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SCIENTIFIC ARTICLE

Subarachnoid meloxicam does not inhibit the mechanical hypernociception on carrageenan test in rats☆

Lanucha Fidelis da Luz Moura[a,*], Silvana Bellini Vidor[b], Anelise Bonilla Trindade[b], Priscilla Domingues Mörschbächer[b], Nilson Oleskovicz[c], Emerson Antonio Contesini[d]

[a] *Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil*
[b] *Postgraduate Program in Veterinary Science, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil*
[c] *Universidade do Estado de Santa Catarina, Florianópolis, SC, Brazil*
[d] *Faculdade de Veterinária da Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil*

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Abstract
Background and objective: Evaluate the antinociceptive effects of subarachnoid meloxicam on the mechanical hypernociception induced by carrageenan in rats.
Methods: Randomized controlled trial. Eighteen adult male Wistar rats underwent a cannula implantation into the subarachnoid space and were randomly divided into two groups: Group I received saline solution 5 μL, while Group II received meloxicam 30 mg. The mechanical hypernociception was induced by intraplantar injection of carrageenan and evaluated using a digital analgesy meter every 30 min during a 4-h period. The results were recorded as the Δ withdrawal threshold (in g), calculated by subtracting the measurement value after treatment from baseline.
Results: The Δ withdrawal threshold mean values were lower in the group of patients treated with meloxicam over all time points between 45 and 165 min, however, there was no statistical significance ($p = 0.835$) for this difference.
Conclusion: Subarachnoid meloxicam at a dose of 30 μg animal^{-1} did not suppress the mechanical hypernociception in a model of inflammatory pain induced by intraplantar administration of carrageenan in rats. The data suggest that other dosages should be investigated the drug effect is discarded.

☆ Work conducted at Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brazil.
* Corresponding author.
E-mail: lanucha_moura@terra.com.br (L.F.L. Moura).

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PALAVRAS-CHAVE
AINE;
Carragenina;
Dor;
Medula espinhal

Meloxicam subaracnoide não inibe a hipernocicepção mecânica no teste da carragenina em ratos

Resumo
Justificativa e objetivo: avaliar os efeitos antinociceptivos do meloxicam subaracnóideo sobre a hipernocicepção mecânica induzida pela carragenina em ratos.
Métodos: estudo randômico e controlado. Dezoito ratos Wistar, machos adultos, foram submetidos à implantação de uma cânula subaracnóidea, e aleatoriamente distribuídos em dois grupos: o Grupo I (GI) recebeu 5 μL de solução salina, enquanto que ao Grupo II (GII) foram administrados 30 μg de meloxicam, ambos pela via subaracnóidea. A hipernocicepção mecânica foi induzida pela injeção intraplantar de carragenina e avaliada com o emprego de um analgesímetro digital a cada 30 minutos durante um período de 4 horas. Os resultados foram registrados como o Δ do limiar de retirada (g), calculado subtraindo-se o valor das mensurações após os tratamentos, do valor basal.
Resultados: os valores médios do Δ do limiar de retirada foram menores no grupo tratado com meloxicam ao longo de todos os momentos de avaliação entre 45 e 165 minutos, contudo não foi demonstrada significância estatística (p = 0,835) para essa diferença.
Conclusão: a administração subaracnóidea do meloxicam na dose de 30 μg.animal^{-1} não foi capaz de suprimir a hipernocicepção mecânica em um modelo de dor inflamatória induzida pela administração intraplantar de carragenina em ratos. Os dados sugerem que outras doses sejam pesquisadas antes que o efeito do fármaco seja descartado.

Introduction

Evidence has shown that besides the known peripheral action nonsteroidal anti-inflammatory drugs (NSAIDs) have a powerful effect on experimental pain that is independent of its anti-inflammatory effects.[1] In addition to its peripheral inhibition of prostaglandin synthesis, a central action of NSAIDs has been suggested by experimental studies in which these drugs demonstrated greater potency by subarachnoid administration compared to systemic administration.[2,3] Studies have shown that both cyclooxygenase (COX) forms are constitutively expressed in the brain and spinal cord of rats,[4] with COX-2 the predominant isoform in the spinal cord dorsal horn.[5] Spinal administration of anti-inflammatory drugs has shown to suppress the reflection of C fibers, inhibit neuronal sensitization in the spinal cord dorsal horn, and attenuate long-term inflammatory pain.[2,6–11]

Meloxicam is an analgesic and nonsteroidal anti-inflammatory drug, which belongs to the phenolic acid class and has a preference for COX-2 isoenzyme.[12] Unlike many other NSAIDs, it has high oral bioavailability and a long half-life, although not free from side effects.[13] Studies of meloxicam administered by spinal pathways are scarce[14–17] and do not assess its effects on acute inflammatory pain. The aim of this study was to evaluate the antinociceptive power of subarachnoid meloxicam on acute pain induced by carrageenan in rats.

