African Journal of Pharmacy and Pharmacology Vol. 5(10), pp. 1327-1330, 15 September, 2011
Available online http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.455
ISSN 1996-0816 ©2011 Academic Journals

Full Length Research Paper

Effect of *Salvia miltiorrhiza* extract on blood lipid, hydroxyproline levels, skeletal muscle Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in swimming exercise rats

Cao Yun[1] and Meng Tao[2]*

[1]Department of Sport, Zunyi Normal University, Zunyi, Guizhou 563002, China.
[2]Department of Physical Training, 3rd Military Medical University, Chongqing 400000, China.

Accepted 16 August, 2011

The purpose of the present study was to examine the effects of aqueous extract of *Salvia miltiorrhiza* on plasma lipid and hydroxyproline levels and skeletal muscle Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in swimming exercise rats. Adult Sprague-Dawley male rats, 3 months of age, were assigned randomly to a sedentary control (n = 10) or exercise (n = 10) group or SME-treatent groups (exercise + SME) (n = 10 in each group). Ten animals in sedentary control group did not receive any exercise. Animals in exercise group received exercise. Animals in SME-treatent groups (exercise + SME) received exercise orally given with *S. miltiorrhiza* extract (50, 100 or 150 mg/kg body weight), respectively. Result showed that aqueous extract of *S. miltiorrhiza* can decrease blood total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-c) and increase high-density lipoprotein cholesterol (HDL-c) levels, enhance hydroxyproline levels in bone, skeletal muscle, heart and lung, and skeletal muscle Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in swimming exercise rats.

Key words: *Salvia miltiorrhiza*, exercise, rat, blood lipid.

INTRODUCTION

Salvia miltiorrhiza Bunge (Danshen root) is a well-known plant used in traditional Chinese medicine to treat various entities, such as cardiovascular disease, angina pectoris, hyperlipidemia and acute ischemic stroke (Zhou et al., 2005; Cheng, 2007). Danshen extracts contain several constituents including water soluble phenolic acids and lipophilic tanshinones (Wang et al., 2007). Danshen contains the lipid-soluble tanshinone I (Tan I), tanshinone IIA (Tan IIA), cryptotanshinone and dihydrotan-shinone as well as the water-soluble danshensu and salvianolic acid B (Sal B) (Adams et al., 2006). Danshen inhibits platelet aggregation and promotes fibrinolysis (Zhou et al., 2005; Chan, 2001).

Physical exercise is a factor that has been suggested to be responsible for the increase in free radical-mediated reactions due to elevation in oxygen consumption and modified reduced nicotinamide-adenine dinucleotide: nicotinamide-adenine dinucleotide phosphate ratio due to lactate production being affected (Lovlin et al., 1987; Alessio and Goldfarb, 1988; Tiidus and Houston, 1994). However, there have been discrepancies among the studies investigating the peroxidative effects of physical exercise. This has been attributed partly to the various methods used to evaluate lipid peroxidation and partly to the exercise model or characteristics of the subjects chosen in these studies (Dekkers et al., 1996).

The purpose of the present study was to examine the effects of aqueous extract of *S. miltiorrhiza* on plasma lipid and hydroxyproline levels and skeletal muscle Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in swimming exercise rats.

MATERIALS AND METHODS

Preparation of aqueous extract of *S. miltiorrhiza*

Roots of *S. miltiorrhiza* was grind into fine powder and extracted with boiling water for 3 h and concentrated to half of the volume by

*Corresponding author. E-mail: mentao013@sina.cn.

Table 1. Effect of *S. miltiorrhiza* extract on blood TC and TG levels in rats.

Group	TC (mmol/L)	TG (mmol/L)	LDL-c (mmol/L)
Sedentary control	2.31 + 0.17	0.62 + 0.05	0.26 + 0.01
Exercise	2.29 + 0.13	0.64 + 0.05	0.25 + 0.02
SME-treatent (50 mg/kg BW)	2.22 + 0.21	0.58 + 0.04	0.22 + 0.02[c]
SME-treatent (100 mg/kg BW)	2.12 + 0.18[c]	0.54 + 0.04[c]	0.2 + 0.01[c]
SME-treatent (150 mg/kg BW)	2.01 + 0.17[c]	0.51 + 0.03[c]	0.18 + 0.02[c]

[c]$P < 0.05$, when compared with exercise group. Body weight (BW).

boiling in a water bath. The resulting extract was cooled and filtered. The filtrate was concentrated up to 100 ml on rotavapour under reduced pressure. The concentrated crude extract was lyophilized into powder (5 g) and used for the study.

