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Extraction, characterization of *Angelica sinensis* polysaccharides and modulatory effect of the polysaccharides and Tai Chi exercise on oxidative injury in middle-aged women subjects

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ABSTRACT

The present study was designed to investigate the effect of *Angelica sinensis* polysaccharides (ASP) and Tai Chi exercise on free radical generation and lipid peroxidation and its modulatory effect on immunity activity in middle-aged women subjects at a dosage levels of 125 mg/kg. ASP was characterized by high performance liquid chromatography (HPLC). HPLC analysis showed that ASP was composed of eight kinds of monosaccharides, namely mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, arabinose and fucose in molar ratios of 1.2:4.5:1:10.5:17.8:37.5:8.7:4.9. Antioxidant activities such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) and lipid peroxidation levels in both ASP and Tai Chi exercise groups were significantly enhanced or reduced compared to the control group. In addition, immunity activities such as serum vascular cell adhesion molecule-1 (s-VCAM-1), interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) levels in both ASP and Ta Chi exercise groups were significantly reduced compared to the control group. It can be concluded that ASP intake and Tai Chi exercise decrease oxidant stress induced injury and improve immunity activities in middle-aged women subjects, indicating a obvious healthy promotion effect.

1. Introduction

The involvement of reactive oxygen species (ROS) in the aging process has been well documented (Berlett & Stadtman, 1997), and an enhanced oxidative stress in the elderly has been reported as being related to several pathologies such as neurodegenerative and vascular diseases (Mariani, Polidori, Cherubini, & Mecocci, 2005). Uncontrolled free radical production in aging is considered to be the result of both increased production of ROS – mainly generated in the organism as byproducts of normal cellular metabolism, especially through the mitochondria pathway (Balaban, Nemoto, & Finkel, 2005; Yu, Yin, Yang, & Liu, 2009) – and lowered antioxidant defenses.

The rhizome of *Angelica sinensis* (Oliv.) Diels (Umbelliferae), known as Dang-gui in Chinese herb, is one of the most important traditional Chinese herbs used as a sedative or a tonic agent (Hsu & Peacher, 1981). Its varieties of pharmacological effects include anti-oxidative, anti-inflammatory and immunomodulatory activities (Cao et al., 2008; Yan et al., 2007). The active components of *Angelica sinensis* (AS) include ferulic acid and polysaccharides, the main components found in the non-aromatic fractions. Liu et al. (2003) had been reported that ASP demonstrated a protective effect against free radical-mediated changes in rats with blood stasis. Long-term oral administration of ASP significantly reduced oxidative injury and improved immunity function.

Despite advances in medicine and clinical care, some age-related degenerative diseases continues to be the leading cause of mortality and morbidity in the world. Physical activity has been shown to reduce atherosclerotic risk factors by improving blood lipid profile, insulin resistance and glucose intolerance and psychosocial well-being; reducing stress, blood pressure and mortality; and aiding weight loss (Chen, Zhong, Zeng, & Ge, 2008; McQuillan, Hung, Beilby, Nidorf, & Thompson, 2001; Ruiz et al., 2005).

Tai Chi is a low impact, moderate-intensity exercise and has been practiced in China for hundreds of years. Recently, Tai Chi has gained considerable popularity as an alternative form of exercise. Tai Chi exercise has been associated with improvements in cardiorespiratory function, balance and muscular strength and endurance, flexibility, relaxation and mood state. In addition, lower blood pressure and improvement in aerobic capacity have been reported in persons with CHD (Yu, Wu, & Niu, 2009).

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The purpose of this study was to investigate the possible modulatory effect of ASP on immunity and antioxidant activity in middle-aged women subjects.

