

EXPERIMENTAL STUDY

Effect on platelet aggregation activity: extracts from 31 Traditional Chinese Medicines with the property of activating blood and resolving stasis

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donic acid (AA) as aggregation inducers, respectively. Aspirin was the positive control.

RESULTS: Lots of the tested TCMs had inhibitory effects with concentration-dependent manner on platelet aggregations induced by various agonists. Especially, some of the TCMs such as Chuanxiong (*Rhizoma Chuanxiong*), Yanhusuo (*Rhizoma Corydalis Yanhusuo*) and Danshen (*Radix Salviae Miltiorrhizae*) showed good anti-platelet aggregation effect similar or higher than that in positive control group.

CONCLUSION: The study provided scientific references that several TCMs such as Chuanxiong (*Rhizoma Chuanxiong*), Yanhusuo (*Rhizoma Corydalis Yanhusuo*) and Danshen (*Radix Salviae Miltiorrhizae*), possess the property of anti-platelet aggregation.

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Key words: Platelet aggregation inhibitors; Adenosine diphosphate; Thrombin; Arachidonic acid; Blood activating stasis removing ; Medicine, Chinese traditional

Abstract

OBJECTIVE: To evaluate the anti-platelet aggregation effects of extracts from 31 Traditional Chinese Medicines (TCM) with the property of activating blood and resolving stasis in terms of TCM theory.

METHODS: The 31 TCMs extracts were prepared using water, 90% ethanol and ethyl acetate, and the effects on anti-platelet aggregation were tested on a platelet aggregation analyzer *in vitro* with adenosine 5'-diphosphate, bovine thrombin and arachi-

INTRODUCTION

Blood stasis pater (BSP) or thrombosis in biomedical terms, which refers to the block in the circulation of blood or meridians,¹ is attributed to a very complicated pathogenesis which mainly involves hemorheology disorder, microcirculatory disturbance, vascular endothelial cell injury and platelet aggregation.² Actually, BSP is considered to be closely related to senile diseases such as atherosclerosis, ischemic heart disease, and stroke,³ as well as rheumatoid arthritis, hyperuricemia, and vari-

ous inflammatory conditions.⁴ Platelet aggregation in blood vessels plays a crucial role in the formation of thrombosis. Inhibiting platelet activation is considered to be an effective way to stop or slow down thrombosis.^{5,6} Therefore, anti-platelet aggregation tests were widely applied to evaluate and investigate cardiovascular disease both in experimental⁷⁻⁹ and clinical observations.¹⁰⁻¹² The adhesiveness and aggregation of platelets will happen due to the impairment of blood vessels or emergency of platelet activation factor, such as adrenaline, adenosine 5-diphosphate (ADP), 5-hydroxytryptamine (5-HT), thromboxane A₂ (TXA₂) and thrombin (THR) besides collagen.¹³ Therefore, there are four main action mechanisms of anti-platelet medicines have been reported such as inhibiting the metabolism of platelet arachidonic acid (AA), increasing the level of platelet cyclic adenosine monophosphate (cAMP), inhibiting the activation of ADP, and blocking the platelet membrane glycoprotein II b/III a.¹⁴

Traditional Chinese Medicines (TCM) have a long history. According to records in *Huang Di Nei Jing* in Chinese,¹⁵ *Shang Han Za Bing Lun*¹⁶ in Chinese and *Jin Kui Yao Lue*¹⁷ in Chinese in the Eastern Han Dynasty, there were several TCMs such as Chuanxiong (*Rhizoma Chuanxiong*) and Yanhusuo (*Rhizoma Corydalis Yanhusuo*) had been used for the treatment of BSS. In Tang and Song Dynasty, more TCMs such as Moyao (*Myrrha*) and Jianghuang (*Rhizoma Curcumae Longae*) were applied to treat BSS. Actually, in *Shen Nong Ben Cao Jing* in Chinese,¹⁸ 83 of 365 TCMs were recorded with the function of activating blood and resolving stasis. However, to date, the systematic investigation and comparison on the anti-platelet aggregation activities of activating blood and resolving stasis TCMs within one study has not been reported.

