Effects of Parecoxib on Pain Threshold and Inflammatory Factors IL-1β, IL-6 and TNF-α in Spinal Cord of Rats with Bone Cancer Pain

Jun Chen, Xiufeng Cong, Xiuzhu Zhan, Zheng Zhou and Wei Zheng

ABSTRACT

Objective: To investigate the effects of parecoxib on pain threshold and inflammatory factors interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in spinal cord of rats with bone cancer pain.

Study Design: An experimental study.

Place and Duration of Study: Department of Oncology, Shengjing Hospital of China Medical University, China, from March 2017 to May 2018.

Methodology: Twenty-four healthy female Sprague-Dawley rats were selected and the bone cancer pain model was inoculated with W256 breast cancer cell into the bone marrow. Rats with bone cancer pain were randomly divided into the model group and the parecoxib group on the 7th day postoperation, with 12 rats in each group. Another 12 rats were taken as the control group. Rats in the parecoxib group were given intraperitoneal injection of parecoxib (8 mg/kg) for 10 consecutive days since the 15th day after operation. Mechanical pain threshold, thermal pain threshold, and the levels of inflammatory factors IL-1β, IL-6 and TNF-α in spinal cord of rats in each group were compared.

Results: On the 14th postoperative day, mechanical pain threshold and thermal pain threshold of rats in the model group and the parecoxib group were significantly decreased compared with those in the control group (p<0.001). After 5 and 10 days of administration, mechanical and thermal pain threshold of rats in the parecoxib group were significantly higher than those in the model group and the control group (p<0.001). After 10 days of administration, levels of IL-1β, IL-6 and TNF-α in spinal cord of the model group were higher than those of the control group (p<0.001); and levels of IL-1β, IL-6 and TNF-α in spinal cord of the parecoxib group were significantly lower than those in the model group and the control group (p<0.001).

Conclusion: Parecoxib can alleviate hyperalgesia in rats with bone cancer pain, increase pain threshold and inhibit the up-regulation of inflammatory factors in the spinal cord. Parecoxib may achieve analgesic effects by down-regulating the expression of IL-1β, IL-6 and TNF-α in the spinal cord.

Key Words: Parecoxib, Bone cancer pain, Rat, Analgesia, IL-1β, IL-6, TNF-α.
with bone cancer pain, so as to reveal the mechanism of parecoxib on bone cancer pain.

**METHODOLOGY**

This study was conducted at the Department of Oncology, Shengjing Hospital of China Medical University, China, from March 2017 to May 2018, after approval from the Hospital Ethical and Research Committee. Thirty-six healthy female Sprague-Dawley rats were selected, with a body mass of 155-178 g, (averaged 165.34 ± 3.72 g).

Then 24 rats were randomly selected for anesthesia, the upper skin of their left hind limb was cut into a small incision of about 1.0 cm, and the tibia was exposed. A 7-gauge needle was rotated and pierced at the upper 1/3 of the tibia and into the marrow cavity for a hole. A 5-μl micro-syringe was used to extract 3 μl of about 4x10^3 W256 breast cancer cell suspension into the bone marrow cavity. The incision was immediately sealed with bone wax. The incision was sutured and a little gentamicin solution was dropped to prevent infection.

After operation, the rats were routinely reared for seven days. After the use of 3% pentobarbital sodium for anesthesia, the limbs were observed under a high-frequency mammography machine. As the upper end of the tibia showed obvious defects, the rat model with bone cancer pain was successfully established. On the 7th day postoperation, the 24 rats with bone cancer pain were randomly divided into the model group and the parecoxib group, with 12 in each group. Another 12 rats were injected with physiological saline instead of W256 breast cancer cell suspension into the bone marrow cavity. A 5-μl syringe was used to extract 3 μl of about 4x10^3 W256 breast cancer cell suspension into the bone marrow cavity as the control group. Rats in each group were given medicine on the 15th day after operation, twice a day for 10 days. Rats in the parecoxib group were given intraperitoneal injection of parecoxib (8 mg/kg), and rats in the control group and the model group were given an equal amount of physiological saline by intraperitoneal injection.

