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Original article

Effect of different component ratio of Astragalus total saponins and Verbena total glycosides on the cerebral infarction area and serum biochemical indicators in the focal cerebral ischemia-reperfusion rat model

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ABSTRACT

Our purpose is to study the effect of different component ratio of Astragalus Total Saponins (ATS) and Verbena Total Glycosides (VTG) on the cerebral infarction area and the serum biochemical indicators in the focal cerebral ischemia-reperfusion rat model. Compared with the model group, different component ratio of ATS and VTG could significantly improve the neurological deficit scores to the focal cerebral ischemia-reperfusion rat model, and the group of 7:3, 6:4, 5:5 got the best results; it could reduce the mortality of rat model to a certain extent, and the group of 5:5 group got the best results; it can significantly reduce the cerebral infarction area, and the group of 7:3, 5:5, 4:6 got the best results; it could significantly reduce the content of TNF-α, and the group of 8:2, 6:4 got the best results; it could significantly reduce the content of NO, and the group of 7:3, 5:5 got the best results; it could significantly increase the content of SOD, and the group of 6:4, 5:5 got the best results. This indicates that different component ratio of ATS and VTG may protect the damage of focal cerebral ischemia-reperfusion rat model to a certain extent, which are compared using the comprehensive weight method and the ratio of 5:5 was proved to be the optimal active ratio.

1. Introduction

Cerebral Ischemia Reperfusion Injury (CIRI) refers to that when blood supply is restored for a certain period of time after cerebral ischemia, its function fails to restore, or even shows more serious brain dysfunction. In modern medical research, the pathogenesis of cerebral ischemia includes energy metabolism disorders, platelet aggregation, vasoconstriction and microcirculation disorders, excitatory amino acid toxicity and so on, and the drugs clinically used for the preventing and treating cerebral ischemia are mainly antiplatelet drugs aspirin, ticlopidine and other drugs. Most cerebral ischemia patients need a long time of medication, but the side effect from long-term medication, drug prices, limitations of treatment course will affect the treatment of the disease (Atta et al., 2017; Halim et al., 2017). Chinese medicine believes that the occurrence of ischemic stroke relates with the Qi stagnation, Qi deficiency, phlegm turbidity, blood stasis, Yin deficiency, heat or Fire and other factors, so, clinically, some therapeutic principles like "tonifying Qi and reinforcing deficiency", "activating blood and resolving stasis", "clearing heat and expelling toxin" and others are adopted to prevent cerebral ischemic diseases. The components compatibility of Chinese medicine has precise clinical application, is safe and effective, and owns controllable quality. QiCao capsule is an effective preparation for clinical treatment of cerebral ischemia, with very good clinical basis. This research used the increase-decrease design of baseline geometric proportion to study the intervene effect of different component ratios of ATS and VTG, the main ingredients in QiCao capsule, on the cerebral infarction area and the serum biochemical indicators in the cerebral ischemia-reperfusion rat model (Miao and Miao, 2012; Miao et al., 2011).

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2. Material and methods

2.1. Experimental animals

SD rat, male, grade SPF, Weight 180–220 g, supplied by Hebei Experimental Animal Center, Animal permit number: 1210020.

2.2. Experimental drugs and reagents

Astragalus Total Saponins, supplied by department of chemistry, Henan University of TCM, content > 50%, batch No.: 111008; Verbena total glycosides, supplied by department of chemistry, Henan University of TCM, content = 50%, batch No.: SWT20120801; Naoluotong capsule, produced by Guangdong Bangmin Pharmaceutical Co., Ltd., batch No.: 120109; Nimodiping tablets, produced by Shandong Xinhua Pharmaceutical Co., Ltd., batch No.: 1107044; Formaldehyde, produced by Tianjin Kermel Chemical Reagent Co., Ltd., batch No.: 20110701; Triphenyl tetrazolium chloride (TTC), produced by Shanghai Shan Pu Chemical Company, batch No.: 20110401; TNF-αkit, produced by R & D company, batch No.: 201211; SOD kit, produced by Nanjing Jiancheng Institute of Biotechnology, batch No.: 20121123; Nitric oxide (NO), produced by Nanjing Jiancheng Institute of Biotechnology, batch No.: 20121117.

2.3. Experimental instrument

UV-2000 UV–Vis spectrophotometer, UNICO (Shanghai) Instrument Co., Ltd.; TGL-16G high-speed refrigerated centrifuge, supplied by Shanghai Anting Scientific Instrument Factory; FA (N)/JA (N) series of electronic balance, Shanghai Minqiao Precision Instrument Co., Ltd; Bio-Rad-680 microplate reader, American Microplate Reader company; Adjustable pipettes, Shanghai Leibo Analysis Instrument Co. Ltd.

