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Effects of Xuezhikang (Red Yeast Rice) on Blood lipids, Hemorheology and the Expression of P65 and Tissue Factor in Atherosclerotic Rats

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ABSTRACT

Objective: To observe the effects of Xuezhikang (red yeast rice) on blood lipids, blood rheology, and expression of P65 and tissue factor, and to explore the anti-atherosclerosis effect and related mechanisms of Xuezhikang (red yeast rice). **Methods:** 32 Wistar rats were randomly divided into normal control group, Xuezhikang treatment group, lovastatin treatment group and atherosclerosis model group (8 in each group). Blood lipids, blood rheology, malondialdehyde (MDA), total antioxidant capacity (T-AOC), and expression of aortic tissue factor (TF) and P65 were measured in each group. **Results:** (1) Both Xuezhikang and lovastatin could reduce blood lipid levels, but there was no significant difference between the two groups; (2) Both Xuezhikang and lovastatin can improve the hemorheology of atherosclerotic rats, but the difference between the two groups is not significant; (3) Compared with lovastatin, Xuezhikang inhibited the expression of TF and P65 in aorta of rats with atherosclerosis; (4) Compared with lovastatin, the Xuezhikang group had lower MDA levels and higher T-AOC. **Conclusion:** Xuezhikang can improve blood lipid levels and hemorheology in rats with atherosclerosis. Compared with lovastatin, Xuezhikang has stronger effects on inhibiting oxidative stress and down-regulating the expression of tissue factor and P65.

1. Introduction

Xuezhikang is a kind of special red yeast rice containing lovastatin and its homologues. It is also rich in various biologically active substances

such as unsaturated fatty acids, flavonoids, ergosterol and alkaloids.^[1] Large-scale clinical trial China coronary secondary prevention study (CCSPS) confirmed that, Xuezhikang can significantly reduce the incidence of non-fatal

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myocardial infarction and coronary heart disease death in patients with coronary heart disease, reduce the need for PCI and / or CABG, and reduce tumor death and total death from various causes.^[2] Compared with foreign secondary coronary heart disease prevention tests such as 4S, CARE and LIPID, although CCSPPS has a smaller reduction in TC and LDL-C, however, there is a clear advantage in reducing the overall mortality rate, the incidence of coronary heart disease events and reducing the need for PCI and CABG, suggesting that Xuezhikang has cardiovascular protection independent of lipid-lowering.^[2] A large number of studies have shown that Xuezhikang also has cardiovascular protection effects such as anti-inflammatory, anti-oxidation, protection of endothelial function and improvement of plaque stability. However, whether Xuezhikang has an effect on blood rheology and tissue factor expression and its mechanism is still unclear.

2. Materials and Methods

2.1 Main Drugs and Reagents

Xuezhikang original drug solution is provided by Peking University Weixin Pharmaceutical Co., Ltd.; Lovastatin by Beijing Wansheng Pharmaceutical Co., Ltd.; High-fat feed is produced by Beijing Keao Xieli Feed Co., Ltd.; Vitamin D3 injection (Shanghai General Pharmaceutical Co., Ltd., Batch No.: 081006); Malondialdehyde (MDA) kit (Nanjing Institute of Bioengineering, Batch No.: 20091222); Total Antioxidant Capacity (T-AOC) Kit (Nanjing Institute of Bioengineering Batch No.: 20091222); TF Rabbit Polyclonal Antibody (Santa Cruz, American); P65 Rabbit Polyclonal Antibody (Santa Cruz, American); β -actin Mouse Monoclonal Antibody (Santa Cruz, American).

2.2 Animal Model Constructing Methods and Grouping

Thirty-two male SPF-grade Wistar rats weighing 200±20 g were provided by the Animal Center of the Chinese Academy of Military Medical Sciences. After two weeks of adaptive feeding in a standard environment, they were randomly divided into control group, Xuezhikang treatment, lovastatin treatment and model group. Rats were induced atherosclerosis using a high-fat diet combined with vitamin D.^[3] The high fat diet formula is: 3% cholesterol, 0.5% sodium cholate, 5% refined sugar, 10% lard and 0.2% propylthiouracil. On the first day of high-fat diet feeding, rats in the model group, Xuezhikang treatment group and lovastatin treatment group were given intraperitoneal injection of vitamin D3 (6 × 10⁵ u/kg body weight). The control group was given normal feed and intraperitoneal injection of equal dose of normal saline. Among them, 8 rats in the control group were given normal saline for

