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Effect of Liangxuehuayu Recipe on hemorheology in rats with blood stasis syndrome

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ABSTRACT

Objective: To investigate the effects of Liangxuehuayu Recipe on hemorheology in rats with blood stasis syndrome induced by mutifactor stimuli. **Methods:** SD rats were divided into control, model, Liangxuehuayu Recipe (high, middle and low dose, 18, 9, 4.5 g/kg accordingly). Except the control group, blood stasis model was established in the rest groups. The hemorheological parameters were measured and compared. **Results:** Blood viscosity at high, moderate and low level in rats with blood stasis significantly increased ($P<0.05$), but blood viscosity at high level and plasma viscosity was significantly decreased in rats induced by some stimuli after Liangxuehuayu Recipe were intra-gastrically administered for 1 weeks ($P<0.01$, $P<0.05$). **Conclusions:** Liangxuehuayu Recipe is effective in improving hemorheology, and has important application value in the prevention of occurrence and development of ischemic stroke.

1. Introduction

Ischemic stroke, also called ischemic cerelral infarction, belongs to the category of "stroke" in traditional Chinese medicine. It is a kind of common ailment and frequently encountered disease with the characteristic of high mortality and disability rate[1-3]. Ischemic stroke has become one of the most detrimental diseases. Traditional Chinese medicine has showed unique academic advantage in the prevention and cure of stroke.

Zhong-Ying Zhou, a expert in Chinese medicine, put forward the etiology and pathogenesis theory of "stagnant heat" on the basis of the ancients' diagnosis and treatment theory and decades of clinical practice experience. Professor Zhou considers that ischemic stroke is similar to many troublesome diseases of internal Chinese medicine. In the process of ischemic stroke, the blood heat and blood stasis exist simultaneously. The effect will be poor if the therapy of removing heat to cool blood or promoting blood to remove stasis is used alone. Over the years, Zhou *et al* has used the therapy of cooling blood to remove stasis with the theory of "stasis hot", and has achieved remarkable curative effect[4,5]. Liangxuehuayu Recipe is the empirical recipe for ischemic stroke formulated by Zhou on the basis of the characteristic of "stasis hot" in ischemic stroke. In this study, we observed the effects of Liangxuehuayu Recipe on hemorheology in rats with blood stasis syndrome, and provided experimental basis for clinical application of this formula.

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2. Materials and methods

2.1. Drugs and reagent

Liangxuehuayu Recipe, which was processed by the center of plant medicine research and development of new medicine, is brown liquid with the batch number of 06011. Mailuoning injection, Nanjing Jinling pharmaceutical factory, Jinling pharmaceutical Co., Ltd, the batch number: 20060106. Activated partial thromboplastin time (APTT) reagent kit, Beijing Shidi Scientific Instruments Company, the batch number: ST20202-23. Fibrinogen (FIB) reagent kit, Beijing Shidi Scientific Instruments Company, the batch number: ST20401-21. Prothrombin time (PT) reagent kit, Beijing Shidi Scientific Instruments Company, the batch number: ST20102-29. Thrombin time (TT) reagent kit, Beijing Shidi Scientific Instruments Company, the batch number: ST20302-22. NOS ELISA reagent kit, ADL Company of the United States, the batch number: 08-10. Adenosine diphosphate glucose pyrophospheralase (ADP), Sigma Company, the batch number: A-2754. Chloralic hydras, A.R, the Chinese Medicine Group Chemical Reagent Co., Ltd, the batch number: 20050525. 0.9% NaCl injection, Nanjing Xiaoying pharmaceutical Co., Ltd, he batch number: 2005102904.

2.2. Experimental animals

Male SD rats in a SPF grade with the weight of 300-400 g which supplied by Shanghai Silaike Experimental Animal Liability Co., Ltd. were selected. The certificate number is SCXK(Shanghai)-2003-0003.

2.3. Instruments

Electrothermal constant-temperature dry box of DHG-9053 A type, Shanghai Medical Thermostatic Equipment Factory. Blood viscosity meter of LG-R-80 series, Beijing Shidi Scientific Instruments Company.

