·基础研究·

Basic Research about Antithrombotic Effect of Geniposide*

LIU Hao[1], ZHANG Hai-yan[1,2], SONG Wei[1], LU Qian[1], LI Fang[1], YANG Ming[1,3]△

(1 Key Laboratory of Modern Preparation of TCM, Ministry of Education, Jiangxi University of TCM, Nanchang 330004, China; 2 Key Lab of Advanced Technology of Materials (Chinese Education Ministry), Southwest Jiaotong University, Chengdu 610003, China; 3 Chengdu University of TCM, Chengdu 611137, China)

ABSTRACT Objective: To make sure the antithrombotic and fibrinolytic effect of geniposide and genipin. Methods: The model of bypass loop thrombosis and carotid artery thrombosis were used to determine CT, BT, PRT, PT and weight of thrombus, then calculated the rate of thrombosis inhibition. Results: Tests showed that geniposide and genipin could remarkably extend time of blood coagulation and hemorrhage and reduce weight of thrombus. Compared with positive drug group(aspirin) genipin and geniposide high dose group had significant difference ($P<0.05$) on anticoagulation and antithrombotic function. Geniposide low dose group had no significant difference on thromblytic activity and antiplatelet aggregation compared with aspirin group ($P>0.05$). Conclusion: Geniposide and its aglycon might have antithrombotic and fibrinolytic function.

Key words: Geniposide; Antithrombotic; Fibrinolytic; Clotting Time; Tail Transection Bleeding Time

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Introduction

Chinese medicine Zhi zi is the dryed fruit of rubiaceae plant Gardenia jasminoides Ellis. Its main effective parts are chemical compounds of iridoid glycosides, such as geniposide and genipin, which is the aglycone of geniposide and widely researched in materials of drug vehicle, such as pH-sensitive hydrogel [1], crosslinking agent of multi-layers of collagen spray-coated on drug-eluting stent [2] and things like that. Current studies show that geniposide and its derivates have the function of antiinflammatory [3], antioxidant [4,5], antiplatelet aggregation [6], diabetes [7] and cardiovascular disease [8-10]. Their pharmacology researches are still going on and on.

1 Materials and Methods

1.1 Animals and Experimental Groups

Experiments were performed on male albino SD rats with average body weight of 200 g and approximate age of 9 months. The temperature in animal room was (25± 2) ℃ with 12 h artifical light. It's airing all the time. Scobicular aspirin and heparin injection came from Hainan Ling Kang Pharmaceutical Co., Ltd.. geniposide(95 %) and genipin(98 %) were gained from Linchuan Zhi Xin Biotechnology Co., Ltd.. Scobicular aspirin, geniposide and genipin were used as injection by solved in saline injection and 1 % PEG. $FeCl_3$ and KOD were solved by DDW.

1.2 Methods

1.2.1 Drug Delivery The experiment only adopted the drug delivery of caudal intravenous injection. There were six groups administered twice a day for 4 days as follows: control group with 5 mg/kg 0.9 % saline injection; positive drug group with 5 mg/kg aspirin or heparin injection; sample group with 5, 10, 20 mg/kg geniposide solution and 15 mg/kg genipin solution.

1.2.2 Clotting Time (CT) and Tail Transection Bleeding Time (BT) 1 h after last administration, blood was gained from venous plexus of inner canthus behind the eyeball by using glass capillary of 1 mm internal diameter. Started recording the time when blood came into the capillary. Put it on the table in a level, break it into two parts every 30 s from one end, pull apart slowly, and observe if there is blood streak at the transaction. If observed, then stop timing.

1 h after last administration, bleeding was induced by section of the extremity of the tail 3 mm from the tip. The tail was maintained in contact with air and gently blotted with filter paper per 15 s during a 10-min period and then every 30 s. The time of stoping bleeding for 1min was noted.

