Randomized Double-blind Placebo-controlled Clinical Trial and Assessment of Fermentation Product of Cordyceps Sinensis (Cs-4) in Enhancing Aerobic Capacity and Respiratory Function of The Healthy Elderly Volunteers

XIAO Yi (肖　毅), HUANG Xi-zhen (黄席珍), and ZHU Jia-shi (朱佳石)

ABSTRACT **Objective**: Cordyceps sinensis (CS) is a popular natural Chinese herbal medicine for invigoration, health preservation and reducing fatigue. Its natural substance has been prepared as a fermentation product of a specific strain of Cordyceps sinensis (Cs-4). Our objective was to assess the effect of Cs-4 on the exercise capacity of the healthy elderly people in a randomized, double-blind, placebo-controlled trial. **Methods**: Thirty-seven healthy, elderly Chinese subjects were randomly assigned to receive either Cs-4 (3 g/day) or identical placebo capsules. Their exercise performance was tested before and after 6 weeks of treatment with a symptom-limited, incremental work rate protocol on a cycle ergometer. Maximum oxygen uptake (VO_2max) was measured using a metabolic chart. Anaerobic thresholds ($VO_2\theta$) were identified by two observers using plots of both VCO_2 vs VO_2 and VE/VO_2 vs time. **Results**: After taking Cs-4 for 6 weeks, VO_2max (1.88±0.13 to 2.00±0.14 L/min; $P=0.050$) and $VO_2\theta$ (1.15±0.07 to 1.30±0.09 L/min; $P=0.012$) were significantly increased, whereas after placebo application they were unchanged. **Conclusion**: These findings support the belief held in China that Cs-4 could improve oxygen uptake or aerobic capacity and ventilation function and resistance to fatigue of elderly people in exercise.

KEY WORDS Cordyceps sinensis, Cs-4, aerobic capacity, ventilation, elderly, exercise

Cordyceps sinensis (Berk) Sace (CS) is a precious natural herbal medicine, which has been used in China for thousands of years for invigoration, health preservation and reduction of fatigue, indicating its effect in tonifying Fei-Shen, and nourishing and reinforcing the body. This natural product of CS is found as a wild fungus growing on the Qinghai-Tibetan Plateau of China at an altitude over 3 000 m. Because of the insufficiency of natural sources, a refined fermentation product (Cs-4) was developed from the mycelial strain Paecilomyces hepialid (Chen et Dai) isolated from wild CS. A close similarity between this fermentation product and natural CS has been demonstrated in terms of their chemical constituents and pharmacological properties[1,2].

An accumulation of oxygen free radicals or "oxidative stress" is believed to affect cellular bio-energetics in aged people; it is also associated with many disease conditions. Studies have suggested that Cs-4 increases the scavenging of oxygen free radicals in both animals and humans by increasing the activity of the enzyme superoxide dismutase (SOD), as it enhances the capacity of scavenging free oxygen radicals[3-5]. Listlessness and repetitive fatigue are clinically common symptoms, even occurring in many "healthy persons", some of them might possibly be related to aging or certain illnesses. Excessive oxygen radicals would damage mitochondria, inhibit the synthesis of bio-energetics and other functions, even induce or initiate the process of cell apoptosis. Treatment with Cs-4 extract (400 mg/kg per day for 7 days) increased the cellular energy state in mice's liver by 12% to 18% as assessed by 31P NMR spectroscopy[6].

In addition, Cs-4 significantly decreased oxygen consumption by mice in a hypoxic environment; the Cs-4-treated mice lived 2 to 3 times longer in the hypoxic environment

Department of Respiratory Disease, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing (100730), China
Correspondence to: XIAO Yi, Tel: 010-65295037, E-mail: xiaoyipumch@yahoo.com.cn

than the controls. These results suggested that treatment with Cs-4 leads to a more efficient utilization of oxygen to support essential physiological mechanisms in these animals. The potential for Cs-4 to reduce oxidative stress and thereby optimize cellular bioenergetics suggests a possible mechanism of action for Cs-4 in overcoming fatigue and other aging-related symptoms and improving the quality of life in patients of chronic disorder. Other experiments denote that Cs-4 could treat chronic cardiac dysfunction and pulmonary diseases, and also increase oxygen free radical scavenging capability and elevate the quality of life[3,4,7,8].

Despite these reports, the ability of Cs-4 to enhance aerobic capacity has not been tested objectively. Our aim was to conduct a small pilot study in China to examine the effect of Cs-4 on aerobic capacity in healthy elderly volunteers. The study design was randomized, double-blind and placebo-controlled. Aerobic capacity was assessed using a symptom limited, incremental work rate protocol on a cycle ergometer to observe Cs-4 on 37 aged healthy volunteer's oxygen uptake and respiratory ventilation function in exercise.