2.4. Experimental methods

Male SD rats with qualified temperature were divided into 6 groups randomly, control group, model group, positive medicine group (Fufangdanshen tablets, 1.08 g/kg), high, middle, low dose group (Liangxuehuayu Recipe, 18 g/kg, 9 g/kg, 4.5 g/kg, dry, the same below). The 6 groups received continuous dosing for 6 days, Until 1 h after final dose, except for the control group, the acute blood stasis models in the other groups were established according to the references[3]: 0.1% adrenergic 0.8 mL/kg, subcutaneous injection, after 2 h, the rats received cold stimulation in ice water for 5 min, and then an injection of equal amount of adrenergic was carried out. 10% chloralic hydras (300 mg/kg, *i.p.*) was used for fixing rats in the overhead position 1 h after dosing at the second day. The blood collection was carried out by carotid artery intubation, and the anticoagulation was carried out at the ratio of 1:9 with 3.8% sodium citrate. The whole blood viscosity, plasma viscosity, blood coagulation time were detected by automatic hemarheology meter.

2.5. Statistical processing

All the data were analyzed by SPSS 17.0. The measurement data were expressed as mean ± standard deviation, and one-way analysis of variance (ANOVA) was used. $P<0.05$ was defined as having statistical difference.

3. Results

As Table 1, 2 and 3 show, high dose of Liangxuehuayu Recipe can obviously reduce the platelet aggregation rate, prolong PT and reduce the content of FIB, it can also enhance the defomation ability of red blood cell, weaken the aggregation ability of red blood cell. In middle and low shear rates, the high dose can reduce the whole blood viscosity and plasma viscosity, The difference reached statistical significance compared with the model group ($P<0.05$, $P<0.01$). The middle dose of Liangxuehuayu Recipe can enhance the defomation ability of red blood cell, weaken the

Table 1

Influence of Liangxuehuayu Recipe on platelet aggregation rate(n=10, $\bar{x}$±SD).

Group	Dose (g/kg)	Platelet aggregation rate (%)	Aggregation inhibition rate(%)
Control group	-	26.67±7.41*	-
Model group	-	34.02±6.68	-
Fufangdanshen group	8	28.06±5.98*	17.52
Low dose group	4.5	33.28±8.53	2.18
Middle dose group	9	28.58±14.68	16.00
High dose group	18	28.12±5.83*	17.34

*$P<0.05$ *vs*. control group.

Table 2
Influence of Liangxuehuayu Recipe on defomation and aggregation ability of red blood cell (n=10, $\bar{x}$±SD).

Group	Dose (g/kg)	Defomation ability		Aggregation ability	
		MAXDI	SSS	MAXD	SS
Control group	–	0.50±0.03*	250.1±15.7*	0.80±0.37*	142.7±39.9**
Model group	–	0.46±0.04	230.0±19.2	1.33±0.50	226.1±72.3
Fufangdanshen group	8	0.51±0.04*	251.3±18.0*	0.82±0.37*	158.9±49.7*
Low dose group	4.5	0.50±0.05	248.4±14.6*	1.13±0.59	185.8±54.5
Middle dose group	9	0.52±0.02**	258.6±14.9**	0.93±0.25*	181.3±39.0
High dose group	18	0.53±0.07*	260.5±28.8*	0.82±0.28*	164.8±43.1*

*P<0.05, **P<0.01 *vs*. control group.

Table 3
Influence of Liangxuehuayu Recipe on blood viscosity (n=10, $\bar{x}$±SD).

