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Effect of β-aescin extract from Chinese Buckeye Seed on chronic venous insufficiency

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The aim of this study was to explore the mechanism of domestic β-aescin treating chronic venous insufficiency through observing its actions on the isolated canine saphenous venous tension, venous pressure, venous return and lymphatic return. The isolated canine spiral saphenous venous tension test was performed to detect the activity of the β-aescin. Furthermore, in the condition of constant canine femoral artery perfusion kept in the extracorporeal circulation, we measured the changes of the canine femoral artery pressure, femoral artery flow and the lymphatic return flow after intravenous injection of the agent. The results showed that when β-aescin was administrated at the dose between $5.0 \times 10^{-5} \sim 5.25 \times 10^{-4}$ mol/L, it increased obviously the contractile tension of the venous to norepinephrine in a dose-dependent manner. With canine femoral artery perfusion kept constant, β-aescin, whose doses were 50 mg and 100 mg, reinforced intently the canine femoral venous tension accelerated the rise of the venous pressure. These finding suggested that domestic β-aescin extracted from Chinese Buckeye Seed had an effect on chronic venous insufficiency by strengthening the venous tension, increasing the venous pressure and promoting venous return and lymphatic return.

1. Introduction

β-Aescin sodium, a triterpene saponin, is one of the major active compounds from the Traditional Herbal Medicine horse chestnut *(Aesculus hippocastanum)* seed, and has been widely used for the treatment of edema and inflammation for many years in European countries (Sirtori 2001). Recently the compound has also been extracted from Chinese Buckeye Seed (*Semen Aesculi*). Therefore, to assess whether domestic β-aescin has an effect on chronic venous insufficiency and provide the experimental evidence for promoting the clinical application of domestic β-aescin in China, we conducted the study to explore the effect of domestic β-aescin on chronic venous insufficiency by observing its actions on the tension of isolated canine saphenous vein, venous pressure, venous return and lymphatic return.

2. Investigations and results

2.1. Effect of β-aescin on the tension of isolated canine saphenous vein

β-Aescin-induced tension of venous strip was expressed as a percentage data of 1×10^{-5}mol/L norepinephrine-induced maximum constriction force. As shown in Table 1, β-aescin remarkably increased contraction tension in the isolated canine saphenous vein strip. Moreover, the contractions tension significantly increased as β-aescin concentration augmented, showing a clearly dose-dependent manner (r = 0.9874).

2.2. Effect of β-aescin on canine femoral venous pressure

Carotid-femoral artery extracorporeal circulation system was conducted to keep canine femoral artery perfusion constant in seven experimental canines. After venous return was blocked by clamping its femoral vein, the pressure of femoral venous increased gradually. Moreover, the pressure returned back to normal immediately following release of venous clamp. After the venous return was blocked, the venous pressure increased significantly in response to two different doses of β-aescin, respectively. However, there were no any electrocardiographic changes in experimental canine during the treatment (Table 2).

Table 1: Effect of β-aescin on the tension of canine isolated saphenous vein

β-Aescin 10^{-5} mol/L	N	Rate of tension changes* (%)
5. 0	8	7.629 ± 10. 037
9. 0	8	12.024 ± 10. 212
16. 2	8	21.426 ± 7. 309
29. 2	8	35.047 ± 9. 314
52. 5	8	41.659 ± 11. 309

y = −19.583 + 35.6721gx r = 0.9874, y = 9.108x-3.767 r = 0.974.

2.3. Effect of β-aescin on canine venous return and lymphatic return

As shown in Table 3, β-aescin could significantly increase canine venous return and lymphatic return flow in seven experimental canines.

Table 2: Effect of β-aescin on canine femoral venous pressure

Groups	Time (min)	Venous pressure during venous clamping (mmHg)					
		0s	10s	20s	30s	45s	60s
50 mg	0	5.8 ± 3.1	11.7 ± 4.2	12.3 ± 4.1	18.1 ± 5.1	20.2 ± 5.3	22.1 ± 5.9
	2	6.2 ± 3.2	12.6 ± 4.1	13.2 ± 4.3	19.9 ± 5.3	21.3 ± 5.5	23.1 ± 6.1
	5	6.8 ± 3.4	13.1 ± 6.6	14.8 ± 3.7	20.7 ± 5.7	22.7 ± 6.1	23.7 ± 6.2
	15	8.2 ± 3.7	14.2 ± 5.4	15.6 ± 4.2	21.2 ± 5.4	23.1 ± 6.3	24.2 ± 5.7
100 mg	0	5.8 ± 3.1	11.7 ± 4.2	12.3 ± 4.1	18.1 ± 5.1	20.2 ± 5.3	22.1 ± 5.9
	2	7.5 ± 2.9	13.3 ± 4.3	14.8 ± 4.6	20.6 ± 4.4	22.4 ± 4.9	24.1 ± 6.2
	5	8.8 ± 3.6	15.7 ± 5.9	16.4 ± 5.3	22.9 ± 4.5	25.1 ± 5.1	25.5 ± 5.7
	15	13.8 ± 3.7	17.2 ± 6.3	19.7 ± 6.4	24.2 ± 5.2	26.5 ± 5.2	26.7 ± 6.1