On the 7th and 14th days postoperation, 5th day after administration (20th day after operation) and 10th day after administration (25th day after operation), the changes of mechanical pain threshold in each group were measured by toe-tenderness method and the changes of thermal pain threshold were measured by hot plate method.

On the 10th day after administration (25th day after operation), rats in each group were measured with mechanical and thermal pain threshold. The rats were deeply anesthetised by intraperitoneal injection of 4% chloral hydrate (400 mg/kg), and the left side of L4-L6 spinal dorsal horn segment was quickly removed, weighed and added with the lysate at a ratio of 200 μL of tissue lysate to 100 mg of spinal cord tissue, and well homogenised on ice. The supernatant was collected after centrifugation and the levels of inflammatory factors IL-1β, IL-6 and TNF-α in the spinal cord tissues of each group were detected by enzyme-linked immunosorbent assay (ELISA).

Data was entered and analysed by using SPSS version 25.0. Continuous data was described as mean ± SD. One-way analysis of variance was used for comparison among the three groups. SNK method was used for comparison between two groups. P-value of less than 0.05 was considered significant.

**RESULTS**

On the 7th day postoperation, there was no significant difference in mechanical pain threshold among the control group, the model group, and the parecoxib group (p=0.213). On the 14th day postoperation, mechanical pain threshold of the model group and the parecoxib group was significantly decreased compared with that of the control group (p<0.001). After 5 and 10 days of administration, mechanical pain threshold of the parecoxib group was significantly higher than that of the model group and the control group (p=0.001, Table I).

On the 7th day postoperation, there was no significant difference in thermal pain threshold among the control group, the model group and the parecoxib group (p=0.982). On the 14th day postoperation, thermal pain threshold of the model group and the parecoxib group was significantly lower than that of the control group (p<0.001). After 5 and 10 days of administration, the thermal pain threshold of the parecoxib group was

**Table I:** Comparison of mechanical pain thresholds at different time points (unit: kg).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of mice</th>
<th>7 days after operation</th>
<th>14 days after operation</th>
<th>5 days after administration</th>
<th>10 days after administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>p-value</td>
<td>Mean ±SD</td>
<td>p-value</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>Control group</td>
<td>12</td>
<td>0.291 ±0.018</td>
<td>0.213</td>
<td>0.304 ±0.027</td>
<td>0.001</td>
</tr>
<tr>
<td>Model group</td>
<td>12</td>
<td>0.278 ±0.016</td>
<td>0.246 ±0.028</td>
<td>0.226 ±0.027</td>
<td>0.231 ±0.033</td>
</tr>
<tr>
<td>Parecoxib group</td>
<td>12</td>
<td>0.283 ±0.020</td>
<td>0.259 ±0.023</td>
<td>0.408 ±0.043</td>
<td>0.313 ±0.044</td>
</tr>
</tbody>
</table>

**Table II:** Comparison of thermal pain thresholds at different time points (unit: s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of mice</th>
<th>7 days after operation</th>
<th>14 days after operation</th>
<th>5 days after administration</th>
<th>10 days after administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>p-value</td>
<td>Mean ±SD</td>
<td>p-value</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>Control group</td>
<td>12</td>
<td>18.87 ±4.82</td>
<td>0.982</td>
<td>18.95 ±4.25</td>
<td>0.001</td>
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<tr>
<td>Model group</td>
<td>12</td>
<td>18.53 ±4.16</td>
<td>11.27 ±3.87</td>
<td>11.94 ±3.01</td>
<td>0.213</td>
</tr>
<tr>
<td>Parecoxib group</td>
<td>12</td>
<td>18.74 ±3.72</td>
<td>11.52 ±3.66</td>
<td>27.18 ±7.95</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data was entered and analysed by using SPSS version 25.0. Continuous data was described as mean ± SD. One-way analysis of variance was used for comparison among the three groups. SNK method was used for comparison between two groups. P-value of less than 0.05 was considered significant.
Table III: Comparison of IL-1β, IL-6 and TNF-α levels in spinal cord of rats in each group after 10 days of administration.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IL-1β (pg/ml)</th>
<th>p-value</th>
<th>IL-6 (pg/ml)</th>
<th>p-value</th>
<th>TNF-α (pg/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>12</td>
<td>80.56 ±17.22</td>
<td>&lt;0.001</td>
<td>383.07 ±74.84</td>
<td>&lt;0.001</td>
<td>89.96 ±6.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model group</td>
<td>12</td>
<td>261.24 ±81.63</td>
<td></td>
<td>867.32 ±93.41</td>
<td></td>
<td>124.23 ±9.38</td>
<td></td>
</tr>
<tr>
<td>Parecoxib group</td>
<td>12</td>
<td>152.73 ±35.57</td>
<td></td>
<td>554.29 ±60.47</td>
<td></td>
<td>60.45 ±5.24</td>
<td></td>
</tr>
</tbody>
</table>