2. Materials and methods

2.1. Preparation of Angelica sinensis polysaccharides

The roots of *Angelica sinensis* were purchased from a Chinese medicine store in Shengyan, and stored at −20 °C. The dried roots (300 g) were grind to fine powder and pre-extracted with 2 L 95% ethanol to remove color materials. The plant residue was extracted with boiling water (4 L) for 3 h twice. The aqueous extract was centrifuged to remove water-insoluble materials and ultrafilted to remove small molecular substances using an ultrafiltion system including a pump and a hollow fiber microporous membrane cartridge (10 cm I.D × 100 cm length) with molecular weight cutoff 5000. The concentrated fraction was collected and washed twice with trichloromethane, twice with methanol to remove lipid and lyophilized to obtain the crude polysaccharide fraction (CKP, brown, 47 g).

2.2. Characterization of Angelica sinensis polysaccharides

The aqueous extract (polysaccharides) of *Angelica sinensis* was passed through a Millex-HV filter unit (13 mm, Millipore, Billerica, MA), and filtered using a 0.45-μm PVDF filter (Millipore) prior to injection onto high-performance liquid chromatograph (HPLC). The HPLC system consisted of, a Shimadzu LC-10AT VP pump, a Rheodyne 7725i injector, a 20-μl sample loop, a Shimadzu RID-10A detector and a Phase Sep-NH2 column (4.6 × 250 mm, 5 μm, Phase Separation Inc., Norwalk, CT). The mobile phase was 85 ml acetonitrile (LC grade, Tedia Co., Fairfield, OH)/15 ml deionised water at a flow rate of 1.0 ml/min. Each sugar was identified using the authentic sugar (Sigma Chemical Co., St. Louis, MO) and quantified by the calibration curve of the authentic compound.

2.3. Subjects

A sample of 90 female participants was evaluated between April and June 2006. Only persons ⩾55 y of age, living independently and generally healthy were invited to take part in the study. Generally healthy persons were defined as those whose physical, medical and mental statuses allowed them to travel and to participate in a comprehensive health examination conducted in a clinical setting (Table 1). Those taking anti-platelet, or antioxidant medication were excluded because of possible interaction with examination result, as were those who used nutritional supplements that potentially raised vitamin and mineral intake above the recommended safe limits. People with conditions that affect dietary absorption, such as Crohn's disease, were also excluded. This study was approved by the committee of ethics in research of Our University and all participants had to provide written informed consent and be available to attend one of the research centres.

2.4. Study design

The subjects were randomized to control group, polysaccharides group or exercise group. Each group contains 30 people. The control group receives usual care, including information on the possible benefits of physical activity. The polysaccharides-treatment groups received oral polysaccharides in a dose of 150 mg/kg body weight. The protocol consisted of taking the polysaccharides 3 times a day before meals for 3 months. Every participant in the exercise group practised Tai Chi twice daily for 30 mins. All participants were asked to continue their normal living routines without restricting their diets or altering their eating habits. We collected blood centrifuged at 3500 rpm for 15 min at 4 °C and supernatants for analysis at 3 months after subjects had fasted for at least 8 h.

Table 1
Information on elderly subjects.

Group	Age (year)	Height (cm)	Body weight (kg)	Heart rate (beats/min)
Control	47.09 ± 2.3	159.77 ± 3.56	63.2 ± 7.83	67.81 ± 4.24
Polysaccharides	49.4 ± 5.0	159.33 ± 3.73	59.9 ± 7.30	62.9 ± 13.6
Tai Chi	49.91 ± 7.1	160.41 ± 3.15	61.2 ± 8.11	64.1 ± 11.62

2.5. Analyses of serum antioxidant enzymes activities and lipid profiles

SOD, GSH-Px, CAT activities and malondialdehyde (MDA) level were determined using commercially available kits and according to the kits' manual. One unit of enzyme activity was defined as the amount of protein needed to decrease the reference rate to 50% of maximum inhibition.

Erythrocyte Na^+/K^+-ATPase activities was measured by an enzymatic method (Bozzo et al., 1990). Plasma GSH level was measured by Beutler's method (Beni, Fioritoni, Salvati, Tentori, & Torlontano, 1973).

