Danhong injection effects on vascular endothelial function in patients undergoing coronary artery stent implantation★

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Abstract

BACKGROUND: Numerous studies have shown that Danshen Root can suppress adhesion and aggregation of neutrophils as well as production of oxygen free radical, can activate blood circulation to dissipate blood stasis. However, there are few studies about precise mechanisms underlying protection of vascular endothelium and anti-thrombosis, especially concerning the mechanism of action following coronary artery stent implantation.

OBJECTIVE: To investigate the influence of *Danhong* injection on vascular endothelial function before and after coronary artery stent implantation in patients with acute coronary syndrome (ACS).

METHODS: A total of 60 ACS patients were randomly assigned to two groups. The patients in the conventional treatment group were subjected to conventional drug treatment following coronary artery stent implantation. The patients in the *Danhong* injection group received conventional drug treatment and *Danhong* injection (20 mL/d) following coronary artery stent implantation. The treatment course was 10 days. Before and after implantation as well as before and after *Danhong* injection treatment, endothelium-derived blood flow-mediated vasodilation, plasma endothelin, thromboxane B2, 6-keto-prostaglandin levels were determined, and the vascular endothelial function were evaluated. Simultaneously, short-term prognosis was observed in patients of both groups.

RESULTS AND CONCLUSION: Plasma endothelin and thromboxane B2 levels were significantly increased, but 6-keto-prostaglandin levels were significantly decreased following stent implantation in patients of both groups compared with that before implantation ($P < 0.05$). Plasma endothelin and thromboxane B2 levels were significantly reduced, but endothelium-derived blood flow-mediated vasodilation and 6-keto-prostaglandin levels were significantly increased in both groups following treatment compared with that following implantation ($P < 0.05$, $P < 0.01$), especially in the *Danhong* injection group ($P < 0.05$). During hospitalization, the incidences of cardiovascular events were diminished in the *Danhong* injection group compared with conventional drug treatment group ($P > 0.05$). Results suggested that *Danhong* injection significantly improves vascular endothelial function, induces vasodilatation, improves blood supply, inhibits platelet aggragation, and shows the tendency of ameliorating short-term prognosis.

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INTRODUCTION

Acute coronary syndrome (ACS) contains unstable angina pectoris, acute myocardial infarction and cardiogenic sudden death, and its precise pathogenesis remains unclear[1]. A previous study has shown that the onset is closely correlated with endothelial dysfunction. Traditional Chinese medicine believed that ACS belongs to “thoracic obstruction”, pathogenesis supergenus asthenia in origin and asthenia in superficiality; therapy consists of the methods of activating blood circulation to dissipate blood stasis and supplementing *qi* and nourishing *yin*. *Danhong* injection is a compound preparation of Danshen root and safflower. A previous study has verified that Danshen root can inhibit adhesion and aggregation of neutrophils as well as production of oxygen free radical, and safflower can activate blood circulation to dissipate blood stasis. Nevertheless, there are few studies about precise mechanisms underlying protection of vascular endothelium and anti-thrombosis, especially concerning the mechanism of action during ACS interventional therapy.

SUBJECTS AND METHODS

Design

A randomized controlled study.

Time and setting

Experiments were performed at the Shenzhou Hospital from September 2002 to June 2009.

Subjects

The ACS patients, who were treated in this hospital from September 2002 to June 2009, were enrolled in this study.

Inclusion criteria: in accordance with WHO standards and Clinical Research Instructions of Traditional Chinese New Drugs for treating Thoracic Obstruction (Coronary Artery Disease Angina Pectoris), formulated by Ministry of Health of China; following observation of clinical symptoms and electrocardiogram, coronary arteriongraphy was used to diagnose coronary artery disease; coronary artery stenosis > 75%; with the indications of stent implantation. Stent implantation was conducted according to a previous method by Cruentzig[2].

Exclusion criteria: ① in combination with infectious disease: hyperpyrexia and usage of inflammatory suppressants; ② acute decompensation cardiac failure; ③ recent history of bleeding wound or surgery; ④ malignant tumor; ⑤ severe liver and kidney dysfunction.

A total of 60 ACS patients were equally and randomly assigned to two groups, aged 46–81 (57±18) years. The patients signed the informed consents. Protocols were approved by Hospital’s Ethics Committee.

Drugs and stent materials: stent is stainless steel

excel ra pamycin-coated stent; product standard number YZB/guo 0370-2007, purchased from Shandong Jiwei Medical Products. The stent presented good pushing, good compliance and good safety and compatibility, could significantly decrease vascular restenosis. *Danhong* injection (Danshen root, safflower, water for injection) was purchased from Jinan Buchang Pharmacy, Z20026866. The main component of *Danhong* included tanshinone (structure formula R1=CH3, R2=H), salvianolic acid (molecular formula $C_{36}H_{30}O_{16}$), safflower red, safflower phenolic glycosides and so on.

