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Protective effect of Shenfu injection on thromboangiitis obliterans model rats

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ABSTRACT

Ethnopharmacological relevance: Thromboangiitis obliterans (TAO) or Buerger's disease is a non atherosclerotic, segmentar inflammatory vasculitis that is incurable at present. Shenfu injection (SFI), a traditional Chinese formulation, have been confirmed to produce protective influences on several organs and limb during ischemia and reperfusion (IR) injury in rats. However, the effects of SFI on TAO remain unclear.

Materials and Methods: Adult male Sprague Dawley rats were randomly divided into sham operated group, TAO model group, SFI 2.5 mg/kg (low dose), 5 mg/kg (medium dose) and 10 mg/kg (high dose) groups ($n = 8$). Rats were intravenously administered SFI 2.5, 5 and 10 mg/kg or saline once per day for 15 days. TAO model was prepared by injecting sodium laurate into the femoral artery of rats. Then we examined the changes of pathological signs, pathologic grading of thrombus, the indexes of hematology, the contents of *thromboxane B2* (*TXB2*), *6-keto-prostaglandin* $F_{l\alpha}$ (*6-K-PGF*$_{1\alpha}$) in plasma following SFI or saline treatment.

Results: More pathological signs of lesions, higher grades of pathological thrombosis, increased blood platelet counts, the increase in the TXB2 and TXB2/6-K-PGF$_{1\alpha}$ ratio, as well as the decrease of 6-K-PGF$_{1\alpha}$ in TAO model group were shown in present experiments; SFI treatment significantly improved the pathological signs of lesions induced by sodium laurate injection, reduced the numbers of thrombus formation, blood platelet counts, the TXB2 and TXB2/6-K-PGF$_{1\alpha}$ ratio but increased the 6-K-PGF$_{1\alpha}$ compared with TAO model group. However, there were no significant alterations in the counts of red blood cell, leucocyte and neutrophil among these groups.

Conclusions: Our preliminary findings first indicated that SFI can produce significant therapeutic effects on experimental Buerger's disease model rats in a dose independent manner. The underlying mechanisms may be due to its modifying hematology, inhibiting platelet aggregation and enhancing anti-thrombotic function of vessel endothelia.

1. Introduction

Thromboangiitis obliterans (TAO) or Buerger's disease is a non atherosclerotic, segmentar inflammatory vasculitis that reaches small and medium size vessels of the limb's extremities. It affects predominantly young male smokers that present distal ischemia, clinically manifested through claudication, rest pain, ulcers and finally gangrene (Quintas and Albuquerque, 2008). More than a century has passed since Buerger's disease was first described in 1908 by Buerger (Buerger, 1908; Espinoza, 2009), but the etiology, pathogenesis and treatment of this disease still remain unclear (Olin, 2000; Quintas and Albuquerque, 2008; Espinoza, 2009). In sodium laurate-induced arterial occlusive disease of rats, the injected sodium laurate is supposed to cause endothelial cell damage that may lead to the aggregation of platelets in peripheral vascular beds (Ashida et al., 1980). The progression of the disease in this model of vasculitis resembles that reported in the patients with TAO (Nielubowicz et al., 1980).

Shenfu injection (SFI) which originated from Shenfu Tang, a traditional Chinese formulation rescuing the patients from collapse by restoring *Yang*, is mainly composed of the extract of *radix ginseng* and *radix aconitum carmichaeli* root. The main components of SFI are *ginsenosides* and *aconitum alkaloids*, a water-soluble active ingredient of *radix aconitum carmichaelii*. Many previous studies have proved that SFI produced protective influences on myocardium (Zheng et al., 2004; Zhang et al., 2005), intestinal mucosal (Xia

Abbreviations: TAO, thromboangiitis obliterans; SFI, Shenfu injection; TXB2, thromboxane B2; 6-K-PGF$_{1\alpha}$ or 6-ketoPGF$_{1\alpha}$, 6-keto-prostaglandin F$_{1\alpha}$; IR, ischemia and reperfusion; SOD, superoxide dismutase; CPK, creatine phosphokinase; MDA, malondialdehyde.

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et al., 2004), skeletal muscle (Xie et al., 2007), liver (Wang et al., 2006; Zhu et al., 2006) and kidney (Yang et al., 2003; Li et al., 2011) during these organs ischemia and reperfusion (IR) injury in rat. In recent years, SFI has also been shown very useful in both prevention and treatment for limb IR injury (Yu et al., 2003; Huang et al., 2007, 2009; Xie et al., 2007). Yu et al. (2003) have observed that SFI enhanced the activity of superoxide dismutase (SOD), reduced the value of serum malondialdehyde (MDA) and creatine phosphokinase (CPK) during IR of human limbs by resisting lipid peroxidation. 10 mL/kg and 20 mL/kg SFI can increase markedly serum SOD activity and decrease MDA contents, suggesting that the SFI can surely alleviate the reinfusion injury of the ischemic limbs, with better effects admitted pre-operations (Huang et al., 2007, 2009). Furthermore, SFI can protect lower limb IR induced myocardial injury in human (Qu et al., 2010).

