Anti-inflammatory and analgesic effects of Yaotuitong Capsules in experimental rats with chemically induced radicular neuritis

Qingchang Xing, Zhenjun Huang, Jianfu Zhang

Abstract

OBJECTIVE: The aims of this study were to determine the anti-inflammatory and analgesic effects of Yaotuitong (translation: low back and leg pain) capsules, a Chinese herbal preparation, and the histological changes it induces in experimental rats with chemically induced radicular neuritis.

METHODS: Wistar rats were randomly divided into normal, model, Western medicine, and traditional Chinese medicine groups (n=24 per group). We surgically duplicated a chemical radicular neuritis model to simulate lumbar intervertebral disc protrusion. Granuloma formation was measured on postoperative days (PODs) 3, 7, 14, and 21. Prostaglandin E2 and 5-hydroxytryptamine (inflammation mediators) levels in the surrounding tissue and the histology of the nerve root were determined on PODs 7 and 14.

RESULTS: Yaotuitong capsules significantly reduced prostaglandin E2 (P<0.01) and 5-hydroxytryptamine (P<0.01) levels in tissue surrounding the nerve root. It also inhibited granuloma formation (P<0.05).

CONCLUSION: Yaotuitong capsules have anti-inflammatory and analgesic effects that can alleviate the discomfort of lumbar intervertebral disc protrusion.

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Key words: Yaotuitong capsules; Radiculitis; Inflammation mediators

INTRODUCTION

Yaotuitong (translation: low back and leg pain) capsules are a Chinese herbal preparation based on a Yaotuitong decoction developed by Professor Jianfu Zhang (Department of Orthopedics, Traditional Chinese Medical University of Henan Province, Zhengzhou, China). According to traditional Chinese medical theory, Yaotuitong capsules can alleviate impediments to the flow of Qi, promote blood circulation, remove meridian obstructions, and eliminate pain. It was therefore thought that it might be used to treat the herniated lumbar disc. We planned to study the effects and the mechanism of action of the Yaotuitong capsules on lumbar disc herniation. Therefore, based on previous anti-inflammatory and analgesic studies, we designed a chemically induced radicular neuritis rat model that simulated lumbar disc herniation. The resulting granuloma formation, levels of inflammatory mediators in tissue surrounding the nerve root, and histopathological changes were evaluated in this model and compared to the same parameters in three other groups of rats.

METHODS

A total of 96 Wistar rats weighing 200-240 g (irrespective of sex) were obtained from the Experimental Animal Center of Henan University of Traditional Chinese Medicine in Zhengzhou, China. The rats were random-
ly divided into four groups: normal rats, untreated model rats, rats treated with Western medicine, and rats treated with traditional Chinese medicine (24 rats per group). All of the rats were housed in separate cages with free access to water and food.

The equipment used included a Sartorius-2452 analytical balance (Sartorius AG, Gottingen, Germany) and a Shimadzu-510 fluorescence photometer (Shimadzu, Tokyo, Japan).

**Model preparation**

The model rats were depilated dorsally 2 days prior to the operation. On the day of surgery, they were anesthetized with sodium pentobarbital (50 mg/kg) via intraperitoneal injection, and a 3 cm midline incision was made on the dorsal skin under strict aseptic conditions using the fourth and fifth lumbar spinous processes as the center. The muscle was bluntly dissected to the paraspinal muscles, and the right lamina and articular process of the fourth and fifth lumbar spinous processes were removed. Using a surgical microscope, the L5 nerve root was carefully exposed. We then placed filter paper (0.6 cm diameter) that had been treated with ter paper (0.6 cm diameter) that had been treated with than 14 days after the model was prepared (P<0.01 versus the development at both 3 days and 7 days). There was significantly less granuloma formation in the traditional Chinese medicine group than in the model group on PODs 14 and 21 (P<0.05). Although there was also less granuloma development in the Western medicine group than in the model group, there was even less in the traditional Chinese medicine group than in the Western medicine group. The difference, however, was not statistically significant. It was clear that the Yaotuitong capsule preparation effectively inhibited granuloma formation around the nerve root (Table 1).

