HIGH VOLTAGE PULSED CURRENT STIMULATION OF THE SCIATIC NERVE IN RATS: ANALYSIS BY THE SFI

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ABSTRACT

Objective: To analyze the efficiency of high voltage pulsed current (HVPC) with early application in three different sites, in the regeneration of the sciatic nerve in rats submitted to crush injury, the sciatic functional index (SFI) was used to assess the functional recovery. Methods: After crushing of the nerve, 57 animals were submitted to cathodal HVPC at frequency of 50Hz and voltage of 100V, 20 minutes per day, 5 days per week. The rats were divided into five groups: control group; ganglion group; ganglion + muscle group; muscle group; and sham group. The SFI was determined weekly for seven weeks, from the preoperative period to the 6th postoperative week. Results: Compared with the control group, the results showed a significantly better performance of group 2 for the first 3 weeks; group 3 showed significantly better performance in the third week; and group 4 showed a significantly negative performance during the 4th and 6th weeks. Conclusion: Early application of HVPC had a positive effect in the treatment of the spinal cord region and the sciatic nerve root ganglion with a dispersive electrode on the contralateral lumbar region or on the gastrocnemius. However, HVPC had a negative effect in the treatment with an active electrode on the gastrocnemius and a dispersive electrode on the contralateral thigh. ***Level of evidence II, Prospective comparative study.***

Keywords: Rats. Sciatic nerve. Crush injury. Electric stimulation. Spinal cord.

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INTRODUCTION

Experiments on recovery after peripheral nerve injuries involve various techniques, both to produce the injury, and in terms of interventions that seek to accelerate the regeneration process.[1-3] The two most common ways of producing this type of injury are transection and crushing. Transection implies sophisticated techniques for the surgical repair of the nerve. On the other hand, when using a device developed to produce the crush injury, it is possible to control weight and time, knowing the severity of the injury that is being produced.[4,5]

A crush injury is a useful modality for the study of peripheral nerve regeneration, as it mimics a type of axonotmesis, leading to distal Wallerian degeneration, but with a good prognosis of functional recovery. Support structures such as the satellite cell, the basement membrane and the supporting connective tissue are preserved.[6,7] There is also injury to the vessels that supply blood for the vital functions of the nerve.[8]

The sciatic nerve of several animals, especially that of rats, is widely used in a large number of experiments for analysis of motor and sensory function.[1,9,10]

Studying the functional recovery of a nerve is equally important as its histological, morphometric and electrophysiological study, since for human beings, return to activities of daily living depends on the recovery of the functions delegated by these nerves. The analysis of rat footprints through the functional index of the sciatic nerve, developed by De Medinacelli et al.[6] and improved by Bain et al.,[9] is a reliable method of evaluation of functional recovery, and can be used to achieve a reliable correlation between morphological and functional regeneration.[10]

Complete sciatic nerve injury in rats produces deficiency of plantar flexion of the ankle, less ankle spread, a tendency to drag the foot due to the decrease in fibular nerve function and an increase in footprint length due to functional alteration of the plantar flexor muscles.[11] The variables analyzed to produce the SFI are: print length, total toe spread and intermediate toe spread. All the parameters are measured the same way in the normal paw and in the injured, or experimental, paw. As a negative indicator of the degree of nerve dysfunction, the SFI can range from "zero" (normal function or absence of dysfunction) to "-100" (complete dysfunction).[9]

All the authors declare that there is no potential conflict of interest referring to this article.