Animals and exercise protocol

Adult Sprague-Dawley male rats, 3 months of age, were assigned randomly to a sedentary control (n = 10) or exercise (n = 10) group or SME-treatent groups (exercise + SME) (n = 10 in each group).

Ten animals in sedentary control group did not receive any exercise. Animals in exercise group received exercise. Animals in SME-treatent groups (exercise + SME) received exercise and were orally given with *S. miltiorrhiza* extract (50, 100 or 150 mg/kg body weight), respectively.

Swimming protocol for exercise: animals were subjected to swimming exercise and were made to swim in a tank with a dimension (150 × 90 × 70) (length × breath × height), filled with water to a depth of 30 to 45 cm, once per day between 08:30 and 9:00 h. Animals were acclimatized by making them to swim for 5 days prior to the commencement of the experimental schedule. The experimental animals were subjected to swimming exercise for 15 days for 30 min.

At the beginning and end of the experiment, blood and urine were collected from fasted animals. Rats were anesthetized using sodium pentobarbital (40 mg/kg, i.p.), and sacrificed. The bone, skeletal muscle, heart and lung were isolated quickly at the time of sacrifice, and one portion was immediately homogenized for subsequent determination of biochemical analysis.

Biochemical analysis

Total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-c) and increase high-density lipoprotein cholesterol (HDL-c) levels were measured by using commercially available kits. All procedures follow the manufacturer's instructions.

Level of hydroxyproline was measured using the test kit (from Nanjing Jiancheng Bio, China). Activity of Na^+-K^+-ATPase was determined by a slight modification of Jones et al. (1979) method. Digoxin-sensitive Na^+-K^+-ATPase activity was determined in a reaction medium containing in final concentrations, 50 mM HEPES, pH 7.5, 3 mM $MgCl_2$, 1 mM EGTA, 120 mM NaCl, 25 mM KC1, 20 mg membrane protein per ml and 3 mM Na_2ATP. The assay was run in duplicate with and without digoxin (700 nM). The inorganic phosphate liberated on the hydrolysis of the γ-phosphodiester bond of ATP was estimated colorimetrically. (Na^+-K^+)-ATPase activity was expressed as µmol inorganic phosphate liberated per mg membrane protein per hour.

Ca^{2+}-Mg^{2+}-ATPase activities were assayed by spectrophotometrically measuring the amount of inorganic phosphate liberated following incubation of the tissue extract with disodium ATP (Sigma, England) as in previous studies (Adebayo and Malomo, 2002).

Statistical analysis

Data are reported as means ± SD. Two-way analysis of variance was performed on all variables. The significance of the difference between the means obtained was determined by the Newman-Keuls test. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Dyslipidemia, including hypercholesterolemia, hypertriglyceridemia, or their combination, is a major risk factor for cardiovascular disease. Generally, dyslipidemia is characterized by increased fasting concentrations of TC, TG and (LDL-c), in conjunction with decreased concentrations of HDL-c (Varady and Jones, 2005). This study represent one of the first training studies to have both exclusively included girls in the subject pool and examined exercise training effects on blood lipids and lipoproteins.

There was no significant difference between blood TC, TG and HDL-c levels of sedentary control group or exercise group. The level of blood TC, TG and LDL-c levels in SME-treatent groups was significantly decreased by treatment of 50, 100 and 150 mg/kg body weight *S. miltiorrhiza* extract. In particular, treatment of higher dose of *S. miltiorrhiza* extract (150 mg/kg) further decreased blood TC, TG and LDL-c content (Table 1).

Exercise resulted in a significant ($P < 0.01$) decrease in blood HDL-c level in comparison to the sedentary control group. After administration of extract of *S. miltiorrhiza* to the exercise animals for 21 days, a significant ($P < 0.05$, $P < 0.01$) increase in blood HDL-c level was noted which was close to the sedentary control level (Table 2). This indicated that treatment of *S. miltiorrhiza* extract was beneficial for reducing blood lipid in training rats.

4-Hydroxyproline or hydroxyproline, is an uncommon amino acid, abbreviated as HYP, e.g., in protein data bank, hydroxyproline differs from proline by the presence of a hydroxyl (OH) group attached to the C (gamma) atom. Other hydroxyprolines also exist in nature, notably 2,3-cis 3,4-trans-dihydroxyproline, which occurs in diatom cell walls, and is postulated to have a role in silica

Table 2. Effect of *S. miltiorrhiza* extract on blood HDL-c level in rats.

Group	HDL-c (mmol/L)
Sedentary control	1.31 + 0.11
Exercise	1.53 + 0.13[b]
SME-treatent (50 mg/kg BW)	1.42 + 0.13
SME-treatent (100 mg/kg BW)	1.51 + 0.12[c]
SME-treatent (150 mg/kg BW)	1.58 + 0.11[d]

[b]$P < 0.01$, when compared with sedentary control group; [c]$P < 0.05$, [d]$P < 0.01$, when compared with exercise group. Body weight (BW).