**Effect of Yaotuitong capsules on inflammatory mediator levels in tissue surrounding the nerve root**

Prostaglandin E$_2$ (PGE$_2$) was soaked in saline and then saponified. The absorbance at 278 nm was determined and expressed as the absorbance value per gram. A second sample of tissue surrounding the nerve root was selected to extract 5-hydroxytryptamine (5-HT) using alkaline n-butanol. The relative fluorescence intensity of the extraction solution was determined using a fluorometric assay with o-phthalaldehyde, with the results expressed as the relative fluorescence intensity per gram. For the histological examination, samples of the L5 nerve root at the surgical side were removed on PODs 7 and 14. They were fixed in 10% formalin and then stored at 4°C for histological analysis.

Data were expressed as the mean ± SD and were analyzed using analysis of variance. Values between groups were compared using the t-test. Statistical analysis was performed using SPSS version 13.0 software (SPSS, Chicago, IL, USA).

**RESULTS**

**Effects of Yaotuitong capsules on stimulus-induced granuloma**

Granuloma development was significant around the stimulus area 14 days after the model was prepared (P<0.01 versus the development at both 3 days and 7 days). There was significantly less granuloma formation in the traditional Chinese medicine group than in the model group on PODs 14 and 21 (P<0.05). Although there was also less granuloma development in the Western medicine group than in the model group, there was even less in the traditional Chinese medicine group than in the Western medicine group. The difference, however, was not statistically significant. It was clear that the Yaotuitong capsule preparation effectively inhibited granuloma formation around the nerve root (Table 1).

**Effect of Yaotuitong capsules on inflammatory mediator levels in tissue surrounding the nerve root**

Prostaglandin E$_2$ and 5-HT levels were significantly increased in local tissue in the model group (P<0.01), suggesting chemical inflammation around the nerve root. Western medicine had strong, rapid anti-inflammatory and analgesic effects (P<0.05 or P<0.01), especially regarding 5-HT. Evidence that traditional Chinese medicine was inhibiting PGE$_2$ appeared on POD 7 and decreased 5-HT levels earlier (P<0.01) (Tables 2, 3).

**Histological examination of the nerve root**

Histologically, there was less inflammation of the nerve root tissue in the Western medicine group than in the model group, but it did not prevent degeneration and necrosis of the nerve roots. In contrast, traditional Chinese medicine significantly delayed the process of degeneration and necrosis of the nerve roots and played a protective role in the rats with chemically induced inflammation and nerve root injury (Figure 1, 2).
Yaotuitong capsules are a pure traditional Chinese medicine preparation modified from Zhang’s decoction. It can alleviate impediments to the flow of Qi, promote blood circulation, remove meridian obstruction, and relieve pain. With these actions, it is considered to reduced levels of nitric oxide and interleukin and inhibition of inflammation. Because their concentrations in tissue directly reflect the degree of inflammation and pain, they are significant factors in the pathological process of producing radicular pain in patients with lumbar disc herniation. Strong evidence indicates that rupture of the annulus fibrosus and the exposed nucleus pulposus stimulates the dural sac and nerve root sleeve, leading to chemically induced radicular neuritis and pain. Venous occlusion and microcirculation disorders impede the supply of oxygen and nutrients to tissues, resulting in accumulation of metabolites. They also increase capillary permeability, exacerbate the inflammatory reaction, and lead to local inflammatory-induced granuloma formation. The keys to treating lumbar disc herniation are to improve the microcirculation, inhibit inflammation around the nerve root, reduce the production/accumulation of inflammatory- and pain-inducing chemicals in local tissue, and prevent damage to nerve roots.

Yaotuitong capsules are a pure traditional Chinese medicine medicine with traditional Chinese and Western medicine. The underlying mechanisms may be related to reduced levels of nitric oxide and interleukin and inhibition of inflammation. Prostaglandin E$_2$ and 5-HT are important mediators of inflammation. Because their concentrations in tissue directly reflect the degree of inflammation and pain, they are significant factors in the pathological process of producing radicular pain in patients with lumbar disc herniation. Strong evidence indicates that rupture of the annulus fibrosus and the exposed nucleus pulposus stimulates the dural sac and nerve root sleeve, leading to chemically induced radicular neuritis and pain. Venous occlusion and microcirculation disorders impede the supply of oxygen and nutrients to tissues, resulting in accumulation of metabolites. They also increase capillary permeability, exacerbate the inflammatory reaction, and lead to local inflammatory-induced granuloma formation. The keys to treating lumbar disc herniation are to improve the microcirculation, inhibit inflammation around the nerve root, reduce the production/accumulation of inflammatory- and pain-inducing chemicals in local tissue, and prevent damage to nerve roots.