In the present study, 31 TCMs with the property of activating blood and resolving stasis were selected based on their traditional medical literature. Their extracts were prepared with 90% ethanol (further liquid-liquid extracted by ethyl acetate) and water. The inhibitory effects on rabbit platelet aggregation were induced by AA, ADP and THR of those extracts were investigated.

MATERIALS AND METHODS

Chemicals and reagents

AA, THR and pentobarbital sodium were obtained from Sigma (St Louis, MO, USA). ADP was the product of Wuhu Huaren Technology Company (Wuhu, Anhui, China). Aspirin and ethanol was purchased from Chengdu Kelong Chemical Reagent Factory (Chengdu, China) and sodium citrate was obtained from Chengdu Aikeda Chemical Reagent Company (Chengdu, China). All other chemicals and reagents were of analytical grade.

Thirty-one raw materials of TCMs were obtained from local drugstores (Chongqing Xhoo Medicin Co., Ltd.,

and Chongqing Hongshengqiao drugstore, Chongqing, China) in Chongqing during autumn of 2012. The voucher specimens of those TCMs were deposited at the School of Chemistry and Chemical Engineering (Chongqing University, Chongqing, China).

Preparation of the extracts

The dried materials of 31 TCMs were ground into fine powder in a pulverizer, respectively. 20 g of the powder was extracted with 60 mL 90% ethanol in an ultrasonic cleanser tank for 20 min, along with reflux extraction for 1 h at 80 °C, filtered and another fresh 60 mL 90% ethanol was added into the residue, repeated the reflux extraction and filtered. The filtrate was combined and dried at 45 °C using a rotary evaporator, and then the residue was re-suspended in 10 mL water and liquid-liquid extraction with 10 mL ethyl acetate for two times. The ethyl acetate extract and the rest solution were separately dried at 45 °C using a rotary evaporator and further dried in an oven at 50 °C. After 90% ethanol extraction, the material residue continued to be reflux extracted by 60 mL × 2 distilled water for twice at 100 °C, filtered and combined the filtrate. The filtrate solution was evaporated by a rotary evaporator to produce aqueous extract. Finally, the aqueous extract, the rest part after ethyl acetate extraction and ethyl acetate extract of 90% ethanol extract were labeled as A1, A2 and A3, respectively. The extraction yields of each extract for 31 TCMs were between 0.03% to 29.10%. The yields of some extracts such as the A2 and A3 extracts of Xuejie (*Sanguis Draconis*) were very low, so the anti-platelet aggregation tests for those extracts were done only with one concentration each. A1 extract was dissolved in phosphate buffer saline, A2 in 60% ethanol and A3 in diluted dimethyl sulfoxide (DMSO) before use, respectively.

Preparation of blood plasma sample

Rabbits, males, weighing [(2.2 ± 0.4) kg] were purchased from Animal farm in Chongqing. All experimental procedures were approved by the Institutional Animal Ethical Committee of Chongqing University and were conducted according to the Guide for the Care and Use of Laboratory Animal of the National Institute of Health (Publication No. 80-23, revised 1996). Rabbit blood samples were collected in 3.8% sodium citrate with the ratio of 9:1 (blood: anticoagulant) from carotid artery after anesthetizing by 1% pentobarbital sodium. Platelet-rich plasma (PRP) was obtained by centrifugation at 93 ×g for 15 min at room temperature, and platelet-poor plasma (PPP) was obtained by further centrifugation from the remaining blood at 2325 ×g for 15 min. The concentration of PRP was adjusted to 3 × 10¹¹/L by PPP.¹⁹

In vitro platelet aggregation assay

Platelet aggregation test was performed using the method described in previous report with some modifications.²⁰ In brief, the level of light transmission was cali-

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