Influence of tramadol on functional recovery of acute spinal cord injury in rats[1]

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Abstract

Purpose: To evaluate the influence tramadol on functional recovery of acute spinal cord injury in rats.
Methods: Ten rats were divided into two groups (n = 5). All animals were submitted by a laminectomy and spinal cord injury at eighth thoracic vertebra. In control group, the rats didn't receive any analgesic. In tramadol group, the rats received tramadol 4mg/Kg at 12/12h until 5 days by subcutaneous. Animals were following by fourteen days. Was evaluated the Basso, Beattie, Bresnahan scale (locomotor evaluation) and Rat Grimace Scale (pain evaluation) at four periods.
Results: There no difference between the groups in locomotor evaluation in all periods evaluated (p>0.05) and in both groups there was a partial recover of function. The tramadol group show a lower pain levels at the first, third and seventh postoperatively days when comparing to the control group.
Conclusion: The tramadol as an analgesic agent don't influence on functional recovery of acute spinal cord injury in rats
Key words: Neuralgia. Pain. Analgesics. Rats.

■ Introduction

Medullar injury is a dramatic event that interference on normal brain function, such as sensory, motor and autonomic functions, and subsequently affects the patient's physical, psychological and social well-being[1,2]. Traumatic spinal cord injury leads not only to motor impairment but also to central chronic pain, making this lesion difficult to treat[3,4]. Chronic compression caused by trauma and subsequent fibrosis may result in loss of motor neurons in the anterior horn[5], but the detailed mechanism of this type of neuronal loss is not fully understood[6].

Despite the surgical and pharmacological resources available, it is not possible to totally reverse the neurological damage after trauma[7]. Surgical therapy is restricted to spinal cord decompression, fragments removal, and spinal stabilization[8]. In drug therapy, studies indicate that medullary lesions can be minimized by drugs, provided they are administered in a short time after the trauma[9].

The use of experimental models has improved the knowledge of the pathophysiology of these lesions, providing new opportunities for therapeutic strategies in vivo[10], using methods of reproduction of acute and chronic spinal cord injury in small rodents, mainly rats[11].

Studies involving neurological injury have become increasingly widespread and frequent in the scientific field, but the precaution and care with the analgesia of the experimental models have not followed the development of the projects, not being noted the adequate application of postoperative analgesic protocol level to the intensity of pain and discomfort caused[12,13].

Tramadol hydrochloride is a clinically effective analgesic widely used in acute and chronic pain conditions, such as neuropathic pain, cancer and postoperative. It acts as a μ-opioid agonist, but also has a variety of other properties that may contribute to its analgesic effect, including inhibition of serotonin and norepinephrine[14,15].

Some studies mention the use of opioids for pain control after the neuropathic injury procedure[12]. However, there is resistance in the use due to the possibility that these medications may influence the results of the study. Thus, this study proposes to evaluate the effect of tramadol on the functional recovery after injury of the acute spinal cord in rats.

■ Methods

This research followed the rules of the Brazilian Law for Animal Care (Law: 11.794/08) and ARRIVE guideline; and it was approved by the Animal Use and Care Committee at the Centro Universitário do Pará, Protocol Nº 06/17.

Ten Wistar male rats (*Rattus norvegicus*) weighing between 250-350 grams and aging between 90-120 days provided from the Animal Colony of the Instituto Evandro Chagas were used. The animals were kept in a vivarium of the Experimental Surgery Laboratory at the Pará State University (Brazil) with a controlled environment with a 12h light and 12h dark cycle. Water and food were provided *ad libitum*. They were randomly divided into two groups (n = 5):

Control Group: Animals' submitted to laminectomy, without analgesic protocol.

Tramadol Group: Animals' submitted to laminectomy, treat with tramadol as analgesic.

All surgical procedures were performed in anesthesia (ketamine 60mg/Kg, xylazine 8mg/kg and fentanyl 0.03mg/Kg, intraperitoneal). Fentanyl was repeat for each 30 minutes until the end of the surgery. All animals underwent the same surgical procedure, with the two

groups differing only in the analgesic protocol used, the surgical procedures were performed by the same researcher. The rats were placed in a horizontal ventral position, then was shaved the thoracic region. Antisepsis was performed with Povidone-iodine.

Microsurgical procedures were performed under a DFVasconcelos© microscope with 40× magnification. A 3-cm incision was performed in the thoraco-lumbar region above the column. The paravertebral muscles were dissected and then the eighth thoracic vertebra was identified. The spinal cord was exposed through a laminectomy of the eighth thoracic vertebra with a precision surgical pliers. The spinal cord injury was performed with a Scalpel blade Nº12. Was performed a complete right hemisection, based on posterior spinal vein, with total depth. The procedure ended with the suture of muscles with 5-0 nylon and skin using 4-0 nylon.

The animals were followed up by 14 days postoperatively. In both groups, enrofloxacin was administrated by subcutaneous at 10mg/Kg once a day until 7 days and lidocaine topical was used in the incision at 12/12h until 5 days. On Tramadol group, the animals received tramadol hydrochloride 4mg/Kg at 12/12h until 5 days by subcutaneous. It was not necessary to perform the bladder massage. The animals were housed in isolated cages after the procedure to avoid injuries and pressure ulcers.

