Mechanism of anti-arterial thrombosis of Dahuangzhecong Fang screened by Ti−Al intermetallic compound porous material

WAN Ling[1], WANG Zhan-yi[2], WEI Xing[1], LI Ji-yang[1], ZHONG Guang-wei[1],
DUAN Xiao-peng[3], HE Fu-yuan[3], JIANG Yao[4], WANG Dong-sheng[1]

1. Xiangya Hospital, Central South University, Changsha 410008, China;
2. Henan Yuzhou Chinese Medicine Hospital, Yuzhou 461670, China;
3. Hunan Traditional Chinese Medicine University, Changsha 410208, China;
4. State Key Laboratory for Powder Metallurgy, Central South University, Changsha 410083, China

Abstract: The mechanism of antithrombotic of Dahuangzhechong Fang separated and purified by Ti−Al intermetallic compound porous material (TAICPM) was researched. Dahuangzhechong Fang, which was isolated and screened by TAICPM, was used to oral rats. At the end of study, their blood and thrombus were collected. The results show that TAICPM with the pore size of 1−5 μm can screen Dahuangzhechong Fang well. Dahuangzhechong Fang can increase 6-keto-PGF1α, lower content of TXD2 and platelet. Dahuangzhechong Fang has good effect to resist arterial thrombosis.

Key words: Ti−Al intermetallic compound porous materials; Dahuangzhecong Fang; arterial thrombosis; isolated Chinese medicine

1 Introduction

Chinese drugs have complex chemical composition. To isolate the active ingredients is an important part of the field of traditional Chinese medicine research. Dahuangzhechong pills with unique effect on blood circulation are widely used all over the internal medicine, surgery, gynaecology and difficult miscellaneous diseases. In order to simplify herbs, Dahuangzhecong Fang was screened by Dahuangzhecong pills as orthogonal test. This experiment separated and purified Dahuangzhechong Fang by homemade Ti−Ai intermetallic compound porous material (TAICPM). In this work, the mechanism of antithrombotic of Dahuang-zhechong Fang screened by self-made TAICPM will be elucidated. Dahuangzhechong pills have better clinical effect significantly in early clinical observations. It can reduce cholesterol, triglycerides, low density lipoprotein, inhibit platelet aggregation and increase high density lipoprotein, inhibit arterial thrombosis and atherosclerosis, improve blood rheology and decrease plasma viscosity. It has significant effect on the anti-arterial thrombosis and superior to aspirin on prevent platelet aggregation [1−4]. In this work, we will clarify the mechanism of Dahuangzhechong Fang for anti-thrombosis.

2 Experimental

2 .1 Materials

Dahuangzhechong Fang contains: rhubarb 30 g, fried Eupolyphaga 3 g, leech 6 g, peach kernel 12 g, gadfly 4.5 g. The decoction was separated and purified by TAICPM with different pore sizes, then precipitated with ethanol to get the decoction concentration of 1 g/mL. FW80-based high-speed universal micro-crushed samples machine was provided by Tianjin Taisite Instrument Co., Ltd.; UV-2102PCS UV visible spectrophotometer was provided by Unico (Shanghai) Analytical Instruments Co., Ltd.; TGL-16GB small universal centrifuges were provided by Shanghai An-ting Scientific Instrument Factory; HMIAS-2000 color medical image analysis system was provided by Wuhan Qianping Imaging Technology Limited Liability Company; TAICPM with the pore size of 1−5 μm, 5−10

μm, 10−15 μm were provided by the State Key Laboratory of Powder Metallurgy, Central South University. Rhein standard (Lot: 0773-9910, The National Institute for the Control of Pharmaceutical and Biological Products), paeoniflorin standard (Lot: 0773-9921, The National Institute for the Control of Pharmaceutical and Biological Products), ferric chloride (FeCl₃) (lot: 20090211 Shanghai Pharmaceutical Group Reagents Ltd.), Thromboxane B2(TXB₂) and 6-keto prostaglandin F1α(6-keto-PGF₁α) radioimmunoassay kits were purchased from Suzhou University, Jiangsu Province Blood Institute. SD male rats (2008A010) weighing 200−240 g were provided by the Experimental Animal Center of Central South University.

