Acute ketoprofen neurotoxicity in spinal cord of rats
Neurotoxicidade aguda do cetoprofeno em medula espinhal de ratos

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ABSTRACT
Objective: To evaluate possible spinal cord injury in rats following different doses of intrathecal ketoprofen. Methods: Animals were divided into four groups of five rats each; 10 µl of an intrathecal solution were injected into the L6-S1 intervertebral space. Ketoprofen 1% was injected in the first group; ketoprofen 0.1% was injected in the second group; ketoprofen 0.01% was injected in the third group; and a sodium chloride 0.9% solution was injected in the Control Group. Histological sections of the cervical, thoracic, lumbar and sacral spine were analyzed to assess the number of neurons showing morphological changes. Variance analysis of repeated measurements was used for statistical comparison between groups. The Wald test was used for multiple comparisons; the Shapiro-Wilk test (p = 0.0904) was used for assess normality of data. The statistical significance was p < 0.05. Results: The 1.0% Dose Group showed a moderate percentage of thoracic (p = 0.0020) and cervical (p = 0.0210) spinal cord changes, compared to the Control Group. The 0.1% Dose Group showed a moderate percentage of cervical spinal cord changes (p = 0.0399) in comparison to the Control Group. There were no significant differences in the lumbar (p = 0.9878) and sacral (0.7300) spinal cord among the groups. Conclusions: Intrathecal injection of ketoprofen in rats, at a concentration equal to or less than 0.01%, may be used in studies aimed to elucidate central analgesia mechanisms produced by this drug.

Keywords: Neurotoxicity syndromes; Spinal cord; Ketoprofen; Analgesia; Rats

INTRODUCTION
The mechanism of antinociceptive effects caused by non-steroidal anti-inflammatory drugs (NSAIDs) is still not fully understood; it is more complex than mere inhibition of prostaglandin synthesis. Many investigations demonstrated that these drugs act centrally, although there has been an emphasis on their peripheral action⁵.

Many studies described the central action of ketoprofen. This drug interacts indirectly with the...
N-methyl-D-aspartate (NMDA) receptor by first acting on hepatic tryptophan 2,3 dioxygenase, thereby increasing serum kynurenic acid levels, which prevents magnesium ions from leaving cells, thus blocking the NMDA receptor, which is then not activated (2-4). If there is no NMDA-receptor activation, there is no nociceptive sensitization and, consequently, there is less pain (5).

Many researches revealed the spinal cord analgesic activity of ketoprofen, which promoted the use of intrathecal ketoprofen in experimental or clinical research (6-7). Before using ketoprofen in this manner, however, it is necessary to check whether ketoprofen injures nervous cells.

**OBJECTIVE**

To assess possibly injury in the spinal cord of rats caused by ketoprofen given intrathecally at different doses.

**METHODS**

Following approval by the Research Ethics Committee, 20 Wistar rats weighing between 300 and 360 g, supplied by the bioterium of the Medical School of the Universidade de São Paulo (USP), were studied. The rats were handled according to National Institutes of Health (NIH) guidelines for the care and use of animals in laboratories (DDHS Publication, NIH, 86-23, 1985). Experiments were conducted in the Medical Investigation Laboratory (LIM, acronym of the Portuguese expression “Laboratório de Investigação Médica”) of the Medical School of USP.

The rats were anesthetized with halothane 2.5%, maintained in anesthesia, placed in prone position with the spine flexed and kept on spontaneous ventilation. Trichotomy and cleaning with an antiseptic solution were performed on the skin of the lumbosacral area skin. The L6-S1 intervertebral space was located by palpating the iliac crests; a brachial plexus access needle was used for the intrathecal injection of 10 µl of 0.9% sodium chloride (saline solution) or of ketoprofen diluted in saline, for three to five seconds. The animals were awakened from anesthesia and sacrificed seven days later.

The rats were divided into four groups of five animals each; 10 µl of a specified solution was given intrathecally to each rat. A Control Group was given saline, the Group 1% was given ketoprofen 1%, the Group 0.1% was given ketoprofen 0.1% and the Group 0.01% was given ketoprofen 0.01%.

Thirty minutes after receiving intrathecal saline or ketoprofen, and also immediately before being sacrificed, the rats underwent a walking test on a flat surface and a gait and leg flexion test upon being raised from that flat surface (8).

The rats were sacrificed seven days after intrathecal injection of saline or ketoprofen, by injecting sodium pentobarbital at 200 mg/kg intraperitoneally, followed by exsanguination and spinal cord fixation. Exsanguination was carried out by needle-puncturing the left ventricle, into which 200 ml of saline at 5 ºC were administered through a roller infusion pump similar to those used in extracorporeal circulation; saline exited through a hole in the right atrium. The same access route was used for infusing 200 ml of paraformaldehyde at 5 ºC. The spinal cord was isolated and kept in buffered neutral formalin 10%, for 48 hours, and, later, it was decalcified for another 48 hours. Macroscopic 5-µm sections were performed along the spinal cord, which were then processed for histology and hematoxylin-eosin stained. Histopathological sections of the cervical, thoracic, lumbar and sacral spine were examined under optical microscopy; all neurons in three non-coinciding random fields at 400 X magnification were examined. Central and/or peripheral chromatolysis, spongiosis, neurolysis and myelin degeneration were investigated.