1.2.3 Arterio－venous Shunt Thrombosis Model 1 h after last administration, paralysed the rats with 0.6 mol·L^{-1} KOD by i.p.(0.3 g·kg^{-1}). Got three polyethylene tutes with two 1mm internal diameter and one 2 mm, connected them and put in a 5 cm line that had been weighed. Then inserted two sides of connected tubes into jugular vein and carotis respectively, while the tubes were full of 0.05 % heparin. Stopped blood circulation when lasted 15 min. Toke the line out and put into oven at 70 ℃ for 2 h and got it weighed. Calculated the net measure of thrombus by total weight reducing line weight. Inhibition rate of thrombosis could be calculated as follows:

$$\text{Inhibition rate of thrombosis} = \frac{\text{thrombus of control group - thrombus of sample group}}{\text{thrombus of control group}} \times 100\%$$

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Author introduction: LIU Hao (1986-), male, master, focus on the research of cardiovascular prevention and cure

△Corresponding author: YANG Ming (1962-), Male, doctorial tutor, E-mail:yangming16@126.com

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1.2.4 Carotid Artery Thrombosis Model 15 min after last administration, paralysed the rats as above. Referencing the method of Kurz [10], separated two sides of common carotid artery with 2cm long. Put two scrap plastic sheets (4 cm× 1.8 cm) under blood vessels to protect tissue around them. 1 h after last administration, put two pieces of filter papers (1 cm× 1 cm) with 20 μL 2.16 mol·L^{-1} $FeCl_3$ on blood vessels. Removed the filter papers 15 min later and observed. 90 min after removing the filter papers, cut down 1cm blood vessel and weighed them.

1.2.5 Whole Blood Clot Dissolution Experiments 30 min after last administration, 1 ml blood was gained from venous plexus of inner canthus behind the eyeball and reserved in PE tube. Then put it in 37 ℃ thermostatic waterbath for 8 h and weighed the remained clot.

1.3 Statistical Analysis

All the data were analyzed by using SPSS 18.0. The values are presented as mean± SEM ($\bar{x}$± s). The differences were considered as statistical significance at P<0.05(ANOVA).

2 Results

2.1 Clotting Time (CT) and Tail Transection Bleeding Time(BT)

Studing from table 1, it's known that, 1) BT: Compared with control group, positive drug group and geniposide middle dose group show significant difference(*P<0.05), geniposide high dose group and genipin group present highly significant difference (**P<0.01). Compared with positive drug group, high dose group shows highly significant difference (△△P<0.01), while genipin group expresses significant difference (△P<0.05). 2) CT: Compared with control group, positive drug group and genipin group show highly significant difference (**P<0.01). Geniposide high dose group have the strongest effect on BT. While aspirin have the strongest effect on CT.

Table 1 The effect of geniposide and genipin on BT and CT ($\bar{x}$± s,n=8)

Group	Dose/(mg·kg^{-1})	BT/s	CT/s
Control	5	68.17± 18.58	50.83± 16.63
Aspirin	5	112.50± 18.72*	111.67± 36.67**
Geniposide	5	82.36± 17.48	53.24± 12.77
	10	114.26± 20.53*	55.51± 18.34
	20	180.17± 26.69**△△	62.33± 14.24
Genipin	15	161.17± 27.49**△	88.67± 20.97**

Note: Compare with control: * P<0.05, * *P<0.01. Compared with positive drug group: △P<0.05, △△P<0.01.
Room temperation was permanent 25 ℃. Subtus tables was the same.

2.2 Arterio-venous Shunt Thrombosis Model

Confirming from table 2, it's known that, compared with control group, each group shows highly significant difference(**P<0.01); compared with positive drug group, high dose group and genipin group express highly significant difference(△△P<0.01). Compared each group on inhibition rate of thrombosis, it shows that: genipin group >high dose group >middle dose group ≈ positive drug group >low dose group.