Table 3. Effect of *S. miltiorrhiza* extract on hydroxyproline levels in bone, skeletal muscle, heart and lung of rats.

Group	Bone (mg/g)	Skeletal muscle	Heart	Lung
Sedentary control	0.038 + 0.004	0.029 + 0.001	0.031 + 0.003	0.036 + 0.002
Exercise	0.041 + 0.003	0.032 + 0.001	0.035 + 0.002	0.039 + 0.003
SME-treatent (50 mg/kg BW)	0.045 + 0.003	0.033 + 0.002	0.038 + 0.002	0.042 + 0.003
SME-treatent (100 mg/kg BW)	0.047 + 0.002[c]	0.037 + 0.002	0.043 + 0.003[c]	0.048 + 0.003[c]
SME-treatent (150 mg/kg BW)	0.05 + 0.003[c]	0.041 + 0.002[c]	0.046 + 0.002[d]	0.054 + 0.004[d]

[c]$P < 0.05$, [d]$P < 0.01$, when compared with exercise group. Body weight (BW).

deposition. Hydroxyproline is also found in the walls of oomycetes, fungus-like protists related to diatoms (Cundy et al., 1983). Hydroxyproline is produced by hydroxylation of the amino acid proline by the enzyme prolyl hydroxylase following protein synthesis (as a post-translational modification). Hydroxyproline is a major component of the protein collagen. Hydroxyproline and proline play key roles for collagen stability. They permit the sharp twisting of the collagen helix. It helps provide stability to the triple-helical structure of collagen by forming hydrogen bonds (Hofman et al., 2011).

Hydroxyproline levels in bone, skeletal muscle, heart and lung were elevated in the exercise group in comparison with the sedentary control group. After treatment with the aforementioned extract to the exercise animals, hydroxyproline levels in bone, skeletal muscle, heart and lung were significantly enhanced in a dose-dependent manner (Table 3). This indicated that treatment of *S. miltiorrhiza* extract could enhance protein collagen level in skeletal muscle and improve bone quality.

Na^+/K^+-ATPase activity is hardly detectable in homogenates of muscle biopsies, because of an excess of Mg^{2+}-ATPase activity. The K^+-dependent hydrolytic cleavage of the artificial substrate 3-0-MFP and its inhibition by ouabain can be used as a measure of Na^+/K^+-ATPase activity in muscle (Barwe et al., 2007). Muscle fibre-type composition and aging (14 to 58 years) had no effect on the K^+-dependent 3-0-MFPase activity (results not shown). The maximal ouabain-binding capacity, that is, the number of Na^+/K^+-ATPase molecules, of normal human skeletal muscle (360 + 70 pmol/g wet wt.; n = 5) agrees with earlier data (Nagel et al., 1987; Dey et al., 2010).

Fluctuation in intracellular Ca^{2+} levels are essential elements for normal cellular activities that are closely connected with the development of cells, mitotic activity, immune response, muscle contraction, endo- and exocytosis or modulation of neuronal cell processes. In animal cells, calcium homeostasis depends on the function of ATP-driven Ca^{2+}-pump that transport cytosolic free calcium into intracellular storage compartments. Amongst them, calcium ATPase (Ca^{2+}-ATPase) of the sarco-(endo-) plasmic reticulum (SERCA), a vesicular integral membrane protein, belongs to the family of P-type ion translocating ATPases. It pumps calcium ion from the cytoplasm into the SR against a large concentration gradient. Much of the earlier work with Ca^{2+}-ATPase was done in the presence of Mg^{2+}-ion and it is a widely accepted view that the complex Mg ATP actually serves as the substrate for the Ca^{2+}-ATPase reaction (Yamada and Ikemoto, 1980; Benech et al., 1991; Møller et al., 2005).

Table 4 shows the activities of skeletal muscle Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase in different group rats. Exercise significantly increased ($P < 0.01$) skeletal muscle Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in the exercise group rats 13 and 12%, respectively, when compared with those of the sedentary control group. With treatment of *S. miltiorrhiza* extract, the skeletal muscle Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in the rats increased from 7.925 + 0.548 and 6.729 + 0.472 to 9.254 + 0.482 and 7.936 + 0.616, and the increasing rate reached 17 and 18%, respectively, when compared with

Table 4. Effect of *S. miltiorrhiza* extract on skeletal muscle Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in rats.