Contrary to lots of clinical case reports of medicine treatment of TAO (Quintas and Albuquerque, 2008), experimental research is relatively limited and much less about the exact ethiopathogenesis of TAO. On the other hand, the effects of SFI on TAO have not been reported so far. Considering that TAO is segmental inflammatory and proliferative lesions of physically small to medium arteries and veins, we speculate that SFI might play its unique role in the prevention and treatment of TAO and conducted investigations to verify this idea.

2. Materials and methods

2.1. Animals and drugs

All animal care and this investigation conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Sprague Dawley male rats (200–250 g) were provided by Medical Experimental Animal Center of Nanchang University. Water and food were given *ad libitum*. Experimental protocols were approved by the Medical Experimental Animal administrative Committee of Jiangxi Province, China. Every effort was made to minimize any discomfort experienced by the subjects. Sodium laurate was obtained from Sinopharm Chemical Reagent Co., Ltd (Xian, China). TXB2 and 6-K-$PGF_{1\alpha}$ detection kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). SFI was the TCM formula of Shenfu Tang, which is for recuperating deleted *yang* and rescuing the patient from collapse, and its main components are *ginsenoside* and *aconitum alkaloid*. It was commercial product from Yaan Sanjiu Pharmaceutical Co., Ltd (Sichuan, China), 10 mL/piece, per milliliter of SFI included 0.5 mg of *ginsenoside* and 0.1 mg of *aconitine*. An HPLC fingerprint data was reported by Jiang et al. (2010).

2.2. Experimental groups and protocol

Adult male SD rats were randomly divided into a total of 5 groups with each 8 animals as follows: the sham operated group, TAO model group, SFI 2.5 mg/kg (low dose), 5 mg/kg (medium dose) and 10 mg/kg (high dose) groups. TAO model was prepared according to the method described previously (Ashida et al., 1980). Briefly, rats were anaesthetized with 10% (w/v) chloral hydrate (3.5 mL/kg, i.p.). The left hind leg was shaved and the femoral artery was exposed by surgical incision and retraction of muscles. Sodium laurate solution (10 mg/mL in normal saline, adjusted to pH 8.0, 0.1 mL per animal) was injected into the left femoral artery while the sham operated group was treated with normal saline as vehicle. All groups were subjected to the surgical procedure. Rats were intravenously administered SFI 2.5, 5 and 10 mg/kg 2 h after sodium laurate injection and once a day for the following 14 days. At the end of the experiment, rats were anaesthetized with 10% chloral hydrate. A blood sample was drawn from the common carotid artery and anti-coagulated with heparin. Plasma was frozen at −80 °C for biochemical analysis.

2.3. Main pathological signs

Progression in the skin temperature, colors, arterial pulse, limb swelling, the scope of gangrene and mummification in the rat hind legs were checked daily after the operation. The degree of the vasculitis was graded according to the system developed by Wang et al. (1996), as follows: 0, normal appearance; I, the affected region was limited to the nail parts; II, lesions confined to the toe; III, lesions limited to the whole paw; IV, confined to the leg below stifle joint; and V, extended to the upper leg above stifle joint.

2.4. Thrombus grade in pathological biopsy

According to previous reported methods (Wang et al., 1996), the right lower affected leg of animals was amputated under anesthesia, fixed with 10% formaline, then decalcified and embedded in paraffin blocks. Cross sections were obtained from several parts of the hip, above and lower ankle and paw and stained with hematoxyline and eosin (H–E). Grading of thrombosis was conducted according to the system developed by Wang et al. (1996): 0, no thrombus; I, one thrombosis; II, two to three thrombosis; III, four or more blood clots.

2.5. Assessment of hematology

Blood cell counts were measured using automatic hematology analyzer (XE-2100, Sysmex Medical Electronics Co., Ltd., Shanghai, China) in the Second Affiliated Hospital of Nanchang University.

2.6. Assessments of thromboxane B2 (TXB2) and 6-keto-prostaglandin $F_{l\alpha}$ (6-K-$PGF_{1\alpha}$) in plasma

The content of TXB2 and 6-K-$PGF_{1\alpha}$ in plasma was determined by radioimmunoassay using a detection kit on the basis of manufacturer's guidelines as described in previous report (Hu, 2010).

2.7. Statistical analysis

All data were analyzed using SPSS 17.0 software (SPSS, Chicago, IL, USA). Significant differences after one way ANOVAs were measured by post hoc Student–Newman–Keuls multiple comparison methods. The Kruskal–Wallis H test was used to compare pathological signs of lesions and thrombus grade of the vasculitis among groups. $P < 0.05$ was considered statistically significant. Data were expressed as means ± SEM (standard error of estimate).

3. Results

3.1. Effect of SFI on the local pathological signs in TAO rats

The whole paw in rats became pale 5 min after injection of sodium laurate by the femoral artery and then went violet within about 5 h. In the meantime, the skin temperature decreased and arterial pulse reduced or lost. On the day 2, the fingers of the affected paw became dark and the dark area extended to the whole upper paw, then the paw was gangrened and then mummified with time elapsed. There were pain, limp and drag-line phenomenon in the affected limb of some animals. Within 15 days after the laurate injection, the gangrened part of the paw of most rats fell off. The most serious lesions were shown in model group, the lightest lesions exhibited in SFI medium- and high-dose groups while sham group did not show the similar lesions. The lesion values in sham