Materials and methods

The experimental protocol was reviewed and approved by the Animal Experimentation Ethics Committee of the institution. Rats were individually housed under controlled temperature (21–24 °C) and light–dark cycle of 12 h, with food and water ad libitum offered for at least 14 days.

The animals were surgically prepared under anesthesia with intraperitoneal injections of ketamine and xylazine (100 and 10 mg kg^{-1}, respectively); then, a cannula was implanted in the subarachnoid space, according to a modification of the technique previously described in the literature.[18] Briefly, the animals were placed in prone position, with the fore and hind limbs fixed in abduction and the region of the head slightly elevated relative to the rest of the body. After skin antisepsis of atlantooccipital region, a vertical incision approximately 2 cm in length was made in the region midline, starting at the point between the ears and extending caudally. The subcutaneous tissue and *biventer cervicis* and *rectus capitis dorsalis* major muscles were removed by blunt dissection. With the muscular retraction, the dura and *cisterna magna* were seen, and after exposure of atlantooccipital membrane, an 18 G needle was used to puncture its central region, until cerebrospinal fluid is seen. A PE-10 polyethylene cannula (#BB31695-PE/1, Scientific Commodities, Lake Havasu City – AZ, USA) was then inserted through the hole and advanced caudally 8.5 cm into the subarachnoid space until it reaches the lumbar enlargement region. Measuring, cutting, and marking of cannulas with enamel paint were conducted prior to the experiment period, with this material individually packaged and sterilized with ethylene oxide. The cranial portion of the cannula was inserted through an 18 G needle, allowing its accommodation in the subcutaneous tissue, in order to emerge from the skin near the top of the head. Muscles and skin were sutured and the catheter external end was occluded with the insertion of a small fragment of dental needle

(30 G × 21 mm). Finally, the outer portion of the cannula was fixed to the skin with sutures. During the period that followed the cannula implantation, the animals were kept in individual plastic boxes under the same conditions of the previous period. On the day following the cannula placement, the animals were assessed for neurological deficits. Those with neurological abnormalities were excluded from the study.

Eighteen Wistar rats, weighing 300–450 g were successfully prepared for the study and, one day after the cannula placement, they were tested for mechanical hypernociception induced by carrageenan. For this purpose, we used a digital analgesy meter (Insight Scientific Equipment Ltda, Ribeirão Preto – SP, Brazil), according to a previously described technique,[19] in which a pressure transducer equipped with a 7 mm^2 polypropylene tip was applied perpendicular to the right plantar surface of the animals, with a linearly increasing pressure. The equipment recorded the force in grams (g) with a precision of 0.1 g. The animal limb stimulation was repeated until obtaining three similar measurements (the difference between the highest and the lowest value was less than 10 g). Thus, the nociceptive behavior was quantified by averaging the three values expressed in grams, which represents the paw withdrawal threshold to the mechanical stimulation at each time point. Limb withdrawal from contact with the tip or shaking and/or licking behavior at the time or immediately after stimulation (flinch) was considered positive response. Ambulation was considered an ambiguous response; therefore, when it occurred at the time of test application, the test was repeated.

About 30 min before starting the assessment, the rats were transferred to the testing place, consisting of acrylic boxes with malleable wire mesh floor, in a quiet room, allowing its acclimatization evidenced by cessation of the grooming and site exploration behavior. Throughout this period, approximately five stimulations of animal limbs were performed, in order to allow their familiarity with the stimulus applied. Subsequently, baseline values from each animal were established.

After registration of baseline values, the animals were manually restrained and the metal fragment the occluding the catheter was removed. Next, the animals were randomized into two groups. Animals in Group I (GI, $n = 9$) underwent subarachnoid administration of 5 μL saline solution, while animals in Group II (GII, $n = 9$) received meloxicam 30 μg diluted in saline to a final volume of 5 μL, by the same route. Solutions were administered with the aid of a Hamilton 10 μL microsyringe (701N, Hamilton Company, Reno – NV, USA) over a period of 30 s, after which, 10 μL sterile saline was injected to flush the catheter.

Immediately after substance administration into subarachnoid space, lambda-carrageenan (0.1 mL of 2.5% carrageenan) was injected into the intraplantar region of the right limb, following the technique previously described in the literature.[20] The carrageenan injection time was recorded as time-zero (T_0), and subsequent evaluations were performed every 30 min during the 4 h post-drug administration to obtain its temporal profile of action. All assessments were performed by an investigator who was blind to animal treatment. Because the animals were tested in the periods before and after the administration of drugs, the results were recorded as the Δ withdrawal threshold (g), calculated by subtracting the measurement value after treatments from the baseline value, and these values were compared.

