Basic Investigations

Effects of Tongmai Huoxue Yin (通脉活血饮) on Tumor Necrosis Factor-alpha in the Acute Cerebral Ischemia Model Rat

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Objective: To observe the interfering action of Tongmai Huoxue Yin (通脉活血饮) on the acute cerebral ischemia model rat.

Methods: Total 60 SD rats, 30 females and 30 males, were randomly divided into 4 groups, sham-operation group, model group, Nimodipine group and Tongmai Huoxue Yin group, 15 rats in each group. The acute cerebral ischemia rat model was duplicated, the middle cerebral artery (MCA) were ligated and the thread was inserted for the rats in the model group, Nimodipine group and Tongmai Huoxue Yin group, for the rats in the sham-operation group, the arteries were separated without ligature and the thread was not inserted. After the modeling has succeed, the water-decocted concentrated solution of 20-fold Tongmai Huoxue Yin clinical dosage was intragastrically administrated in a dose of 3 mL / 100 g · d divided into twice, 1.5 mL / 100 g once. Distilled water 3 mL / 100 g divided into twice, 1.5 mL / 100 g once, for the rat in the model group, Nimodipine suspension 3 mL / 100 g (0.6 mg / 100 g) for the Nimodipine group and 3 mL / 100 g · d (5.4 g / 100 g) for the Tongmai Huoxue Yin group, no drugs for the sham-operation group. And changes of tumor necrosis factor-alpha (TNF-α) contents in the serum and brain tissue were investigated.

Results: Compared with the model group, compared with the sham-operation group, serum TNF-α content at 5 h of focal cerebral ischemic ischemia in the model group started to increase and reached to the high peak at 12 h, but in both the Tongmai Huoxue Yin group and the Nimodipine group decreased in varying degrees at the same time; compared with the sham-operation group, brain TNF-α content at 6 h of focal cerebral ischemic ischemia in the model group started to increase and reached to the high peak at 12 h, but in both the Tongmai Huoxue Yin group and the Nimodipine group decreased in varying degrees, with the most obviously decreased at 24 h of ischemia. Tongmai Huoxue Yin could significantly decrease TNF-α content in the brain tissue.

Conclusion: Tongmai Huoxue Yin has a protective action on acute cerebral ischemia injury in the rat.

Keywords: Tongmai Huoxue Yin; acute cerebral ischemia model; TNF-α

Tumor necrosis factor-alpha (TNF-α), a more important inflammatory factor in cerebral injury, is closely related with ischemic cerebrovascular disease. How to alleviate inflammatory response after cerebral injury is one of research hot points at present. In the study, on the basis of successful duplication of acute cerebral ischemia rat model, the interfering action of Tongmai Huoxue Yin (通脉活血饮) on the acute cerebral ischemia model rat was investigated and the mechanism was explored.

MATERIALS AND METHODS

Drugs and Reagents
Tongmai Huoxue Yin is composed of Dang Gui (Radix Angelicae Sinensis), E Jiao (Colla Corii Asini), Chuan Xiong (Rhizoma Chaunxiong), Dan Shen (Radix Salviae Miltiorrizae), Tian Ma (Rhizoma Gastrodiae), Dan Nan Xing (Arisaema cum Bile), Shen Jin Cao (Herba Lycopodii), Luo Shi Teng (Caulis Trachelospermi), Chuan Niu Xi (Radix Cyathulae), etc. (Batch No: 20050703). All the medicinal materials were purchased once from Henan Provincial Crude Drug Company. Preparation: The medicinal materials were immerged in water of four times of the totalvolumes and boiled gently for 40 min × 2, and then the filtrates were combined and concentrated into 2.7 g crude drugs/mL. Each rat was intragastrically administrated 3 mL /100 g · d divided into twice, and the concentrated drug solution was added respectively with distilled water in the ratio 5:1, 2:1 and 1:1, prepared as high dose (2.25 g/mL), middle dose (1.8 g/mL), low dose (1.35 g/mL), and poured respectively into a bottle and kept in a refrigerator at 4 °C; Nimodipine (20 mg/granule), (Batch No:H10910080), was supplied by Hena Provincial Medical Company, which was ground and prepared as 20% suspension with distilled water (one table Nimodipine was added into 100 mL distilled water); protease K 20 μg/mL (Zhengzhou City Kangqiao Bio-technique Co. Ltd); TNF-α kit (Beijing Dongya Immuno-technique Institute, Batch No:2004924).

