PREEMPTIVE ANALGESIC EFFECT OF LIDOCAINE IN A CHRONIC NEUROPATHIC PAIN MODEL

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Abstract – Preemptive analgesia inhibits the progression of pain caused by surgical lesions. To analyze the effect of lidocaine on postoperative pain relief, we performed compression of the right sciatic nerve in Wistar rats and observed the differences on behavior between the group that received lidocaine and the group that was not treated with the local anesthetics pre-operatively. Group 1 was not operated (control); group 2 underwent the sciatic nerve ligature without lidocaine; group 3, underwent surgery with previous local infiltration of lidocaine. Group 2 showed significantly longer scratching times with a peak on day 14 post-operative (p=0.0005) and reduction in the latency to both noxious (p=0.003) and non-noxious (p=0.004) thermal stimulus. Group 3 presented significantly shorter scratching times (p=0.004) and longer latency times when compared to Group 2. Preemptive use of lidocaine 2% can potentially reduce the postoperative neuropathic pain associated with sciatic nerve compression.

KEY WORDS: pain, postoperative, sciatic neuropathy, analgesia, lidocaine, experimental animal models.

Efeito analgésico preemptivo da lidocaína em modelo de dor crônica neuropática

Resumo – A analgesia preemptiva inibe a progressão da dor causada por lesão cirúrgica. Para analisar o efeito da lidocaína na diminuição da dor pós-operatória, submetemos ratos Wistar a compressão cirúrgica do nervo ciático e observamos diferenças em alguns padrões de comportamento entre o grupo tratado com lidocaína pré-operatória e o grupo não-tratado com o anestésico local. O grupo 1 não foi operado (controle); o grupo 2, submetido a ligadura do nervo ciático sem lidocaína, apresentou significativo aumento do tempo de coçar-se com um pico no 14° pós-operatório (p=0.0005) e redução na latência para os estímulos térmicos nocivo (p=0.003) e não-nocivo (p=0.004); o grupo 3, operado com a droga preemptiva, demonstrou significativo decréscimo no tempo de coçar-se (p=0.004) e maiores tempos de latência quando comparados aos do grupo 2. O uso preemptivo da lidocaína 2% pode, potencialmente, reduzir a dor neuropática pós-operatória associada à compressão do nervo ciático.

PALAVRAS-CHAVE: dor pós-operatória, neuropatia ciática, analgesia, lidocaína, modelos animais.

Neuropathic pain is characterized by a dull or burning sensation in the setting of hyperalgesia and allodynia and is often represented as a chronic pain[1,2]. Chronic pain usually cannot be controlled by regular treatments. It is well known for its devastating impact in the emotional and cognitive behaviour[3] and follows the acute postoperative pain in 10–50% of patients after regular surgeries[4].

This can be explained by the increased pain responsiveness and neural sensitization developed after the surgical lesion leading to an exaggerated postoperative pain, allodynia and persistent pain, which can be clinically associated with slow recovery, movement restriction, pulmonary embolism and higher blood pressure[5,6].

Preemptive analgesia may be used before the painful

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stimulus in an attempt to achieve inhibition or at least reduction of pain intensity after surgical procedures. It works by preventing central sensitization, which is responsible for the secondary development of pain[7]. The animal model of Chronic Constriction Injury[8] has been used to reproduce the characteristics of the human neuropathic pain secondary to injury, evidenced by an overreaction to the thermal stimuli, excessive limb movement and asymmetric thermal sensitivity in the extremities. Physiologically, aberrant action potentials known as ectopic discharges are conducted via activation of sodium channels along the injured nerve[9]. Therefore local anesthetics like lidocaine, blocking sodium channels, could suppress ectopic discharges[10]. Two approaches have been described to assess the preemptive analgesic effect: the PRE vs. NO treatment design, where the study will compare preoperative treatment and nontreatment groups; and the PRE vs. POST, which aims to prove that the pre-treatment is more effective than the same drug given at the end of the surgery[11]. Although preemptive analgesia does not consist in a novel management anymore, its clinical application is still not consensual, and there is not a definition of the most reliable experimental model to test its efficacy[7,11,12].