**DISCUSSION**

### Chemical radiculitis

Chemical radiculitis and intervertebral foramen venous obstruction are significant factors in the pathological process of producing radicular pain in patients with lumbar disc herniation. Strong evidence indicates that rupture of the anulus fibrosus and the exposed nucleus pulposus stimulates the dural sac and nerve root sleeve, leading to chemically induced radicular neuritis and pain. Venous occlusion and microcirculation disorders impede the supply of oxygen and nutrients to tissues, resulting in accumulation of metabolites. They also increase capillary permeability, exacerbate the inflammatory reaction, and lead to local inflammatory-induced granuloma formation. The keys to treating lumbar disc herniation are to improve the microcirculation, inhibit inflammation around the nerve root, reduce the production/accumulation of inflammatory- and pain-inducing chemicals in local tissue, and prevent damage to nerve roots.

### DISCUSSION

**Table 1** Effect of Yaotuitong capsules on inflammation-induced granuloma formation (*x±s, n=6*)

<table>
<thead>
<tr>
<th>Group</th>
<th>Inflammatory granuloma formation at PODs 3 – 21 (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>Model</td>
<td>10.18±1.18</td>
</tr>
<tr>
<td>Western medicine</td>
<td>11.70±2.54</td>
</tr>
<tr>
<td>Traditional Chinese</td>
<td>9.22±1.51</td>
</tr>
</tbody>
</table>

Notes: *P*<0.05, **P**<0.01, compared with the model group at the same time point.

**Table 2** Effect of Yaotuitong capsules on PGE$_2$ levels in tissue surrounding the nerve root (*x±s, n=6*)

<table>
<thead>
<tr>
<th>Group</th>
<th>PGE$_2$ levels on PODs 3 – 21 after preparation of the model (absorbance value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>Normal</td>
<td>1.84±0.35</td>
</tr>
<tr>
<td>Model</td>
<td>3.31±0.12*</td>
</tr>
<tr>
<td>Western medicine</td>
<td>2.84±0.31a</td>
</tr>
<tr>
<td>Traditional Chinese</td>
<td>3.04±0.42a</td>
</tr>
</tbody>
</table>

Notes: PGE$_2$: prostaglandin E$_2$. *P*<0.01, **P**<0.001, compared with the normal group at the same time point; *P*<0.05, **P**<0.01, compared with the model group at the same time point.

**Table 3** Effect of Yaotuitong capsules on 5-HT levels in tissue surrounding the nerve root (*x±s, n=6*)

<table>
<thead>
<tr>
<th>Group</th>
<th>5-HT levels on PODs 3 – 21 after preparation of the model (relative fluorescence intensity/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>Normal</td>
<td>26±5</td>
</tr>
<tr>
<td>Model</td>
<td>50±9*</td>
</tr>
<tr>
<td>Western medicine</td>
<td>34±8*</td>
</tr>
<tr>
<td>Traditional Chinese</td>
<td>38±8*</td>
</tr>
</tbody>
</table>

Notes: 5-HT: 5-hydroxytryptamine. *P*<0.01, **P**<0.001 compared with the normal group at the same time point; *P*<0.05, **P**<0.01, compared with the model group at the same time point.

**DISCUSSION**

Chemical radiculitis and intervertebral foramen venous obstruction are significant factors in the pathological process of producing radicular pain in patients with lumbar disc herniation. Strong evidence indicates that rupture of the anulus fibrosus and the exposed nucleus pulposus stimulates the dural sac and nerve root sleeve, leading to chemically induced radicular neuritis and pain. Venous occlusion and microcirculation disorders impede the supply of oxygen and nutrients to tissues, resulting in accumulation of metabolites. They also increase capillary permeability, exacerbate the inflammatory reaction, and lead to local inflammatory-induced granuloma formation. The keys to treating lumbar disc herniation are to improve the microcirculation, inhibit inflammation around the nerve root, reduce the production/accumulation of inflammatory- and pain-inducing chemicals in local tissue, and prevent damage to nerve roots. Yaotuitong capsules are a pure traditional Chinese
Figure 1 Histological image of nerve root in rats on postoperative day (POD) 7 (× 400)
A Normal group: Nerve roots show intact structure and tight arrangement of fibers. Long Fei’s nodules are visible. B Model group: Nerve root fibers appear edematous and thicker, axons have partially disappeared, and most of the myelin sheath has disintegrated. C Western medicine group: Axons have partially disappeared, and demyelination of nerve fibers is observed. D Traditional Chinese medicine group: Partial nerve fiber demyelination is visible.
A Model group: Nerve root degeneration and necrosis are found with inflammatory infiltration. B Western medicine group: Discontinuous axonal remnants are visible. C Traditional Chinese medicine group: Nerve cells have survived in the ganglia.