6 weeks, and given normal saline (300 mg/Kg/day) for 6 weeks. 8 rats in the model group were fed with high-fat diet for 6 weeks, and then given normal saline (300 mg/Kg/day) for 6 weeks. 8 rats in Xuezhikang treatment group, 6 weeks after high-fat feeding, were given Xuezhikang (300mg/Kg/day) for 6 weeks; 8 rats in the lovastatin treatment group were given lovastatin (2.5 mg/Kg/day) for 6 weeks after 6 weeks of high-fat diet. Animals in each group were fasted overnight, anesthetized with intraperitoneal injection of pentobarbital sodium, the abdominal aorta was isolated, and arterial blood was punctured below the bifurcation of the abdominal aorta. 5 ml of whole blood was added with heparin (20 U/ml) for anticoagulation, and 1 ml of whole blood was anticoagulated with 3.28% sodium citrate (the volume ratio of sodium citrate to whole blood was 1:9).

2.3 Blood Lipid Determination

Heparin anticoagulated whole blood was centrifuged at 3000 rpm for 10 min to take plasma. Plasma triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were detected enzymatically using a Hitachi 7600-110 automatic biochemical analyzer.

2.4 Blood Rheology Determination

Heparin anticoagulation was performed, and both the whole blood high-cut viscosity and low-cut viscosity were measured by LBY2N6A self-cleaning rotary viscometer (Beijing Plyson Co., Ltd.); Whole blood was centrifuged at 3000 rpm for 10 min, plasma was taken, and plasma viscosity was measured. The deformation index (DI) of red blood cells in whole blood was measured in the range of 50-1000 s⁻¹ by using LBY2-BX2 laser diffractometer (Beijing Plyson Co., Ltd.); Hematocrit (HCT) was measured using a micro-pressure tube.

2.5 MDA and T-AOC Detection

Rat plasma MDA and T-AOC levels were measured according to the kit instructions. Plasma MDA levels were measured by TBA. Plasma T-AOC is reflected by detecting the level at which Fe³⁺ is reduced to Fe²⁺.

2.6 The Expression of Aortic Tissue Factor (TF) and P65

Take 100 mg of aortic tissue, 1 ml of tissue lysate, homogenize, centrifuge at 13 °g for 20 min at 4 ° C, detect the protein concentration by Bradford method, and add an equal volume of 2×SDS gel loading buffer to 100 µg of protein/lane. Mix and boil for 5-10 min to denature the protein, centrifuge at 12000 rpm for 5 min, then load in the order. 80V laminated glue, 120V separation gel,

electrophoresis separation of protein, 200mA2h transfer film. 5% skim milk powder was blocked for 4 h, primary antibody was added, and the membrane was washed 3 times for 15 min at 4 °C for 8 h. The horseradish peroxidase-labeled secondary antibody was added for 1 h, and the membrane was washed 3 times with PBST for 15 min. And after electrochemiluminescence with enhanced chemiluminescence (ECL), the strip was placed in an Alpha Imager™ 2200 image analysis processing system. Using Alpha Ease 40 analysis software, the computer directly scans and determines the integrated optical density value of the developed strip. The integrated optical density value (IDV) of the strip indicates the expression level of the protein, and the relative expression of the target protein in each sample is calculated (TF/β-actin and P65/β-actin).

3. Statistical Processing

Using SPSS13.0 software, the measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). The mean difference between groups was tested by one-way ANOVA. $P < 0.05$ was used to indicate the difference between the groups.

4. Results

4.1 Changes in Blood Lipids

Compared with the control group, TC, LDL-C and HDL-C were significantly increased in the atherosclerosis model group ($P < 0.05$), but there was no difference in TG between the two groups. Compared with the model group, the plasma levels of TG, TC and LDL-C in the Xuezhikang and lovastatin-treated rats were significantly

lower than those in the model group ($P < 0.01, 0.05, 0.05$), but there was no significant change in HDL-C. Compared with the lovastatin group, the mean values of TG, TC and LDL-C in the Xuezhikang group were lower, but the difference was not statistically significant. The results are shown in Table 1.