Group	Na^+-K^+-ATPase (umol Pi/mg protein/h)	Ca^{2+}-Mg^{2+}-ATPase
Sedentary control	7.032 + 0.521	6.032 + 0.391
Exercise	7.925 + 0.548[b]	6.729 + 0.472[b]
SME-treatent (50 mg/kg BW)	8.174 + 0.412	7.165 + 0.417[c]
SME-treatent (100 mg/kg BW)	8.829 + 0.632[c]	7.382 + 0.529[c]
SME-treatent (150 mg/kg BW)	9.254 + 0.482[d]	7.936 + 0.616[d]

[b]$P < 0.01$, when compared with sedentary control group; [c]$P < 0.05$, [d]$P < 0.01$, when compared with exercise group. Body weight (BW).

those of the exercise group. This indicated that treatment of *S. miltiorrhiza* extract could stimulate ion transportation in skeletal muscle and increase contractability of skeletal muscle.

REFERENCES

Adams JD, Wang R, Yang J, Lien EJ (2006). Preclinical and clinical examinations of Salvia miltiorrhiza and its tanshinones in ischemic conditions. Chin Med., 1: 3.

Alessio HM, Goldfarb AH (1988) Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. J. Appl. Physiol., 64: 1333-1336

Barwe SP, Kim S, Rajasekaran SA, Bowie JU, Rajasekaran AK (2007). Janus Model of The Na,K-ATPase β-Subunit Transmembrane Domain: Distinct Faces Mediate α/β Assembly and β-β Homo-oligomerization. J. Mol. Biol., 365: 706-714.

Benech JC, Galina A, de Meis L (1991). Correlation between Ca2+ uptake, Ca^{2+} efflux and phosphoenzyme level in sarcoplasmic-reticulum vesicles, Biochem. J., 274: 427–432.

Chan TY (2001). Interaction between warfarin and danshen (Salvia miltiorrhiza). Ann Pharmacother, 35(4): 501-504.

Cheng TO (2007). Cardiovascular effects of Danshen. Int. J. Cardiol., 121(1): 9–22.

Cundy T, Bartlett M, Bishop M, Earnshaw M, Smith R, Kanis JA. (1983). Plasma hydroxyproline in uremia: Relationships with histologic and biochemical indices of bone turnover. Metab. Bone Dis. Relat. Res., 4: 297-303.

Dekkers JC, Van Doornen LJP, Kemper HCG (1996). The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. Sports Med., 21: 213-238.

Dey K, Chakraborti T, Roy S, Ghosh B, Kar P, Chakraborti S (2010). Identification, purification and partial characterization of a 70 kDa inhibitor protein of Na^+/K^+-ATPase from cytosol of pulmonary artery smooth muscle. Life Sci,, 86: 473-481.

Hofman K, Hall B, Cleaver H, Marshall S (2011). High-throughput quantification of hydroxyproline for determination of collagen. Anal. Biochem., 417: 289-291.

Jones LR, Besch HR, Flemming JW, McConnaugtey MM Watanabe AM (1979) Separation of vesicles of cardiac sarcolemma from vesicles of cardiac sarcoplasmic reticulum. Comparative biochemical analysis of component activities. J. Biol. Chem., 254: 530.

Lovlin R, Cottle W, Pyke I, Kavanagh M, Belcastro AN (1987) Are indices of free radical damage related to exercise intensity. Eur. J. Appl. Physiol., 56: 3l3-316

Møller JV, Nissen P, Sørensen TL-M, le Maire M (2005). Transport mechanism of sarcoplasmic reticulum Ca^{2+}-ATPase pump, Curr. Opin. Struct. Biol., 15: 387–393.

Nagel G, Fendler K, Grell E, Bamberg E (1987). Na^+ currents generated by the purified (Na^+ + K^+)-ATPase on planar lipid membranes. Biochim Biophys Acta (BBA) – Biomembranes, 901: 239-249.

Tiidus PM, Houston ME (1994) Antioxidant and oxidative enzyme adaptations to vitamin E deprivation and training. Med. Sci. Sports Exerc., 26: 354-359.

Varady KA, Jones PJH (2005). Combination diet and exercise interventions for the treatment of dyslipidemia: an effective preliminary strategy to lower cholesterol levels? J. Nutr., 135: 1829–1835.

Wang X, Morris-Natschke SL, Lee KH (2007). New developments in the chemistry and biology of the bioactive constituents of Tanshen. Med. Res. Rev., 27(1): 133–148.

Yamada S, Ikemoto N (1980). Reaction mechanism of calcium-ATPase of sarcoplasmic reticulum, J. Biol. Chem., 255: 3108–3119.

Zhou L, Zuo Z, Chow MSS (2005). Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J. Clin. Pharmacol., 45(12): 1345–1359.