The aims of this study are: to assess the feasibility of the Chronic Constriction Injury model in a PRE vs. NO treatment design; to test the efficacy of lidocaine 2% as a preemptive drug in this combined model; to identify when its effect is more evident within the postoperative observation period.

METHOD
Animals
All the experiments described in this study were conducted according to the guidelines of the International Association for Study of Pain (IASP)[13], with the use of a restricted number of animals, avoidance of definitive incapacities and relief of the symptoms caused by the experiments as much as possible. The study was approved by the Animal Ethics Committee of the Federal University of Ceará. Eighteen Wistar rats, weighing about 300g were distributed into 3 different groups consisting of 6 animals each. All rats were kept in an approved laboratory with a 12-hour day/12-hour night cycle. The animals were fed mouse chow and water ad libitum. The animals in group 1 did not undergo any surgical procedure; the group 2 comprised animals that were submitted to common sciatic nerve constrictive ligature with no preoperative lidocaine treatment, and the rats in group 3 underwent the surgical procedure, receiving lidocaine perioperatively, just before the sciatic constriction.

Common sciatic nerve ligature surgery
Under anesthesia (phenobarbital sodium 50 mg/kg intraperitoneal) and aseptic technique, the right sciatic nerve was exposed at the high-thigh level. Animals in group 2 received 0.4 mL of saline solution (placebo) over the sciatic nerve for 10 minutes before its ligature. In group 3, the rats had their right sciatic nerves washed in 0.4 mL of lidocaine 2% without vasoconstrictors, for 10 minutes before the chronic constriction injury. After that, a surgical injury was performed on the sciatic nerves of both experimental groups 2 and 3. The dorsum of the nerve was carefully freed from surrounding connective tissues at a site near the trochanter just distal to the point at which the posterior biceps semitendinosus (PBST) nerve branches off the common sciatic nerve. Using honed (no. 5) jewelers forceps, the nerve was fixed in its place by pinching the epineurium on its dorsal aspect, taking care not to press the nerve against underlying structures. A mononylon 4.0 suture was inserted into the nerve with a 3/g curved, reversed-cutting mini-needle and tightly ligated so that the dorsal 1/3–1/2 of the nerve thickness was trapped in the ligature. The wound was then closed using silk suture. The procedure was performed according to the technique described by Bennett and Xie[8]. A sham surgery was performed in the left leg of the animals in the control group (group 1), with exposure of the common sciatic nerve, but no injury to it. After the exposure of the nerve, the wound was closed with the use of silk suture.

Behavioral observation
The behavior pattern considered as a sign of neuropathic pain in rats was the scratching activity[14]. The scratching times were collected during observation sessions carried for 28 days, beginning on day zero (pre-op) and followed by the postoperative (post-op) days 2, 3, 7, 14, 21 and 28. Each animal was observed for 30 minutes, after 5 minutes of adaptation to the environment of the observational box.

Thermal test
Response to thermal stimuli was also analyzed. Tests using non-noxious (40°C) and noxious (46°C) stimulation were performed involving immersion of the rat's hind paw in a bath until it was observed the withdrawal or struggle[15]. After 15 seconds of exposure (cut-off), the thermal stimulus was removed even if the animal did not experience any of the behaviors mentioned above and the withdrawal latency to the thermal stimuli was determined. The tests were performed on day zero (preoperative day) and on postoperative days 2, 3, 7, 14, 21 and 28.

Statistical analysis
The analysis of the results was carried out with the use of the software NCSS/PASS (Cintze J. NCSS and PASS. Number Cruncher Statistical Systems, 2001. Kaysville, Utah). In order to evaluate the differences in times of scratching as well as in the latency to noxious and non-noxious stimuli, the differences between the times obtained in the post-op observational day and in the pre-op observation were used. These differences were reported as ΔScr for scratching behavior and ΔLat for latency to thermal stimuli. The differences of ΔScrs and ΔLats among

Table. Hind paw withdrawal latency to noxious (46°C) and to non-noxious (40°C) thermal stimuli in each group. Medians and interquartile ranges were used.