METHODS

Subjects

Thirty-seven elderly volunteers, 58 to 78 years old, were enrolled in this study, 23 males and 14 females. They were recruited from centers and clubs for aged individuals in a suburban area in Beijing, P. R. China. They were non-smokers and with no history, symptoms, or signs of active cardiovascular, pulmonary, or muscular-skeletal diseases. A resting ECG was obtained to ensure that they showed no evidence of myocardial ischemia. The volunteers signed the letter of informed consent in their native language over the risks and benefits of participation in the study. Under double-blind conditions, they were randomly assigned to either the Cs-4 group (18 subjects, age: 63.9 ± 5.0 years, female : male = 6 : 12, weight: 69.0 ± 2.1 kg, height: 1.66 ± 0.08 m) or placebo group (19 subjects, age: 66.7 ± 5.3 years, female : male = 8 : 11, weight: 65.1 ± 2.6 kg, height: 1.63 ± 0.06 m), according to a randomization table. There were no differences in their basic characteristics. The subjects were instructed to keep their former life style unchanged as far as possible during the trial, including dietary habit, exercise, and other routine activities, and not to take any other medicinal herbs and drugs during the trial.

Study Methods

This study was designed as a randomized, placebo-controlled, double-blind clinical trial, to test effects of Cs-4 on exercise capacity in healthy elderly volunteers. The enrolled subjects were randomly assigned to two treatment groups and received either Cs-4 (3 g per day) or identical placebo capsules for 6 weeks. Cs-4 and identical placebo capsules were manufactured by Jiangxi Guoyao Company. Exercise performance was tested before and after the 6-week treatment according to the methods described below.

Incremental Work Rate

Aerobic capacity was measured using a symptom-limited, incremental work rate (IWR) protocol on an electromagnetically braked cycle ergometer (EM-840; Siemens, Germany). Maximum oxygen uptake (VO_2max) was determined by exhaled gas analysis using a metabolic measurement system with portable work rate controller (METAMAX, Cortex Biophysik GmbH, Germany). This system calculated a range of physiological variables every 30 seconds. After 5 min of unloaded cycling, the work rate was increased by 25 watt every 2 min. The subjects were asked to maintain a pedaling cadence of 60 r/min. The ECG was continuously monitored and blood pressure recorded intermittently during each study. Exercise was stopped when any of the following criteria were met: (1) Achievement of maximal heart rate (fCmax) according to age (fCmax = 220 − age); (2) Borg rating of perceived exertion (RPE) >17; (3) Development of exercise-limiting symptoms, e.g. chest pain, headache, or dizziness, etc.; (4) Systolic blood pressure > 230 mm Hg or diastolic blood pressure > 120 mmHg etc.; (5) Decrease in systolic blood pressure despite increasing work rate; (6) Serious arrhythmia; (7) ECG signs of myocardial ischemia, i. e. ST segment depression > 1 mm; (8) Occurrence of a plateau in VO_2max despite increasing work rate.

Exercise Performance Evaluation and Metabolism Measurements

Maximal exercise performance was assessed immediately before the trial and after six weeks of treatment with either Cs-4 and placebo. Values for maximum oxygen uptake (VO_2max), maximum work rate (W max), fCmax and maximum ventilation (VEmax) were derived from the output of the metabolic measurement system every 30 seconds. The anaerobic thresholds ($VO_2\theta$) were determined independently by two blinded, experienced observers using an approach previously reported. Each observer was provided with a set of randomly coded data including, for each subject, a plot of VCO_2 vs VO_2, a plot of VE/VO_2 vs time, and a table of data for the whole test was averaged every 10 seconds. The observers chose the threshold primarily from the plot of VCO_2 vs VO_2 and verified their choice with the additional data. For each threshold determination, a confidence level ranging from 1 (low) to 4 (high) was recorded. The mean difference between all of the thresholds for the two observers was 10 ml/min with a standard deviation of 50 ml/min. The observers disagreed by more than 100 ml/min in only 13 out of 66 cases and these differences were resolved in every case by further independent review of the data. Venous blood lactate was measured before the exercise and 3 min after each exercise test.

Since no reference values exist for VO_2max in aged Chinese, the reference values of Jones and Campbell were adopted[9]. The normal $VO_2\theta$ was assessed by reference to predict VO_2max.