2.2 Animal grouping and treatment

Fifty male SD rats were randomly divided into 5 groups: 10 rats in normal group; 10 in model group; 10 in aperture of TAICPM 1−5 μm group; 10 in the aperture 5−10 μm group; and 10 in the aperture of 10−15 μm group. After calculating according to mass, rats of three TAICPM groups received the Dahuangzhecong Fang soup processed by TAICPM with 0.01 mL/g soup orally, 2 times a day in one week, about crude drug 4 g per day. Normal group and model group received an equal volume of normal saline. Before 12 h of the experiment oral each group Dahuangzhechong Fang soup once, then repeated one time before modeling it. Intraperitoneal anesthesia injected the rat by 2% pentobarbital sodium 0.0025 mL/g, according to the ferric chloride-induced carotid artery thrombosis model [9,10]. The rat bilateral carotid arteries were isolated. Then after 1 h small piece of filter paper (1 cm×1 cm) which includes 20 μL 2.16 mmol/L FeCl₃ was deposited on the left carotid artery. In the control group, equal volume of normal saline was used instead of FeCl₃ solution. The right carotid artery was separated about 1.5 cm length. We remove the paper after FeCl₃ paper on it for 0.5 h, and then blood was obtained in the right carotid artery after 1.5 h. Lastly, the modeled carotid artery was cut down, put on filter paper to sucking the excess blood, dried, weighed and placed in 10% formalin to fix it.

2.3 Detection

Radioimmunoassay was used to determine TXB-2 and 6-Keto-PGF1a. Each tube was added to standard (or sample) 100 μL, 125I-TXB-2 100 μL, or antibody 100 μL according to the specification. Then put it into 4 °C refrigerator for 24 h after mixture sufficient and kept it at room temperature for 15 min, centrifuged for 15 min under 4 °C 1500 g, pumped supernatant, and then measured TXB-2 and 6-Keto-PGF1a. Morphology of carotid artery thrombosis was observed by routine HE staining and under light microscope.

2.4 Statistical methods

Data are mean ± standard deviation ($\bar{x}$±s), between two groups using t-test. Among groups comparison used one-factor analysis of variance (LSD). Results are applied by SPSS11.0 statistical analysis software.

3 Results and discussion

3.1 Adsorption and desorption ability of different types of porous material to rhein and paeoniflorin

Wet packed with column volume 25 mL and diameter-height ratio of 1:8, the sample concentration was 0.75 g crude drug per mL liquid, sampled 5 BV, standing up till the adsorption saturation after sampled 0.5 h. The impurity was washed with 3 BV water, and then eluted by 70% ethanol 8 BV. The sample effluent, water lotion and alcohol eluent were sub-collected with the flow rate of the process 3 BV/h. The content of rhein substance was determined, and the absorption rate and recovery rate were calculated according to

$$E=(C_0-C_r)/C_0\times100\% \tag{1}$$

$$Y=[C_dV_d/(C_0-C_r)V]\times100\% \tag{2}$$

where E is the adsorption rate, Y is the recovery rate, C_0 is the sample liquid concentration of rhein (mg/mL), C_r is the effluent liquid after sampled the concentration of rhein (mg/mL), V is the sample solution volume (mL), C_d is the concentration of rhein when desorption equilibrium (mg/mL), and V_d is the desorption solution volume (mL).

While the dynamic desorption parameters were investigated, their evaluation is the purity of rhein.

$$P=(C_dV_d/M_d)\times100\% \tag{3}$$

where P is the purity of rhein, C_d is the elution concentration of rhein (mg/mL), V_d is the elution volume (mL), and M_d is the eluate mass after evaporation (g).