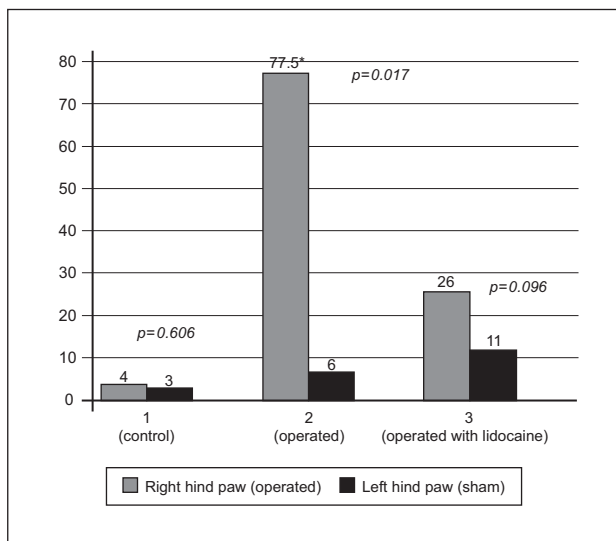| Group	Noxious stimulus (46°C)	Non-Noxious stimulus (40°C)	p
1 (Not-operated control)	3.5 (3−4)	13 (12.2−13.8)	0.001
2 (No-preemptive drug)	1.5 (1.2−2.5)	5.5 (5−6.5)	0.01
3 (Preemptive drug)	8 (6−8.2)	9.5 (7.5−11)	0.109

Fig 1. Scratching times in the right and left hind paws. Mirror pain in group 3 suggested by the non-significant difference between the operated and the sham paws. The times are represented in seconds. Medians were used.
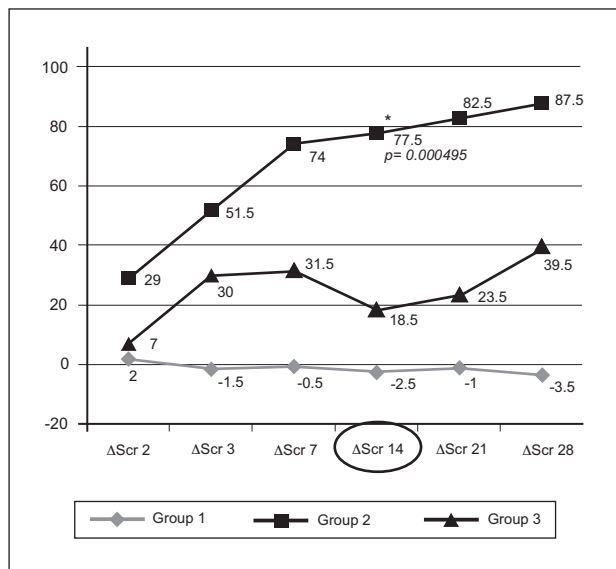

*Fig 2. Scratching behavior in the right (operated) hind paws of the three study groups. ΔScr is the difference of the duration of scratching between the given day and the baseline (pre-op) observation. The times are represented in seconds. Medians were used. *post-hoc analyses revealed no significant difference between groups 2 and 3 in ΔLat 7, 21 and 28. For all other comparisons, p<0.04 was achieved. The maximum difference among the 3 groups was seen in ΔLat 14. This was also the greatest difference between groups 1 and 2 and between groups 2 and 3 separately (p=0.0038 and p=0.03 respectively).*

the three groups in each day of observation were assessed by a Kruskal-Wallis One-Way ANOVA test. The analysis between two groups was performed with a post-hoc T-test. The significance of the differences the right and left hid paws was assessed by the non-parametric Mann-Whitney U for Difference in Medians test. Values of $p < 0.05$ were considered as significant.

RESULTS
Group 1
The animals in group 1 showed median scratching times of 4 and 3 seconds, respectively in the right and left leg, which demonstrate that there is no significant difference in these values (Fig 1). This is a normal pattern since this is an expected behavior in normal animals and does not correspond to pain. Regarding the the thermal tests, the median times of withdrawal latency to noxious stimulus (46°C) was 3.5 seconds, and to non-noxious stimulus (40°C) was 13 seconds, evidencing an expected significant difference (Table).

