

Impact of Lycium Barbarum Polysaccharide and Danshensu on vascular endothelial growth factor in the process of retinal neovascularization of rabbit

Xue-Min Tian¹, Rui Wang¹, Bai-Ke Zhang¹, Chun-Lei Wang¹, Hao Guo¹, Shi-Jin Zhang²

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¹Department of Ophthalmology, No.153 Hospital of Liberation Army, Zhengzhou 450042, Henan Province, China

²Department of Traditional Chinese Medicine, People's Hospital of Zhengzhou, Zhengzhou 450003, Henan Province, China

Correspondence to: Shi-Jin Zhang, Department of Traditional Chinese Medicine, People's Hospital of Zhengzhou, Zhengzhou 450003, Henan Province, China. zzwyszj@163.com

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Abstract

• **AIM:** To discuss the impact of Lycium Barbarum Polysaccharide (LBP) and Danshensu purified from Traditional Chinese Medicine (TCM) on vascular endothelial growth factor (VEGF) of rabbits with retinal neovascularization.

• **METHODS:** Forty rabbits were divided into normal control group, model control group, LBP group and Danshensu group. Animals in the normal control group were fed in the normal oxygen environment. Animals in the other three groups were put into the environment with 70% oxygen for 5 days in order to build the model of oxygen-induced vascular proliferation retinopathy. And then different TCM extract was injected into the abdominal cavities of these animals. After 7 days, the VEGF content of in the serum of rabbit was measured by double antibody sandwich method.

• **RESULTS:** Data analysis indicated that VEGF content was as follows: Danshensu group was lower than model control group ($12.92 \pm 3.84 \text{ ng/L}$ vs $19.32 \pm 4.15 \text{ ng/L}$, $P < 0.05$); LBP group and normal control group were lower than model control group ($12.92 \pm 3.84 \text{ ng/L}$, $9.26 \pm 1.61 \text{ ng/L}$ vs $19.32 \pm 4.15 \text{ ng/L}$, $P < 0.01$); total blood viscosity, plasma viscosity, cholesterol content, fibrinogen content and triacylglycerol content after peritoneal injection of LBP and Danshensu were obviously lower than before injection.

• **CONCLUSION:** TCM extract-LBP and Danshensu can prominently reduce the content of VEGF in the process of vascular proliferative retinopathy of rabbit; can prevent the occurrence of retinal microvascular disease by improving partial oxygen-deficient environment or affecting all kinds of new growth factor.

• **KEYWORDS:** Lycium Barbarum Polysaccharide; Danshensu; vascular endothelial growth factor; retinal neovascularization; rabbit

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INTRODUCTION

Retinal neovascularization disease, *i.e.* vascular proliferative retinopathy is a common clinical eye disease which can cause blinding. Its main clinical manifestation is retinal neovascularization, however, its formation mechanism is not clear until now^[1], and currently there are no ideal cure measures or methods. Study found that many growth factors such as vascular endothelial growth factor (VEGF), endothelin, endothelin, *etc.* have a close relation with the breaking of blood-retinal barrier^[2-4]. Among these growth factors, VEGF should be valued particularly for its unique biological effects. VEGF is a kind of soluble protein which can promote vascular endothelial cell division and proliferation and also can improve vasopermeability, and it can adjust itself and other retinal cells' proliferation and function by synthesis many kinds of growth factor. In addition, VEGF plays a key role in the process of pathological and physiological neovascularization.

The effects of lowering VEGF level by Lycium Barbarum Polysaccharide (LBP) and by Danshensu to treat retinal neovascularization disease and bring down VEGF level were observed and compared. Then this paper further discusses the impact of VEGF on retinal microvascular lesions in the process of retinal neovascularization.

MATERIALS AND METHODS

Materials The experiment was conducted in Animal Experimental Center of Zhengzhou University from July, 2011 to March, 2012. Laboratory animal: Forty NZW rabbits in clean grade, (Certificate number: 0005318), 4 months old, half male, half female, raised by Animal Experimental Center of Zhengzhou University. Medicine: Compound Danshen injection (Jiangxi Tianshikang Traditional Chinese Medicine Co., Ltd., China; Content of Danshensu: 1.86mg/mL; lot number: 011104-1); LBP (Shaanxi Ruikang

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Bioengineering Co., Ltd., China; molecular weight: 1112100 D; lot number: 980340); Reagent: Monoclonal rabbit anti-mouse VEGF antibody (bought from Beijing Xinxingtang Biotechnology Co., Ltd., China).