3.2 Results of screened porous materials by dynamic adsorption and desorption experiment

Figures 1 and 2 show that the dynamic adsorption rates of three porous materials are decreased with increasing sample volume. The effect of 1−5 μm TAICPM on the adsorption rate of flavonoids are conspicuous than that of the 5−10 μm and 10−15 μm (P <0.05), and 1−5 μm TAICPM desorption capacity is larger than that of 5−10 μm and 10−15 μm (P<0.05). Therefore, 1−5 μm TAICPM was proved to be suitable for rhein purification.
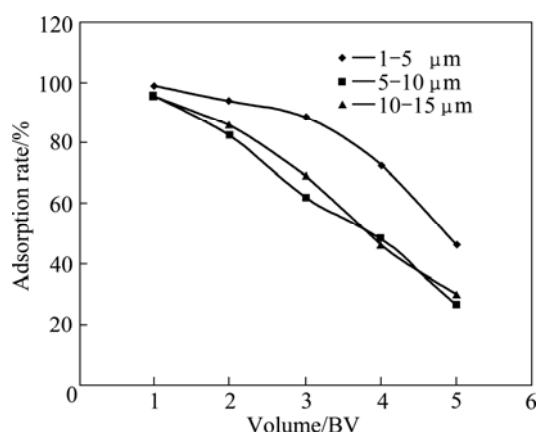
Fig. 1 Dynamic adsorption curves of different types of TAICPM on rhein substance
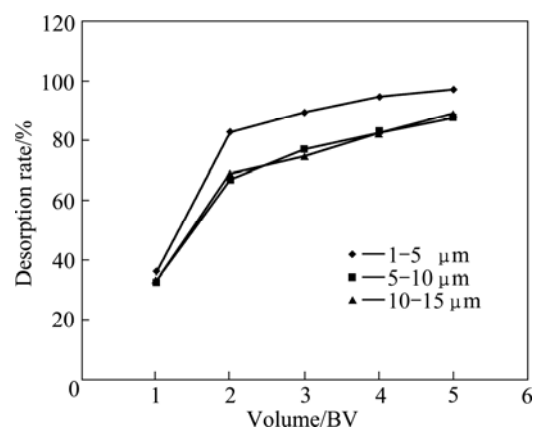
**Fig. 2** Dynamic desorption curves of different types of TAICPM on rhein

3.3 Dahuangzhecong Fang anti-artery thrombosis

3.3.1 Thrombus morphology

Figure 3(a) shows the modeled vessel from the group of TAICPM. Compared with Fig. 3(b), incomplete thrombosis or partial dissolution and light vascular endothelial injury are observed. Figure 3(b) shows the modeled vascular from model group rats, which shows the thrombosis, totally occlusion of blood vessels, severely vascular endothelial injury, continuously and closely thrombosis. Figure 3(c) shows the normal rats modeled vessel, which shows no thrombosis, smooth and continuous endothelial without any damage.

