The preliminary study of the mechanism and efficacy of non-steroidal anti-inflammatory drug treatment on nerve root type cervical spondylosis

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This study aims to evaluate the cyclooxygenase-2 (COX-2) protein expression in spinal cord dorsal horn of rats with nerve root type cervical spondylosis (NRCS) and the clinical efficacy of different non-steroidal anti-inflammatory drugs (NSAIDs) treatment on Chinese patients with NRCS. 24 Sprague-Dawley (SD) rats were randomly divided into normal health group and NRCS model group. The COX-2 protein expression in spinal cord dorsal horn of rats was detected by immunohistochemical staining after administration. 52 NRCS patients were divided into celecoxib treatment group and diclofenac treatment group to compare the efficacy of different NSAIDs. The efficacy of NSAIDs on NRCS was assessed by visual analog scale (VAS) test. Compared with normal health group, the expressions of COX-2 protein in spinal cord dorsal horn increased significantly than in the NRCS model group. According to the results of VAS test, we found out that celecoxib is more effective than diclofenac sodium. Clinical drug therapy for NRCS can give priority to specific COX-2 inhibitors. Over expression of COX-2 might be a potential pathological mechanism of NRCS.

Key words: Nerve root type cervical spondylosis, non-steroidal anti-inflammatory drugs, clinical observation, cyclooxygenase-2.

INTRODUCTION

Nerve root type cervical spondylosis (NRCS) is usually caused by stimulation and oppression of the cervical nerve root due to retrograde degeneration of the small intervertebral joints and Luschka joints (uncinate vertebral joints). It is a common disease in clinic. NRCS patients may have symptoms of cervical stiffness, rest rained movement, arm pain and numbness of the fingers which may affect patients’ normal work and daily life (Allan and Binder, 2007). In recent years, conservative treatment for NRCS has been adopted by modern medicine for most patients, while surgical treatment has also been used for a small number of patients who were conservatively treated with unsatisfactory effect or lingered by obstinate disease with operation indications. It was reported that extradural injection of non-steroidal anti-inflammatory drugs (NSAIDs) is a conservative treatment to relieve inflammation and edema of the nerve root. However, the therapeutic mechanism of the NSAIDs and pathological mechanism of NRCS are still unclear. In the present work, we detected the cyclooxygenase-2 (COX-2) protein expression in spinal cord dorsal horn of NRCS rats by immunohistochemical staining. Moreover, we also compared the efficacy of celecoxib and diclofenac on 52 NRCS patients.

MATERIALS AND METHODS

The general kit of immunohistochemistry (ICH) and monoclonal antibody of COX-2 were obtained from SANTA CRUZER Biotechnology Company in USA.

Laboratory animals

Twenty-four Sprague-Dawley (SD) rats, aged>16 months, weighing
(252 ± 25) g, of either gender, were provided by the Experimental Animal Center of the Putian University between July 2010 and November 2011. The rats were housed under controlled conditions (room temperature, 22 ± 2°C).

Establishment of NRCS rats model

The rats in model group were anesthetized by injecting intraperitoneally 40 mg/kg of pentobarbital sodium. Dorsal neck was shaved and a longitudinal incision about 1.5 cm was followed. The dorsal muscles were separated and reserved. Spinal processes, inter spinal ligaments, part of superior and inferior articular processes between C6 and 7 levels were cut off. When the movement degree between the neighboring superior and inferior laminae was increased obviously after operation, the incision was then closed. The successful models were confirmed by evaluating X-ray films and the motion function with oblique board test 3, 4 and 5 mo, respectively, after the operation. For rats in the normal health group, they were closed after opening without further operation.

Detection of COX-2 protein expression

Rats were anaesthetized with 10% of urethane and then sacrificed by transcardiac perfusion with phosphate-buffered saline (PBS) followed by separating their lumbar spinal cord immediately, cryoprotected by immersion in 30% sucrose for 24 h at 4 to 8°C and frozen in a tissue-freezing medium. The brains were cut on a freezing microtome, into six adjacent series of 30-μm-thick coronal sections. The paraffin section was general deparaffinated to water, and the section was placed in 10% of hydrogen dioxide at room temperature for 5 to 10 min to blockade endogenous peroxidase. Then, PBS was used to washout the section for 3 times and one time was for 2 min. The pamcreatin fluid was added on the section and incubated at 37°C for 20 min. Then, the PBS was used to washout for 3 times.

The blood serum that was found in the section was incubated for 15 min at ambient temperature. The blood serum was poured and added 50 μl of antibody of COX-2, then stored at 4°C overnight. The PBS was used to washout the section for 3 times and general antibody of 2 was added for 15 min at ambient temperature. The PBS was used to washout the section and dropped wise the fluid labeled by horse-radish enzyme for 15 min at room temperature. Then, the PBS was used to washout the section for three times. Dimethylaminoazobenzene (DAB) fluid was added on the section and control colouration under the microscope. The distilled water was used to poach, and the campeachy was used to stain and mount.

Patients

56 NRCS patients were taken as the objects of study. Among 56 patients who volunteered to participate in the intervention treatment, there were 24 men and 32 women with a proportion of 1: 1.33. The age ranks and average age was (52 to 71) and (61.80 ± 4.20), respectively. 56 NRCS patients were averaged randomized into Celecoxib group and Diclofenac Sodium group. Their height, weight, gender, age and average course of disease and X-ray staging among groups are comparable.