4.2 Blood Rheology

Compared with the control group, the plasma viscosity and whole blood high-cut and low-cut viscosity of the model group were significantly increased, and the red blood cell deformation index was significantly decreased ($P < 0.05$). Xuezhikang and lovastatin can reduce the plasma viscosity and whole blood viscosity of rats with atherosclerosis and increase the deformability of red blood cells, the difference is significant ($P < 0.05$). There were no significant differences in plasma viscosity, whole blood viscosity and erythrocyte deformability between Xuezhikang group and lovastatin group. There was no significant difference in hematocrit between the groups. The results are shown in Table 2.

4.3 Plasma T-AOC and MDA

The plasma MDA levels of the rats in each group after 6 weeks of drug intervention are shown in Figure 1. Compared with the control group, the MDA level in the plasma of the model group was significantly increased ($P < 0.05$). Both Xuezhikang and lovastatin reduced plasma MDA levels in atherosclerotic rats ($P < 0.05$). Plasma MDA levels in the Xuezhikang group were significantly lower than those in the lovastatin group ($P < 0.05$). The plasma T-AOC levels of the rats in each group after 6 weeks of drug

Table 1. Plasma levels of TC, TG, HDL-C and LDL-C in each group (\pm SD, mmol/L)

Groups	TG	TC	LDL-C	HDL-C
Control Group	0.64±0.06	1.82±0.18	0.17±.003	0.64±0.06
Model Group	0.67±0.19	12.75±1.61 ^a	5.36±1.02 ^a	1.12±0.32 ^a
Lovastatin Group	0.31±0.11 ^b	8.79±1.75 ^b	3.80±1.08 ^b	0.96±0.33
Xuezhikang Group	0.29±0.16 ^b	7.68±1.02 ^b	3.42±0.56 ^b	0.94±0.31

Notes: a $P < 0.05$ compared with the control group; b $P < 0.05$ compared with the model group; c $P < 0.05$ compared with the lovastatin group.

Table 2. Blood flow characteristics of each group of rats (\pm SD)

Groups	Plasma viscosity (mPa·s)	whole blood viscosity (mPa·s)			Red blood cell deformability	Hematocrit
		Shear rate=50/S ⁻¹	Shear rate=100/S ⁻¹	Shear rate=150/S ⁻¹		
Control Group	1.56±0.15	4.72±0.68	4.34±0.64	3.99±0.48	0.36±0.01	0.42±0.01
Model Group	1.81±0.16 ^a	5.47±0.41 ^a	4.91±0.39 ^a	4.58±0.33 ^a	0.31±0.02a	0.41±0.01
Lovastatin Group	1.68±0.16 ^b	4.66±0.59 ^b	4.17±0.45 ^b	3.97±0.39 ^b	0.35±0.01b	0.41±0.01
Xuezhikang Group	1.54±0.20 ^b	4.50±0.26 ^b	4.04±0.19 ^b	3.87±0.20 ^b	0.35±0.01b	0.41±0.02

Notes: a $P < 0.05$ compared with the control group; b $P < 0.05$ compared with the model group; c $P < 0.05$ compared with the lovastatin group.

intervention are shown in Figure 2. Plasma T-AOC was significantly lower in the model group compared with the control group ($P < 0.05$). Both Xuezhikang and lovastatin increased plasma T-AOC in atherosclerotic rats ($P < 0.05$). The plasma T-AOC of Xuezhikang group was higher than that of lovastatin group ($P < 0.05$).

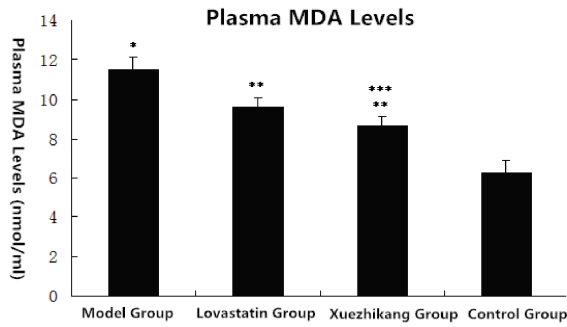


Figure 1. Plasma MDA Levels of rats in each group

Notes: * $P < 0.05$ compared with the control group; ** $P < 0.05$ compared with the model group; *** $P < 0.05$ compared with the lovastatin group.