Also the percentage of VO_2max of this group of Chinese elderly volunteers was calculated as a percentage of the VO_2max of healthy young Western people prior to treatment. The formulae are as follows: Predicted VO_2max of a healthy young Western man $= 4.2 - 0.032 \times$ body weight; Predicted VO_2max of a healthy young Western woman $= 2.6 - 0.014 \times$ body weight

Statistical Analysis

Descriptive statistics were expressed as means ± standard deviations. For measurements before and after the treatment period Students' t test was used for paired data. A P-value<0.05 was considered significant.

RESULTS

Thirty-seven healthy elderly subjects were randomly assigned to receive either Cs-4 ($n=18$) or placebo ($n=19$). Four subjects (two in each group) dropped out for certain reasons. In addition, in the placebo group, one subject experienced vomiting, and two subjects were suspected of developing myocardial ischemia during the end-of-trial exercise tests. These subjects did not complete their end-of-trial evaluations. The remaining 30 subjects completed the study (16 receiving Cs-4 and 14 receiving placebo).

Changes of Maximum Work Rate

At baseline assessment there was no difference in Wmax between the two groups ($P = 0.986$). There were no statistically significant changes in Wmax after 6 weeks of either Cs-4 or placebo treatment. These results are shown in Table 1. After normalizing the individual data by their pre-treatment values, a power analysis for the percentage change of the maximal work load

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under the study protocol showed that a minimum of 48 subjects in each group would be needed to explore a significant difference at the level of $P<0.05$ for comparison between the two groups.

Table 1. Changes in Maximum Work Rate in The Two Groups ($\bar{x}\pm s$)

Group	*n*	Maximum work rate (watt)		*P*	Percentage change (%)
		Baseline	6-week		
Placebo	14	123.2±10.6	117.9±9.2	0.272	−1.19±4.77
Cs-4	16	123.4±6.6	128.0±6.4	0.083	+4.77±3.18
	P	0.992	0.367		0.244

Changes of VO_2max

VO_2max was similar in the two experimental groups during pretreatment IWR exercise (Table 2). VO_2max did not change after a 6-week placebo treatment. In contrast, VO_2max was increased significantly after Cs-4 therapy for 6 weeks. This reflects an average 7.0% increase in VO_2max from the pretreatment baseline. Although no significant differences in post-treatment VO_2max were found between the two groups' *t* test analysis ($P=0.257$). However, a power analysis showed that a minimum of 45 subjects would be needed in each group to reveal a difference between the groups at a significant level of $P<0.05$. Prior to treatment, the group of Chinese elderly volunteers had their VO_2max as about 94% (placebo) or 95% (Cs-4) of the predicted VO_2max of healthy young Western people (Table 2). After treatment, subjects receiving Cs-4 had their VO_2max increased to predicted VO_2max, i. e. increased, while the controls showed no change.

Table 2. Changes in VO_2max in the Two Groups and Comparison with Predicted VO_2max of Healthy Young Western People ($\bar{x}\pm s$)

Group	*n*	VO_2max (L/min)		*P*	Percentage change (%)	Predicted VO_2max of healthy young Western people (%)	
		Baseline	6-weeks			Baseline	6-weeks
Placebo	14	1.80±0.12	1.79±0.11	0.805	−0.05±2.84	94.3±4.8	93.8±4.7
Cs-4	16	1.88±0.13	2.00±0.14	0.050	+6.96±3.46	95.6±5.9	103.0±5.9
	P	0.649	0.257		0.129		

Increases in METs

At baseline assessment, there was no difference in METs between the two groups. METs did not change after six weeks of treatment with placebo. By contrast, METs increased significantly after six weeks of Cs-4 treatment. Thus, there was an average 8.3% increase in METs from the pretreatment baseline in the Cs-4 group.

Table 3. Changes in METs in The Two Groups ($\bar{x}\pm s$)

Group	*n*	METs		*P*	Percentage change (%)
		Baseline	6-weeks		
Placebo	14	7.77±0.41	7.83±0.38	0.804	−1.73±3.27
Cs-4	16	7.68±0.42	8.26±0.44	0.017	+8.29±3.33
	P	0.885	0.473		0.171

Changes of VEmax

At baseline assessment, there was no difference in VEmax between the two groups ($P=0.481$). VEmax did not change after six weeks of treatment with placebo ($P=0.675$). By contrast, VEmax increased significantly after six weeks of Cs-4 treatment ($P=0.049$). Thus, there was an average 10.4±4.5% increase in VEmax from the pretreatment baseline in the Cs-4 group. A difference between the two groups after treatment was not found. However, based on the variances of pretreatment values, a power analysis showed that a minimum of 28 subjects would be needed in each group to reveal a difference in VEmax at the level of $P<0.05$.