Table 1 Mean difference, standard error, and *p*-value of Δ withdrawal threshold (g) in rats from GI and GII at different time points of hypernociception induced by intraplantar injection of carrageenan.

Time points	Estimated mean difference	Standard error	*p*
30–60 min	−4.13	3.38	0.914
30–90 min	−5.60	3.82	0.813
30–120 min	−7.59	2.96	0.234
30–150 min	−13.64	4.03	0.055
30–180 min	−15.30	4.24	0.036
30–210 min	−23.50	3.68	<0.001
30–240 min	−27.76	2.53	<0.001
60–90 min	−1.47	2.42	0.998
60–120 min	−3.46	2.58	0.872
60–150 min	−9.51	3.13	0.104
60–180 min	−11.16	3.67	0.104
60–210 min	−19.37	2.71	<0.001
60–240 min	−23.63	2.88	<0.001
90–120 min	−1.99	2.84	0.996
90–150 min	−8.04	2.67	0.111
90–180 min	−9.70	3.35	0.136
90–210 min	−17.90	2.71	<0.001
90–240 min	−22.16	2.99	<0.001
120–150 min	−6.06	2.41	0.254
120–180 min	−7.71	3.81	0.497
120–210 min	−15.92	2.22	<0.001
120–240 min	−20.18	2.80	<0.001
150–180 min	−1.65	3.37	1.000
150–210 min	−9.86	2.60	0.025
150–240 min	−14.12	3.02	0.004
180–210 min	−8.21	2.36	0.046
180–240 min	−12.47	3.03	0.013
210–240 min	−4.26	2.48	0.677

Data are expressed as mean ± standard deviation. The Δ withdrawal values were compared using variance analysis with repeated measures and two factors, with GI or GII group as the fixed factor and the time (every 30 min) as the repetition factor. For analysis, an unstructured correlation matrix between time points was supposed. Analyses were performed with SAS software version 8.0 for Windows, and the level of significance was determined at $p < 0.05$.

Results

A mean increase of the Δ withdrawal threshold occurred over time, with statistically higher values in assessments at 210 and 240 min compared to other time points ($p < 0.05$). There were differences between other time points, always with a mean increase of Δ withdrawal threshold in the course of the experiment, as shown in Table 1.

The mean values of the Δ withdrawal threshold were lower in the group treated with meloxicam over all time points between 45 and 165 min, although not statistically

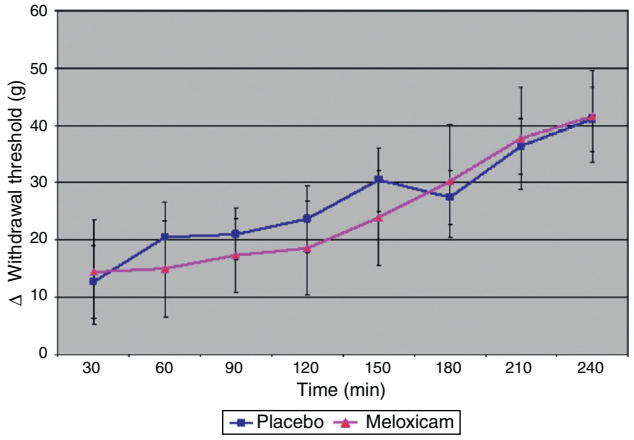

Figure 1 Mean values of Δ withdrawal threshold ± standard deviation (g) in different time points of mechanical hypernociception in rats after SA administration of saline solution (GI) or meloxicam (GII).

significant ($p = 0.835$) for this difference (Fig. 1). Mean differences in the Δ withdrawal threshold occurred between time points within each group ($p < 0.001$).

During antinociception evaluation, two rats, both from GI, showed clinically differentiated responses after induction of hyperalgesia. These animals presented with eye discharge, significant prostration, with vocalization at rest and reluctant to rest the limb subjected to carrageenan application, suggesting the presence of severe pain. The antinociceptive evaluation for these rats showed a high degree of difficulty compared to others, as the animals did not allow force to be exerted against the plantar surface during measurements. In these cases, the animals raised their affected limb, following the tip movement, not exerting pressure resistance. As a result, these animals showed high levels of withdrawal threshold, which were not consistent with what could be observed clinically.