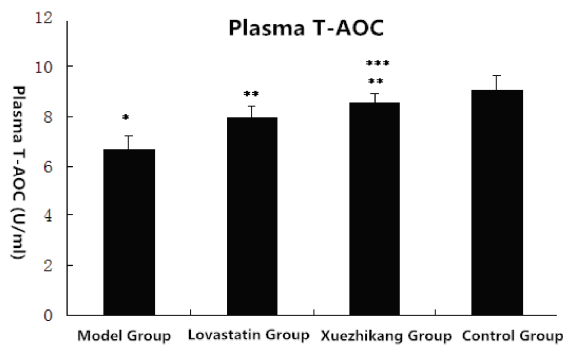


Figure 2. Total plasma antioxidant capacity (T-AOC) of rats in each group

Notes: * $P < 0.05$ compared with the control group; ** $P < 0.05$ compared with the model group; *** $P < 0.05$ compared with the lovastatin group.

3.4 The Expression of Aortic Tissue Factor (TF) and P65

The expression of aortic TF in each group of rats is shown in Figure 3. Compared with the control group, the expression of aortic TF in the model group was significantly increased ($P < 0.05$). Compared with the model group, the expression of aortic TF in the Xuezhikang group and the lovastatin group was significantly lower ($P < 0.05$). Compared with lovastatin, Xuezhikang inhibited the expression of TF in rat aorta ($P < 0.05$). The expression of P65 in the aorta of each group was shown in Figure 4. Compared with the control group, the expression of P65 in the aorta

of the model group was significantly increased ($P < 0.05$). Compared with the model group, the expression of P65 in the aorta of the Xuezhikang group and the lovastatin group was significantly lower ($P < 0.05$). Compared with lovastatin, Xuezhikang inhibited the expression of P65 in rat aorta ($P < 0.05$).

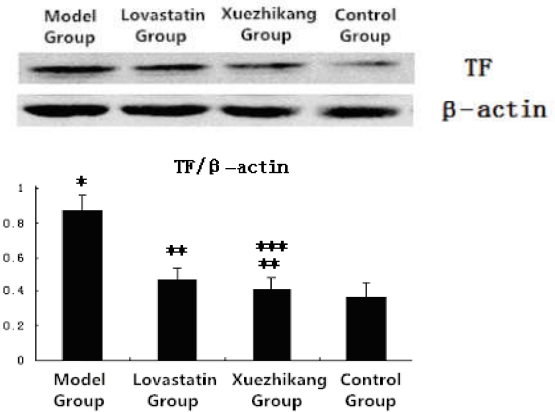


Figure 3. Expression of aortic TF in rats of each group after 6 weeks of drug intervention

Notes: * $P < 0.05$ compared with the control group; ** $P < 0.05$ compared with the model group; *** $P < 0.05$ compared with the lovastatin group.

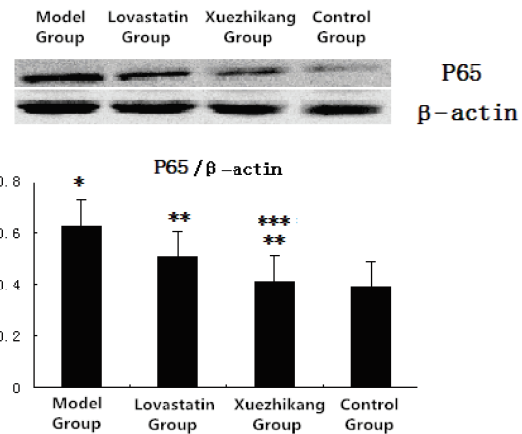


Figure 4. Expression of aortic P65 in rats of each group after 6 weeks of drug intervention

Notes: * $P < 0.05$ compared with the control group; ** $P < 0.05$ compared with the model group; *** $P < 0.05$ compared with the lovastatin group.

5. Discussion

The main component of Xuezhikang is lovastatin and its homologues, which reduces the synthesis of cholesterol and accelerates the clearance of LDL by increasing the activity of low-density lipoprotein (LDL) receptors on the

surface of hepatocytes through feedback regulation, thereby achieving a blood lipid regulation effect that lowers serum cholesterol.^[4] In addition to statin lipid-lowering drugs, Xuezhikang also contains various components such as unsaturated fatty acids and sterols, which have synergistic effects, which makes it have better lipid-lowering effect than the same dose of lovastatin.^[4] This research confirmed that Xuezhikang can reduce plasma TG, TC and LDL-C levels. In this research, Xuezhikang's lipid-lowering effect was similar to that of lovastatin, suggesting that lovastatin is the main effective lipid-lowering component of Xuezhikang.

