

Effects of the coadministration of Huatuo Zaizao pills and aspirin on hemorrheology and blood coagulation in rabbits

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Abstract. Huatuo Zaizao pills (HT) is a compound used in Chinese medicine for the treatment of cerebrovascular diseases. The present study aimed to investigate the effects of the coadministration of HT and aspirin on hemorrheology and blood coagulation in rabbits. Rabbits were randomly divided into the control, HT, aspirin and HT plus aspirin groups (n=5 animals per group). The rabbits were treated with HT at a dose of 1 g/kg, aspirin at a dose of 5 mg/kg or HT (1 g/kg) plus aspirin (5 mg/kg) administered by gavage. Blood samples were collected prior to treatment and at 2 or 4 h after treatment. Compared to the control group, there were no significant changes in whole blood viscosity (WBV), plasma viscosity (PV), activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT) and fibrinogen content (FIB) in the HT or the aspirin group. Coadministration of HT and aspirin prolonged APTT, PT and TT and decreased WBV and PV. The findings indicated that coadministration of HT and aspirin exerted a more potent effect on hemorrheology and blood coagulation, compared to each agent administered alone, and may be a novel approach to the prevention and treatment of hemorrheological disorders.

Introduction

Stroke remains a major healthcare problem, with a significant human and economic toll. According to the World Health Organization, 15 million people suffer from stroke worldwide each year. Of these, 5 million succumb to the stroke and a further 5 million become permanently disabled (1). Primary and secondary prevention plays a significant role in reducing the burden of stroke. Aspirin remains the cornerstone of

primary and secondary stroke prevention (2) and is increasingly being used in the prevention and treatment of ischemic stroke. However, it may also be accompanied with severe gastrointestinal complications, including bleeding, perforation and ulceration. Whereas a lower dose of aspirin may reduce its risks, it may also be accompanied by lower effectiveness. Furthermore, an increasing number of reports on aspirin resistance has led to a growing concern among clinicians and patients, regarding the efficacy of aspirin treatment. Based on a recent meta-analysis *in vitro* or laboratory studies, aspirin resistance is a factor independent of future vascular events (3). It was reported that aspirin fails to inhibit platelet aggregation in 5-55% of individuals and this is an important cause of clinical aspirin resistance or failure (4,5).

Hemorrheological disorders play an important role in the pathogenesis and development of several diseases, such as ischemic stroke, coronary artery disease and diabetic complications. It was reported that promoting blood circulation to relieve blood stasis may improve hemorrheological events. Traditional Chinese medicine (TCM), which is mostly based on formulations, has attracted increasing attention as a complementary therapeutic method to western medicine (6).

Huatuo Zaizao pills (HT), a pure natural preparation derived from plants, is a TCM formulation marketed in China. In 1985, it was formally listed in the key scientific and technical projects during the sixth Five-Year Plan by the National Commission for Science and Technology. Over the past few years, studies that are only partly published in the Chinese medical literature, on the pharmacognosy, pharmaceutics and pharmacology of HT, have been conducted by 14 medical research institutions. HT is widely used in the treatment and prevention of cerebrovascular diseases, apoplexy and hemiplegia. The aim of the present study was to evaluate the effects of the coadministration of HT and aspirin on hemorrheology and blood coagulation in rabbits.

Materials and methods

Animals. New Zealand rabbits, weighing 2.5-3.0 kg (provided by the Experimental Animal Center of Shandong Engineering Research Center for Natural Drugs), were housed in conventional cages with free access to food and water, at a temperature of 22.0±2.0°C and a relative humidity of 50±5%, with a 12-h light-dark cycle. Prior to the experiment, the rabbits were

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Table I. Effects of the coadministration of Huatuo Zaizao pills (HT) and aspirin on whole blood viscosity (WBV) at low shear rate.