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Physiotherapy plays an important role in post-injury rehabilitation, seeking to recover neuromuscular function with the use of therapeutic instruments with a regenerative purpose. Attempts to boost nerve regeneration through electric currents, ultrasound and low-power laser, have been in use for a long time; nevertheless, there is not yet any consensus regarding the best intervention.[2,3,12]

High voltage pulsed current (HVPC) stimulation is used in physiotherapeutic treatments for the drainage of edema fluid and to reduce pain, among others, yet so far there are no data on its use in nerve regeneration. HVPC can be described qualitatively as being a double-peaked (or twin-peak) monophasic pulsed current. It presents a pulse duration that can range from 5 to 100 microseconds, with almost instantaneous rise and exponential falls, high peak voltage, high voltage (above 100V), frequency ranging from 2 to 100Hz enabling relatively pleasant stimulation, able to reach the sensory and motor nerve fibers, as well as those responsible for conducting nociceptive impulses.[13,14]

Under the electrode with negative polarity, the high voltage current has shown the following effects: it stimulates the granulation tissue, increases the blood flow with reduction of edema and necrotic tissue, proliferation of fibroblasts, production of collagen and migration of neutrophils and epidermal cells. Under the electrode with positive polarity, the current stimulates epithelization, blood coagulation and blockage of small veins, denatures proteins, reduces the mast cells in ulcers and reduces the migration of macrophages to the ulcer bed.[15,16]

The aim of this study was to analyze the efficiency of treatment with HVPC at three different sites, with early application in the regeneration of the sciatic nerve submitted to crush injury, and evaluated through the SFI, in rats. Hypothesis: HVPC would be efficient in accelerating the regeneration of the sciatic nerve of rats after crush injury.

MATERIAL AND METHOD

The research project was approved by the Committee of Animal Experimentation Ethics of the School of Medicine of Ribeirão Preto of the Universidade de São Paulo and was developed at the Bioengineering Laboratory of the same institution according to the Animal Experimentation Ethics.

The study subjects were 78 male Wistar rats (Rattus norvergicus albinus), with body weight ranging between 180g and 245g, provided by the vivarium of Universidade de São Paulo - Ribeirão Preto Campus. The animals were confined in cages of 0.15m^2, with five animals per cage, maintained in a 12-hour photoperiod, with temperature and humidity maintained by air conditioning, minimum noise, solid feed and water "*ad libitum*", remaining under observation for a period of two days, before their use in the experiment. The final sample was composed of 57 animals. This discrepancy occurred due to complications over the course of the experiment.

The surgical procedure was carried out under anesthesia with 10% ketamine (0.1ml/100g of body weight) and 2% xylazine (0.1ml/100g of body weight) administered by intramuscular route. The lateral region of the right thigh was shaved, as were the sites where the electrodes were to be positioned for stimulation with the aforesaid current. The animal was positioned in left lateral decubitus and fixed to the operating table, followed by antisepsis with povidine iodine and circular delimitation of the operating field with fenestrated sterile drape. The sciatic nerve of the right thigh was approached through a longitudinal rectilinear cutaneous incision, on the lateral side of the thigh, extending from the greater trochanter to the knee. The surgeon opted for the intermediate nerve segment, situated 5mm above its division into the three main branches (fibular, tibial and sural). Once the animal's nerve was exposed, it was transferred to the deadweight device, especially designed to produce the crush injury with a load of 5kg for 10 minutes.[5] The surgical wound was closed with suturing of the tissues in planes, then the area was washed with antiseptic solution and 20% iodized alcohol. After this the animals were returned to their original cages. The analgesic and anti-inflammatory drug ketoprofen was administered by intramuscular route for 3 consecutive days (3.5mg/kg) after production of the injury.

After the surgical procedure, the animals were split into 5 groups identified as follows: group 1 (Control) n=12: sciatic nerve injury/group 2 (Ganglion) n=13: sciatic nerve injury submitted to HVPC with application of the active electrode on the right sciatic nerve root ganglion area and of the dispersive electrode on the contralateral lumbar region (Figure 1)/group 3 (Ganglion + Muscle) n=11: sciatic nerve injury submitted to HVPC with application of the active electrode on the right sciatic nerve root ganglion area and of the dispersive electrode on the right gastrocnemius (Figure 2)/group 4 (Muscle) n=11: sciatic nerve injury submitted to HVPC with application of the active electrode on the right gastrocnemius and dispersive electrode on the contralateral thigh (Figure 3)/group 5 (Sham) n=10: sciatic nerve injury submitted to sham HVPC.