The parameters analyzed were Basso, Beattie, Bresnahan (BBB) scale[16] and Rat Grimace Scale[17]. Both scales were performed at first, third, seventh and fourth quarter postoperatively days. The BBB scale[16] is a locomotor evaluation performed by the filming of the motricity of each rat during five minutes, three times at least. This test was performed along a 50×20 cm open field cage, lined with ethylene vinyl acetate to avoid slip action, by only a trained researcher. The BBB scale ranging from 0 – No observable movement of the hindlimbs to 21 - Consistent plantar stepping and coordinated gait, consistent movement of the toes; paw position is predominantly parallel to the body during the whole support stage; consistent trunk stability; consistent tail elevation.

The rat grimace scale[17] consists of four facial "action units" (orbital tightening; nose/cheek flattening; ear changes; and whisker change) scored on a 0-2 scale for their prominence in still photographs taken from digital video of mice in either a baseline or pain condition. For the time-course study, 120 images were collected from 10 rats at each of the 4 time points (24 hour, 3, 7 and 14 days) and were analyzed by an experienced blinded observer.

The software BioEstat© 5.3 was used. All data were expressed as means ±standard deviation. Student's t test was used to compare two groups in both scales (intergroup comparisons). The Kruskal-Wallis test, followed by Dunn post hoc test correction when necessary, was used to comparing the intragroup evolution. The Pearson's correlation test was used to determine the association between BBB scale and rat grimace scale. Statistical significance was assumed at $p< 0.05$.

■ Results

All animals survived during the study period. Table 1 presents the mean and standard error of mean BBB and rat grimace scales. Regarding the BBB scale (Figure 1), in the control group at the 14th postoperatively days the animals have a better score than the first and third postoperatively days ($p<0.05$). The same result was identified on tramadol group. There were no significant differences between the groups at the four analyzed periods ($p>0.05$).

Table 1 – Mean scores of BBB and rat grimace scales according groups.

Parameter	Postoperatively day			
	First	Third	Seventh	Fourth quarter
BBB scale				
Control group	2.00 ±1.87	5.20 ±2.77	7.40 ±5.32	12.20 ±3.70
Tramadol group	1.80 ±3.03	6.00 ±4.47	10.00 ±6.16	13.00 ±3.39
Rat grimace scale				
Control group	1.13 ±0.08	1.06 ±0.10	0.60 ±0.10	0.25 ±0.11
Tramadol group	0.37 ±0.32	0.20 ±0.21	0.16 ±0.13	0.14 ±0.12

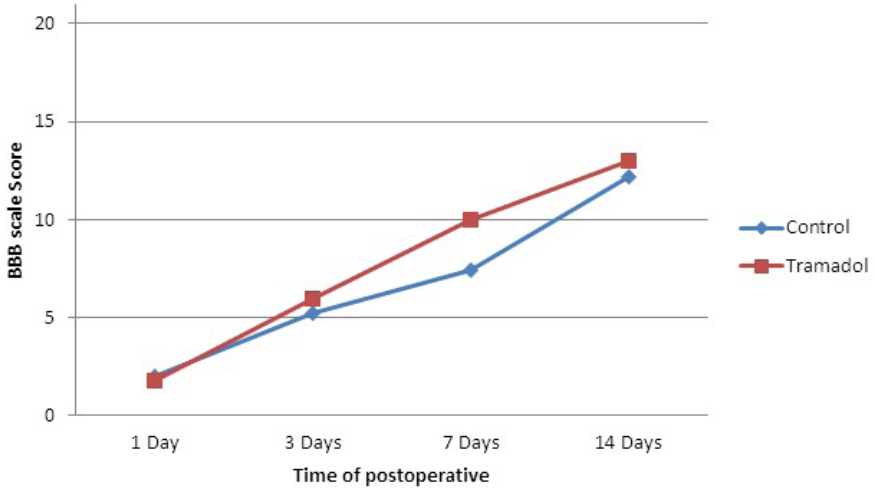

Figure 1 – Evolution of score of Basso, Beattie, Bresnahan (BBB) scale according each group.

Regarding the rat grimace scale (Figure 2), there were no significant differences in the tramadol group at the four analyzed periods ($p>0.05$). In control group, in the first and third postoperatively days have a higher pain level than the 14th postoperatively days ($p<0.0001$). There were no significant differences between the groups at the 14th postoperatively days ($p>0.05$), however at the first, third and seventh postoperatively days the tramadol group show a lower score the control group ($p<0.0001$).

