Effect of angong niuhuang wan on serum lactate dehydrogenase isoenzymes in rats with cerebral infarction

Tang Yi-shan, Pan Xin-hua, Wang Pei-xun, Wang Ning-sheng

Tang Yi-shan, Pan Xin-hua, Wang Pei-xun, Wang Ning-sheng, Institute of Clinical Pharmacology, Guangzhou University of Traditional Chinese Medicine, Guangzhou 510405, Guangdong Province, China

Correspondence to: Tang Yi-shan*, Master, Associate investigator, Lecturer, Institute of Clinical Pharmacology, Guangzhou University of Traditional Chinese Medicine, Guangzhou 510405, Guangdong Province, China tangyishan@tom.com

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Abstract

BACKGROUND: Angong niuhuang wan consists of mercury and arsenic, which has drawn the attention on its safety. It is necessary to carry on the research on evaluation in its availability and safety.

OBJECTIVE: To study the mechanic differences of angong niuhuang wan in the organic body physiologically and pathologically.

DESIGN: Randomized controlled experiment based on the experimental animals.

SETTING: Institute of clinical pharmacology affiliated to a university.

MATERIALS: The experiment was performed in Institute of Clinical Pharmacology affiliated to Guangzhou University of Traditional Chinese Medicine from March to April in 2001. Guangdong Medical Experimental Animal Center provided 24 SD male rats, weighted varied from 250 g to 300 g.

METHODS: SD rats were randomized into 4 groups, named as normal group, normal & angong niuhuang wan group(normal & wan group), model of cerebral infarction group(model group) (The middle cerebral artery embolism was induced in rats by photochemistry.) and model of cerebral infarction group & angong niuhuang wan group(model & wan group), 6 rats in each group. Medication instruction: gastric feeding was applied once daily, 0.13 g/kg, totally for 7 days.

MAIN OUTCOME MEASURES: contents of lactate dehydrogenase (LDH) isoenzymes, LDH1.

RESULTS: Contents of serum LDH1, in normal & wan group were significantly increased than those in normal group (P < 0.01), of which, the values of LDH1, LDH2, LDH3, LDH4 were (17.02 ± 3.46)%, (14.70 ± 1.18)% , (15.47 ± 0.13)% successively in normal & wan group, were (11.23 ± 2.07)% , (15.25 ± 0.90)% , (12.86 ± 0.90)% successively in normal group. Content of serum LDH1(1.55 ± 2.60)% in model & wan group was significantly increased compared with that in model group (10.93 ± 2.10)% (P < 0.01). The contents of LDH1 were (22.62 ± 3.00)% and (28.18 ± 1.18)% respectively in two groups, indicating significantly reducing (P < 0.01).

CONCLUSION: The organic injury of angong niuhuang wan is less in pathological state of focal cerebral infarction than that in physiological state, suggesting that the mechanic differences of angong niuhuang wan in organic body present in physiological and pathological states.


INTRODUCTION

Angong niuhuang wan( wan) originates from Treatise on Differentiation and Treatment of Epidemic Febrile Diseases written by Wu Ju-tong in Qing dynasty, composed of 11 herbs, named as niuhuang (Calculus Bovis), saxinqiao (Pulvis Cornus Buchuli) concentrated powder, shexiang (Moschus), zhenzhu (Margarita), zhusha (Cinnabaris), xionghuang (Realgar), huangqin (Rhizoma Coptidis), huangqg (Radix Scutellariae), zuizi (Fruuits Gardeniae), yuqin (Radix Curcumae), bingpian (Borneolum) [1], etc. It acts on clearing away heat, detoxification, resolving phlegm and opening orifice, and presents significant therapeutic effects on emergent treatments of severe cerebral injury. Since the main components of the formula are cinnabar(HgS) and realgar(As2S3), the safety of the formula is questioned, which has become the main obstacle in the export of patent Chinese herbal medicine. This experiment was to observe the mechanic differences of angong niuhuang wan in organic body in physiological and pathological states so as to provide evidences for its availability and safety.

MATERIALS AND METHODS

Materials

The experiment was accomplished in Institute of Clinical Pharmacology affiliated to Guangzhou University of Traditional Chinese Medicine from March to April in 2001, and the rat model of cerebral infarction was prepared in Oncomia Institute of Zhongshan University. Animals and group divisions: Totally 24 SD male rats, weighted varied from 250 g to 300 g, were employed, which were healthy and in common grade, provided by Guangdong Medical Experimental Animal Center(Oblicified No. 2001A024) . Four groups were randomized, named as normal group, normal & angong niuhuang wan group(normal & wan group), model of cerebral infarction group (model group) and model of cerebral infarction group & angong niuhuang wan group(model & wan group), 6 rats in each group.

Drugs and reagents: angong niuhuang wan (provided by First Guangdong Foshan Pharmaceutical Factory) 2 Pentobarbital sodium 20 g/L. 3 Disodium-tetrachloride-fluorescein (rose Bengal B) 30 g/L. 4 Sodium carboxymethyl cellulose (CMC-Na) solution 3 g/L. 5 Regents required for the assays of lactate dehydrogenase (LDH) isoenzymes with agarose gel electrophoresis method[2]. Main instruments: There were 360-argon-ion-laser(Nanjing Electron Tube Factory), craniotomies, agarose gel electrophoresis apparatus and UPV GDS7600 gel image-forming system.

