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EXPERIMENTAL STUDY

Microvascular pathological features and changes in related injury factors in a rat acute blood stasis model

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Abstract

OBJECTIVE: To examine the microvascular pathological characteristics and changes in related injury factors in a rat model of acute blood stasis.

METHODS: A total of 75 Sprague-Dawley rats were divided randomly and equally into a control group and four experimental groups assessed at different times after the induction of stasis (0, 1, 3 or 6 h after stasis) (n = 15). The acute blood stasis model was established through rat tail-vein injection of high-molecular-weight dextran. After Electrocardiograph (ECG) detection at predetermined times (0, 1, 3 and 6 h after induction of stasis), the rats were sacrificed and blood and cardiac samples were harvested for analysis. Hematoxylin-eosin (HE) staining and transmission electron microscopy were used for histopathological detection; an enzyme linked immunosorbent assay (ELISA) was used to detect thromboxane B2 (TXB2) and 6-keto-prostaglandin F1α (6-Keto-PGF1α) concentrations; a real-time polymerase chain reaction (PCR) reaction system was used to detect intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule1 (VCAM-1) mRNA expression; western blotting was used to detect vascular endothelial cadherin (VE-cadherin) protein expression.

RESULTS: The ST segment in the ECG showed gradual elevation after induction of stasis and continued elevation at a high level at 3 and 6 h. The HE staining showed changes in myocardial cell necrosis and tissue dissociation after the induction of stasis, along with inflammatory infiltration. Results of transmission electron microscopy showed immediate changes in blood stasis and lumen occlusion in the microvasculature, along with endothelial cell swelling. After the induction of stasis, TXB_2 concentrations gradually increased while 6-Keto-$PGF_{1\alpha}$ concentrations were immediately significantly reduced. The TXB_2/6-Keto-$PGF_{1\alpha}$ ratio was maintained at a high level. ICAM-1 mRNA expression showed an unstable elevation while VCAM-1 mRNA expression was significantly reduced after the induction of stasis. Compared with the control group, VE-cadherin protein expression increased at 0 and 3 h after the induction of stasis, while no change occurred at 1 and 6 h.

CONCLUSION: The pathological manifestations of acute blood stasis are microvascular blood retention, lumen stenosis and even occlusion. The condition is also called "blood coagulation and weep" in Traditional Chinese Medicine. The blood stasis model resulted in the injury and necrosis of endothelial cells and cardiomyocytes, along with the presence of an imbalance of vasomotor factor levels, platelet activation, and increases in the expression of adhesion molecules and endothelial barrier dysfunction, which corresponds to "blood failed to nourish" in Traditional Chinese Medicine.

Key words: Blood stasis; Acute-phase reaction; Microvessels; Endothelial cells; Medicine, Chinese traditional

INTRODUCTION

Blood stasis is a common pathological mechanism in Traditional Chinese Medicine (TCM), but is also the common pathological basis of cardiovascular disease. The concept of blood stasis includes two types: poor blood stasis and blood running outside meridians. This paper focuses on poor blood stasis. The TCM classic *Huang Di Nei Jing* does not propose the concept of "blood stasis",[1] but repeatedly mentions "blood coagulation and weep" to highlight the pathological mechanisms of blood barriers. Around 220 AD, the *Shang Han Lun* officially proposed "blood stasis" as a disease.[2] In the 14th century, *Pu Ji Fang* stressed that blood stasis plays a major role in the development of chronic diseases.[3] TCM physicians Ye Tianshi in the 17th century and Wang Qingren in the 18th century highlighted the significance of blood stasis in the vein in disease. Previous theories correlated the collateral vasculature and the microvasculature,[4] in terms of the nature of blood stasis, and from a modern scientific point of view, blood stasis is closely related with the microcirculation.[5] High-molecular-weight dextran (HMWD) features a high molecular weight and high viscosity, and can form a stable bridge between erythrocytes, for which the surface force is greater than the surface repulsion of the electric charge, thereby inducing erythrocyte aggregation, platelet aggregation, and an increase in blood flow resistance. An infusion of HMWD for 5 min will result in microcirculatory dysfunction and a gradually worsening microcirculatory situation.[6] Therefore, it is the ideal drug to model for poor blood stasis. In this study, we conducted a tail-vein injection of HMWD in rats, as an acute blood stasis model, to study the pathological characteristics of the microvasculature, and changes in related injury factors. The objective of this study was to assess the physiological changes of blood stasis, a classic TCM pathological state.

MATERIALS AND METHODOLOGY

Animals

A total of 75 male Sprague-Dawley rats of specific-pathogen-free grade (Beijing, Certificate No. SCXK 2012-0001), weighing 280-300 g, 10 weeks old, were provided by the Laboratory Animal Center, Chinese PLA General Hospital. The rats were permitted a 3 d acclimatization period with a normal feeding regime before the experiment. Rats were fed and treated in accordance with the guidelines of the animal experiments committee of PLA General Hospital. Protocols and surgical procedures were approved by the PLA General Hospital Animal Ethics Committee.

Acute blood stasis model in rats

After 3 days of acclimatization, the rats were anesthetized using an intraperitoneal injection of 0.3% sodium pentobarbital (1 mL/100 g body weight; Sigma, St. Louis, MO, USA), and then fixed in a supine position. Electrodes were connected to the limbs to monitor the electrocardiogram (ECG). The acute blood stasis model was established through a rat tail-vein injection of 6% HMWD (0.8 mL/100 g body weight; HMWD 500, Amresco, OH, USA). After the induction of stasis, the rats curled and were agitated, and demonstrated a reduced response to external stimuli. ST segment elevation proved that the induction of stasis was successful.

Experimental groups

The rats (n = 75) were divided randomly and equally into a control group, and four experimental groups assessed at different times after the induction of stasis (0, 1, 3 or 6 h after stasis) (n = 15). The control group rats were injected with saline (0.8 mL/100 g body weight) through the tail vein, and the remaining procedures were consistent with the other experimental groups. ECG was assessed at predetermined times (0, 1, 3 and 6 h after the induction of stasis), and abdominal aortic blood and cardiac samples were harvested from each group.

Optical microscopy

Two rats demonstrating successful induction of stasis were selected randomly in each group. The rats underwent carotid artery intubation. The heart was perfused using 4% paraformaldehyde and was removed. The excised hearts underwent 10% formalin fixation and conventional dehydration until transparent. The hearts were embedded in paraffin wax, and sectioned at a thickness of 5 μm. Hematoxylin-eosin (HE) staining was then conducted. Briefly, the paraffin sections underwent conventional dewaxing followed by hematoxylin staining. The sections were then washed using water, and underwent 1% hydrochloric acid alcohol differentiation and anti-blue and eosin staining. Sections were dehydrated until transparent and then mounted using neutral gum and observed using a light microscope.

Transmission electron microscopy

Three rats were selected randomly in each group. Abdominal aortic blood samples were harvested and the heart was removed immediately and placed in an ice bath. A piece of tissue (1 mm × 1 mm × 2 mm) was removed from the left and right ventricle, respectively. The specimens were fixed using 2.5% glutaraldehyde for 2 h and were then washed using phosphate buffer