3.3.2 Determination of thrombus

Compared with the normal group, the model group has thrombosis, heavier thrombus dry weight ($P<0.01$). Compared with the model group, the three treatment groups can significantly inhibit thrombosis, shorten the length of the thrombus and lighten the thrombus dry weight ($P<0.05$). In three treatment groups, the 1−5 μm group can significantly decrease the dry weight of thrombus ($P<0.05$), which was shown in Fig. 4.
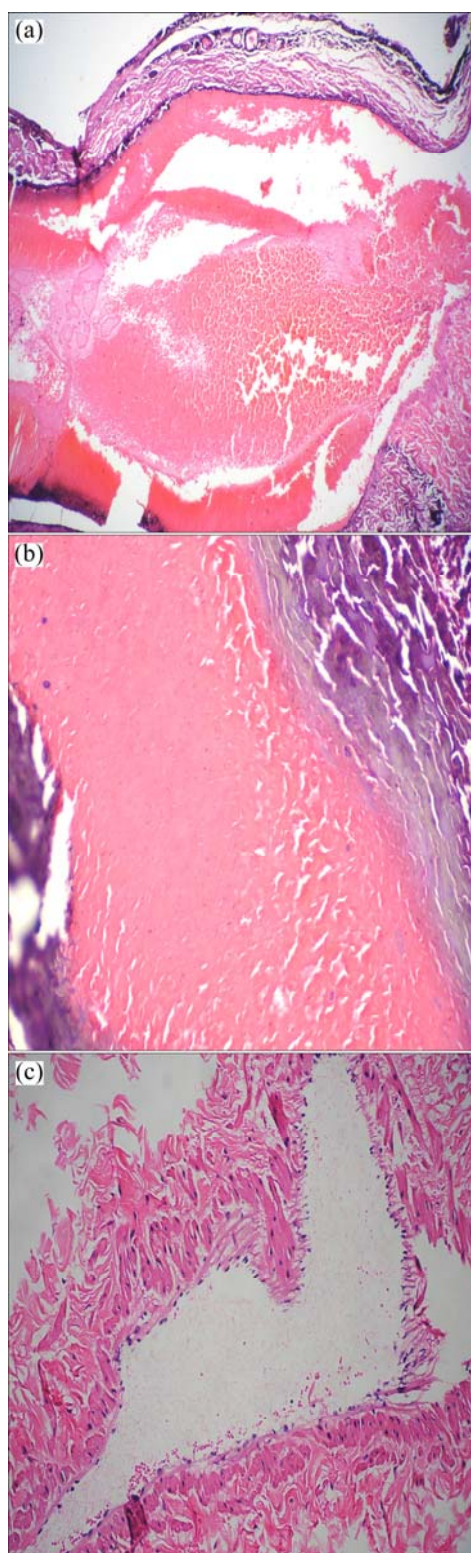
Fig. 3 Morphological characteristics of carotid artery of each group mice: (a) TAICPM group; (b) Model group; (c) Normal group

3.3.3 Determination of platelet count

Compared with the normal group, the model group shown increased platelet counts ($P<0.01$). Compared with the model group, the three treatment groups

significantly reduced platelet counts ($P<0.05$). Table 1 and Fig. 5 show in the three treatment groups, 1−5 μm group can significantly lower platelet counts ($P<0.05$).
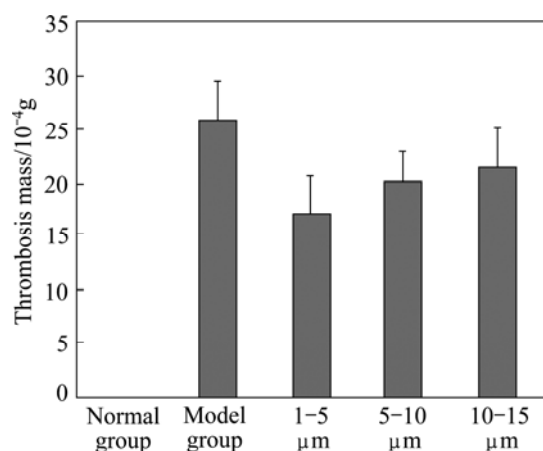

Fig. 4 Comparison of each group thrombus dry mass

Table 1 Comparison of platelet counts in each group

Groups	Number of cases	Platelet count/10^9L^{-1}
Normal group	10	685.19±105.35
Model group	10	1485.26±188.38◆
1−5 μm group	10	729.12±119.65▲★
5−10 μm group	10	847.37±98.26▲
10−15 μm group	10	983.74±102.61▲★

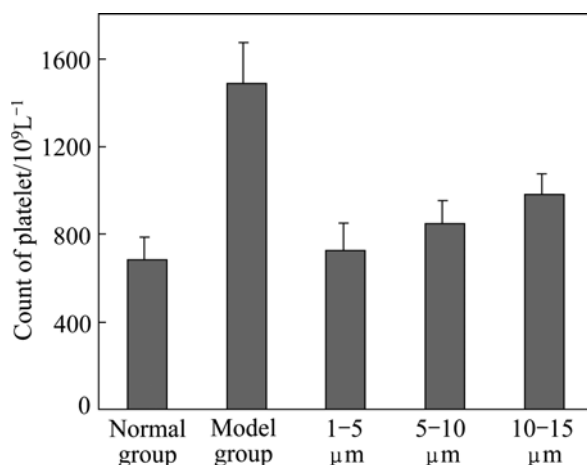Compared with normal group, ◆$P<0.01$; compared with model group, ▲$P<0.01$; with 1−5 μm group, ★$P<0.05$.