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Experimental Animal and Grouping
Total 60 SD healthy clean rats, weighing 230±20 g, 30 females and 30 males, were supplied by Henan Provincial Experimental Animal Center (License No: 050906), which were divided into 4 groups by simple randomization: sham-operation group, model group, Nimodipine group and Tongmai Huoxue Yin group, 15 rats in each group. They were raised in a room at 22±2 °C and relative humidity 60% with routine forage and free access to water. Before the experiment, the rats were raised in a room at 20–25 °C for adapting to the circumstance for one week, with individual cage, standard forage, tap water.

Duplication of Acute Cerebral Ischemia Rat Model
According to the classical modeling method, the rat was weighted and anesthetized with intraperitoneal injection of 10% chloral hydrate in 0.3 mL / 100 g body weight (BW), and then was fixed on an operative table in a supine position. After the hair was cut, an incision on the middle of the neck was made and the right skin, common carotid artery, external carotid artery, internal carotid artery (ICA) and branches were separated, and the distal end of the right common carotid artery was ligated and a thread was prepared for ligature of the proximal end of the common carotid artery; The original segment of the external carotid artery was ligated at the fork of the external carotid artery and the internal carotid artery, and the pterygopalatine artery, a branch of the internal carotid artery was ligated, and the branching arteries parallel to the internal carotid artery were cauterized with electrocaugulation. A thread embolus with a end of 0.26 mm in diameter was inserted into the oblique incision on the common carotid artery about 0.5 cm from the fork into ICA, passing through the middle cerebral artery (MCA) original end reaching to the proximal end of cerebral anterior artery to block the blood flow of MCA, and the prepared thread on the common carotid artery was tightly ligated, and the embolus thread was fixed and the superfluous thread was removed. The skin was sutured and the wound was disinfected with iodophor. The died rats were rejected. For the rats in the sham-operation group, the arteries were separated without ligature and the thread was not inserted, and other manipulations were same as those in other operation groups.

Judgment of Successful Model
After awaking, the animal model was judged with Longa’s 5-point assessing method, 0 point: no nerve defect symptoms; 1 point: The left anterior limb was unable to completely extend; 2 points: The body rotated towards the left; 3 points: The body fell toward the left; 4 points: unable to walk or coma. The rats with 1–4 points were regarded as effective model.

Administration Methods
The water-decocted concentrated solution of 20-fold Tongmai Huoxue Yin clinical dosage was intragastrically administrated in a dose of 3 mL / 100 g • d divided into twice, 1.5 mL / 100 g once. Distilled water 3 mL / 100 g • d was intragastrically administrated, 1.5 mL / 100 g once, for the rat in the model group, Nimodipine suspension 3 mL / 100 g • d (0.6 mg / 100 g) for the Nimodipine group and 3 mL / 100 g • d (5. 4g / 100 g) for the Tongmai Huoxue Yin group.

Determination of Indexes
After intragastrical administration for 5 days, and on the 6th day, 1 h before the final administration the acute cerebral ischemia rat model was duplicated, and 6 h, 12 h and 24 h after modeling, blood was taken from abdominal aorta respectively for determination of TNF-α contents.

Six hours, 12 h and 24 h after modeling, 3 rats were taken respectively and after anesthesia, they were decapitated, the right brain was taken with the cerebellum and medulla oblongata removed, homogenized in a ice-bath, and centrifuged in 3500 r/min for 10 min. Then the supernatant was taken for determination of TNF-α contents with radioimmunoassay.