Groups	WBV (mPa.s, 10 sec ⁻¹)		
	0 h	2 h	4 h
Control	7.24±1.04	7.49±0.94	7.51±0.71
HT	7.45±0.66	7.31±0.87	6.75±0.62
Aspirin	7.01±0.91	7.07±0.60	6.75±0.52
HT + aspirin	7.19±0.78	7.32±0.92	5.90±0.59 ^{a,b}

Data are expressed as the means ± SEM (n=5). One-way ANOVA was used to analyze the significant differences among the different groups. The comparison between two groups was performed with the Student's unpaired t-test. Compared to control, ^aP<0.05. Compared to prior to treatment, ^bP<0.05.

Table II. Effects of the coadministration of Huatuo Zaizao pills (HT) and aspirin on whole blood viscosity (WBV) at high shear rate.

Groups	WBV (mPa.s, 150 sec ⁻¹)		
	0 h	2 h	4 h
Control	3.61±0.82	3.70±0.56	3.60±0.47
HT	3.61±0.33	3.75±0.47	3.41±0.71
Aspirin	3.51±0.29	3.47±0.10	3.50±0.18
HT + aspirin	3.53±0.26	2.92±0.30 ^{a,b}	2.99±0.34 ^{a,b}

Data are expressed as the means ± SEM (n=5). One-way ANOVA was used to analyze the significant differences among the different groups. The comparison between two groups was performed with the Student's unpaired t-test. Compared to control, ^aP<0.05. Compared to prior to treatment, ^bP<0.05.

allowed to acclimatize for 1 week. All animal experiments were conducted with the approval of the Yantai University Committee for the Care and Use of Laboratory Animals.

Drugs and chemicals. HT were purchased from Guangzhou Qixing Pharmaceutical Co., Ltd. (Guangzhou, China). Aspirin was obtained from Bayer HealthCare Manufacturing S.r.l. (Beijing, China). Urethane was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Experimental design. Twenty New Zealand rabbits were randomly assigned to 4 groups (n=5 rabbits per group): control group rabbits were treated with distilled water (1 ml/kg) by gavage; HT group rabbits were treated with HT at a dose of 1 g/kg by gavage; aspirin group rabbits were treated with aspirin at a dose of 5 mg/kg by gavage; HT plus aspirin group rabbits were treated with HT at a dose of 1 g/kg, plus aspirin at a dose of 5 mg/kg by gavage. Blood samples were collected from the carotid aortas prior to treatment and at 2 or 4 h after treatment. Blood was collected into plastic tubes with 3.8% sodium citrate (citrate:blood=1:9, v/v) for the evaluation of whole blood viscosity (WBV). Plasma was then separated from blood by centrifugation at 3,000 x g for 10 min for the detection of plasma viscosity (PV) and plasma anticoagulation.

Viscosity determination. A total of 800 µl blood or plasma was used to determine the viscosity with a blood viscometer

(LBY-N6COMPACT; Beijing Precil Instrument Co., Ltd, Beijing, China) at 37°C. WBV was measured at the shear rates of 10 and 150 sec⁻¹. PV was measured at the shear rate of 150 sec⁻¹.

Plasma anticoagulation assay. Activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT) and fibrinogen content (FIB) were assessed with commercial kits following the manufacturer's instructions with a coagulometer (NC-050; Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China). APTT was determined by incubating 50 µl of plasma with 50 µl of APTT-activating agent for 3 min at 37°C, followed by the addition of 50 µl CaCl₂. PT was determined by incubating 50 µl of plasma solution for 3 min at 37°C, followed by the addition of 100 µl of thromboplastin agent. TT was determined by incubating 50 µl of plasma solution for 3 min at 37°C, followed by the addition of 100 µl of thrombin agent. FIB was determined by incubating 10 µl of plasma with 90 µl of imidazole buffer for 3 min at 37°C, followed by the addition of 50 µl of FIB agent.

Data analysis. Data were analyzed using SPSS 17.0 statistical software and are expressed as the means ± SEM. One-way ANOVA was used to analyze the significant differences among the different groups. Comparisons between two groups were performed with the Student's unpaired t-test. P<0.05 was considered to indicate a statistically significant difference between groups.

Table III. Effects of the coadministration of Huatuo Zaizao pills (HT) and aspirin on plasma viscosity (PV).