Blood was analyzed for total cholesterol (TC), triacylglycerol, low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) in serum. The TC and triacylglycerol concentrations were measured enzymatically (Homma et al., 2003) on a Hitachi 7450 analyzer (Hitachi, Japan). The LDL-c concentrations were determined by homogeneous turbidimetric test (Yu, Che, Ma, & He, 2009), and HDL-c concentrations were determined by homogeneous enzymatic colorimetric assay (Zhao et al., 2007) on the same analyzer.

The levels of s-VCAM-1 were measured using a commercially available ELISA kit from R&D Systems using the manufacturer's published protocol. The secretion of IL-6, IL-1β and TNF-α in serum were measured by means of ELISA using commercial test kits.

2.6. Statistical analysis

The statistical analyses were performed using STATA software version 7 and SPSS software version 10.0 (SPSS, Chicago, IL, USA). Significance was set at a $P < 0.05$; P values were unadjusted for multiple testing. Estimates were provided with the 95% confidence interval between brackets.

3. Results and discussion

3.1. Analysis of composition of Angelica sinensis polysaccharides

On the basis of HPLC of standard samples (Fig. 1), HPLC analysis showed ASP was composed of eight kinds of monosaccharides, namely mannose (retention time 2.531), rhamnose (retention time 5.214), glucuronic acid (retention time 7.056), galacturonic acid (retention time 10.323), glucose (retention time 12.513), galactose (retention time 17.672), arabinose (retention time 22.681) and fucose (retention time 27.209) in molar ratios of 1.2:4.5:1:10.5:17.8:37.5:8.7:4.9 (Fig. 2).

3.2. Effect of polysaccharides and Tai Chi on serum SOD, CAT and GSH-Px activities in middle-aged women subjects

To assess whether antioxidant enzyme expression has been affected by polysaccharides and Tai Chi, serum SOD, CAT and GSH-Px activities were measured in middle-aged women subjects. As shown in Table 2, a significant increase in antioxidant enzyme

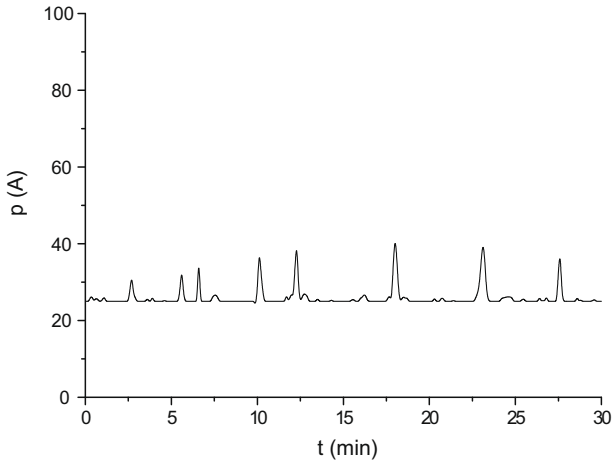

Fig. 1. HPLC of standard sample.

activities was observed in polysaccharides and Tai Chi groups ($P < 0.01$) compared to control group.

3.3. Effect of polysaccharides and Tai Chi on serum GSH and MDA levels in middle-aged women subjects

To assess whether non enzymatic antioxidant defense system and lipid peroxidation level have been affected by polysaccharides and Tai Chi, serum GSH and MDA levels were measured in middle-aged women subjects. As shown in Table 3, a significant increase in serum GSH level was observed in polysaccharides and Tai Chi groups ($P < 0.01$) compared to control group. In addition, a significant decline in serum MDA level was observed in polysaccharides and Tai Chi groups ($P < 0.01$) compared to control group. Furthermore, the effect of Tai Chi exercise was more potent than polysaccharides treatment (Table 3).

3.4. Effect of polysaccharides and Tai Chi on serum Na^+–K^+-ATP enzyme activity in middle-aged women subjects

Table 4 showed effect of polysaccharides and Tai Chi on serum Na^+–K^+-ATP enzyme activity in middle-aged women subjects. As shown in Table 4, a significant increase in serum Na^+–K^+-ATP enzyme activity was observed in polysaccharides and Tai Chi groups ($P < 0.01$) compared to control group.