Processing of Data
The data were expressed as mean ± standard deviation (X ± s), and the data were processed with single factor analysis of variance in SPSS 11.0 statistical software, and the comparison between the two groups was done by Chi-square test.

RESULTS
Effect of Tongmai Huoxue Yin on Serum TNF-α Content in the Rat with Focal Acute Cerebral Ischemia
As showed in Table 1, compared with the sham-operation group, serum TNF-α content at 5 h of focal cerebral ischemic ischemia in the model group started to increase and reached to the high peak at 12 h, but in both the Tongmai Huoxue Yin group and the Nimodipine group decreased in varying degrees at the same time.

Effect of Tongmai Huoxue Yin on Brain TNF-α Contents in the Rat with Focal Acute Cerebral Ischemia
As indicated in Table 2, compared with the sham-operation group, brain TNF-α content at 6 h of focal cerebral ischemic ischemia in the model group started to increase and reached to the high peak at 12 h, but in both the Tongmai Huoxue Yin group and the Nimodipine group decreased in varying degrees, with the most obviously decreased at 24 h of ischemia.
**DISCUSSION**

“Acute cerebral ischemia” belongs to the category of “apoplexy” in TCM, originating from the cognition of “Neijing” for cause of disease and pathogenesis of apoplexy. Before the Tang dynasty, it was considered that it was caused by “internal deficiency”, stressing “external wind” as main, and after the Tang Dynasty, “internal wind” was stressed. The authors hold that during apoplexy, deficiency, fire, wind, phlegm, qi and blood are influenced and acted mutually, but in acute stage, deficiency and blood stasis are principal, qi deficiency, blood stasis and channel obstruction are main pathogenesis of acute ischemic apoplexy.

Researches indicate that TNF-α expression level after craniocerebral injury is closely related with inflammatory response. \(^3\)\(^6\) In ischemia and inflammation, neutrophil, astrocyte astroglia and vascular endotheliocyte in the central nervous system all produce TNF-α, which can directly promote aggregation of neutrophil towards the injury area, leading to extension of local inflammation and exacerbating injury of cerebral tissues. \(^7\) An experiment indicates that after craniocerebral injury, the high expression of TNF-α is more obvious at the early stage. \(^8\) TNF-α gene expression in the brain tissue of local ischemia are strengthened during ischemia.

Tongmai Huoxue Yin has functions of invigorating qi and promoting blood flow. Clinically, it is used for treatment of acute cerebral ischemia with better therapeutic effects. It is composed of Dang Gui (Radix Angelicae Sinensis) and E Jiao (Colla Corii Asini) as monarch drugs have functions of promoting blood flow and enriching the blood. Chuan Xiong (Rhizoma Chaunxiong) and Dan Shen (Radix Salviae Miltiorrizae) have functions of activating blood circulation and removing blood stasis, removing stagnation of blood. Dang Gui (Radix Angelicae Sinensis) combined with Gou Qi Zi (Fructus Lycii) can promote blood circulation and functions tonifying the blood and nourishing yin, producing new blood. Tian Ma (Rhizoma Gastrodii) has functions of calming the endopathic wind and relieving convulsion, calming the liver and suppressing yang hyperactivity of the liver and subduing the exuberant yang of the liver-yang, expelling the wind and clearing the collaterals. Dan Nan Xing (Arisaema cum Bile), Shen Jin Cao (Herba Lycopodii), and Luo Shi Teng (Caulis Trachelospermii) have functions of calming the endopathic wind and relieving convulsion. Chuan Niu Xi (Radix Cyathulae) and Luo Shi Teng (Caulis Trachelospermii), etc. In the prescription, Dang Gui (Radix Angelicae Sinensis) and E Jiao (Colla Corii Asini) as monarch drugs have functions of promoting blood flow and enriching the blood. Chuan Xiong (Rhizoma Chaunxiong) and Dan Shen (Radix Salviae Miltiorrizae) have functions of activating blood circulation and removing blood stasis, removing stagnation of blood. Dang Gui (Radix Angelicae Sinensis) combined with Gou Qi Zi (Fructus Lycii) can promote blood circulation and functions tonifying the blood and nourishing yin, producing new blood. Tian Ma (Rhizoma Gastrodii) has functions of calming the endopathic wind and relieving convulsion, calming the liver and suppressing yang hyperactivity of the liver and subduing the exuberant yang of the liver-yang, expelling the wind and clearing the collaterals. Dan Nan Xing (Arisaema cum Bile), Shen Jin Cao (Herba Lycopodii), and Luo Shi Teng (Caulis Trachelospermii) have functions of activating blood flow, clearing the collaterals, resolving sputum, calming the endopathic wind and relieving convulsion. Chuan Niu Xi (Radix Cyathulae) makes the blood flow down as a guide drug. Clinical experimental study indicates that the main compose, Dan Shen (Radix Salviae Miltiorrizae), can significantly increase the caliber of microangium and relieve edema of endothelial cells in treatment of cerebral convulsion. \(^9\)