Table 3: Effect of β-aescin on canine venous return and lymphatic return (X ± SD, N = 7)

Dose	Femoral venous flow (ml/min)		Lymphatic return flow (ml/min)	
	Pre-administration	Post-administration	Pre-administration	Post-administration
50 mg	78.7 ± 11.1	107.9 ± 16.7*	5.6 ± 2.1	8.1 ± 2.3*
100 mg	78.7 ± 11.1	118.6 ± 17.2*	5.6 ± 2.1	9.5 ± 2.5*

* $P < 0.05$ vs. Pre-administration

3. Discussion

Aescin, a natural mixture of triterpene saponins, has α and β isomers, among which β-aescin is the main active component. β-Aescin was originally extracted from horse chestnut *(Aesculus hippocastanum)* seed to treat exudative inflammation (Sirtori 2001). In Russia, Japan and other countries of Europe, β-aescin is a medication drug in clinic for the treatment of haemorrhoids and edema caused by traumatism, cerebral vascular accident and post-operation. Moreover, β-aescin has an effect on venous diseases, such as varicose vein of lower limb and venous reflux disease (Montopoli et al. 2007; Wang et al. 2009; Carrasco and Vidrio 2007; Kushida et al. 2003). Although β-aescin used in our study was isolated from Chinese Buckeye Seed (*Semen Aesculi*), it also increased venous tension and venous pressure, accelerated the rise of the venous pressure, promoted venous return and lymphatic return flow according to our *ex vitro* and *in vivo* results. These data confirmed that domestic β-aescin extracted from *Semen Aesculi* had similar pharmacological effects to that from horse chestnut seed (Robert 2003; Bielanski and Bielanski 2003). Correspondingly, our results suggested that domestic β-aescin might be a potential candidate agent for chronic venous disease in clinic.

Many studies have proven that β-aescin has an effect on chronic venous insufficiency, which might be associated with an increase in prostaglandin F2a and corticosteroids, an inhibition in prostaglandin G1, and an improvement in cell and blood vessel permeability (Hu et al. 2004; Li et al. 2009). Furthermore, β-aescin increases venous contractile activity by enhancing the sensitivity of calcium ion channels and other inflammation-related molecules such as 5-hydroxytryptamine (5-HT) (Sirtori 2001; Kushida et al. 2003; Bielanski and Bielanski 2003). In inflammatory processes, poor oxygen supply to where it matters induces a deficiency in mitochondrial oxidative phosphorylation reaction and ATP content. Moreover, some inflammation factor actions including prostaglandin (PG) release, platelet-activating factor (PAF) accumulation, and neutrophil adhesion and activation, mediate venous stagnation and edema under inflammation conditions. Nevertheless, β-aescin antagonizes the reduction of ATP content and augmentation of phospholipase A2 (PLA2) that stimulates the secretion of the potent inflammatory mediator precursors. Thus, the mechanism of β-aescin on protecting vein and reducing oedema was due to, at least in part, its beneficial role on inflammation.

4. Experimental

4.1. Main reagents

β-Aescin sodium was purchased from Wuhan Aimin Pharmaceutical Company (China). Modified Krebs-Henseleit buffer (mM) (NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25; glucose 11) was freshly prepared to obtain following final concentration: 5.0, 9.0, 16.2, 29.2, 52.5×10^{-5} mol/L.

4.2. Main equipment

An isolated organ perfusion device was provided by the Instrument Factory of Tongji Medical College (China). It was composed of the peristaltic pump, temperature controller, organ bath, polyethylene or silicone catheter tube, three-way valve, etc. Desktop balance recorder and tension transducer (L6510) were obtained from LINSEIS company (Germany). The electrocardiogram machine (HXD-3B) was purchased from Shanghai Medical Electronic Instrument Factory. Electronic peristaltic pump (LDB-M) was made by Zhejiang Xiangshan Dingshan instrument Factory. Physiological polysomnography, Magnetic Flow meter and other equipment were also used in our experiment.

4.3. Animals

Healthy mature mongrel canines without gender restrictions, weighing from 15 to 30 kg, were provided by the Laboratory Animals Department of Tongji Medical College, Huazhong University of Science and Technology (China). All canines passed animal quarantine, and had a balanced diet with tap water *ad libitum*.