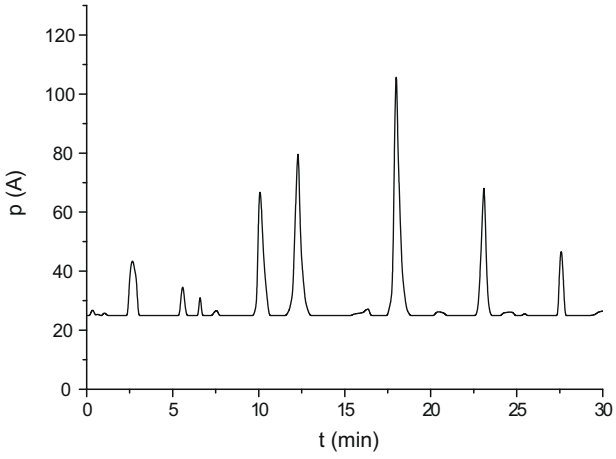

Fig. 2. HPLC of ASP.

Table 2
Serum SOD, CAT and GSH-Px activities in middle-aged women subjects.

Group	SOD (U/ml)	CAT (U/ml)	GSH-PX (U/ml)
Control	295.3 ± 22.2	13.27 ± 0.83	5.24 ± 0.15
Polysaccharides	437.4 ± 28.41[a]	25.16 ± 1.94[a]	8.23 ± 0.25[a]
Tai Chi	461.2 ± 33.4[a]	24.11 ± 1.41[a]	9.63 ± 0.44[a]

[a] $P < 0.01$, compared to control group.

Table 3
Serum GSH and MDA levels in middle-aged women subjects.

Group	GSH (mg/L)	MDA (nmol/ml)
Control	309.1 ± 21.4	17.28 ± 2.11
Polysaccharides	407.5 ± 34.3[a]	11.05 ± 1.14[a]
Tai Chi	431.2 ± 25.4[a]	13.17 ± 1.03[a]

[a] $P < 0.01$, compared to control group.

Table 4
Serum Na^+–K^+-ATP enzyme activity in middle-aged women subjects.

Group	Na^+–K^+-ATP enzyme activity (μmol Pi/10^7RBC h)
Control	2.815 ± 0.029
Polysaccharides	3.013 ± 0.014[a]
Tai Chi	3.097 ± 0.033[a]

[a] $P < 0.01$, compared to control group.

Table 5
Serum TC, TG, LDL-c and HDL-c levels in middle-aged women subjects.

Group	TC (mmol/L)	TG (mmol/L)	LDL-c (mmol/L)	HDL-c (mmol/L)
Control	3.73 ± 0.09	4.01 ± 0.07	1.09 ± 0.11	93.43 ± 2.83
Polysaccharides	3.35 ± 0.11[a]	3.72 ± 0.08[a]	0.87 ± 0.06[a]	118.38 ± 1.02[a]
Tai Chi	3.41 ± 0.08[a]	3.64 ± 0.05[a]	0.91 ± 0.04[a]	113.66 ± 0.79[a]

[a] $P < 0.01$, compared to control group.

Table 6
Serum s-VCAM-1, IL-1β, IL-6 and TNF-α levels in middle-aged women subjects.

Group	s-VCAM-1 (ng/ml)	IL-1β (ng/ml)	IL-6 (ng/ml)	TNF-α (ng/ml)
Control	479.3 ± 7.44	0.63 ± 0.04	1.11 ± 0.7	3.24 ± 0.12
Polysaccharides	458.69 ± 8.49[a]	0.55 ± 0.02[a]	0.91 ± 0.02[a]	2.81 ± 0.06[a]
Tai Chi	446.48 ± 4.31[a]	0.57 ± 0.03[a]	0.87 ± 0.05[a]	2.93 ± 0.09[a]

[a] $P < 0.01$, compared to control group.