Methods

Preparation for model of cerebral infarction in rats: Anesthesia was done with intra-abdominal injection with pentobarbital sodium 40 μg / g( body weight). A slight arch incision was done perpendicularly from the midpoint between the eye and ear on the same side, about 1.5 cm. The temporal muscle was separated till to the conjunction with zygomatic bone and was cut at the posterior 1/2. The squamoparietal protrubance was exposed anterior and superior to the oval foramen. A bony window was made, 5 mm in diameter, by a dental drill at 2 mm anterior end to the conjunction between zygomatic arch and temporal squamotemporal bone. The middle cerebral artery was exposed by opening dura mater of brain. After injected rose Bengal B from femoral vein(25 mg/kg), the photocoductive beam from laser(560 nm in wave, 300 W in power) was radiated on middle cerebral artery for 10 minutes, and suture on the incision was followed[1].

Medication instruction: In normal & wan group and model & wan group, angong niuhuang wan was prescribed for gastric feeding once daily, 0.13 g/kg(mixed evenly with 3 g/K CMC-Na in a proper dosage), totally for 7 days. In normal and model groups, physiological saline of equal dosage was prescribed for gastric infusion.

Index assays: Blood was collected from the orbit after 7 days and the serum was separated[1](be careful to avoid hemolysis). Agarose gel
RESULTS
Quantitative analysis of the experimental animals
Totally 24 rats were included in the experiment, which was randomized into 4 groups, 6 rats in each. All the rats entered the analysis of the results.

Effect of angong niuhuang wan on serum LDH isoenzymes in rats with cerebral infarction (Table 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH 1</th>
<th>LDH 2</th>
<th>LDH 3</th>
<th>LDH 4</th>
<th>LDH 5</th>
<th>LDH 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nomal</td>
<td>11.25 ± 0.70</td>
<td>8.26 ± 0.90</td>
<td>12.86 ± 0.90</td>
<td>30.10 ± 1.20</td>
<td>37.53 ± 2.70</td>
<td>19.20 ± 2.70</td>
</tr>
<tr>
<td>Model</td>
<td>13.30 ± 1.20</td>
<td>10.10 ± 1.70</td>
<td>13 ± 2.10</td>
<td>70.10 ± 9.20</td>
<td>10 ± 2.80</td>
<td>18 ± 2.80</td>
</tr>
<tr>
<td>Nomal &amp; Model</td>
<td>17.02 ± 0.46</td>
<td>14.70 ± 0.81</td>
<td>18 ± 15.47</td>
<td>3.13 ± 22.97</td>
<td>56.8 ± 24.94</td>
<td>0.41 ± 0.33</td>
</tr>
</tbody>
</table>

DISCUSSION
LDH is one of the key enzymes of glycolysis and distributed in obvious histological speciality, LDH, and LDH is mostly distributed in cardiac muscle, kidney and erythrocytes, LDH mostly in spleen, pancreas, thyroid gland, adrenal gland and lymph node and LDH, and mostly in skeleton muscles and liver. When normal disorders happen, isoenzymes released in blood are in various percentages, therefore, the changes of serum LDH isoenzyme spectrum can reflect the location of sickness and severity of injury sensitively and precisely. For instance, LDH, and LDH are increased significantly in cardiac embolism, viral or rachitic myocarditis and Keshan disease; the general activity of LDH and LDH increased remarkably during injury of hepatic cells and the activity of LDH increased during kidney damage.

In the experiment, serum LDH level in normal & angong niuhuang wan group was increased significantly compared with the control \( P < 0.01 \), indicating that long-term taking angong niuhuang wan in normal physiological state can induce a certain of injury to cardiac muscle, kidney, erythrocytes and spleen. Only serum LDH was increased \( P < 0.01 \) and LDH decreased significantly \( P < 0.01 \) in model & angong niuhuang wan group compared with model group, suggesting that angong niuhuang wan causes less damage in the pathological state of focal cerebral infarction than that in normal physiological state. It is to infer that Cinnabar and Realgar in angong niuhuang wan, manifest rather lower toxicity in proper pathological state, by probably coordination compound formed with some endogenous components. Wang et al.\(^2\) synthesizes in imitation arsenic-cysteine coordination compound, LDG, 650 mg/kg, that is lower than AsG. LDG, 150 mg/kg, explaining the antagonism of cysteine to arsenic toxicity. In recent years, the researches of the biological effects on arsenic have deepened to molecule and gene and have discovered that micro-arsenic provides some positive reactions, its toxic activity intensity is not only related to its dosage and existed state, but also to antagonism or coordination with other substances and physiological and pathological states in individual.

To conclude, the injury of angong niuhuang wan in the pathological state of focal cerebral infarction is less than that in normal physiological state, suggesting the different effects of angong niuhuang wan on the organic body in physiological and pathological states.

REFERENCES