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SCIENTIFIC ARTICLE

Local analgesic effect of tramadol is not mediated by opioid receptors in early postoperative pain in rats



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KEYWORDS

Tramadol;
Postoperative pain;
Opioid receptors;
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Abstract

Background and objectives: Tramadol is known as a central acting analgesic drug, used for the treatment of moderate to severe pain. Local analgesic effect has been demonstrated, in part due to local anesthetic-like effect, but other mechanisms remain unclear. The role of peripheral opioid receptors in the local analgesic effect is not known. In this study, we examined role of peripheral opioid receptors in the local analgesic effect of tramadol in the plantar incision model.

Methods: Young male Wistar rats were divided into seven groups: control, intraplantar tramadol, intravenous tramadol, intravenous naloxone-intraplantar tramadol, intraplantar naloxone-intraplantar tramadol, intravenous naloxone-intravenous tramadol, and intravenous naloxone. After receiving the assigned drugs (tramadol 5 mg, naloxone 200 µg or 0.9% NaCl), rats were submitted to plantar incision, and withdrawal thresholds after mechanical stimuli with von Frey filaments were assessed at baseline, 10, 15, 30, 45 and 60 min after incision.

Results: Plantar incision led to marked mechanical hyperalgesia during the whole period of observation in the control group, no mechanical hyperalgesia were observed in intraplantar tramadol group, intraplantar naloxone-intraplantar tramadol group and intravenous naloxone-intraplantar tramadol. In the intravenous tramadol group a late increase in withdrawal thresholds (after 45 min) was observed, the intravenous naloxone-intravenous tramadol group and intravenous naloxone remained hyperalgesic during the whole period.

Conclusions: Tramadol presented an early local analgesic effect decreasing mechanical hyperalgesia induced by plantar incision. This analgesic effect was not mediated by peripheral opioid receptors.

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PALAVRAS-CHAVE

Tramadol;
Dor no
pós-operatório;
Receptores opioides;
Ratos Wistar

O efeito analgésico de tramadol não é mediado por receptores opioides na dor de ratos no pós-operatório imediato**Resumo**

Justificativa e objetivos: Tramadol é conhecido como um fármaco analgésico de ação central, usado para o tratamento de dor moderada a grave. O efeito analgésico local foi demonstrado, em parte devido ao efeito semelhante ao anestésico local, mas outros mecanismos permanecem obscuros. O papel dos receptores opioides periféricos no efeito analgésico local não é conhecido. Neste estudo, examinamos o papel dos receptores opioides periféricos no efeito analgésico local de tramadol em modelo de incisão plantar.

Métodos: Ratos Wistar, jovens e machos, foram divididos em sete grupos: controle, tramadol intraplantar, tramadol intravenoso, tramadol intraplantar-naloxona intravenosa, tramadol intraplantar-naloxona intraplantar, tramadol intravenoso-naloxona intravenosa e naloxona intravenosa. Após receberem os medicamentos designados (5 mg de tramadol, 200 mg de naloxona ou NaCl a 0,9%, os ratos foram submetidos à incisão plantar, e os limiares de retirada após estímulos mecânicos com filamentos de von Frey foram avaliados no início do estudo e nos minutos 10, 15, 30, 45 e 60 após a incisão.

Resultados: A incisão plantar levou à hiperalgesia mecânica acentuada durante todo o período de observação no grupo controle; hiperalgesia mecânica não foi observada nos grupos tramadol intraplantar, tramadol intraplantar-naloxona intraplantar e tramadol intraplantar-naloxona intravenosa. No grupo tramadol intravenoso, um aumento tardio do limiar de retirada (após 45 minutos) foi observado, os grupos tramadol intravenoso-naloxona intravenosa e naloxona intravenosa permaneceram hiperalgésicos durante todo o período.

Conclusões: Tramadol apresentou efeito analgésico local inicial, diminuindo a hiperalgesia mecânica induzida pela incisão plantar. Esse efeito analgésico não foi mediado por receptores opioides periféricos.