Table 2 The comparison of thrombus weight between each group ($\bar{x}$± s, n=8)

Group	Dose/(mg·kg^{-1})	Dry weight/g	Inhibition rate of thrombosis /%
Control	5	0.00327± 0.000198	-
Aspirin	5	0.00154± 0.000206 * *	52.905%
Geniposide	5	0.00196± 0.000336* *	40.061%
	10	0.00162± 0.000385* *	50.459%
	20	0.000710± 0.000120 * *△△	78.287%
Genipin	15	0.000560± 0.0000810* *△△	82.875%

2.3 Carotid Artery Thrombosis Model

State from table 3 present that, compared with control group, positive drug group shows significant difference (*P<0.05), while high dose group shows HSD(**P<0.01).

2.4 WB Clot Dissolution Experiments

Study from table 4 show that, compared with control group, each group shows HSD (**P<0.01); compared with positive drug group, high dose group presents SD(△P<0.05).

3 Discussion

Table 3 The comparison of thrombus weight between each group ($\bar{x}\pm$ s, n=8)

Group	Dose/(mg·kg^{-1})	Dry weight /g
Control	5	0.00290± 0.000863
Aspirin	5	0.00161± 0.000340*
Geniposide	5	0.00268± 0.000522
	10	0.00244± 0.000481
	20	0.00139± 0.000230* *

Table 4 The effect of each group on thrombolysis ($\bar{x}\pm$ s n=8)

Group	Dose/(mg·kg^{-1})	Clot weight/g
Control	5	0.303± 0.0692
Aspirin	5	0.120± 0.0389**
Geniposide	5	0.145± 0.0478**
	10	0.0927± 0.0153**
	20	0.0558± 0.00923**△

The study showed that, geniposide and its aglycone could prolong BT and CT, play antithrombotic role by reducing thrombus weight and have thrombolysis effect. This was coincided with the research of Suzuki Y[6]et al. Now it was known that antiplatelet aggregation effect of geniposide and genipin was related with their repression on PLA2 activity in vitro, which caused the release of arachidonic acid inhibited. This research had progress on their antiplatelet aggregation study in vivo with arterio-venous shunt thrombosis model. Preliminary study found that genipin had stronger antithrombotic effect than geniposide. And there was some kind of dose-effect relationship. Besides, the study of whole blood clot dissolution experiments showed that, geniposide and genipin had the effect of fibrinolytic activity, which might not only be related with its effect of protecting and promoting endotheliocyte growth [11,12], but also promote some tissues plasminogen activators that weren't clear [13]and inhibite thrombin-induced VWF release and P-selectin translocation in HUVECs in a dose- and time-dependent manner [14]. VWF is a protein that is essential for platelet adhesion and aggregation [15,16]. Less P-selectin on the surface of endothelial cells would decrease leukocyte adhesion to the vessel wall[17].

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京尼平苷的抗血栓基础研究 *

刘 昊[1] 张海燕[1,2] 宋 伟[1] 芦 乾[1] 李 芳[1] 杨 明[1,3△]

(1 江西中医学院现代中药制剂教育部重点实验室 江西 南昌 330004；
2 西南交通大学材料先进技术教育部重点实验室 四川 成都 610003；3 成都中医药大学 四川 成都 611137)

摘要 目的：明确京尼平苷和京尼平的抗血栓和纤溶的作用效果。方法：用旁路循环血栓形成模型和颈总动脉血栓模型，测定 CT、BT、PRT、PT，全血溶栓，计算血栓形成抑制率。结果：试验表明，京尼平苷及其苷元能显著延长凝血、出血时间，减少两个血栓模型的血栓重量，京尼平苷高剂量组与阳性药组相比，溶栓作用具有显著性差异（P<0.05），优于阳性药；京尼平、京尼平苷抗凝血及抗血栓作用与阿司匹林组相比无显著差异（P>0.05）。结论：实验表明中药栀子能延长凝血和出血时间，可能有一定的抗血栓和溶栓作用。

关键词：京尼平苷；抗血栓；纤溶；凝血时间；出血时间

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作者简介：刘昊（1986-），男，硕士，主要从事中药新制剂研究

△通讯作者：杨明，E-mail：yangming16@126.com

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