Discussion

The effectiveness of conventional NSAIDs, COX-2 inhibitors, and monoclonal anti-prostaglandin E2 as anti-inflammatory agents in experimental models using carrageenan is well described in the literature.[20–22] The actions were traditionally assigned to peripheral prostaglandin inhibition, which play an important role in nociceptor sensitization at the site of injury,[23] as carrageenan intraplantar injection induces a significant increase of COX-2 expression, as well as prostaglandin E2 production.[24] However, the relative amounts of each isoform expressed in different tissues vary and may be modulated in pathological conditions. Thus, in contrast to other organs, the rat's normal brain, as well as the spinal cord, expresses more COX-2 than COX-1,[25] and data confirm its role in the sensory processing of pain.[26] Thus, a series of experimental evidence currently suggests that NSAIDs exert their analgesic action also by activity on the central nervous system, in addition to its well-known peripheral action.[25]

Several drugs have been administered by spinal route in an attempt to prove such a mechanism, and the lack of meloxicam consistent effects on spinal nociception induced in the experimental model used contradicts the findings of other authors who reported antinociceptive activity after spinal use of other NSAIDs.[2,14,15,20,27–32] It is noteworthy, however, that although there are a variety of studies evaluating the antinociceptive power of COX-2 administered in the subarachnoid space, studies specifically investigating the effects of meloxicam are still scarce. These studies have found an inhibitory effect on the wind-up phenomenon in vitro,[33] as well as on nociception induced by capsaicin or formalin.[34] Additionally, a synergistic analgesic effect with morphine was found after its SA administration in animals with experimental visceral pain.[15] In a previous study,[17] a dose similar to the one used in the present study (30 μg animal^{-1}) was used to investigate the antiallodynic effects of SA meloxicam. The authors, however, used an experimental model of neuropathic pain in diabetic mice, differing from this study inflammatory pain model. However, the drug effects were demonstrated with the dose used, which did not occur in this study. Observing carefully the data shown in Fig. 1, one can see that the group receiving meloxicam showed mean values of Δ withdrawal threshold lower than those shown by the group receiving saline solution during all time points between 45 and 165 min.

The intraplantar carrageenan inflammation is characterized by a biphasic behavior with respect to swelling. The early phase (0–1 h) has been attributed to the release of histamine, 5-hydroxytryptamine, and bradykinin, so the effectiveness of NSAIDs in this period has been questioned. In the late phase (1–6 h), on the other hand, a high production of PGs has been verified.[35] However, hyperalgesia seems to develop in parallel with the increase of COX-2 spinal levels, and its peak only occurs within 4 h after carrageenan injection.[36] Looking at Fig. 1, however, one can see that the effects of meloxicam on hyperalgesia occurred precisely in the period prior to the 4 h of assessment, with significantly higher values of Δ withdrawal threshold after 165 min of administration, which differs from such statements.

However, this response curve behavior has also been observed in studies using carrageenan model, which reported anti-hyperalgesic thermal effects with SA administration of SC58125 – a selective COX-2 inhibitor – only during the first 170 min of evaluation.[37] As thermal hyperalgesia has been shown to be similarly mediated by the action of spinal COX-2,[38] studies that characterize the pattern of spinal COX-2 expression in carrageenan-induced inflammation, hitherto seemingly nonexistent, may help to elucidate such observations. A significant increase in the hypernociception intensity, characterized by increased Δ withdrawal threshold, especially in assessments at 210 and 240 min, was observed in both experimental groups. These findings may be explained by other authors who report that the repetition of mechanical stimulation can produce an increased sensitivity of the stimulated area.[39] Thus, the administration of meloxicam also failed to prevent the increase of hypernociception over time.

Among the different factors that may have influenced this study outcomes, the establishment of an adequate dose has emerged as an essential need whose importance may have been decisive for the data presented here. Currently, few studies of subarachnoid meloxicam involve administration protocols distinct from that recommended in this study,

such as continuous infusion techniques[16] or its association with opioids.[15] The need to determine a dose that shows consistent effects, or even the absence of such effects, was based on the extrapolation of results obtained in these studies and their suitability to the needs of this work. Thus, based on previous studies that showed satisfactory results using 30-μg animal^{-1} of subarachnoid meloxicam on experimental neuropathic pain in diabetic animals,[17] a similar dose was recommended in order to observe its effects on inflammatory hyperalgesia.

Neuropathic pain, however, is a complex syndrome involving inflammatory and immune theories yet to be understood, and whose hyperalgesia results from both neural and non-neural tissue involvement and is associated with Aβ and Aδ fibers activation.[40] However, the nociceptive system complexity has shown that different sensory pathways are activated after minimal changes in the nature of a painful process,[41] leading to believe that different doses of the same drug may be required for suppression of pain of different origins. Such a possibility can be readily seen in the present study, as a dose capable of controlling neuropathic hyperalgesia did not achieve the same results as that of inflammatory origin, thus demonstrating the need for further studies with different doses of the drug.

Conflicts of interest

The authors declare no conflicts of interest.

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