Groups	PV (mPa.s, 150 sec ⁻¹)		
	0 h	2 h	4 h
Control	1.35±0.23	1.51±0.39	1.46±0.22
HT	1.28±0.08	1.46±0.21	1.36±0.16
Aspirin	1.40±0.28	1.36±0.19	1.32±0.22
HT + aspirin	1.31±0.25	1.11±0.05	0.91±0.16 ^{a,b}

Data are expressed as the means ± SEM (n=5). One-way ANOVA was used to analyze the significant differences among the different groups. The comparison between two groups was performed with the Student's unpaired t-test. Compared to control, ^aP<0.05. Compared to prior to treatment, ^bP<0.05.

Table IV. Effects of the coadministration of Huatuo Zaizao pills (HT) and aspirin on activated partial thromboplastin time (APTT).

Groups	APTT (sec)		
	0 h	2 h	4 h
Control	15.00±2.33	13.74±2.56	14.24±2.38
HT	14.24±2.64	13.20±2.49	14.58±2.84
Aspirin	14.86±1.83	13.24±2.19	12.82±2.40
HT + aspirin	14.22±1.38	14.54±2.06	18.26±2.45 ^{a,b}

Data are expressed as the means ± SEM (n=5). One-way ANOVA was used to analyze the significant differences among the different groups. The comparison between two groups was performed with the Student's unpaired t-test. Compared to control, ^aP<0.05. Compared to prior to treatment, ^bP<0.05.

Results

Effects of the coadministration of HT and aspirin on WBV.

The effects of the coadministration of HT and aspirin on WBV are presented in Tables I and II. Compared to the control group, treatment with HT or aspirin alone exerted no effects on WBV. At 4 h following coadministration of HT and aspirin, the WBV of the HT plus aspirin group was significantly decreased at low shear rate (P<0.05). At 2 and 4 h following coadministration of HT and aspirin, the WBV of the HT plus aspirin group was significantly decreased at high shear rate (P<0.05).

Effects of the coadministration of HT and aspirin on PV.

At 2 and 4 h following treatment with HT or aspirin alone, there were no significant differences in PV in the HT or the aspirin group, compared to that in the control group (P>0.05). Compared to the control group or prior to treatment, coadministration of HT and aspirin significantly reduced PV at the shear rate of 150 sec⁻¹ (P<0.05, Table III).

Effects of the coadministration of HT and aspirin on plasma coagulation parameters.

As shown in Tables IV-VII, at 4 h following the coadministration of HT and aspirin, APTT, PT and TT were significantly increased when compared to those in the control group (P<0.05). Compared to the control group, treatment with HT or aspirin alone exerted no effects on

plasma coagulation parameters (P>0.05). Furthermore, there were no significant differences in FIB content among the 4 groups.

Discussion

Hemorheology studies blood flow in relation to pressure, flow volume and resistance in blood vessels, as well as WBV, PV and the disorders of blood coagulation parameters (APTT, PT, TT and FIB level). Several prospective trials confirmed that the increased WBV is a major risk factor for ischemic stroke and heart disease (7,8) and the rheologic changes may affect the clinical outcome of patients with cerebral or coronary arterial diseases (9,10). Recent data also demonstrated that >80% of strokes are ischemic and the main factors underlying the pathomechanism of brain ischemia are arterial obstruction and microcirculatory stasis. Previous studies reported a significantly elevated PV, erythrocyte aggregation and impaired red blood cell deformability in ischemic stroke patients (11). Data obtained from several studies confirmed that all ischemic strokes are associated with significant rheological abnormalities, independent of the subtype (12,13). It is possible that in patients with acute ischemic stroke, increased WBV leads to a further reduction of cerebral vascular flow in regions of already compromised flow (14). Therefore, hemorrheological parameters may be important in the development of brain ischemia.

Table V. Effects of the coadministration of Huatuo Zaizao pills (HT) and aspirin on prothrombin time (PT).