2.4. Experimental methods

SPF-grade SD healthy rats, male, weight 200–220 g, were taken and randomly and uniformity divided into 13 groups, and every group was 15 rats, including sham operation group (SG), model group (MG), Naoluotong capsule group (NLT), nimodiping group (NMP), and ATS and VTG groups with different component ratios of 10:0, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 0:10. The drug disposition method: the dose of the different component ratios of ATS and VTG was all 200 mg/kg. In details, the dose of 10:0 group was (ATS 200 mg and VTG 0 mg)/kg, 8:2 group (ATS 160 mg and VTG 40 mg)/kg, 7:3 group (ATS 140 mg and VTG 60 mg)/kg, 6:4 group (ATS 120 mg and VTG 80 mg)/kg, 5:5 group (ATS 100 mg and VTG 100 mg)/kg, 4:6 group (ATS 80 mg and VTG 120 mg)/kg, 3:7 group (ATS 60 mg and VTG 140 mg)/kg, 2:8 group (ATS 20 mg and VTG 160 mg)/kg, and 0:10 group (ATS 0 mg and VTG 200 mg)/kg. Besides, the dose of Naoluotong capsule group was 500 mg/kg (equivalent to 10 times of clinical dose), and Nimodiping group 20 mg/kg (equivalent to 10 times of clinical dose). Each group of drugs were formulated with distilled water. Each treatment group was fed with corresponding drugs, and the Sham operation group and the model group were given equal volume of distilled water, in which the filling volume was 0.2 ml/10 g once a day and lasted for 7 days.

One hour after the last administration (Fasting 12 h), it's the time to build the MCAO model of each model group.

The rats were anesthetized by intraperitoneal injection with 10% chloral hydrate (0.3 ml/100 g), supine fixed, cut in mid-line and slant left of neck, layer-by-layer separated and exposed the left common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA), ligatured CCA and ECA. After using arterial clamp to clip the telecentric end of ICA, made an incision at the bifurcation of ECA and ICA and inserted smooth nylon thread with the tip coated with silicone rubber (the diameters of line body and head were 0.28 mm and 0.38 ± 0.02 mm respectively, with a mark at 20 mm from the line head) at a depth of about 20 mm, which can achieve middle cerebral artery occlusion and therefore lead to cerebral ischemia. Suture the skin at the ligation entrance with Nylon line left 1–2 cm at outside. After 2 h, gently pulled the line head that left at outside until facing a little resistance, to achieve reperfusion. During the process of brain ischemia and reperfusion, the room temperature should be kept at 23–25 °C. (For sham operation group, it only required to expose the left side of the blood vessel without inserting nylon thread). It can be deemed as a successful model when the ipsilateral Horner syndrome and that the contralaterally upper extremity was more paralyzed than lower limbs appear after the rats awake. Initially 195 rats were included in experiment, 77 rats died after the operation, and eventually 118 rats entered the final analysis.

After anesthesia awake, the rats received the neural function deficit scoring (NS) (Neuroethology criteria used the Zealonga scoring method. Score 0: No symptoms of neurological deficits, normal activities; Score 1: those who cannot fully extend the contralateral forepaw; Score 2: a circular motion to the right when crawling; Score 3: when walking, the body dumped by the side of hemiplegia; Score 4: not spontaneously walk, the loss of consciousness; Score 5: Death.). 24 h after surgery, the blood was taken to measure the contents of TNF-α, SOD and NO. Decapitated and rapidly stripped brain tissue, and cut the rest coronal region after removing the olfactory bulb, cerebellum and low brain steminto five slices. In TTC staining, after staining, the non-ischemic region was rosy red and the infarct region was white (Ishaq and Jafri, 2017). Took pictures by digital camera, and used the NIS-Elements AR 4.10.01 to calculate the areas of the total five brain slices (including 10 planes) and the infarction region, as well as the percentage of infarction region in total area.

2.5. Statistical analysis

Data analysis was made using SPSS 17.0 for windows. The measurement data was expressed by mean ± SD. ANOVA was adopted in analysis and comparison between the groups. Those with equal variances shall be tested by the smallest used least significant difference (LSD) method, while those with heterogeneous variance

Table 1
Effect of the neurological deficit scores and the mortality ($\bar{x} \pm s$).

Group	n	Dose (g kg^{-1})	NS	Mortality (%)
SG	15	–	0.0 ± 0.0**	0.0
MG	8	–	3.1 ± 0.4	46.7
NMP	9	0.02	1.8 ± 0.8**	40.0
NLT	9	0.5	1.9 ± 0.6*	40.0
10:0	8	ATS:VTG = 0.2:0	1.9 ± 0.6*	46.7
8:2	8	ATS:VTG = 0.16:0.04	1.9 ± 0.4*	46.7
7:3	9	ATS: VTG = 0.14:0.06	1.4 ± 0.5**	40.0
6:4	9	ATS: VTG = 0.12:0.08	1.7 ± 0.5**	40.0
5:5	10	ATS: VTG = 0.1:0.1	1.5 ± 0.5**	33.3
4:6	9	ATS: VTG = 0.08:0.12	2.0 ± 0.7*	40.0
3:7	8	ATS: VTG = 0.06:0.14	2.0 ± 0.7*	46.7
2:8	8	ATS: VTG = 0.04:0.16	1.9 ± 0.6*	46.7
0:10	8	ATS: VTG = 0:0.2	2.0 ± 0.7*	46.7

Note: Compared to the model group.
* $P < 0.05$.
** $P < 0.01$.