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Introduction

Tramadol, (1-RS,2RS)-2-[(dimethyl-amino)-methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride, is an analgesic drug used mainly for treatment of moderate to severe, as well as acute and chronic pain.^{1,2} It presents a weak opioid effect and has other different mechanism of action through the decrease of the reuptake of monoaminergic neurotransmitters (5-hydroxy-tryptamine and noradrenaline).³ Additionally it presents an analgesic effect in peripheral nerves, which is, in part, local anesthetic-like.⁴⁻⁶

The peripheral analgesic effect of tramadol has been also showed in humans after infiltration for third molar extraction, minor surgical procedures in children and as adjuvant to local anesthetics to reduce postoperative pain.⁷⁻¹² Peripheral opioid receptors have long been described and their role in analgesia in animals and humans demonstrated,¹³⁻²⁰ but little is known about peripheral opioid mediated effect of tramadol.

This study aimed to evaluate the role of opioid receptors in the analgesic effects of intraplantar tramadol in a model of postoperative pain.

Materials and methods**Animals**

The experiments were performed after the approval of the Bioethics Committee of the Hospital of Clinics of The

University of São Paulo Faculty of Medicine and according to the Committee for Research and Ethical Issues of the IASP.²¹ All experiments were performed on Wistar male rats, weighing 250 g, supplied by own breeding facilities of the University of São Paulo Faculty of Medicine. The total number of animals used in the study was 35 rats. All behavioral experiments were conducted between 9:00 am and 12:00 pm. All animals were housed in pairs, in cages with bedding and free access to food and water.

Plantar incision

The rat hind paw plantar incision model was performed as previously described.²² Briefly, rats were anesthetized with 2% to 3% isoflurane delivered *via* nose cone. The plantar aspect of the right hind paw was prepared in a sterile manner with a 10% povidone-iodine solution and draped. A 1 cm longitudinal incision was made with a number 11 blade through skin and fascia of the plantar aspect of the paw of the rat, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The flexor muscle was elevated and incised longitudinally and the muscle origin and insertion remain intact. After hemostasis with gentle pressure, the skin was opposed with two simple sutures of 5-0 monoylon.

Mechanical hyperalgesia

Rats were placed on an elevated plastic mesh floor covered with a clear plastic cage top. The animals were allowed to

ambulate, explore, and eventually rest lying on the mesh. For testing pain behaviors, calibrated von Frey filaments (thin plastic filaments with calibrated forces) were applied underneath the cage adjacent to the wound. Each filament was applied once and continuing until a withdrawal occurred, which was considered as the withdrawal threshold. Filaments with bending forces of 5.9; 9.8; 13.7; 19.7; 39.2; 58.8; 78.4; 98 147, 255, 588 and 980 mN were used in the study.

Experimental design

After assessment of the pre incision withdrawal thresholds for mechanical hyperalgesia, animals were divided into seven experimental groups of five animals each: 1 – Control, 50 μ L of 0.9% NaCl solution were injected in the plantar aspect of the right hindpaw, 5 min later another 50 μ L of 0.9% NaCl solution were reinjected; 2 – Intraplantar tramadol (Ipl-tramadol), 50 μ L of 0.9% NaCl solution were injected in the plantar aspect of the right hindpaw and 5 min later 5 mg (20 mg/kg) of tramadol dissolved in 50 μ L of 0.9% NaCl were administered in the plantar aspect of the right hindpaw; 3 – Intravenous tramadol (Iv-tramadol), 50 μ L of 0.9% NaCl solution were injected in the plantar aspect of the right hindpaw and 5 min later 5 mg of tramadol were injected in the dorsal vein of the penis; 4 – Intravenous naloxone-intraplantar tramadol (Iv-naloxone + Ipl-tramadol), 200 μ g naloxone injected in the dorsal vein of the penis 5 min before 5 mg of tramadol dissolved in 50 μ L of 0.9% NaCl in the plantar aspect of the right hindpaw; 5 – Intraplantar naloxone-intraplantar tramadol (Ipl-naloxone + Ipl-tramadol), 200 μ g naloxone was injected in the right hindpaw 5 min before intraplantar tramadol; 6 – Intravenous naloxone-intravenous tramadol (Iv-naloxone + Iv-tramadol); 200 μ g naloxone injected in the dorsal vein of the penis 5 min before 5 mg of tramadol dissolved in 50 μ L of 0.9% NaCl injected in the dorsal vein of the penis; 7 – Naloxone (Iv-naloxone), 200 μ g naloxone injected in the dorsal vein of the penis and 50 μ L of 0.9% NaCl solution injected in the paw. All injections were done under general anesthesia with 2%–3% isoflurane. Animals were kept anesthetized and 5 min after second injection they were submitted to plantar incision. As soon as the animals recovered, the tests were started and performed 10, 15, 30, 45 and 60 min after recovery from anesthesia. At the end of the experiments the animals were submitted to euthanasia with lethal dose of sodium thiopental.