The variance analysis for repeated measures was used for statistical comparisons, considering the percentage of altered neurons in each rat as the variable. The Wald-Wilk (p = 0.0904) test was applied to verify normality of data. Statistical significance was p < 0.05.

**RESULTS**

None of the animals showed altered gait or leg flexion on tests conducted on the day saline or ketoprofen solutions were injected intrathecally or on the day the animals were sacrificed. The structural change found was central chromatolysis, characterized by a condensed, hyperchromatic nucleus and a vacuolated cytoplasm with homogeneous pink areas.

Chart 1 shows the number of rats and the neurons found in each spinal cord section and the number of neurons that presented alterations of any kind. Figure 1 shows the percentage of rats with altered neurons in each spinal cord region.

**Chart 1. Number of rats of each group with neurons found (E) and number of altered neurons (A) in each spinal region**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cervical E</th>
<th>Thoracic E</th>
<th>Lumbar E</th>
<th>Sacral E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>0.01%</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>0.1%</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1.0%</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>
The percentage of altered neurons in each spinal cord section was calculated for each rat. Table 1 and Figure 3 show the means and standard deviations of these percentages in each spinal cord region. Group 1.0% showed a higher mean percentage of neuron alterations compared to the Control Group in the thoracic (p = 0.0020) and cervical (p = 0.0210) regions. Group 0.1% showed the mean percentage of alterations over the Control Group in the cervical spinal cord (p = 0.0399). There were no significant differences between groups in the lumbar (p = 0.9878) and sacral (0.7300) regions.

**DISCUSSION**

Drugs must cross the blood-brain barrier and reach the cerebrospinal fluid to act on the central nervous system. Most drugs, however, do not easily cross this membrane, resulting in low cerebrospinal fluid concentrations; high systemic doses are, therefore, required to reach a minimal cerebrospinal fluid concentration, which is frequently insufficient to induce an adequate pharmacological effect.

In rats, NSAIDs at doses that are insufficient for blocking pain when given systemically cause adequate analgesia when given intrathecally. Such central effects appear to be due to a direct effect on spinal nociceptive processes, which may be mediated by independent mechanisms from those that cause peripheral analgesia and anti-inflammatory effects(9).

NSAIDs cross the blood-brain barrier with difficulty; nevertheless, their presence in the cerebrospinal fluid is required for central analgesia. Ketoprofen is significantly lipophilic and, therefore, crosses the blood-brain barrier much more easily compared to most NSAIDs(10). Even so, cerebrospinal fluid concentrations are low and may take more than 30 minutes to be detected by laboratory tests in human adults and children(11-14).
Introducing ketoprofen directly into the cerebrospinal fluid makes it possible to study its central action, as increased concentrations may be attained at the action sites. A major problem is that ketoprofen or adjuvant substances may injure neurons; it is therefore essential to learn which concentration of a drug injected directly into the cerebrospinal fluid will not cause neuronal injury.

Results clearly show that injecting 10 μl of saline into the intrathecal space of rats produces mild changes in a small number of neurons. Injecting the same volume of ketoprofen diluted in saline at 0.01% resulted in similar alterations as those found in controls; higher concentrations of ketoprofen resulted in a statistically significant increase in the number of neurons presenting alterations, particularly in the cervical and thoracic spinal cord. Injection into the intrathecal space was performed at the point of transition between the sacral and lumbar spinal cord – an area in which the spinal cord has already ended.

In the process characterized as central chromatolysis, the cell body undergoes tumefaction, Nissl bodies disappear in the central portion of the cell, and the nucleus is pushed to its periphery. Central chromatolysis is an axonal reaction where changes appear to reflect reversible changes in cell metabolism that include ischemia; these changes are interpreted as a state of heightened metabolic activity that favors axonal regeneration.(15-17).

Using the expression of c-fos as a marker, experiments in which ketoprofen is applied systemically in rats and intrathecally in rats and mice result in central analgesia(7, 18). In rats, the analgesic power of spinally administered NSAIDs was 100 to 1,000-fold higher compared to that attained by the systemic route; this raises the possibility of using these drugs by the intrathecal route(19), with reversal obtained by using atropin(20).

The analgesic effect of ketoprofen is not yet fully understood; there may be peripheral and central mechanisms involved. Evidence has shown that action sites are located in the dorsal horns of the spinal cord, in medial rostral bulbar areas and in the periaqueductual gray matter.(6,21)

CONCLUSIONS

Under the conditions of this study, we concluded that intrathecal injection of ketoprofen in rats at concentrations equal to or below 0.01% may be used in research aimed to elucidate the central analgesic mechanisms of this drug.

REFERENCES