Intervention methods: the patients in the conventional treatment group were subjected to conventional drug treatment following coronary artery stent implantation (Drug was used in accordance with Treatment Instruction of Two-level Prevention of Coronary Heart Disease and Acute Coronary Syndrome). The patients in the *Danhong* injection group received conventional drug treatment and *Danhong* injection (main components were Danshen Root and safflower) (20 mL/d) following coronary artery stent implantation. The treatment course was 10 days.

Plasma endothelin, thromboxane B2, 6-keto-prostaglandin levels were determined using radioimmunity assay. Human plasma endothelin kit was supplied by the Institute of Radioimmunity, Technology Development Center, General Hospital of Chinese PLA.

Thromboxane B2, 6-keto-prostaglandin F1α kit was obtained from the Research Room of Thrombus and Hemostasis, Suzhou Medical College.

Statistical analysis

The data were analyzed using SPSS 13.0 software. Measurement data were expressed as Mean ± SD. The difference among groups was compared using *t* -test. The correlation among data was analyzed utilizing correlation analysis. A value of $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered extremely statistically significant.

RESULTS

Quantitative analysis of participants

A total of 60 patients were included in the final analysis.

Comparison of basic data of both groups (Table 1).

Table 1 Comparison of basic data and risk factors in both groups ($\bar{x}\pm s$)

Group	Male (*n*/%)	Age (yr)	Diabetes (*n*/%)	Systolic pressure (mmHg)
Danhong injection	17/60.00	54.21±16.23	6/20.00	135.81±13.37
Conventional treatment	17/60.00	55.87±19.05	7/23.33	137.01±19.91

Group	Diastolic pressure (mm Hg)	Serum creatinine (mmol/L)	LDL-C (mmol/L)	TC (mmol/L)
Danhong injection	85.61±16.30	135.56±20.31	2.12±0.99	5.23±3.22
Conventional treatment	86.03±26.01	140.21±18.23	2.53±0.89	5.31±2.24

LDL-C: low density lipoprotein-cholesterol; TC: total cholesterol; 1 mm Hg=0.133 kPa

There was no significant difference in basic data between two groups, with comparability.

Comparison of plasma endothelin, thromboxane B2, 6-keto-prostaglandin levels (Tables 2, 3).

Table 2 Measurement results of vascular endothelial excreted factors in patients of both groups before and after stent implantation ($\bar{x}\pm s$, ng/L)

Group	Preimplantation		
	Endothelin	6-keto-prostaglandin	Thromboxane B2
Danhong injection	96.32±29.12	38.54±31.03	87.50±26.11
Conventional treatment	87.13±32.32	29.21±29.87	77.11±21.34

Group	Postimplantation		
	Endothelin	Thromboxane B2	6-keto-prostaglandi
Danhong injection	107.12±25.22[a]	32.23±36.91[a]	92.24±27.93[a]
Conventional treatment	96.23±25.62[a]	25.44±17.01[a]	81.97±27.07[a]

[a]$P < 0.05$, *vs.* preimplantation

Table 3 Measurement results of vascular endothelial excreted factors in patients of both groups before and after Danhong treatment ($\bar{x}\pm s$, ng/L)

Group	Pretreatment		
	Endothelin	6-keto-prostaglandin	Thromboxane B2
Danhong injection	96.32±29.12	38.54±31.03	87.50±26.11
Conventional treatment	87.13±32.32	29.21±29.87	77.11±21.34

Group	Posttreatment		
	Endothelin	Thromboxane B2	6-keto-prostaglandin
Danhong injection	67.12±25.22[ab]	49.23±46.11[ab]	42.24±21.33[ab]
Conventional	82.23±25.62	37.44±17.01	51.87±28.08

[a]$P < 0.05$, *vs.* pretreatment; [b]$P < 0.01$, *vs.* conventional treatment group

Plasma endothelin and thromboxane B2 levels were significantly increased following stent implantation in both groups, but 6-keto-prostaglandin levels were significantly decreased ($P < 0.05$).

Following 10 days of treatment, plasma endothelin and thromboxane B2 levels were decreased, but 6-keto-prostaglandin levels were significantly increased ($P < 0.05$), and there were significant differences between *Danhong* injection and conventional treatment groups ($P < 0.01$).

Comparison of adverse events in both groups

There was no significant difference in death rate, incidence rate of recurrent angina pectoris and myocardial infarction between *Danhong* injection and conventional treatment groups ($P > 0.05$) (Table 4).