Methods Forty rabbits were randomly divided into normal control group, model control group, LBP group and Danshensu group. Rabbit vascular proliferative retinopathy model was built through modeling. The animals in normal control group were raised in normal environment, while the other 3 groups were put in hermetic containers wherein oxygen partial pressure was controlled at 70% and kept for 5 consecutive days; next all the rabbits were raised in normal oxygen environment for 7 days, then Optical Coherence Tomography (OCT) was utilized to determine whether the model was successfully built or not. The successfully modeled animals were raised in normal environment. The LBP group was given peritoneal injection by the standard of 10mg/kg per day; the Danshensu group was given peritoneal injection of 10mL/kg compound Danshen injection per day. Normal control group and normal model group were given peritoneal injection of Normal Saline by the same volume per day. Seven days later, eyeball blood was taken from all the experimental animals, and double antibody sandwich method was used to measure the content of VEGF in rabbit serum. Before feeding, venous blood was taken from rabbit ears. After using heparin to prevent coagulation, blood viscosity was detected by IS-C automatic blood viscosity survey meter. And OLYMPus-Au 400 automatic biochemical analyzer was used to measure the cholesterol level, fibrinogen level and triacylglycerol level of rabbit venous blood.

Main observational indexes: each rabbit group's VEGF content in serum; total blood viscosity, plasma viscosity, cholesterol content, fibrinogen content and triacylglycerol content in venous blood in the process of rabbit retinal neovascularization.

Statistical Analysis All the experimental data were analyzed by SPSS 13.0 software, and physiologic index were expressed as mean±SD, measurement data difference between groups was compared by *t*-test.

RESULTS

Experimental animals grouping Forty included laboratory rabbits were divided into 4 groups, which were normal control group, model control group, LBP group and Danshensu group, 10 rabbits per group, and all of them were included in the results analysis.

VEGF contents in rabbit serum of each group Data analysis found that VEGF contents in rabbit new vessel were as follows: Danshensu group was lower than model control group (12.92±3.84ng/L vs 19.32±4.15ng/L, $P<0.05$); LBP group and normal control group were lower than model control group (12.92±3.84ng/L, 9.26±1.61ng/L vs 19.32±4.15ng/L, $P<0.01$) (Table 1).

Table 1 VEGF contents in rabbit serum ($\bar{x} \pm s$, $n=10$)

Groups	VEGF(ng/L)
normal control	9.26±1.61
model control	19.32±4.15 ^d
LBP	15.47±2.71 ^a
Danshensu	12.92±3.84 ^b

^a $P<0.05$, ^b $P<0.01$ vs model control group; ^d $P<0.01$ vs normal control group.

The test results of blood viscosity and blood-fat level before and after peritoneal injection of TCM extract in the process of rabbit retinal neovascularization were shown in Table 2.

Data analysis indicated that the impacts of LBP and Danshensu on each physiologic index in the process of rabbit retinal neovascularization were as follows: total blood viscosity, plasma viscosity, cholesterol content, fibrinogen content and triacylglycerol content after peritoneal injection of LBP and Danshensu were obviously lower than those before injection. This showed that LBP and Danshensu significantly reduced total blood viscosity, plasma viscosity, cholesterol content, fibrinogen content and triacylglycerol content in the process of rabbit retinal neovascularization.

DISCUSSION

VEGF, which is called vascular permeability factor, is a kind of vascular endothelial cell mitogen with high degree of specificity, and is also a kind of autocrine/paracrine growth factor. VEGF, after combined with its receptor, can increase vasopermeability, stimulate multiplication, migration of endothelial cell and formation of intracorporal blood vessel. Some research indicated that as long as the drug VEGF Trap, which functioned on VEGF receptor, combined with VEGFR-2, it can inhibit the expression of VEGFR-2, which can restrain the formation of dog retinal new vessels effectively.