4.4. Detection of contractile tension of isolated spiral saphenous venous strip

Experimental canines were anesthetized with sodium pentobarbital (30 mg/kg body weight, iv). Then the saphenous vein of anesthetized canines was carefully exposed. After a proximal saphenous vein strip with 2 cm was selected, all attached strap muscle, surrounding fascia and connective tissues were removed. Then, the spiral saphenous venous strip was fixed in organ baths containing 10 ml of modified Krebs-Henseleit solution. Organ bath solutions were maintained at 37 ± 1 °C and continuously aerated with 95% O_2 and 5% CO_2. The spiral saphenous venous strip was balanced in organ baths for 60–120 min. Before the experiment started, the spiral saphenous vein strip was preload with 50 mg. The tension activity during isometric contractions was recorded through a tension transducer connected to the automatic balance recording meter. 1×10^{-5}mol/L norepinephrine-induced maximal contraction was normalized as 100%. Accordingly, β-aescin-induced contractile tension values were expressed as a percentage of norepinephrine-induced maximum force. Additionally, another dose was not added until the saphenous venous strip had no response to the previous dose. Referring to guiding principles for preclinical studies of New drug and Traditional Chinese medicine (Pharmacy, Pharmacology and Toxicology) as well as previous studies, the dose range and dose group assignment were determined. According to preliminary experiment, we had five dose groups in our study and final concentration of β-aescin was 5.0, 9.0, 16.2, 29.2, 52.5×10^{-5} mol/L, respectively.

4.5. Determination of canine femoral venous pressure

Experimental canines were anesthetized with sodium pentobarbital (2 mg/kg body weight, iv), and their back was fixed, breathing naturally. The canine femoral artery was perfused through a peristaltic pump which was used for establishing a carotid-femoral artery extracorporeal circulation system. When the proximal part of the canine femoral vein was blocked, vein pressure was measured through a pressure transducer inserted via small branching saphenous veins. Meanwhile, the continually changing electrocardiogram (EKG) of the canine was monitored. β-Aescin was given to canine via the femoral artery in peristaltic pump. The femoral vein was clamped for 1 min before and 2, 5, 15 min after 50 mg or 100 mg β-aescin administration in the canine at indicated time points. The femoral venous pressure changes were observed and all related parameters were recorded in the polygraph.

4.6. Determination of canine venous return and lymphatic return

Canines were anesthetized with sodium pentobarbital (2 mg/kg body weight, iv), and then fixed for skin preparation. The venous pressure and arterial pressure were observed by jugular vein and carotid artery intubation, respectively. Thoracotomy was performed along the third intercostal space, and then thoracic duct was separated and inserted a polyethylene catheter. Lymphatic fluid volume was recorded by measurement of lymph volume per minute. Femoral venous flow was determined with an Electromagnetic Flow meter. The continually changing electrocardiogram (EKG) of the canine was monitored. All experiments started 30 min after surgery, and all related parameters were recorded in the polygraph. Doses of 50 or 100 mg β-aescin were given to the canine brachial vein via infusion pump (0.4 ml/min × 15 min, constant rate)

4.7. Statistical analysis

Comparison between the groups were made by student *t* test, results were expressed as means ± SD. Significance was accepted at $P < 0.05$.

References

Bielanski TE, Piotrowski ZH (1999) Horse-chestnut seed extract for chronic venous insufficiency. J Fam Pract 48: 171–172.

Carrasco OF, Vidrio H (2007) Endothelium protectant and contractile effects of the antivaricose principle aescin in rat aorta. Vasc Pharmacol 47: 68 – 73.

Hu XM, Zhang Y, Zeng FD (2004) Effects of sodium beta-aescin on expression of adhesion molecules and migration of neutrophils after middle cerebral artery occlusion in rats. Acta Pharmacol Sin 25: 869 –875.

Kushida M, Takeuchi T, Fujita A, Hata F (2003) Dependence of Ca^{2+}-induced contraction on ATP in alpha-toxin-permeabilized preparations of rat femoral artery. J Pharmacol Sci 93: 171–179.

Li YY, Wang YG, Liu XY (2009) Progress on Aescin. J Neurol Neurorehabil (in Chinese) 6:147–150.

Montopoli M, Froldi G, Comelli MC, Prosdocimi M, Caparrotta L (2007) Aescin protection of human vascular endothelial cells exposed to cobalt chloride mimicked hypoxia and inflammatory stimuli. Planta Med 73: 285 – 288.

Robert WF (2000) Three treatments for chronic venous insufficiency: Aescin, hydroxyethylrutoside, and daflon. Angiology 51: 197–205.

Sirtori CR (2001) Aescin: pharmacology, Pharmacokinetics and therapeutic profile. Pharmacol Res 44: 183–193.

Wang T, Fu F, Zhang L, Han B, Zhu M, Zhang X (2009) Effects of aescin on acute inflammation and the immune system in mice. Pharmacol Rep 61: 697–704.