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Improving abnormal hemorheological parameters in aging guinea pigs by water-soluble extracts of *Salvia miltiorrhiza* Bunge

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Abstract

Salvia miltiorrhiza Bunge, known as Danshen in Chinese traditional medicine is effective at promoting blood circulation and removing (or decreasing) blood stasis. In the present study, we selected aging, 24-month-old guinea pigs as the animal experimental models and fed them a diet containing 75, 100 or 150 mg/(kg day) of water-soluble extract components of *Salvia miltiorrhiza* Bunge (WSm) for 28 days, respectively, in order to evaluate the effects of WSm on their abnormal hemorheological parameters.

The results showed that the blood biochemical parameters of the aging guinea pigs remained unaffected by orally given WSm compared to the controls, except that the fibrinogen levels of the group fed the high dose of WSm (150 mg/(kg day)) decreased. Aging guinea pigs fed a low dose of WSm (75 mg/(kg day)) showed no significant difference in hemorheological parameters. However, feeding of WSm at 100 mg/(kg day) (medium dose), significantly reduced erythrocyte membrane MDA levels, which probably increased erythrocyte deformability and decreased erythrocyte flow resistance, though no improvement in erythrocyte aggregation, blood viscosity, and blood viscoelasticity could be observed. Furthermore, when the dose reached 150 mg/(kg day) of WSm (high dose), a significant decrease in whole blood viscosity was observed at high, medium and low shear rates. Blood viscosity and viscoelasticity exhibited significant improvement in oscillatory measurements. Also, we found that the oxygen transport efficiency of whole blood increased.

Keywords: *Salvia miltiorrhiza*; Danshen; Aging; Blood viscosity; Erythrocyte deformability

1. Introduction

Hemorheology is the study of the flow of blood in relation to the pressure, flow volume, and resistance in blood vessel and includes blood viscosity, erythrocyte deformability, erythrocyte aggregability, and blood platelet aggregation. Over the past three decades, hemorheological impairment, in such forms as a rise in blood viscosity, plasma viscosity, fibrinogen levels, erythrocyte aggregation, and impaired erythrocyte deformability, has been observed in patients with cardiovascular diseases (Lowe et al., 2002; Lipowsky, 2005; Steiner et al., 2005), stroke (Ratnayake et al., 2000; Grasso, 2004), and hypertension (Sandhagen, 1999; Lip et al., 2001). Patients with neuropathies such glaucoma, Alzheimer's disease, and even hearing impairment show a marked association with hemorheological abnormalities (Hamard et al., 1992; Wen et al., 2000; Solert et al., 2000). We do not intend to discuss the extent of association between hemorheological abnormalities and diseases in this study. However, it is known that such abnormalities generally reflect the physiological stress leading to disease occurrence.

Danshen (*Salvia miltiorrhiza*, Sm) is widely used as a traditional Chinese medicine with a number of physiological benefits. Zhao et al. (1996) found that a *Salvia miltiorrhiza* injection could

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Nomenclature

AI	aggregate index of erythrocyte
DI	deformability index of erythrocyte
Hct	hematocrit
Hgb	hemoglobin
MCV	mean corpuscular cell volume of erythrocytes
MDA	malondialdehyde
Sm	*Salvia miltiorrhiza* Bunge
T_E	oxygen transport efficiency (or oxygen delivery index) of blood
T_K	erythrocyte rigidity (or internal viscosity of erythrocyte)
WSm	water-soluble extract components *of Salvia miltiorrhiza* Bunge

Greek letters

β	flow resistance of erythrocytes suspension
γ	shear rate of steadily flow
η	viscosity

scavenge the oxygen free radicals generated from an ischemia-reperfusion injury in the myocardium as effectively as SOD. Ding et al. (2005) demonstrated that *Salvia miltiorrhiza* and its major ingredient danshensu and salvianolic acid B significantly inhibited TNF-α induced increases in endothelial cell permeability. Additionally, Danshen is used in the treatment of hyperlipidemia and acute ischemic stroke in China (Wu et al., 2004; Ling et al., 2005). Although several studies have reported its beneficial effects in promoting blood circulation and removing (or decreasing) blood stasis, its detailed mechanism is not yet fully understood. Patients with hemorheological abnormalities are at a high risk physiologically and may develop cardiovascular diseases and become susceptible to cerebrovascular accidents and hypertension.