3.5. Effect of polysaccharides and Tai Chi on serum TC, TG, LDL-c and HDL-c levels in middle-aged women subjects

To assess whether blood lipid metabolism has been affected by polysaccharides and Tai Chi, serum TC, TG, LDL-c and HDL-c levels were measured in middle-aged women subjects. As shown in Table 5, a significant decrease in serum TC, TG and LDL-c level was observed in polysaccharides and Tai Chi groups ($P < 0.01$) compared to control group. In addition, a significant increase in serum HDL-c level was observed in polysaccharides and Tai Chi groups ($P < 0.01$) compared to control group.

3.6. Effect of polysaccharides and Tai Chi on serum s-VCAM-1, IL-1β, IL-6 and TNF-α levels in middle-aged women subjects

To assess whether the immunity activity has been affected by polysaccharides and Tai Chi, serum s-VCAM-1, IL-1β, IL-6 and

TNF-α levels were measured in middle-aged women subjects. As shown in Table 6, a significant increase in serum s-VCAM-1, IL-1β, IL-6 and TNF-α levels was observed in polysaccharides and Tai Chi groups ($P < 0.01$) compared to control group.

4. Discussion

ROS are capable of causing lipid peroxidation, the oxidation of amino acid residues, the formation of protein–protein cross-links, and DNA oxidative damage. Under normal physiological conditions, ROS can be scavenged by the cellular defending systems, but under certain pathological conditions, the dynamic balance between the generation and elimination of ROS may be broken, causing increases in cellular ROS levels. High levels of ROS may do oxidative damage to various cellular components, and result in cell death (Choi & Koo, 2003; Liu et al., 2007).

Tai Chi is relatively inexpensive, as it requires no special facility or equipment, and can be performed either indoors or outdoors. Tai Chi can be performed by persons of all ages, regardless of previous exercise experience or ability (Hong, Li, & Robinson, 2000) and (Taylor-Piliae, 2003). Tai Chi exercise was reported to be positively associated with perceived self-efficacy and physical function among older adults (Harmer, Perrett, Cowen, & Goodwin, 2001; Li et al., 2001), suggesting that Tai Chi exercise may promote exercise adherence (Harmer et al., 2001). In the present study, Tai Chi exercise can reduce oxidative stress and improve antioxidant enzymes activities. This findings are in agreement with ‘s results. Our study of the effect of Tai Chi exercise on serum s-VCAM-1, IL-1β, IL-6 and TNF-α levels in middle-aged women suggests that the exercise is effective in improving immunity function. These results have been described in several species including humans (http://autoimmunedisease.suite101.com/article.cfm/tai_chi_enhances_immune_function; Irwin, 2007).

Recently, pharmacological researches disclosed that the ASP possessed substantial anti-inflammatory actions (Cao et al., 2008) and antioxidant (Yan et al., 2007). In the present work, we demonstrated that ASP could reduce oxidative stress and improve blood lipid metabolism in middle-aged women. The immunity-stimulating activity by antioxidants treatment has been found associated with oxidative stress (Alves de Sousa et al., 2007; Chi, Chen, Wang, Xiong, & Li, 2008; Niu, Wu, Yu, & Wang, 2008). Several markers of excess oxidative stress, such as an increase in ROS, accumulation of oxidized products like protein carbonyls from protein oxidation and aldehydes and isoprostanes from lipid peroxidation, confirms the direct role of antioxidants in the oxidative damage associated with some age-related degenerative diseases (Kemp et al., 2002; Meydani, 1999). The use of antioxidants has been recognized as an effective method in minimizing pathological and toxic effects associated with oxidative stress (Ariel et al., 2002; Poliandri, Cabilla, Velardez, Bodo, & Duvilanski, 2003). In this work, we could assume that ASP enhanced immunity function, at least in part, by reducing oxidative stress and stimulating expression and function of antioxidant enzymes. The effect can be comparable to those of Tai Chi exercise.

Based on the results presented above, we can come to the conclusion that ASP have potential of enhancing immunity activity and reducing oxidative injury.

Little is known regarding the synergistic effect between Tai Chi exercise and ASP. This will be further studied in our later work.

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