The HVPC application started 24 hours after the surgical procedure, lasting 3 weeks on a daily basis for 20 minutes, 5 days a week, in the evening. The intramuscular application of 10% ketamine (0.1ml/100g of body weight) and 2% xylazine (0.1ml/100g body weight) was required to keep the animal immobile for fixation on the procedure board. After this the carbon electrodes (1cm^2) with hydrosoluble gel were placed in the positions determined according to the application groups and fastened

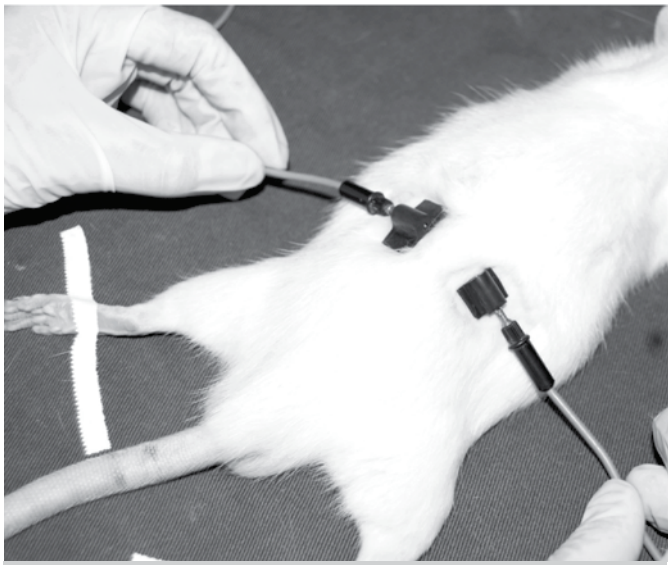

Figure 1. *Positioning of the electrodes in Group 2 (right side active - cathode; left side dispersive).*

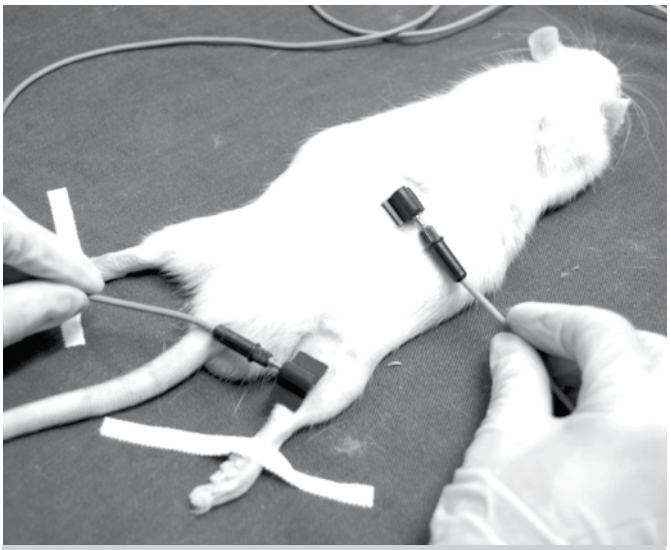

Figure 2. *Positioning of the electrodes in Group 3 (lumbar region active - cathode; paw dispersive).*

