Comparison on Anticoagulation and Antiplatelet Aggregation Effects of Puerarin with Heparin Sodium and Tirofiban Hydrochloride: An In Vitro Study

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ABSTRACT Objective: To detect the anticoagulation and antiplatelet effects of different concentrations of puerarin, heparin sodium and tirofiban hydrochloride on the blood samples of healthy volunteers by Sonoclot coagulation and platelet function analyzer. Methods: Peripheral blood samples were extracted from 20 healthy volunteers, followed by adding different concentrations of puerarin, heparin sodium and tirofiban hydrochloride. Samples were detected for activated clotting time (ACT), clot rate (CR) and platelet function (PF) by Sonoclot coagulation and platelet function analyzer instrument. Results: For puerarin and heparin sodium, the values of ACT gradually increased, and the values of CR and PF gradually decreased with increasing in drug concentration. There was a linear (or log linear) relationship between ACT, CR, PF value and drug concentration (P<0.01). Corresponding to each value, a regression equation was obtained. For tirofiban hydrochloride, the values of ACT and CR had no significant changes, while PF values gradually decreased with concentration increasing. There was also a linear relationship between PF values and concentrations of tirofiban hydrochloride (P<0.01). Under the same ACT values, the puerarin corresponding CR values (CR = e^{-0.0086ACT+4.3}, P<2.2e-16) were always higher than the corresponding values (CR = e^{-0.0028ACT-2.78}, P-value<2.2e-16) of heparin sodium. For high concentrations of puerarin (e.g. 3.8 mg/600 μL) and tirofiban hydrochloride (e.g. 0.8 μg/600 μL), PF values had no significant difference. However, PF values for high puerarin concentration had a larger variance. Conclusions: Puerarin has similar anticoagulant and antiplatelet effects with the heparin sodium, and may have a lower hemorrhage risk than heparin sodium when obtained the same anticoagulation effect in the concentration range of this experiment. In addition, for high concentration, puerarin had the same antiplatelet function as tirofiban hydrochloride but with a larger individual variability.

KEYWORDS puerarin, heparin, tirofiban hydrochloride, in vitro, Sonoclot coagulation analyzer, anticoagulation, antiplatelet aggregation

Puerarin is a flavonoid glycoside derived from the canes or roots of leguminous plant kudzu [Pueraria lobata (Willd.) Ohwi]. It is a vasodilator with the effects of expanding coronary artery and cerebral vessel, reducing myocardial oxygen consumption, improving microcirculation, and inhibiting platelet aggregation. Animal studies showed that puerarin could inhibit 5-hydroxytryptamine (5-HT) release in platelet-mediated by thrombin induction.1

Sonoclot coagulation and platelet function analyzer is mainly used for the coagulation and platelet function test in vitro. At present, this instrument has gradually become an important, accurate and fast clinical hemostatic inspection tool in cardiovascular surgery, liver transplant and other surgeries of massive hemorrhage.2-4 The parameters include activated clotting time (ACT), the liquid-remained time of blood sample, clot rate (CR), the rate of fibrin formation (indirectly reflects the level of fibrinogen), and platelet function (PF), which reflects platelet function.
and platelet function analyzer *in vitro* to compare the anticoagulant/antiplatelet effects between Puerarin Injection and Heparin Sodium Injection/ Tirofiban Hydrochloride Injection, and to explore the anticoagulation and antiplatelet effects of puerarin.

**METHODS**

**Source of Specimen**

Twenty healthy volunteers from Beijing Fuwai Hospital were included, 9 males, 11 females, aged 25–50 years old (38 $\pm$ 6 years old). They had not taken any drug in the previous month, and there were no women who were during pregnancy, menstrual period or lactation period. All the healthy volunteers signed their informed consents and agreed to provide blood samples for experiments in March 2016 in this study.

**Sample Collection**

Fasting venous blood (3 mL) was taken from each participant in a resting state, then 0.109 mol/L sodium citrate in a ratio of 9:1 was added to every blood sample, and each blood sample was gently mixed well. After that, 500 $\mu$L of anticoagulant blood was taken with a 1000 $\mu$L manual pipette each time and injected into a 1 mL Eppendorf tube. The lid of the tube was closed to prevent water evaporation.

**Apparatus and Reagents**

Sonoclot analyzer was purchased from the Viscell Company, UK. The 0.109 mol/L sodium citrate solution (equal to 32 g/L sodium citrate containing two molecules of water of crystallization) was purchased from Beijing Greiner Bio-one Suns Limited Company, China. Glass beads-ACT kit (gb-ACT kit) was purchased from the Sienco Company, CO, USA. The calcium chloride solution (concentration of 0.25 mol/L) was configured by our laboratory. Puerarin Injection was purchased from Baiyun Mountain in Guangzhou Tianxin Pharmaceutical Limited Company. Heparin Sodium Injection was purchased from Qianhong in Changzhou Biochemical Pharmaceutical Co., Ltd. Tirofiban Hydrochloride Injection was purchased from Iroko Cardio Australia Pty Ltd, Australia.

**Experimental Protocol**

The Eppendorf tube filled with 500 $\mu$L anticoagulant blood was rewarmed to 37 °C, then added with 100 $\mu$L different concentrations of each injection (puerarin, heparin sodium or tirofiban hydrochloride) respectively. After gently mixed well, 20 $\mu$L 0.25 mol/L calcium chloride solution was added for recalcification, then 360 $\mu$L blood sample was injected into a kit box with a manual pipette. Turning on the blood sample mixed button immediately, at 10 s after the lid were covered. The instrument ran automatically. The ACT, CR and PF values of glass beads coagulant was measured on blood samples spiked with increasing concentrations of the 3 different injections [puerarin (0, 2.5, 3.0, 3.2, 3.4, 3.6, 3.8 mg/600 $\mu$L), heparin sodium (0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 IU/600 $\mu$L) or tirofiban hydrochloride (0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 $\mu$g/600 $\mu$L)] respectively.

Based on literature and preliminary experiment results, Puerarin Injection initial concentration (2.5 mg/600 $\mu$L) is roughly 5 times of the highest clinical dose, Heparin Sodium Injection initial concentration (0.1 IU/600 $\mu$L) is roughly equivalent to the highest clinical dose, and Tirofiban Hydrochloride Injection initial concentration (0.1 $\mu$g/600 $\mu$L) is roughly half of the highest clinical dose.

**Statistical Analysis**

The values of ACT, CR and PF were presented as mean and standard deviation ($\bar{x}$ ± s). All reported P values were two-sided with significance set at P<0.01. All statistical analysis were performed using R (version 3.2.3, with package stats). A correlation analysis of the values of ACT, CR and PF under different concentrations of each injection was performed and a regression equation was created. The slop of linear regression and its significance for linear relationship were used to verify the impact on the above metrics. A log-linear regression was used to model the relationship between ACT and CR for different injections to compare their CR values under the same ACT value. A paired t-test was performed to compare the impacts of two injections on PF values.

**RESULTS**

**Correlation between Values of ACT, CR, PF and Concentrations of Puerarin**

The values of ACT, CR, PF measured under different concentrations of puerarin are summarized in Table 1. The results showed that the values of ACT gradually increased, the values of CR and PF gradually decreased with increasing concentrations of puerarin. There was a linear relationship between ACT, CR, PF values and concentrations of puerarin (P<0.01). Furthermore, corresponding to each index, a linear regression equation was obtained (Figure 1).