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

WAN Ling1, WANG Zhan-yi2, WEI Xing1, LI Ji-yang1, ZHONG Guang-wei1, DUAN Xiao-peng3, HE Fu-yuan3, JIANG Yao4, WANG Dong-sheng1

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

Received 1 July 2011; accepted 8 October 2012

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 Dahuangzhechong 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

Foundation item: Project (2010FA32370) supported by The Ministry of Science and Technology of China; Project (2009BAI80B04) supported by the National Science and Technology Support Program of China; Project (2008WK3002) supported by Hunan Provincial Science and Technology Department, China

Corresponding author: WANG Dong-sheng; Tel: +86-731-84327369; E-mail: wdsh66@yahoo.com.cn

DOI: 10.1016/S1003-6326(12)61768-3
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 Dahuangzheccong Fang soup processed by TAICPM with 0.01 mL/g soup orally, twice 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 Dahuangzheccong 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 artery was isolated. Then in 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 isolated. Then 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-PGF₁α. 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-PGF₁α. Morphology of carotid artery thrombosis was observed by routine HE staining and under light microscope.

2.4 Statistical methods

Data are mean ± standard deviation (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

\[ E = \frac{(C_0 - C_d)}{C_0} \times 100\% \]  
\[ Y = \frac{C_d V_d}{(C_0 - C_d) V} \times 100\% \]

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

\[ P = \frac{(C_0 V_d / M_d) \times 100\%} \]

where \( P \) is the purity of rhein, \( C_0 \) 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.

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 \( \mu \)m group can significantly lower platelet counts \( (P<0.05) \).

Table 1 Comparison of platelet counts in each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of cases</th>
<th>Platelet count/10^9L (^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>10</td>
<td>685.19±105.35</td>
</tr>
<tr>
<td>Model group</td>
<td>10</td>
<td>1485.26±188.38</td>
</tr>
<tr>
<td>1–5 ( \mu )m group</td>
<td>10</td>
<td>729.12±119.65 ( \uparrow )( \downarrow )</td>
</tr>
<tr>
<td>5–10 ( \mu )m group</td>
<td>10</td>
<td>847.37±98.26 ( \uparrow )( \downarrow )</td>
</tr>
<tr>
<td>10–15 ( \mu )m group</td>
<td>10</td>
<td>983.74±102.61 ( \uparrow )( \downarrow )</td>
</tr>
</tbody>
</table>

Compared with normal group, \( \uparrow P<0.05; \) compared with model group, \( \uparrow P<0.01; \) with 1–5 \( \mu \)m group, \( \downarrow P<0.05. \)

Table 2 Comparison of each group plasma TXB-2 and 6-keto-PGF1\( \alpha \)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of cases</th>
<th>6-keto-PGF1( \alpha )/ (pg·mL(^{-1} ))</th>
<th>TXB-2/ (pg·mL(^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>10</td>
<td>155.38±15.54</td>
<td>74.23±11.64</td>
</tr>
<tr>
<td>Model group</td>
<td>10</td>
<td>91.27±10.23 ( * )</td>
<td>145.17±18.45 ( * )</td>
</tr>
<tr>
<td>1–5 ( \mu )m group</td>
<td>10</td>
<td>139.37±10.27 ( ** )</td>
<td>79.39±12.73 ( ** )</td>
</tr>
<tr>
<td>5–10 ( \mu )m group</td>
<td>10</td>
<td>122.68±14.38 ( \uparrow )( \downarrow )</td>
<td>93.24±9.85 ( \uparrow )( \downarrow )</td>
</tr>
<tr>
<td>10–15 ( \mu )m group</td>
<td>10</td>
<td>117.53±11.21 ( ** )</td>
<td>103.06±11.36 ( ** )</td>
</tr>
</tbody>
</table>

Compared with normal group, \( \downarrow P<0.05; \) compared with model group, \( \uparrow P<0.05; \) with 1–5 \( \mu \)m group, \( \downarrow P<0.05. \)

Fig. 4 Comparison of each group thrombus dry mass

Fig. 5 Comparison of each group platelet counts

3.3.4 Plasma TXB-2 and 6-keto-PGF1\( \alpha \) 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-PGF1\( \alpha \) \( (P<0.01) \). Compared with the model group, the three treatment groups show decreased TXB-2 and increased 6-keto-PGF1\( \alpha \) \( (P<0.01) \), and 1–5 \( \mu \)m group most significantly than others \( (P<0.05) \).

Fig. 6 Charts of each group plasma TXB-2, and 6-keto-PGF1\( \alpha \) 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) \( \text{FeCl}_3 \) 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-PGF1\( \alpha \)). The refined Dahuangzhechong 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 1\( \mu \)m-5\( \mu \)m shows the best comprehensive separation results, providing basis for our further study of TAICPM in separating Dahuangzhechong fang.
References