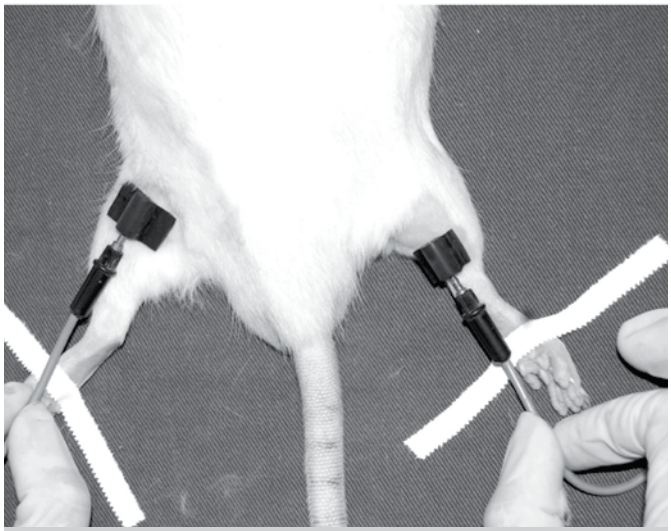

Figure 3. *Positioning of the electrodes in Group 4 (right paw active - cathode; left paw dispersive).*

with elastic bands, prior to the current stimulation. In this experiment we used the Ibramed® Neurodyn High Volt device with the following parameters: negative polarity (cathodic), frequency of 50 Hz and voltage above 100V. At the end of the 6th week the animals were identified, weighed and sacrificed using an overdose of anesthetic.

Footprints were recorded for all the animals through video filming, before the production of the injury, one week after the injury and so on, periodically, once a week, for seven consecutive weeks. Before the first footprint recording, the animals were taught to walk on the treadmill at speed. The images obtained in the filming sessions were adapted to the ideal size using Adobe Photoshop software (version CS3®), and edited for use of the functional gait analysis computer program (AFNP – functional analysis of the peripheral nerves)[10] where the SFI Parameters are calculated according to the method proposed by Bain et al.[9]

RESULTS

The animals tolerated the surgical procedure well, and on the following day, presented alterations in the paw and in their walking pattern corresponding to those described by Costa et al.[11] During the experiment, some animals were excluded and others died due to complications, such as: death of the animals during the surgical procedure; animals discarded because of failure to walk on the track and amputation of toes or necrosis of the paw (due to sciatic nerve injury); death of animals during the anesthesia for stimulation. The animals that completed the study gradually reacquired the ability to walk normally over time, with adequate weight bearing and toe spread over the injured paw.

A total of 399 footprints were analyzed: group 1 = 84 footprints (n=12 x 7 weeks); group 2 = 77; group 3 = 77; group 4 = 77; group 5 = 70.

During the preoperative phase (week 0), the mean SFI values were: group 1 = 3.59 (variation: -17.26 to 27.67); group 2 -0.96 (variation: -22.94 to 24.8); group 3 = -2.7 (variation:-12.14 to 7.21); group 4 = -3.85 (variation: -16.44 to 11.8); group 5 = 7.56 (variation: -20.88 to 0.53). There was no statistical difference in this period between or among the groups, keeping in mind that the animals were still intact. In week 1, the mean SFI values were: group 1 = -96.53 (variation: -111.3 to -52.7); group 2 = -78.33 (variation: -105.9 to -9.48); group 3 = -96.48 (variation:-114.5 to -28.11); group 4 = -101.6 (variation: -109 to -90.91); group 5 = -98.81 (variation: -118.5 to -80.71). There was significant difference between groups 1 and 2 $p=0.01$. In week 2, the mean SFI values were: group 1 = -89.09 (variation: -107.3 to -65.41); group 2= -70.33 (variation: -103 to -32.7); group 3 = -86.56 (variation:-101.1 to -38.56); group 4 = -88.36 (variation: -105.1 to -53.72); group 5 = -87.87 (variation: -111.6 to -71.07). There was significant difference between groups 1 and 2 $p=0.01$. In week 3, the mean SFI values were: group 1 = -59.91 (variation: -89.28 to -33.45); group 2 = -37.37 (variation: -85.46 to -9.88); group 3 = -43.64 (variation:-83.12 to 1.24); group 4 = -55.99 (variation: -92.17 to -15.55); group 5 = -62.89 (variation: -98.02 to -12.46). There was significant difference between: groups 1 and 2 $p<0.01$, groups 1 and 3 $p=0.03$. In week 4, the mean SFI values were: group 1 = -43.75 (variation: -72.01 to -11.65); group 2 = -29.51 (variation: -52.53 to 1.38); group 3 = -29.29 (variation:-61.47 to 9.63); group 4 = -61.06 (variation: -85.13 to -44.82); group 5 = -53.11 (variation: -71.3 to -32.16). There was significant difference between groups 1 and 4 $p=0.02$. In week 5, the mean SFI values were: group 1 = -33.15 (variation: -71.02 to 4); group 2 = -17.84 (variation: -34.2 to 1.35); group 3 = -33.19 (variation:-52.27 to -4.75); group 4 = -32.51 (variation: -48.53 to -20.39); group 5 = -33.2 (variation: -66.12 to -2.8). There was significant difference between groups 1 and 2 $p=0.03$. In week 6, the mean SFI values were: group 1 = -17.54 (variation: -43.69 to 3.39); group 2 = -12.88 (variation: -34.33 to 1.56); group 3 = -20.96 (variation:-34.78 to 0.39); group 4 = -32.13 (variation: -96 to 0.64); group 5 = -19.1 (variation: -42.91 to 13.6). There was significant difference between groups 1 and 4, $p=0.04$.