Fig. 5 Charts of platelet counts

3.3.4 Plasma TXB-2 and 6-keto-PGF1α determination

Table 2 and Fig. 6 show that compared with the normal group, the model group shows increased plasma TXB-2 and decreased 6-keto-PGF1α ($P<0.01$). Compared with the model group, the three treatment groups shows decreased TXB-2 and increased 6-keto-PGF1α ($P<0.01$), and 1−5 μm group most significantly than others ($P<0.05$).

Table 2 Comparison of each group plasma TXB-2 and 6-keto-PGF1α

Groups	Number of cases	6-keto-PGF$_1$α/ (pg·mL^{-1})	TXB-2/ (pg·mL^{-1})
Normal group	10	155.38±15.54	74.23±11.64
Model group	10	91.27±10.23◆	145.17±18.45◆
1−5 μm group	10	139.37±10.27▲★	79.39±12.73▲★
5−10 μm group	10	122.68±14.38▲	93.24±9.85▲
10−15 μm group	10	117.53±11.21▲★	103.06±11.36▲★

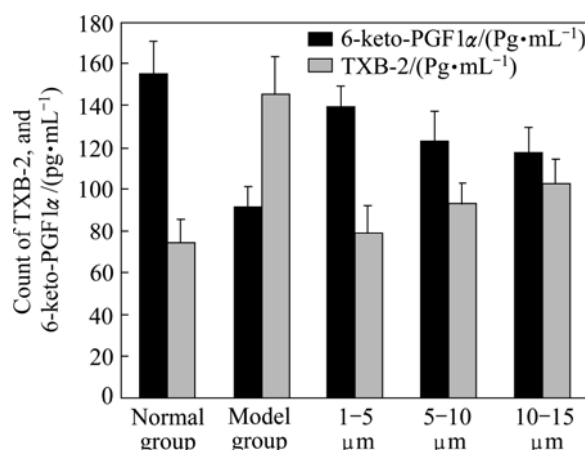Compared with normal group, ◆$P<0.05$; compared with model group, ▲$P<0.05$; with 1−5 μm group, ★$P<0.05$.

Fig. 6 Charts of each group plasma TXB-2, and 6-keto-PGF1α measurement

4 Conclusions

1) TAICPM is a non-gel porous material excluding exchange groups and including the pore structure. It has characteristics as large surface area, adsorption capacity, good selectivity, anti-acid and convenient regenerating.

2) FeCl$_3$ over coat vessel was used to copy animal thrombosis model. We observed three parameters such as the thrombus morphology, thrombus weight, platelet count, anti-coagulation activity and fibrinolysis activity (TXB-2, and 6-keto-PGF1α). The refined Dahuang-zhechong Fang separated by TAICPM has good effect on anti-arterial thrombosis.

3) By comparing the separation of three different aperture filtration material in reducing thrombus weight and platelet count and regulating vaso-motor factor, etc., it was found that the porous material with the pore size of 1μm-5μm shows the best comprehensive separation results, providing basis for our further study of TAICPM in separating Dahuangzhecong fang.