Correlation analysis showed intense negative correlation between the BBB scale and rat grimace scale in control group [r(Pearson): -0,78 IC (95%) -0,53 – -0,91; $p<0.0001$] and a great negative correlation between the BBB scale and rat grimace scale in tramadol group [r(Pearson): -0,51 IC (95%) -0,02 – -0,80; $p=0.04$]

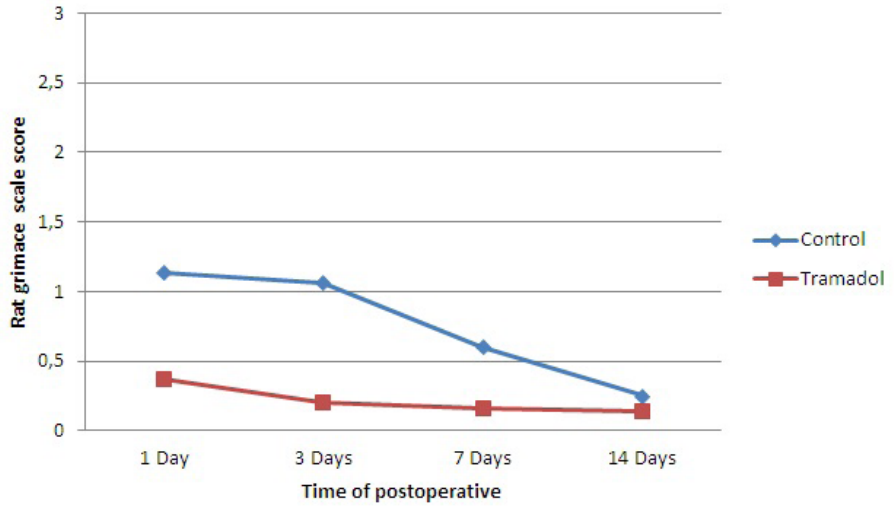

Figure 2 – Evolution of score of rat grimace scale according each group.

■ Discussion

Models of nerve damage in animals have been developed over the last 25 years to mimic the clinical manifestation of neuropathic pain. Normally, they have focused on damaging nerves that innervate the hind paw, as this is likely to show changes in sensory function via measurement of withdrawal reflexes in response to mechanical, thermal or chemical stimulation[12,18]. Regardless of the method applied, trauma-induced neuropathic models are typically invasive and give rise to perioperative nociceptive signaling of surrounding tissue. Despite this, the use of appropriate post-operative analgesics is not performed as rigorously as it should be, despite being an irrefutable example from an animal welfare perspective and minimizing experimental variance due to stress induced by pain[12,13,19]. In this study, no difference statistically significant was identified, considering the functional outcome of the BBB scale in any of the evaluated time, showing no influence of tramadol in this injury protocol.

The pain, in the short term, serves to protect an individual from harm, but in the long term can become a debilitating condition. The purpose of pain is to alert an individual to withdraw from immediate tissue damaging stimuli and to prevent further damage to the site of injury during the healing process[20]. This fact was observed in this study, whereas, both GC and GT groups showed inversely proportional relationships between the rat Grimace score (the pain scale) and the BBB scale (functional scale), suggesting that the lower the pain, the greater the function of the affected member.

Philips *et al.*[21] evaluated the neuropathic pain in a cervical radiculopathy model in rats and verified the persistence of pain after injury on days 1 and 7, which confirms the verified data in this study, where the GC presented higher score in the rat grimace scale compared to the GT until the 7th postoperative day, not being identified between the groups on the 14th day. Although rat grimace scale most sensitively detects pain at acute time points[17,22-24], the disappearance of facial grimace is not necessarily indicative of the resolution of spontaneous pain[17], whereas natural adaptations have led prey species, like rodents, to inhibit facial grimace as soon as possible so that they do not become the target of predators[25]. By hiding painful expressions,

pain may persist for longer than is detectable by rat grimace scale. This hypothesis has been already suggested previously for visceral, inflammatory, and neuropathic conditions[17,24]. Other tests of spontaneous behaviors, like conditioned place preference and activity monitoring, also fail to capture robust pain behaviors after acute time points[26].

In the GT, tramadol was administered postoperatively in order to avoid the pain felt by the animal during anesthetic awakening. Previous work showed that inflammatory cytokine levels increase in the spinal cord after injury as early as 1 h after nerve root compression[27,28] being indicated preemptive, postoperative analgesics or even early after injury, and this was verified in the GT group, which presented lower values in the Grimace score in the first 24 hours, in relation to the control group.

Despite the apparent lack of applicability of the rat grimace scale for animal care staff in evaluating pain levels on a day-to-day basis, the use of facial coding to detect pain has clinical utility in refining our understanding of acute pain[29-34]. This study confirms the importance of applying a pain scale in spinal cord injury procedures reaffirming that cited by Sperry *et al*.[33], as a useful modality to predict long periods in clinical pain models. Although the non-invasive collection of rat grimace scale data is simple to execute, its scoring can be a labor-intensive process[17]. Recent advances in real-time rat grimace scale[32] and convolutional neural networks for automated facial grimace scoring[34] provide methods to streamline its use in both clinical and pre-clinical research applications.

Some limits of this study must be highlight, we don't evaluated the effect of tramadol on chronic stage of spinal cord injury, and was not performed inflammatory markets, however it don't invalided this study because we proved that tramadol don't affect the functional recovery, so new studies could evaluate the effect of tramadol or others opioids' drugs on chronic spinal cord injury.

■ Conclusion

The tramadol attenuates pain in a model of spinal cord injury without altering the functional evaluation of the injured member.

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