The results presented by the groups over the seven weeks of follow-up are described in Table 1 and in Figure 4.

Table 1. *Comparison between the groups at each time – level of significance (p<0.05).*

Groups			Time	p- value
1	-	2	0	0.52
1	-	3	0	0.38
1	-	4	0	0.30
1	-	5	0	0.14
1	-	2	1	0.01
1	-	3	1	1.00
1	-	4	1	0.49
1	-	5	1	0.76
1	-	2	2	0.01
1	-	3	2	0.73
1	-	4	2	0.93
1	-	5	2	0.87
1	-	2	3	<0.01
1	-	3	3	0.03
1	-	4	3	0.59
1	-	5	3	0.69
1	-	2	4	0.05
1	-	3	4	0.05
1	-	4	4	0.02
1	-	5	4	0.21
1	-	2	5	0.03
1	-	3	5	0.99
1	-	4	5	0.94
1	-	5	5	1.00
1	-	2	6	0.51
1	-	3	6	0.63
1	-	4	6	0.04
1	-	5	6	0.84

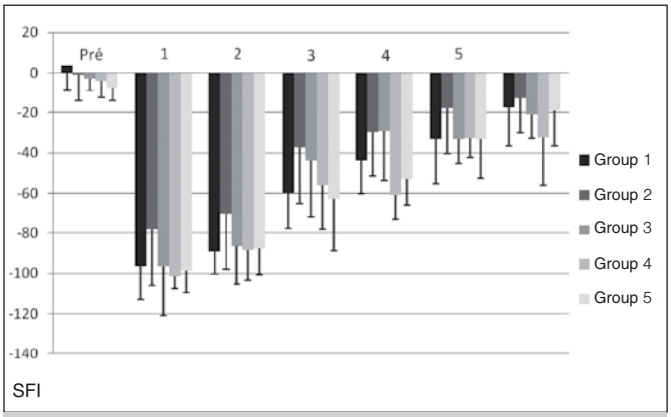

Figure 4. *Performance of the SFI of the groups over the weeks (Means and Standard Deviation).*

DISCUSSION

In our study we employed the crush model[5] that caused severe injury to the sciatic nerve, yet maintained the necessary structures for growth of the axon, enabling its regeneration.

Since nerve regeneration is dependent on revascularization,[17,18] applying HVPC for this purpose could provide findings similar to those of other studies[15,16] that evidenced the efficacy of HVPC in promoting revascularization at the site under the cathode.

The hypothesis presented in this study appears to have been confirmed mainly by the performances presented by group 2, due to the significantly superior difference in relation to the performances of the control group throughout the intervention period. The results presented by group 2 in the present study corroborate the findings of Shamir et al.[19] and Rochkind et al.[20] demonstrating widespread applicability of HVPC with the objective of regenerating the sciatic nerve based on stimulation of the ganglion of its root.