Groups	PT (sec)		
	0 h	2 h	4 h
Control	7.40±0.50	7.12±1.00	7.18±0.44
HT	7.70±1.23	7.26±1.33	7.82±1.43
Aspirin	7.46±1.35	7.28±1.37	7.72±1.45
HT + aspirin	7.68±1.05	8.06±1.77	9.66±1.55 ^{a,b}

Data are expressed as the means ± SEM (n=5). One-way ANOVA was used to analyze the significant differences among the different groups. The comparison between two groups was performed with the Student's unpaired t-test. Compared to control, ^aP<0.05. Compared to prior to treatment, ^bP<0.05.

Table VI. Effects of the coadministration of Huatuo Zaizao pills (HT) and aspirin on thrombin time (TT).

Groups	TT (sec)		
	0 h	2 h	4 h
Control	14.12±1.54	14.60±2.08	14.94±2.48
HT	14.3±1.94	14.16±2.33	16.40±2.81
Aspirin	13.32±0.86	14.84±1.93	13.20±1.29
HT + aspirin	13.82±1.36	15.20±1.74	18.52±2.17 ^{a,b}

Data are expressed as the means ± SEM (n=5). One-way ANOVA was used to analyze the significant differences among the different groups. The comparison between two groups was performed with the Student's unpaired t-test. Compared to control, ^aP<0.05. Compared to prior to treatment, ^bP<0.05.

Table VII. Effects of the coadministration of Huatuo Zaizao pills (HT) and aspirin on fibrinogen content (FIB).

Groups	FIB (g/l)		
	0 h	2 h	4 h
Control	3.30±0.46	3.32±0.42	3.40±0.36
HT	3.20±0.34	2.94±0.28	3.02±0.34
Aspirin	3.34±0.54	3.20±0.46	3.00±0.30
HT + aspirin	3.32±0.47	3.26±0.54	2.98±0.46

Data are expressed as the means ± SEM (n=5). One-way ANOVA was used to analyze the significant differences among the different groups. The comparison between two groups was performed with the Student's unpaired t-test.

WBV is an important parameter in hemorrheology, which reflects the rheological properties of blood and blood cells. The major determinants of WBV are PV, hematocrit and the properties of red blood cells, including erythrocyte deformability and erythrocyte aggregation (15). WBV is a reflection of the intrinsic resistance of blood to flow in vessels. WBV abnormalities have been associated with increased risk of ischemic stroke and the reduced blood fluidity may expedite tissue ischemia in atherosclerotic diseases (14). PV is a factor that significantly influences the blood perfusion velocity through the capillaries. The viscosity of capillary blood is closely related to PV, together with all the consequences of

blood perfusion in the microcirculation (16). Lower capillary blood viscosity results in higher blood perfusion velocity in the macrocirculation. In the present study, treatment with HT or aspirin alone exerted no effects on WBV or PV. However, at 4 h following the coadministration of HT and aspirin, WBV and PV were significantly decreased. The results demonstrated that HT and aspirin may have a synergetic effect on decreasing WBV and PV.

Under normal physiological conditions, a balance exists between factors that promote and those that retard coagulation. Coagulation disorders may lead to an increased risk of clotting. PT and APTT are performance indicators reflecting the effi-

cacy of both the intrinsic and extrinsic coagulation pathways. Shortening of PT and APTT reflects an increased tendency to clot (17). In our study, the coadministration of HT and aspirin significantly prolonged APTT, PT and TT. However, treatment with HT or aspirin alone exerted no effects on plasma coagulation parameters. The results indicated that the synergistic effect of HT and aspirin on WBV and PV may be correlated with both extrinsic and intrinsic coagulation systems.

In conclusion, our study has demonstrated that treatment with HT or low-dose aspirin alone exerted no effects on hemorrheology and blood coagulation. However, the coadministration of HT and aspirin prolonged APTT, PT and TT and decreased WBV and PV. The findings indicated that this coadministration exerted a more potent effect on hemorrheology and blood coagulation, compared to each agent administered individually, and may be a novel approach to the prevention and treatment of hemorrheological disorders.

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