References

[1] WEI Xing, WANG Zhan-yi, LI jie-yang, HE Fu-yuan, DUAN Xiao-peng, ZHONG Guang-wei, JIANG Yao, WANG Dong-sheng. Study of dahuangzhechong pills on anti-arterial thrombosis with the orthogonal design [J]. Journal of Chinese Medicinal Materials, 2012, 35(1): 106−111. (in Chinese)

[2] WANG Dong-sheng, CHEN Pills-ping, HE Shi-lin, XIE Qin-zhi, FU Bin, LI Xin, CAO Xing-yu. CHEN Yan. Comparison of plasm and serum pharmacologic methods in evaluating the effect of dahuang zhechong capsule on platelet aggregation [J]. Chinese Journal of Thrombosis and Hemostasis, 2005, 1l(1): 5−8. (in Chinese)

[3] WANG Dong-sheng, CHEN Pills-ping, HE Shi-lin, XIAO Chang-jiang, TANG Fa-qing. Mechanism study on Dahuangzhechong pill antiplatelet activation [J]. China Journal of Traditional Chinese Medicine and Pharmacy, 2008, 23(9): 114−117. (in Chinese)

[4] WANG Dong-sheng, CHEN Pills-ping, TANG Fa-qing, FENG Jun-tao, HE Shi-lin, HUANG Xi-lian, XIAO Chang-jiang. Effect of Dahuangzhechong pills on arterial thrombosis in rabbits [J]. J Central South University Med Sci, 2009, 34(9): 919−925. (in Chinese)

[5] HUANG Hu-jun, HE Yue-hui, JIANG Yao, HUANG Bai-yun, XU Nan-ping. Preparation and characteristics of Ti−Al based alloy porous material [J]. Chinese Journal of Materials Research, 2007, 4(21): 18−23. (in Chinese)

[6] WU Zhi-feng, HE Yue-hui, JIANG Yao, TANG Lie-ming, HUANG Bai-yun, XU Nan-ping. Corrosion resistance of porous TiAl alloy in hot hydrochloric acid solution [J]. Materials Science and Engineering of Powder Metallurgy, 2007, 12(5): 310−315. (in Chinese)

[7] JIANG Yao, HE Yue-hui, HUANG Bai-yun, LIN Jun-pin, YANG Fan. Progress in research on Ti−Al intermetallic compound porous material [J]. Materials China, 2010, 29(3): 18−22. (in Chinese)

[8] GAO Lin, HE Yue-hui, JIANG Yao, HUANG Bai-yun, LIU C T. Preparation and properties of microporous TiAl intermetallic compound filter membrane [J]. Materials Science and Engineering of Powder Metallurgy, 2005, 10(4): 216−219. (in Chinese)

[9] KURZ K D, MAIN B W, SANDUSKY G E. Rat model of arterialthrombosis induced by ferric chloride [J]. Thromb Res, 1990, 604: 269−280.

[10] TANG Ying-hong, LIANG Yan, YANG Jing, ZHANG Shu-ping, DENG Chang-qing. Changes of TXA2 and PGI2 content and anticoagulative and fibrinolytic activity in the plasma in a rat model of topical FeCl3-induced carotid artery thrombosis [J]. Chinese Pharmacological Bulletin, 2006, 22 (11): 1353−1355. (in Chinese)

钛铝金属间化合物多孔材料筛选
大黄蛰虫方抗动脉血栓形成机理

万 玲[1]，王战义[2]，魏 星[1]，李佶阳[1]，钟广伟[1]，段晓鹏[3]，贺福元[3]，江 垚[4]，王东生[1]

1. 中南大学 湘雅医院，长沙 410008；
2. 河南禹州市中医院，禹州 461670；
3. 湖南中医药大学，长沙 410208；
4. 中南大学 粉末冶金学院，长沙 410083

摘　要：探讨经过钛铝金属间化合物多孔材料分离筛选的大黄蛰虫方抗血栓形成机理。将通过钛铝金属间化合物多孔材料分离筛选的大黄蛰虫方干预大鼠，收集血液、血栓。结果表明，钛铝金属间化合物多孔材料能有效分离筛选大黄蛰虫方，以孔径为 1−5 μm 的效果最优。大黄蜇虫方能升高 6-keto-PGF1α，降低 TXD2，降低血小板含量，有良好的抗动脉血栓形成作用。多孔材料处理过的药物具有良好的抗血栓形成作用。
关键词：Ti−Al 金属间化合物多孔材料；大黄蛰虫方；动脉血栓形成；中药分离筛选

(Edited by YUAN Sai-qian)