As regards the aspect of the start of intervention, beginning the treatment on the first postoperative day may have accelerated the nerve regeneration process, as observed in the case of group 2. These results are similar to those of other studies where pulsed current stimulation (electrical stimulation [ES] or functional electrical stimulation [FES]) was applied at an early stage, in sciatic nerve regeneration.[12, 21] On the other hand, the performance of the other groups of this study, despite having the same intervention period, did not keep up with the positive performance of the above mentioned group.

As regards the evaluation method, the SFI of rats, in this study we opted to use the filming method developed in our laboratory.[10,22] The chosen option made it easier to capture ideal images for evaluation in the first weeks after surgery, enabling the early functional evaluation of the individuals.

In view of the hypothesis that HVPC could accelerate functional recovery when compared to the group without stimulation (group 1), only the performances presented by group 2 are in accordance with the hypothesis initially proposed. The findings of group 3 and of group 4 presented performance standards similar to those of group 1 and group 5.

Observing the behaviors of groups 2 and 3 of this study, stimulation with the active electrode (cathode) occurred in the same region, therefore it would be possible to assume that they would present similar standards of performance. However, we can suggest that the superior performance of group 2 has occurred due to the application distance between the electrodes, which was considerably different from one group to the other. The shorter distance between the electrodes in group 2 may have exerted influence on the efficacy of the stimulation, and not only due to the specific location of the active electrodes. Bettany et al.[23] noted that HVPC, depending on its application site, as well as on the frequency and voltage applied, may or may not influence the blood flow of the stimulated region. In dealing with a polarized current, there is the formation of ions under the electrode, which although dissipating rapidly due to the short space of time in which the current flows, influence the stimulation of electrolytic reactions in its interior.[15,16] Therefore, when the electrodes are positioned closer to one another, these effects are facilitated, and can be more intense. In this regard, it is possible to infer that the performances of groups 3 and 4 have not been significantly superior to the performance of group 1, on account of the considerable distance between the active and dispersive electrodes.

In relation to the performance of group 3, two aspects should be considered: a possible late positive effect; and the non-maintenance of the significantly positive effect from the 3rd postoperative week. In relation to the possible late effect of group 3 in the 3rd week, the performance climbed to a significantly higher level. So far the literature consulted has not

yielded any surveys demonstrating late effects with HVPC. As regards the non-maintenance of the superior performance in relation to group 1 from the 3rd week, if, on the one side, this may simply be a random occurrence, on the other side, it may be justified by the interruption of the stimulation in the same week. Both aspects suggest that further surveys could explore the occurrence of these effects.

On the other hand, it is necessary to also consider that HVPC has a negative late effect, as suggested by the significantly inferior performance of group 4, in the 4th and 6th weeks. Such findings have characteristics that are similar to those of the study by Baptista et al.[24], using TENS (Transcutaneous Electrical Nerve Stimulation) in an attempt to accelerate sciatic nerve regeneration after crush injury. The histological results of the two groups showed signs of impaired regeneration. Thus the negative results of group 4, in the 4th and 6th weeks, supported by the survey of Baptista et al.,[24] suggest that this type of stimulation not only failed to improve the group's performance, but also contributed to the negative effects in sciatic nerve regeneration. Finally, the group 5 was under the effect of simulated stimulation, therefore its performance was similar to that of the control group, as the animal did not suffer the effect of the applied current.

CONCLUSIONS

HVPC proved efficient in the treatment of crush injury to nerves, when applied at any early stage on the area of the spinal cord and of the sciatic nerve root ganglion, with the dispersive electrode placed in the same contralateral region;

HVPC demonstrated a late effect when applied at an early stage on the area of the spinal cord and of the sciatic nerve root ganglion, with the dispersive electrode placed on the gastrocnemius.

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