VEGF plays an important role in the formation and development of retinal neovascularization. Currently it was considered that VEGF is a key stimulation factor of formation of eye newvessels [5]. Injection of VEGF siRNA plasmid is a common adopted method for retinal neovascularization disease in clinic, main methods include partial spot injection, subconjunctival injection, subretinal injection and intravitreal injection [6-9]. Anti-VEGF treatment has double function of anti-neovascularization and effusion, through inhibition of VEGF to improve vasopermeability and further improve eye sight [10]. Chinese wolfberry was used to repair liver, improve vision, prevent and cure cataract and other ophthalmology disease in traditional and modern medicine. Having various biological activities like hypoglycemic effect, hypolipemic, antioxidation, inhibition of apoptosis, LBP is one of effective components of Chinese wolfberry [11]. It has the function of removing blood stasis and relieving pain. Its main chemical component is water soluble Danshensu. At present, the application of Danshensu in diabetic retinopathy is frequently reported. The pathological

Table 2 Test results of blood viscosity and blood lipid level before and after peritoneal injection in the process of rabbit retinal neovascularization

Test items	LBP		Danshen	
	Before injection	After injection	Before injection	After injection
Total blood viscosity	4.59±0.26	3.86±0.21 ^b	4.48±0.21	3.93±0.21 ^d
Plasma viscosity	2.35±0.22	1.86±0.09 ^b	2.47±0.26	1.79±0.12 ^d
Total cholesterol(mmol/L)	6.56±0.31	5.61±0.43 ^b	6.35±0.27	5.73±0.46 ^d
Fibrinogen(g/L)	5.95±0.28	5.08±0.34 ^b	5.84±0.23	5.26±0.48 ^d
Triacylglycerol(mmol/L)	2.14±0.22	1.52±0.18 ^b	2.52±0.26	1.47±0.16 ^d

t=2.885-4.587, ^b*P*<0.01 vs before peritoneal injection of LBP; *t*=2.885-4.587, ^d*P*<0.01 vs before peritoneal injection of Danshen.

basis of diabetic retinopathy is the damage of blood-retina barrier, protein and water in serum coming into substance layer of retina, which leads to remarkable expansion of the extracellular space of retinal cells and retinal thickening^[12,13]. Danshen injection contains an effective component-Danshensu which can remove blood stasis. Danshensu has functions such as eliminating free radical, suppressing the ultra-oxidation of free radical on biomembrane lipid layer, lessening formation of lipid peroxidation and accelerating the eliminating of lipid peroxidation.

The study investigates the impact of LBP and Danshensu retorted from TCM on VEGF of rabbit which has retinal neovascularization, and compares the physiologic index such as blood viscosity and blood-fat level, *etc.* before and after peritoneal injection of TCM extract in the process of rabbit retinal neovascularization. The results show that VEGF contents were as follows: Danshensu group was lower than model control group (12.92±3.84ng/L vs 19.32±4.15ng/L, *P*<0.05); LBP group and normal control group were lower than model control group (12.92±3.84 ng/L, 9.26±1.61 ng/L vs 19.32±4.15 ng/L, *P*<0.01). Total blood viscosity, plasma viscosity, cholesterol content, fibrinogen content and triacylglycerol content after peritoneal injection of LBP and Danshensu were obviously lower than before injection.

In retinal neovascularization disease, the newly-grow micrangium is composed of mere endothelial cell, without muscular layer and exosporium, lacking structural support. Meantime, vascular wall is relatively thin and connections of cytoplasm are not close. All these might be important pathological basis of clinical manifestations of retinal neovascularization disease such as haemorrhage and effusion. The increase of VEGF and abnormal pathological new vessels might lead to maldistribution of tightly-connect proteins and increase of retinal effusion^[14]. Experimental results show that LBP and Danshensu can prominently reduce VEGF content and then reduce the occurrence rate of rabbit retinal neovascularization.

Experimental results show that both LBP and Danshensu can reduce VEGF content of rabbit having vascular proliferative retinopathy, and the function of Danshensu is better than LBP. Therefore, in future clinical application, we should further explore the intervention mechanism and function of TCM extract on retinal neovascularization from multi-disciplinary perspectives such as morphology, molecular biology, pathology, *etc.*

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