Table 4 Comparison of show-term prognosis between both groups (*n*/%)

Group	n	Death	Angina pectoris	Recurrent myocardial infarction
Danhong injection	30	1/3	17/57	2/7
Conventional treatment	30	1/3	19/63	3/10

DISCUSSION

Previous studies have suggested that ACS occurrence is associated with plaque instability and secondary thrombosis[3-4]. Its onset basis is due to breakage of instable coronary artery atheromatous plaque, secondary platelet aggregation and thrombosis under the co-action of inflammatory stimulation, mechanism stress, and abnormal vascular endothelial function. Cytokines play important roles in onset mechanism of ACS. Cytokines are soluble protein or micromolecule polypeptide synthesized by body immunocytes and non-immunocytes. Cytokine function contains induction of inflammatory reaction, regulation of immune response, histocyte proliferation, influence of hematopoiesis function and neuroendocrine-like effects. Plasma endothelin, thromboxane B2 and 6-keto-prostaglandin have been concluded as key cytokines that can mediate body non-specific inflammatory reactions. To measure above-described cytokine levels in plasma has been common indices of exploring occurrence, development and diagnosis of above-mentioned diseases.

Vascular endothelium is not only the barrier and liner of vessels, but the regulatory tissues with specific differentiation, and controls and regulates the tension of vascular smooth muscle by releasing diastolic and systolic substances[5]. Endothelin is a kind of vasoconstrictor substance by Japanese scholar Yanagisawa *et al*[6]. At present, it is found that ET-1, ET-2, ET-3 and VIC existed in living bodies. Human vascular endothelial cells only produce ET-1. ET is strongest presently known angiotonics. It has systolic effects on vessels of each organ. Under physiological condition, plasma endothelin did not show circulatory hormone action. However, under some pathological conditions, oversynthesis and release of ET are closely correlated with occurrence and development of diseases. Thus, it is of great significance to explore ET properties and functions in prevention and treatment of these disease[7]. A present study has confirmed that endothelin is released by damaged endothelial cells, but conversely damages vessels and myocardial tissues, which is a hallmark of lesion degree[8]. In addition, endothelin can prolong the time of action potential of cardiomyocytes, induce early after-depolarization. The cardiac electrophysiological activity of endothelin may exhibit important effects on sudden death due to coronary artery disease[9].

Results from this study (Table 2) indicated that plasma endothelin levels were significantly increased following stent implantation in both groups ($P < 0.05$), which suggested that stent implantation could lead to an increase in plasma endothelin levels in ACS patients. This is probably correlated with interventional surgery induced vascular endothelium. The patients were subjected to a significant damage to vascular endothelial function following stent implantation. ET1 levels were decreased following 10 days of Chinese medicine treatment ($P < 0.05$), which demonstrated that postoperation treatment could suppress plasma endothelin levels in ACS patients, *i.e*. inhibit the strongest vaso-excitor material, regulate vascular endothelial function, resulting in relaxing vessels and improving blood supply. Moreover, the manifestation was significant in the *Danhong* injection group compared with the conventional treatment group ($P < 0.01$). These suggested that *Danhong* can remarkably ameliorate vascular endothelial function in ACS patients following stent implantation, with the tendency of improving short-term prognosis.

TXA2 is strongest platelet aggregation and angiotonics, whereas PGI2 is strongest platelet aggregation inhibitors and vessel relaxor. They are active substances with contrary effects, and show dynamic equilibrium under normal conditions. This equilibrium plays important effects on maintaining environmental stability in bodies. Multiple clinical and basic studies have shown that platelet activation plays a key role in the process of unstable angina pectoris and coronary thrombosis of acute myocardial infarction[10-11]. Platelet activation is composed of platelet adherence, aggregation and release. Thromboxane A2 (TXA2) and prostaglandin I2 (PGI2) are a pair of bioactive substance of regulating platelet aggregation in bodies. Their equilibrium is a main factor for platelet function, but TXA2 is unstable in bodies, whose half-life period is only 37 seconds[12], and then rapidly degrades into inactive TXB2. PGI2 can relax vessels, inhibit platelet aggregation, whose half-life period is 2 minutes, and then rapidly degrades into stable 6-keto-PGF1α. Therefore, TXA2 and PGI2 contents can be determined by measuring TXB2 and 6-keto-PGF1α levels. ACS induces an increase in plasma TXA2, and resulting in enhanced platelet aggregation[13], which simultaneously damages vascular endothelial cells, reduces PGI2 synthesis and secretion of vascular endothelial cells, induces a decrease in its inhibitory effects on platelet aggregation, and then platelet develops activation and aggregation.