Group	Dose (g/kg)	Whole blood viscosity				Plasma viscosity (/100s)
		High shear rates (/200s)	Middle shear rates (/30s)	Middle shear rates (/5s)	Low shear rates (/1s)	
Control group	–	5.60±1.16	7.24±1.79*	12.9±3.6*	28.1±9.9*	1.44±0.28*
Model group	–	6.68±1.20	9.19±1.63	16.6±3.7	37.7±10.1	2.09±0.70
Fufangdanshen group	8	5.62±0.92*	7.25±1.50*	12.9±3.5*	27.1±9.6*	1.51±0.41*
Low dose group	4.5	6.46±1.65	8.40±2.38	15.7±3.9	39.1±9.3	1.86±0.51
Middle dose group	9	6.35±0.94	8.12±1.40	14.8±5.0	33.0±11.9	1.82±0.64
High dose group	18	5.95±0.96	7.41±1.56*	12.3±3.0*	27.6±7.5*	1.57±0.26*

*P<0.05, **P<0.01 *vs*. control group.

aggregation ability of red blood cell. The difference reached statistical significance compared with the model group (P<0.05, P<0.01).

4. Discussion

Blood stasis syndrome refers to the patho-physiological state of blood stagnation, delayed blood flow, blockade in blood vessel, and is one of the most common clinical syndromes. Professor Zhou indicated that stasis includes blood stasis and static blood in the state of "stagnant heat". Blood stasis refers to the delayed and impeded blood flow, local stagnation, while the static blood refers to a kind of pathological product, both can be reciprocal causation. Previous study[4,5] revealed that the "stasis" in traditional Chinese medicine was closely related to the dysfunction of blood system. The dysfunction of blood system was also the basis of pathology and pathological performance of ischemic stroke. The patients with ischemic stroke usually showed the characteristic of "blood stasis" of pre-thrombosis or thrombosis state. Therefore, improvement of abnormal hemarheology, is the common therapeutic principle for traditional Chinese medicine and western medicine in the treatment of ischemia stroke.

Liangxuehuayu Recipe consists of cortex moutan, radix raeoniae rubra, acorus gramineus, cornu bubali and rhubarb. It's a basic recipe for the treatment of ischemia stroke according to the theory of "stagnant heat". The cortex moutan and radix raeoniae rubra have the effect of cooling, activating blood and scattering stasis[6,7]. Total paeony glycoside, the key ingredient of radix raeoniae rubra, can prolong the cogulation time, inhibit the formation of thrombus. Paeonol, the key ingredient of cortex moutan, has been proved to be the calcium channel blocker, and has the spasmolysis for vascular smooth muscle and the effect of anti-free radical, analgesia, sedation and anti-convulsion[8]. Therefore, it has important significance in the prevention and control of ischemic brain damage. Cornu bubali and rhubarb have the effect of removing heat to cool blood, with the complementary of the two drugs, the function of cooling blood to remove stasis will be more effective[9-11]. Chinese scholars have carried out a series of studies on the therapy of traditional Chinese medicine on hemorheology in rats with blood stasis syndrome[12-16]. Zhao *et al*[17] explored the effect of the effective components group of Xiaoshuantongluo formula (XECG) on acute blood stasis rat model, the results showed that XECG significantly reduced ADP-induced platelet aggregation, but little influence on the whole blood viscosity, plasma viscosity and erythrocyte aggregation rate. XECG extended PT and TT slightly, but had no effects on APTT and FIB content. The results suggest that the role of XECG of anti-aggregation is more prominent. Deng *et al*[18] established a rat model of blood stasis and heat accumulation syndrome in accordance with the traditional Chinese medicine theoretic features. The results showed that compared with the model group, the length of ecchymosis in the tail obviously decreased in rats of the danshen root group, the blood velocity score of the microcirculation obviously increased, the whole blood viscosity, hematocrit,

and erythrocyte electrophoretic time obviously decreased, red blood cell deformation index obviously increased.

The results of this study shows that after the intervention of Liangxuehuayu Recipe, the abnormal hemorheology which caused by compound factors improved obviously: the obvious reduction of whole blood viscosity and plasma viscosity in low and middle shear rates, and can also enhance the defomation ability of red blood cell, weaken the aggregation ability of red blood cell, and improve coagulation function.

In conclusion, Liangxuehuayu Recipe can play the role of treatment according to the main pathological characteristics of blood stasis, and its effect of improving hemorheology has important application value in the prevention of occurrence and development of ischemic stroke.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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