINIVET. During the first 36 hours, the patient received analgesia with 16 µg/h fentanyl (2 mg/kg per minute) by intravenous continuous infusion. It also received 16 micrograms of tramadol (2 mg/kg) to 240 µg of Dipyrone (30 mg/kg) subcutaneously during 5 days. Antibiotic prophylaxis was performed by intravenous injection of 49 µg of enrofloxacin (5 mg/kg) during 7 days. The dog’s owners received instructions about rest, monitoring of urinary function and mild physical exercises.

2.9 Stem cells

The stem cells from adipose tissue were isolated using enzymatic digestion, according to Rebelatto and Strutt [22, 23]. They were transplanted into the epidural space, in the amount of 1.2 x 10⁶ cells/ 50 µL, with a volume of 10 µL/min and after injection of 50 µL, the needle was maintained into the epidural space for five minutes to prevent any reflux [24].

2.10 Electrical stimulation therapy

The low-intensity electrical stimulation was applied only once after surgery, on subsensorial (~500µA) level with rectangular waves. The mean voltage applied was 30 mV, and the burst frequency was modulated from 5-20Hz. The carrier frequency was 1 kHz with 200 µs and 800 µs in active and inactive pulse period. Each modulated frequency was applied during 5 minutes in the following order: 5Hz, 10Hz, 15Hz and 20Hz (20 min total application period) with approximately 15% duty cycle, where 14% consisted of standard duty cycle and 1% of reverse polarity to prevent charge build-up and possible biochemical changes during treatment [25].

The electrodes used were acupuncture needle type (disposable) to lower the tissue impedance to less than 1 kΩ [26] with high current density, positioned in the interspinous space, above (anode) and below (cathode) the location of stem cells transplantation.

3. Results

Fig. 1 shows the EMG wavelet response before (Fig. 1A) and 30 days after (Fig. 1B) surgery and therapy procedures with stem cells and LIES. From the analysis of EMG signal, it is possible to detect improvements in motor response of the patient. As seen in Fig. 1A, no electrical signal was captured at pre-treatments evaluation. In Fig. 1B it is possible to observe the electrical muscle response with increased signal amplitudes along several frequency bands, mainly between 50 and 200 Hz, which is typical for healthy muscle response. The EMG_RMS were 3.3 µV_RMS and 157 µV_RMS, pre and post treatment (30 days interval), respectively.
There is a noticeable difference in motor responses before and after surgical decompression, SC therapy and LIES. Fig. 1A represents the register of EMG activity before surgery. In that moment, there was no EMG signal. This was expected due to SCI in the animal, hindering the transmission of nervous signal from the spinal cord to vastus lateralis muscle. However, the Fig. 1B represents the EMG signal on the same muscle, 30 days after the decompressive surgery, SC transplantation and LIES. The signal was softened by wavelet processing due to its ability to filter and maintain the same signal characteristics with noise reduction, as it was reported by Hussain [27]. The difference between the signals in Fig. 1A and 1B are in accordance with our hypothesis that combination of SC and LIES promotes the axon growth in the injured spinal cord contributing to restoration of the motor function [28].

Fig. 2 shows that EMD decreased between the pre and post treatment evaluations. Neurological diseases such as SCI can result in changes in the EMD due to reduction in the diameter of axons, and the consequent reduction of the conduction speed. The application of LIES accelerates the axon regeneration and improves the specificity of sensory reinnervation [5, 29, 30]. Also, the application of additional therapy, such as stem cell transplantation which increases the nerve fiber count and accelerates the functional recovery [31], may be
responsible for the decrease of the EMD after the treatments.

Esposito et al. [32] also used the EMG and MMG during voluntary contraction and muscle relaxation and during electrical stimulation to evaluate the components of the EMD and potential differences in delay in patients affected by myotonic muscular dystrophy compared to healthy subjects. In the evaluated muscles (tibialis anterior and vastus lateralis), all components of delay in both phases (contraction and relaxation) showed an increase in patients affected by the disease (50%; p<0.05) compared to healthy.

The stem cell transplantation is more effective in acute stages and sub-acute tissue injury, while in later stages often does not show functional benefits [33]. Stem cell transplantation may lead to axon remyelination and reconnection of neural pathway [34]. We hypothesized that stem cell transplantation after surgical decompression, followed by LIES, can promote physiological changes that lead to neural remodeling in excitable tissues (muscular, neural and glandular). The LIES induces neuron growing and elongation in the direction of the cathode [35].

In previous studies in dogs with thoracolumbar intervertebral disc disease, Kim [36] showed that decompression surgery was less effective (N\text{\textsubscript{\text{tot}}} = 25; U\text{\textsubscript{\text{nsuccess}}} = 12) than when the surgery was combined with SC transplantation (N\text{\textsubscript{\text{tot}}} = 9; U\text{\textsubscript{\text{nsuccess}}} = 2). Moreover, Ashour et al. [37] reported very promising results with combining the LIES and stem cells transplantation in repair of sciatic nerve crush injury in rats. They showed that both, LIES as SC transplantation, could accelerate and promote the nerve functional regeneration in a period of 8 weeks. They analyzed the nerve conduction velocity (mm/s), showing that SC transplantation group (34.87 ± 1.3 mm/s) and LIES group (34.96 ± 1.29 mm/s) had the nerve conduction velocity significantly higher than SCI control group (26.71 ± 1.3 mm/s) [37].

Our results may be associated with the importance of LIES for SC differentiation [38-40] as shown by Gu in study where in vitro low-frequency (~20 Hz) electrical stimulation promoted proliferation and contributed to the differentiation of SC [41]. Liu [39] showed the same in patients with multiple sclerosis. He combined LIES with transplantation of SC and the results showed not only increased SC differentiation, but also the promoted myelin repair [39].

5. Conclusion

The results of this study demonstrated a positive effect of hybrid therapy involving stem cells and low-intensity electrical stimulation after surgical decompression in SCI dog. Low-intensity electrical stimulation promotes physiological changes due to neuroplasticity and stem cells promote axonal growth in spinal cord injury, contributing to the restoration of its function and preventing demyelination of the distal axons. There is an obvious difference in motor responses before and after the applied procedures, as can be seen from patient's electromyography recordings.

The study of electromechanical delay allows quantification of the level of dysfunctions caused by disease, natural evolution and effectiveness of pharmacological interventions. Our results showed a decrease in Δt\text{ES-MMG} from 8.25ms to 5.75ms.

Both results confirm that the therapies were effective, but we cannot claim them as conclusive because it was an experimental study with a single model. Further studies are required with a larger number of participants to prove our hypothesis and provide more details about the influence of these therapies on the sensory-motor recovery.

References