In our previous studies, we demonstrated that chronic administration of the water-soluble extract of *Salvia miltiorrhiza* Bunge (WSm) ameliorated CCl_4-mediated hepatic damage. This effect was related to the antioxidant properties of WSm, which decreased hepatic thiobarbituric acid-reactive substance (TBARS) and replenished GSH levels (Lee et al., 2003a,b). In addition, WSm also showed improvement in the hemodynamic state (including portal venous pressure, superior mesenteric artery blood flow, cardiac index, and total peripheral resistance) in bile duct ligation rats (Lee et al., 2003b). Our earlier studies (Lee et al., 2003a,b, 2006) demonstrated WSm's potential to protect against oxidant damage, a fact which should be taken into account in exploring its pivotal role in the pathogenesis and progression of relevant diseases. However, studies concerning WSm modulation of hemorheological parameters are scarce. Therefore, the objective of this work is to investigate the effect of WSm in the improvement of aging-induced hemorheological impairment. Additionally, a possible mechanism behind changes in hemorheological parameters is advanced and discussed in detail.

2. Materials and methods

2.1. Animals and treatment

After 1 week of acclimation, 48 guinea pigs, 24-month-old, were randomized and divided into four groups: a control group ($n = 12$) received a normal diet and sterile water, and three experimental groups representing low, medium, and high doses of WSm ($n = 12$, respectively for each group), respectively, received an equivalent diet and water with different amounts of WSm dried powder, i.e. low dose: 75 mg/(kg day); medium dose: 100 mg/(kg day) and high dose: 150 mg/(kg day). The experiment lasted 28 days after oral administration of WSm. At experiment end, all animals were sacrificed to collect blood samples from their hearts for subsequent hemorheological measurements. The animal experimental protocols were approved by the Animal Ethics Committee of the Taipei Medical University (no: LAC-94-0010).

2.2. Preparation of WSm

The aqueous extract of Sm (WSm) was prepared as described in our earlier report (Lee et al., 2003a,b) with some modification. The powder of Sm (400 g) root was mixed with three volumes of distilled water at room temperature with continuous shaking overnight. After filtration, the WSm was concentrated and lyophilized with an approximate yield of 51%. The WSm was stored at −20 °C until further use. The 20 μL of WSm (20 mg/mL, dissolved in distilled water) was analyzed by Hitachi HPLC system equipped with a photodiode array detector (L-2450) and a BioSil Aqu-ODS-W column (4.6 mm i.d. × 250 mm). The mobile phase was composed of distilled water (solvent A) and methanol (solvent B) with linear gradient elution from 0% solvent B (0 min) to 100% solvent B (50 min) and hold for 10 min. The flow rate was 1.0 mL/min. The wavelength was set at 290 nm.

2.3. Analysis of WSm constituents

Twenty microliters of WSm (20 mg/mL in distilled water) was analyzed by Hitachi HPLC (high performance liquid chromatography) system equipped with a photodiode array detector (L-2450) and a BioSil Aqu-ODS-W column (4.6 mm i.d. × 250 mm). The mobile phase was composed of distilled water (solvent A) and methanol (solvent B) with linear gradient elution from 0% solvent B (0 min) to 100% solvent B (50 min) and stand for 10 min. The flow rate was 1.0 mL/min. The wavelength was set at 290 nm. Analysis of the standard compounds of danshensu (β-3,4-dihydroxyphenol lactic acid) and salvianolic acid B was carried out under the same conditions.

2.4. Hematological measurements

Fresh blood samples from guinea pigs were collected into plastic test tubes containing EDTA (1.5 mg/mL) as an anticoag-