The blood viscosity reflects the inherent resistance of blood flow in the blood vessels. When the blood viscosity increases, the blood flow velocity slows down, and thrombosis is easy to occur and promote the formation of atherosclerotic plaque. Whole blood viscosity is determined by hematocrit, plasma viscosity, erythrocyte aggregation, and erythrocyte deformability, while plasma viscosity is primarily determined by fibrinogen, macromolecular lipoprotein, and blood lipids. The aggregation of red blood cells can be represented by low shear rate whole blood viscosity, which is related to the concentration of bridging proteins in the blood such as fibrinogen and macromolecular lipoprotein.^[5] The increase in plasma viscosity and erythrocyte aggregation in AS rats is caused by an increase in blood lipid, fibrinogen, and lipoprotein concentrations. The deformability of red blood cells is determined by the composition of the erythrocyte membrane and the skeletal protein of red blood cells.^[6] Studies have shown that plasma cholesterol concentration, lipid peroxidation and other factors can affect the composition and structure of red blood cell membrane, change the deformability of erythrocyte membrane, affecting the rheological properties of blood.^[7] Compared with the control group, the plasma viscosity and whole blood viscosity of the AS group increased, and the red blood cell deformability decreased; There was no significant difference in hematocrit between the two groups, suggesting that the increase in whole blood viscosity is mainly related to plasma viscosity, erythrocyte aggregation and erythrocyte deformability. In this research, plasma cholesterol and MDA concentrations were elevated in the AS group, while T-AOC was decreased, which may be the main cause of the decrease in the deformability of red blood cells. The effect of Xuezhikang on blood viscosity is mainly related to its effect on lowering blood lipid levels and fibrinogen concentration, and the effect of increasing red blood cell deformability is mainly related to its effect of lowering plasma cholesterol, reducing lipid peroxidation and increasing T-AOC. The effect of Xuezhikang on the plasma viscosi-

ty, whole blood viscosity and erythrocyte deformability of AS rats was not stronger than that of the same dose of lovastatin, suggesting that the abnormality of hemorheology in AS rats is mainly caused by dyslipidemia.

In this research, compared with the control group, the AS model rats had elevated MDA levels and decreased T-AOC, which was associated with increased expression of P65 in AS rats. Xuezhikang and lovastatin improve oxidative stress in AS rats, lower MDA in plasma and increase T-AOC levels. Compared with lovastatin, the blood lipids in the Xuezhikang group had lower MDA content and higher T-AOC. Oxidative stress causes lipid peroxidation, endothelial dysfunction, smooth muscle migration and proliferation, degradation of extracellular matrix, and promotes the formation and progression of AS.^[8] In addition, oxidative stress can lead to platelet activation, increased expression of tissue factor, decreased red blood cell deformability, increased plasma and whole blood viscosity, and the body is in a hypercoagulable state, promoting the formation of arterial thrombosis.^[9] What's more, Xuezhikang is rich in unsaturated fatty acids, flavonoids, ergosterol, alkaloids and other substances with obvious antioxidant effects, which can improve blood coagulation by inhibiting platelet aggregation, improving blood rheology and inhibiting the expression of tissue factor. This research suggests that Xuezhikang has a stronger antioxidant effect than lovastatin.

Tissue factor is a promoter of the coagulation cascade and is closely related to the occurrence and progression of atherosclerosis. In addition to coagulation function, it also has the effects of promoting inflammation and regulating angiogenesis.^[10] Compared with the control group, the expression of aortic TF in the AS model group was significantly increased. Xuezhikang and lovastatin can inhibit the expression of TF in aortic atherosclerosis rats, and the inhibitory effect of Xuezhikang is stronger than that of lovastatin. The 5' upstream promoter of the tissue factor structural gene includes 2 activated protein-1 (AP-1), 1 κB, 3 early growth response-1 (Egr-1) and 5 Sp1 binding sites.^[11] Among them, the nuclear transcription factor NF-κB plays an important regulatory role in the expression of tissue factor in atherosclerotic disease, and c-Rel/P65 is the major NF-κB subtype that regulates the expression of tissue factor.^[7] Compared with the control group, the expression of P65 in the aorta of the AS group was significantly increased. Xuezhikang inhibited the expression of P65 in aorta of AS rats more than the same dose of lovastatin.

6. Conclusion

Xuezhikang can reduce blood lipid levels in AS rats and

improve blood rheology. Compared with the same dose of lovastatin, Xuezhikang has stronger antioxidant capacity and can better inhibit the expression of aortic TF and P65.

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