Drugs

Tramadol hydrochloride and naloxone hydrochloride were provided by Cristália Prod. Quim. Farm, São Paulo, SP, Brazil.

Statistical analysis

All data were analyzed by Prism 5.0 software (GraphPad Software, Inc., USA). Withdrawal threshold to mechanical stimuli are non-continuous and were analyzed with nonparametric tests. The data were expressed as median and interquartile range. Differences were determined by

Kruskal–Wallis test followed by Dunn's *post hoc* test for comparing paw withdrawal threshold.

Results

Plantar incision led to mechanical hyperalgesia during the whole period of observation. In the control group the median withdrawal threshold to von Frey filament decreased from 980 to 39 mN after 10 min and 98 mN after 60 min, in the intraplantar tramadol group the median withdrawal threshold was 980 mN during the whole period, in the intravenous tramadol group median decreased from 980 to 59 mN after 10 min and increased after 45 min to 255 mN and in the intraplantar naloxone-intraplantar tramadol the median withdrawal threshold was 980 mN throughout the 60 min of observation (Fig. 1).

In the intravenous naloxone-intraplantar tramadol group the median withdrawal threshold ranged from 588 to 980 mN throughout all period of observation, in the intravenous naloxone-intravenous tramadol group, median went from 980 to 59 mN, staying hyperalgesic at 60 min and in the intravenous naloxone, median went from 980 to 20 mN at 60 min of injection, the median was different from control from 15 min to 60 min (Fig. 2).

Discussion

The use of experimental pain models is under scrutiny due to the partial failure of translation of results obtained experimentally to clinical and real situations.^{23,24} The development and search for models that mimic clinical scenarios is very important. In this study, we used the plantar incision model, a postoperative pain model, being used for the last

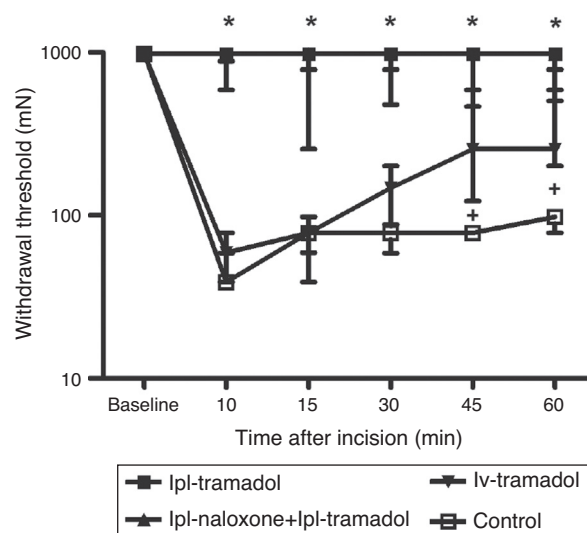


Figure 1 Withdrawal thresholds to mechanical stimulus after intraplantar or intravenous tramadol. Withdrawal thresholds were higher in the Ipl-tramadol and Ipl-naloxone + Ipl-tramadol groups, Iv-tramadol presented a late analgesia after 45 min of injection. Data presented as median \pm interquartile interval (25th–75th percentile); $p < 0.05$. *Ipl-tramadol and Ipl-naloxone + Ipl-tramadol groups different from control, +Iv-tramadol different from control.