Group 2
Comparing the scratching times between the right and left side, it was observed that the side where the ligature was performed (right) displayed a significantly longer time (Fig 1). These animals that underwent right common sciatic nerve ligature without preemptive lidocaine presented an increasing curve of scratching values from post-op day 2 to post-op day 28 (Fig 2). They showed significantly longer scratching times in the operated leg than those in group 1, and significantly shorter scratching times than those in group 3. The peak of these differences in scratching times was seen in post-op day 14 (Fig 2).

Thermal tests presented median times of withdrawal of the hind paw to noxious stimulus of 1.5 seconds, and 5.5 seconds to non-noxious stimulus (Table). This group achieved significantly lower latency times to both noxious and non-noxious stimuli in comparison with the other two groups. The withdrawal latency to the thermal stimuli presented the maximal reduction in the post-op day 14 (Figs 3 and 4).

Group 3
In group 3 (lidocaine group), the scratching times of both sides progressively increased from the post-op day 2
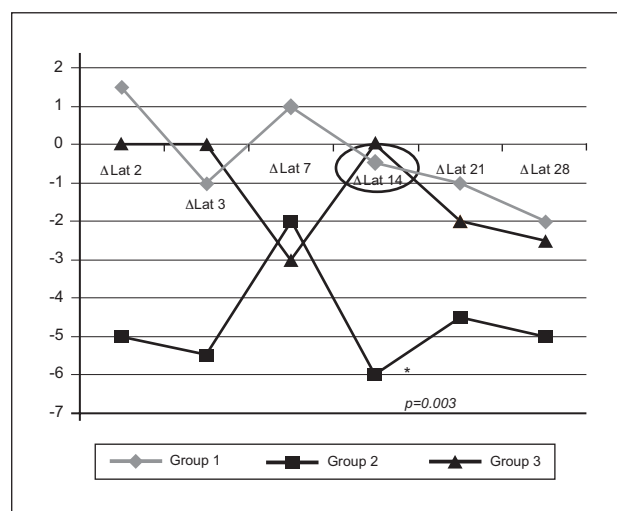
Fig 3. Hind paw withdrawal latency to noxious (46°C) thermal stimuli among the three study groups. ΔLat is the difference of latency between the given day and the baseline (pre-operative) observation. The times are represented in seconds. Medians were used. *-ΔLat 14 presented the maximum difference among the three study groups. Post-hoc analyses showed p<0.037 for all comparisons (except for ΔLat 7 between groups 2 and 3) between Groups 1 and 2 as well as between Groups 2 and 3.
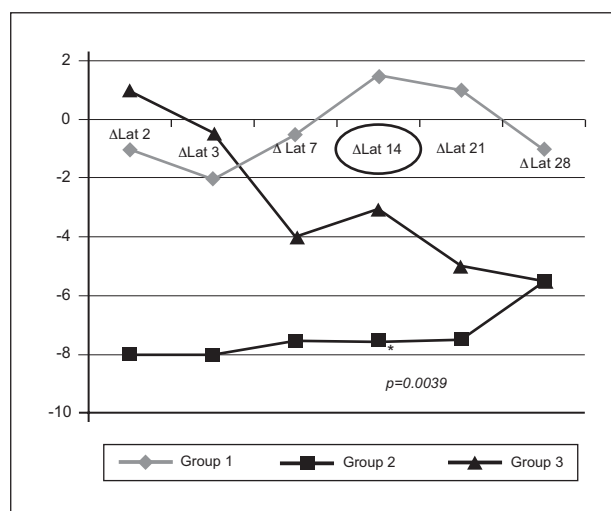
Fig 4. Hind paw withdrawal latency to non-noxious (40°C) thermal stimuli among the three study groups. ΔLat is the difference of latency between the given day and the baseline (pre-operative) observation. The times are represented in seconds. Medians were used *post-hoc analyses found no significant difference between groups 2 and 3 in ΔLat 7, 21 and 28. For all other comparisons, p<0.04 was achieved. The maximum differences between groups 1 and 2 and between groups 2 and 3 were seen in ΔLat 14 (p=0.0038 and p=0.03 respectively).

until the post-op day 28 with a marked decrease at day 14 (Fig 2). They showed median scratching times of 26 and 11 seconds, respectively on the right and left sides with no significant difference between the two sides, suggesting a mirror pain pattern (Fig 1). Comparing the scratching times on the right side (operated leg) among the three study groups, the animals in group 3 had significantly shorter scratching times than those in group 2 (without lidocaine), but longer than the time displayed by the control group 1 (Fig 2).