Drug intervention

Random, open and self control test were adopted in drug intervention treatment, in which 26 patients in celecoxib group took celecoxib tablet at the dosage of 100 mg, †bid, course of treatment: 3 weeks. 30 patients in diclofenac sodium group took diclofenac sodium tablets at the dosage of 50 mg, †bid, course of treatment: 3 months. Visual analog scale (VAS) records were made before and after administration. Patients with severe adverse reactions discontinued the administration.

Evaluation of efficacy

VAS (Majani et al., 2003) scoring was used for evaluation of the therapeutic effects before and after the therapy. Among the score, 0 to 10 (0 was no pain, 10 was acute pain), the value indicated the painful intensity and mental assault degree. Less than 3 scores indicated good, 3 to 4 basically satisfied and ≥ 5 poor. The VAS scores of the patients were evaluated before and after the therapy.

Statistical analysis

The database was set up with the Statistical Package for Social Sciences (SPSS) 16.0 software package for analysis. Data were represented as mean ± standard deviation (SD). The means of different groups were compared with student’s t-test. P<0.05 was considered as statistically significant.

RESULTS

ICH results

Compared with normal health control group, the COX-2 protein expression increased significantly in the NRCS group (t=7.0716, P<0.001) (Table 1 and Figure 1). These results suggested that increased expressions of COX-2 protein might play an important role in the mechanism of NRCS generation.

VAS score results

After three months treatment of the 56 cases, the therapeutic results are as shown in Table 2. Compared with the VAS scores before administration, the VAS scores after administration decreased significantly in each group. Meanwhile, the VAS scores after administration in the celecoxib treatment group were less than that in the diclofenac treatment group (t=-14.0602, P<0.0001).

DISCUSSION

In rat cerebral cortex, a selective expression of COX-2 was shown. COX-2 immunoreactivity was found especially in a subpopulation of excitatory neurones in allocortices, hippocampus and amygdala. Immunoreactivity was compartmentalised to dendritic arborisations. Moreover, COX-2 protein is present in dendritic spines, which are specialized structures in synaptic signaling. The developmental profile of COX-2 expression coincided with the critical period for activity-
Table 1. The comparison of COX-2 positive cells between NRCS model group and Normal health control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>COX-2 positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRCS model</td>
<td>12</td>
<td>0.2604 ± 0.0353</td>
</tr>
<tr>
<td>Normal health control</td>
<td>12</td>
<td>0.1728 ± 0.0244</td>
</tr>
</tbody>
</table>

Figure 1. COX-2 positive cells of NRCS model group and normal health control group. A: COX-2 immunoreactivity in NRCS model rat; B: COX-2 immunoreactivity in normal health control rat.

Table 2. The comparison of VAS score between celecoxib treatment group and diclofenac treatment group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>VAS (before administration)</th>
<th>VAS (after administration)</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celecoxib</td>
<td>26</td>
<td>8.6 ± 1.2</td>
<td>1.4 ± 0.3</td>
<td>29.6807</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>26</td>
<td>8.4 ± 1.3</td>
<td>3.5 ± 0.7</td>
<td>16.9221</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

dependent synaptic remodeling. Results of Kaufmann et al. (1996) indicated that COX-2 and its diffusible prostanoid products, may play a role in postsynaptic signaling of excitatory neurones in cortex and associated structures. In contrast to COX-1, COX-2 is admittedly constitutively expressed in brain and spinal cord, but at the same time highly regulated by different influencing factors, like ischaemia, immunomodulators and cytokines, structural brain damages, toxic agents and during maturational processes (Hoffmann et al., 2000; Kuslich et al., 1991). In our present work, we found out that the COX-2 protein expression was higher in the NRCS rats than normal healthy rats. The results indicated that COX-2 might play an important role in the process of NRCS.

NSAIDs inhibit cyclooxygenase (COX) activity, thereby suppressing the synthesis of proinflammatory prostaglandins. The identification and molecular-biological characterization of an inducible COX isoform (COX-2) in inflammatory cells lead to the hypothesis that a selective inhibition of COX-2 would result in relief of inflammation and pain without causing the COX-1-dependent side effects (gastrointestinal ulceration, platelet dysfunction and kidney damage) of conventional NSAIDs (Bensen et al., 2000; Goldstein et al., 2001; Silverstein et al., 2000).

NRCS can be treated by selected NSAIDs in the light of the overall analysis of symptoms and signs. The therapeutic effects have been shown to be satisfactory. However, since the samples size is small, it is difficult to exactly identify the therapeutic effect of the NSAIDs treatment. Therefore, it is highly necessary that strict clinical observation indexes and a universally used standard for the therapeutic effect should be worked out in the future.

**Conclusion**

In the present work, animal results showed that COX-2 protein expression was higher in the NRCS rats than normal healthy rats. The over expression of COX-2 protein might be a potential mechanism of NRCS generation. Clinical results with the selective COX-2
inhibitors show a better safety profile than non-selective COX inhibitors. Clinical use after drug registration will be decided on further role of this new class of drugs in NRCS therapy and on new fields of clinical use.

REFERENCES