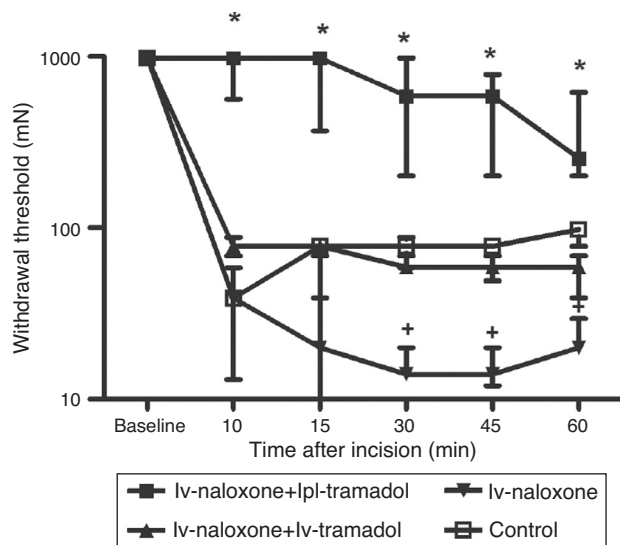


Figure 2 Withdrawal thresholds to mechanical stimulus after naloxone with or without tramadol. Withdrawal thresholds were higher in the Iv-naloxone + lpl-tramadol group, Iv-naloxone + lv-tramadol was not different from control and withdrawal thresholds were lower in naloxone group than control after 25 min of injection. Data presented as median \pm interquartile interval (25th–75th percentile); $p < 0.05$. *Iv-naloxone + lpl-tramadol different from control, +naloxone different from control.

two decades and that brings to the experimental environment a relevant clinical situation, the postoperative pain. The prominent clinical characteristic of postoperative pain is mechanical hyperalgesia or mechanical allodynia, and we decided to observe this pain modality.

Present results showed an analgesic effect of intraplantar tramadol in the postoperative pain model used. The analgesic effect lasted throughout the whole period of observation and led to higher paw withdrawal thresholds than intravenous tramadol. Tramadol is known to be an atypical drug, presenting different mechanisms of action to achieve analgesia. When used systemically, tramadol is metabolized by cytochrome P450 (CYP) 2D6, and its major metabolite is mono-*o*-desmethyl-tramadol (M1), responsible for the systemic analgesic effect.^{3,25} Its analgesic effect is observed 20–40 min after oral intake.²⁶ In the present study, intravenous tramadol decreased hyperalgesia 45 min after injection, the expected time for tramadol systemic analgesia. However, intraplantar tramadol in the same dose blocked hyperalgesia from the beginning throughout all observation period. These results indicate that the parental drug, tramadol, presents an early analgesic effect expressed right after its injection, effect that seems to be local, since the analgesic effect observed after intravenous route did not induce early analgesia. The observed local effect seems not to be mediated by opioid receptors, no reduction in analgesia was observed after naloxone neither by intraplantar nor intravenous routes. In another study analgesic effect of tramadol in the same post-operative pain model was antagonized with naloxone, but they used the intraperitoneal and intrathecal routes, and analgesic effects in the central nervous system is due to the classic decrease in monoamines

reuptake and opioid mediated effects.^{3,26,27} Although our results did not find peripheral opioid action, we cannot rule out a local opioid analgesic effect of tramadol occurring later. It has been demonstrated that there is an increase in the expression of μ opioid receptor (MOR) synthesized in the dorsal root ganglia, then trafficked to nerve endings after intraplantar carrageenan.²⁸ Up regulation of MOR in dorsal root ganglia was higher one day after injection and decreased at day three. Intraplantar carrageenan induces pain behavior that subsides four hours after injection. Post-operative pain in the plantar incision model peaks at day one and lasts up to five days, when thermal and mechanical thresholds are similar to baseline.²⁰ MOR expression may occur later in this pain model what can affect peripheral opioid receptor role. An interesting data observed occurred in the intravenous naloxone group, where thresholds were lower than in control group, indicating that naloxone antagonized endogenous opioids in the first hour after incision, leading to an increase in the pain behavior after mechanical stimulus.

Local analgesic effect of tramadol has been shown to be similar to local anesthetic.^{4–6,29,30} The mechanisms involved in this peripheral action are still not well understood. Weak anti-inflammatory effect has been proposed for tramadol analgesia and calcium dependent effect.^{31,32} Interestingly tramadol also acts as an agonist of the transient receptor potential vanilloid-1 (TRPV1) channel, maybe presenting, at some extent, a pronociceptive effect.³³

The results of this study indicate that tramadol presents an early local analgesic effect decreasing mechanical hyperalgesia induced by plantar incision. This effect is not mediated, in the first hour, by peripheral opioid receptors.

Conflicts of interest

The authors declare no conflicts of interest.

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