The study shows that compared with the sham-operation group, TNF-α contents in serum and brain tissue in the model group started to increase at 6 h of local cerebral ischemia and reached to high peak at 12 h, indicating release of TNF-α contents in serum and brain tissue at initial stage is normal response of the organism, with no more general stress noxious factor, not inducing injuring action, but if the release reaches to a certain degree, the noxious stress factor will significantly increase, obviously influencing cerebral infarction. \(^10\) Compared with the model group, TNF-α contents in serum and brain tissue in both the Tongmai Huoxue Yin group and

**Table 1.** Effect of Tongmai Huoxue Yin on serum TNF-α content in the rat with focal acute cerebral ischemia (ng/mL, \(\bar{x} \pm s, n=3\))

<table>
<thead>
<tr>
<th>Group</th>
<th>6 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operation</td>
<td>0.113±0.029</td>
<td>0.116±0.032</td>
<td>0.122±0.024</td>
</tr>
<tr>
<td>Model</td>
<td>0.200±0.066</td>
<td>0.217±0.031</td>
<td>0.212±0.031</td>
</tr>
<tr>
<td>Tongmai Huoxue Yin</td>
<td>0.129±0.049</td>
<td>0.150±0.070</td>
<td>0.150±0.060</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>0.135±0.050</td>
<td>0.153±0.065</td>
<td>0.161±0.064</td>
</tr>
</tbody>
</table>

Notes: Compared with the model group, *\(P<0.05\), **\(P<0.01\).

**Table 2.** Effect of Tongmai Huoxue Yin on brain TNF-α contents in the rat with focal acute cerebral ischemia (ng/mL, \(\bar{x} \pm s, n=3\))

<table>
<thead>
<tr>
<th>Group</th>
<th>6 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operation</td>
<td>1.710±0.494</td>
<td>1.700±0.338</td>
<td>1.634±0.449</td>
</tr>
<tr>
<td>Model</td>
<td>2.321±0.436</td>
<td>2.377±0.625</td>
<td>2.272±0.605</td>
</tr>
<tr>
<td>Tongmai Huoxue Yin</td>
<td>1.872±0.036</td>
<td>1.835±0.466</td>
<td>1.700±0.527</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>1.945±0.289</td>
<td>1.858±0.451</td>
<td>1.797±0.423</td>
</tr>
</tbody>
</table>

Notes: Compared with the model group, *\(P<0.05\), **\(P<0.01\).
Nimodipine decreased in varying degrees, particularly the most decreased at 24 h, with no significant difference between the Tongmai Huoxue Yin group and the Nimodipine group.

In brief, Tongmai Huoxue Yin can play protective action on acute cerebral ischemia model rat through decreasing TNF-α contents in serum and brain tissue, with a same effect as Nimodipine.

REFERENCES


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