Results from the present study (Table 2) have indicated that thromboxane B2 levels were increased, but 6-keto-prostaglandin levels were diminished following implantation in both groups ($P < 0.05$). These suggested that the mechanical stimulation of coronary artery stent implantation contributes to platelet aggregation and vasoconstriction, and confirmed that vascular endothelial function was damaged once following surgery in patients undergoing stent implantation. Thromboxane B2 levels were reduced, whereas 6-keto-prostaglandin levels were increased following 10 days of Chinese medicine treatment ($P < 0.05$). These indicated that postoperative curative effects are thought to take place through inhibition of platelet aggregation and improvement of vasomotoricity in ACS patients, and it was more significant in the *Danhong* injection group compared with the conventional treatment group ($P < 0.01$). *Danhong* could significantly improve vascular endothelial function in ACS patients following stent implantation, induce vasodilatation, improve blood supply, inhibit platelet aggregation, and show the tendency of ameliorating short-term prognosis. The mechanisms may be as follows: protection of vascular endothelial cells, increased PGI2 synthesis and secretion; inhibitory effects of endothelial cells against

platelet aggregation are increased, which induces a decrease in TXA2 production to reach a new dynamic equilibrium.

The interventional therapy of coronary artery obviously improves the prognosis of ACS patients[14]. The case-fatality rate of coronary artery disease was not significantly decreased. In particular, the high incidence rate of cardiac events should be solved following stent implantation such as acute and subacute thrombosis. It is proposed that the mechanisms may be associated with abnormal secretion of vascular endothelium-derived factors following endothelial damage, which may result in poor feedback of intercellular layer smooth muscle and capillary vessels on ischemia-diastole[15-18]. Present coronary artery disease second-level prevention ABC schedule and ACS treatment instructions did not show above-mentioned mechanism drugs and measures[15-18].

ACS belongs to traditional Chinese medicine "thoracic obstruction", whose pathogenesis is *Qi* stagnation and turbid phlegm, cardiac blood stasis, besides *Qi*-blood *Yin* and *Yang* deficiencies as well as visceral dysfunction. *Danhong* injection activates blood circulation to dissipate blood stasis and promotes blood circulation and collaterals. Western medicine studies suggested that main components of *Danhong* include tanshinone, salvianolic acid, safflower red, safflower phenolic glycosides and so on. *Danhong* can protect vascular endothelium, promote vascular regeneration and resist hemagglutinin, and has been widely used in clinical therapy for cardio-cerebrovascular disease. Nevertheless, there are few studies concerning action mechanisms of endothelial functional lesion in ACS patients following stent implantation.

Results from this study have shown that *Danhong* dramatically promotes vascular endothelial function in ACS patients following stent implantation, with a tendency of improving short-term prognosis. Therefore, the protective effects on plaque endothelial function at the target vessel lesion and other regions following stent implantation may be a major mechanism of *Danhong* improving symptom and long-term prognosis following stent implantation. This action may be take place through multiple pathways, and this is the superiority of many beneficial compound components of *Danhong*.

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丹红注射液对冠状动脉支架置入患者血管内皮功能的影响*

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摘要

背景：众多研究证实丹参能抑制中性粒细胞的黏附、聚集及氧自由基的产生，具有活血化瘀的疗效，但目前对于其保护血管内皮以及抗血栓形成具体机制，尤其是在冠状动脉支架置入后作用机制的研究很少。

目的：观察丹红注射液对急性冠脉综合征患者冠状动脉支架置入前后血管内皮功能的影响。

方法：采用随机对照方法将 60 例急性冠脉综合征患者分为 2 组，常规治疗组在冠状动脉支架置入后给予常规药物治疗；丹红组在冠状动脉支架置入后给予常规药物治疗基础上加用丹红注射液 20 mL/d，疗程 10 d。支架置入前后及丹红治疗前后测定内皮依赖性血流介导的血管舒张反应、血浆内皮素、血栓烷 B2、6-酮-前列腺素水平，双重评价其血管内皮功能；同时观察两组患者近期预后。

结果与结论：与支架置入前比较，支架置入后两组患者血浆内皮素、血栓烷 B2 均明显提高，6-酮-前列腺素水平明显下降($P < 0.05$)。与支架置入后比较，两组治疗后血浆内皮素、血栓烷 B2 均明显降低($P < 0.05$，$P < 0.01$)；内皮依赖性血流介导的血管舒张反应、6-酮-前列腺素明显提高($P < 0.05$，$P < 0.01$)，丹红组更明显($P < 0.05$)。住院期间丹红组心血管事件发生较常规治疗组略有下降，但差异无显著性意义($P > 0.05$)。结果表明丹红注射液可显著改善支架置入急性冠脉综合征患者血管内皮功能，使血管舒张，改善供血，抑制血小板聚集，且有改善近期预后的倾向。

关键词：介入治疗；急性冠脉综合征；血管内皮功能；丹红注射液；支架置入；心血管植入物

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