Figure 2 Pathological image of nerve root in rats on POD 14 (× 400)
serve as important hallmarks of anti-inflammatory and analgesic efficacy. In this study, we successfully replicated a chemical radicular neuritis model in rats to simulate lumbar disc herniation. Stimulus-induced granuloma development and the concentrations of these inflammatory mediators in the nerve root and surrounding tissue were determined at several time points after the model was prepared. Results of this study showed that PGE2 and 5-HT levels were significantly elevated in local nerve root tissue after the surgery to prepare the model rats. Inflammatory granuloma formation was visible, suggesting that the pathological process of lumbar disc herniation involves chemically induced radicular neuritis. Yaotuitong capsules effectively reduced the levels of inflammatory mediators in nerve roots and surrounding tissue, prevented inflammatory granuloma formation, and inhibited inflammation. Histopathological analysis of the nerve root confirmed that Yaotuitong capsules significantly delayed the process of nerve root degeneration and necrosis and protected nerve roots against injury caused by chemically induced inflammation.

The pharmacological effects of Yaotuitong capsules are determined by its active ingredients, which include the following: Ma Qian Zi (Semen Strychni), Yan Hu Suo (Rhizoma Atractylodis), Ru Xiang (Frankincense), MoYao (Myrrh), Dan Shen (Radix Salviae Miltiorrhizae), Quan Xie (Scorpions), Wu Gong (Scolopendra), Cang Zhu (Rhizoma Atracylodis), Bai Shao (Radix Paeoniae Alba), Dang Gui (Radix Angelicae Sinensis), and Radix Glycyrrhizae. Studies have shown that strychnine and other alkaloids in Strychnus, Radix Salviae Miltiorrhizae, and Radix Paeoniae Alba selectively excite spinal cord tissue, improve skeletal myasthenia, and adjust the imbalance between the vertebral column and the medullary spinal cord. Rhizoma Atractylodis has analgesic effects in modern pharmacological studies with a potency of total alkaloids of approximately 40% of morphine’s efficacy and an analgesic effect greater than that of aspirin. Myrrh and Radix Salviae Miltiorrhizae dilate capillaries, reduce blood viscosity, and improve the microcirculation. Radix Salviae Miltiorrhizae delays degeneration of lumbar intervertebral discs; its mechanism may be related to improving the local microcirculation of the intervertebral disc tissue, reducing venous stasis, and regulating the immune response. β2-Amine alcohol in Rhizoma Atractylodis can alleviate cell hypoxia. Total glucosides of peony have immunomodulatory and significant analgesic effects. Methanol extract of Glycyrrhizae FM100 in Radix Glycyrrhizae reacts synergistically with total glucosides of peony to create antispasmodic and analgesic effects. Pharmacological studies also found steroid hormone-like anti-inflammatory effects of Radix Glycyrrhizae, where the anti-inflammatory potency of glycyrrettinic acid was one-tenth that of hydrocortisone. Safflower can expand blood vessels; it antagonizes epinephrine- and norepinephrine-induced vascular congestion while improving the local microcirculation around the disc. All of the ingredients in the Yaotuitong prescription can alleviate chemical inflammation of the nerve root.

In summary, Yaotuitong capsules can reduce the effects of chemically induced radicular neuritis. It exhibits anti-inflammatory and analgesic effects and effectively alleviates inflammation-induced nerve root injury. These effects may explain the pharmacological mechanisms underlying the response to treatment of lumbar disc herniation with Yaotuitong capsules. Our findings provide experimental evidence to support clinical application and promotion of the use of Yaotuitong capsules.

REFERENCES