The withdrawal latency was 8 seconds to the noxious stimulus and 9.5 seconds to the non-noxious stimulus, achieving no significant difference (Table 1). However, the hind paw withdrawal latency to the noxious stimulus showed to be significantly longer than in group 2 (Fig 3).

DISCUSSION

Selecting the proper animal model for the study of neuropathic pain represent one of the most important tools to the further comprehension of mechanisms related to the initiation and progression of pain. Bennett and Xie[8] developed one of the most reliable models, the sciatic nerve Chronic Constriction Injury. In an attempt to get rid of the pain, operated rats express spontaneous behaviors manifested as licking, scratching and even tissue-mutilation of the operated hind paw. Since the paw lost its sensory innervations there is no nociceptive input to prevent the animal from expressing autonomy on the paw[16]. It is still not clear whether animals who receive treatment before the trauma show less posttraumatic pain than the

ones who receive the medication after the lesion, but the present data document that local pretreatment with lidocaine achieves a marked role preventing hyperalgesia and allodynia. The scratching time on the left side, which did not suffer the sciatic lesion, has been shown to be influenced by the lesion on the contralateral limb in group 3. The phenomenon known as mirror pain could not be prevented by lidocaine in this study (Fig 1). Whether this will be clinically significant, further studies might be needed. However, it has been successfully demonstrated the inhibition of the mirror neuropathic pain in rats with the administration of opioid in the prefrontal ventrolateral orbital cortex[17]. Animals in group 2, submitted to nerve lesion with no preemptive lidocaine administration, showed significantly higher scratching times when compared to the non-operated animals in group 1 as well as when compared to the rats in the preemptive drug group 3 (Fig 2). The reduction of scratching times achieved by the animals in group 3 demonstrates a potential advantage of the drug as a preemptive analgesic in the study model. Interestingly, the analgesia response in rats pretreated with lidocaine demonstrated a peak on postoperative day 14, which was also observed in the thermal tests (Figs 3 and 4), suggesting a long-lasting preemptive effect. Another possibility could be that the rats would be presenting some sort of centrally-mediated sensory decline (i.e. hypoesthesia and hypoalgesia) due to the previous lesion. It is known that noxious stimuli may significantly alter somatosensory perception of healthy subjects and chronic pain patients[18].

The withdrawal latency to noxious stimulus in group 2 was significantly shorter than those presented on both groups 1 and 3 (Fig 3), suggesting that the latency described for the group 2 was probably secondary to the development of hyperalgesia. On the other hand, the longer withdrawal latency to noxious stimulus revealed in the group pretreated with lidocaine might have resulted from the development of hypoalgesia secondary to the nerve lesion or to the block of the afferent pathways of pain mediated by the drug. During the post-operative day 14, the latency to noxious stimulus in the lidocaine- treated group was even higher than in the control group (Fig 3).

The lesion of the sciatic nerve was associated with a shorter latency to non-noxious stimulus, as observed in groups 2 and 3 (Fig 4). The change in the response time is possibly due to the development of allodynia in the operated groups. The achievement of hyperalgesia and allodynia suggest that the Chronic Constriction Injury model was successfully reproduced here and that is feasible in combination to the PRE vs. NO treatment design. The group that received pretreatment with lidocaine showed longer latency time when compared to animals that did not receive the treatment, demonstrating the capacity of the drug to inhibit the development of allodynia. Such results support the data obtained in from the scratching analysis, demonstrating the potential of lidocaine in blocking the development of postoperative chronic neuropathic pain.

From our data, we conclude that the combination of the Chronic Constriction Injury model and the PRE vs. NO treatment design is feasible and successfully reproduce the main features of the chronic neuropathic pain. We also suggest that the preemptive use of lidocaine 2% is effective in reducing the levels of postoperative chronic neuropathic pain in Wistar rats, based on the results of thermal tests and scratching times.

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