significantly higher than that of the model group and the control group (p<0.001, Table II).

After administration for 10 days, the levels of IL-1β, IL-6 and TNF-α in the spinal cord of the control group, the model group and the parecoxib group were significantly different (p<0.001); levels of IL-1β, IL-6 and TNF-α in spinal cord of the model group were higher than those of the control group (p<0.001); and levels of IL-1β, IL-6 and TNF-α in spinal cord of the parecoxib group were significantly lower than those in the model group and the control group (p<0.001, Table III).

DISCUSSION

Parecoxib is the first selective cyclooxygenase-2 (COX-2) inhibitor that can be injected intravenously and intramuscularly. It is now widely used for the prevention and treatment of moderate to severe pain. Studies have shown that parecoxib can reduce the need for opioid analgesics in surgical patients. Moreover, 40 mg parecoxib is more effective than 4 mg morphine in the treatment of postoperative pain. Parecoxib is recommended for the treatment of cancer pain. However, the mechanism of action of parecoxib against cancer pain is still not clear.

In chronic pain, glial cells can mediate the release of various pro-inflammatory factors. These factors, represented by IL-1β, IL-6 and TNF-α, play an important role in the development of chronic pain. Nocuous stimulation can cause up-regulation of IL-1β, IL-6 and TNF-α expression. IL-1β can induce the expression of various inflammatory pain-causing substances such as IL-6, TNF-α, etc.; and its degree of elevation is closely related to pain. IL-6 has a pain-inducing effect on the peripheral or central nervous system and stimulates the massive release of COX-2. Previous studies have found that inflammatory factors such as IL-1β, IL-6 and TNF-α in the spinal and dorsal ganglia of animal models participate in and mediate the occurrence of bone cancer pain. Inflammatory factors, such as IL-1β, IL-6 and TNF-α may affect and amplify the spinal cord’s perception of noxious stimuli, eventually leading to hyperalgesia, which plays an important role in the occurrence and maintenance of pain sensitivity in patients with bone cancer pain. The experiments showed that the levels of IL-1β, IL-6 and TNF-α in the spinal cord of the model group were higher than those of the control group after 10 days of administration. The levels of IL-1β, IL-6 and TNF-α in the spinal cord of the parecoxib group were significantly lower than those of the model group and the control group. This indicates that parecoxib inhibits the secretion and release of inflammatory factors in the spinal cord of rats with bone cancer pain, thereby reducing the activation and sensitisation of primary afferent neurons, and finally reducing the cancer pain.

The authors believe that parecoxib has a good analgesic effect on bone cancer pain. It is speculated that parecoxib may have an analgesic effect by eliminating inflammation and reducing inflammatory swelling. It is worth mentioning that because bone cancer pain is the result of a combination of factors, the analgesic effect of parecoxib on bone cancer pain may also involve other mechanisms. In addition, the results of animal models are still far from those of the human, so the analgesic mechanism of parecoxib remains to be further studied.

CONCLUSION

Parecoxib can alleviate hyperalgesia in rats with bone cancer pain, increase the pain threshold and inhibit the up-regulation of inflammatory factors in the spinal cord. Parecoxib may achieve analgesic effects by down-regulating the expression of IL-1β, IL-6 and TNF-α in the spinal cord.
REFERENCES