Table 4. Changes in VEmax in The Two Groups ($\bar{x} \pm s$)

Group	n	VEmax (L/min) Baseline	6-weeks	P	Percentage change (%)
Placebo	14	52.1±3.9	51.3±4.0	0.675	−0.86±3.75
Cs-4	16	56.7±4.8	60.4±4.6	0.049	+10.40±4.45
P		0.481	0.152		0.093

Changes of Anaerobic Threshold

$VO_2\theta$ was assessed for the subjects in the two groups during pretreatment IWR exercise. Our results demonstrated that no significant differences existed in $VO_2\theta$ between the two groups (Table 5). At week 6, $VO_2\theta$ did not change after 6 weeks of placebo treatment. But it was significantly increased in the Cs-4 group from pretreatment ($P = 0.012$), reflecting a 12.6±4.3% increase in $VO_2\theta$. After normalizing the individual by their pre-treatment values, a power analysis for percentage changes in $VO_2\theta$ showed significant differences between the two groups.

Table 5. Changes in $VO_2\theta$ in The Two Groups ($\bar{x} \pm s$)

Group	n	$VO_2\theta$ (L/min) Baseline	6-weeks	P	Percentage change (%)
Placebo	14	1.20±0.05	1.18±0.07	0.406	−2.40±2.41
Cs-4	16	1.15±0.07	1.30±0.09	0.012	+12.60±4.28
P		0.559	0.320		0.006

DISCUSSION

Cordyceps sinensis has been traditionally believed to have the property to invigorate "Fei" and "Shen" [it must be understood that the concept in traditional Chinese medicine (TCM) of the Fei (lung) and Shen (kidney) does not conform to West anatomical and physiological concepts]. These pharmacologic functions of natural herbs were based on the philosophy and theory of TCM. In an effort to explore health-promoting function of Chinese herbs with modern clinical protocol and exercise-metabolism examination technology, our data clearly suggest that Cs-4 treatment increased maximal oxygen uptake, maximal METs, maximal ventilation, and gas exchange $VO_2\theta$ in this group of Chinese elderly healthy subjects. These results indicate enhanced muscle fitness for exercise and improvement of ventilation capacity in normal Chinese elderly subjects following 6 weeks of Cs-4 treatment.

We found enhanced aerobic capabilities during incremental work after 6 weeks of Cs-4 administration. Accelerated glycolysis to supply bio-energy for working muscles increase steeply production of lactate causing lactic acidosis, which is buffered by bicarbonate above a particular metabolic rate. The bicarbonate buffering-derived CO_2 causes the increase in CO_2 output to be greater than the increase in O_2 uptake[10,11], as we have found the significant increase in anaerobic threshold. This bicarbonate buffering led to insignificant changes in blood lactate after Cs-4 treatment.

Cs-4 and the parental natural CS are composed of a great deal of chemical components, as summarized previously by Zhu, et al[1,2]. Like other natural products, Cs-4 have many biologically active chemical components. From it protein-bound polysaccharides that contain large numbers of mannitol residues and a 45-KDa polysaccharide with an unique molar ratio of three kinds of monosaccharide (galactose, glucose, and mannose) have been isolated and proved to be effective in reducing blood lipids and glucose or suppressing tumor size in vitro[12-14]. Water-soluble components of Cs-4 were shown to be responsible for the improvement of hepatic energy condition[6]. Alcohol-soluble substances were also proven to be effective in several pharmacologic areas, such as lowering serum lipids, and improving cardiovascular and immune functions[15-17]. Micro-quantities of CS (3′-deoxyadenosine) and 2′-dexyadenosine have also been found in natural CS and Cs-4. These compounds and their precursors, adenosine, have shown beneficial effects on the cardiovascular system. In addition, other chemical components, such as flavones, non-hormonal sterols, cyclic dipeptides, trace elements, and many others, all show a separate activity or synergistic activities that benefit the health of humans[18-20].

However, direct relationships between each chemical component and therapeutic effects are yet to be established, and here the effect is probably related to the function of Cs-4 in scavenging oxygen free radicals, and increasing maximal work rate, VO_2max, METs, and $VO_2\theta$ following Cs-4 treatment. What was found in this study may attribute to reduced oxidative pressure on mitochondria, the cellular organelle of bio-energetic process of oxidation-phosphorylation and respiratory chain. This may induce increased ATP levels which have recently documented in mice after Cs-4 treatment[6].

To this end, this study has demonstrated the power of Cs-4 to improve aerobic capacity supporting the belief held in China that Cs-4 has the potential for improving capacity for exercise and resistance to fatigue. A further study involving more subjects remains to be done to further reveal significant post-treatment differences between the two groups. Also, exercise endurance test at a constant sub-maximal work rate would be necessary to verify the truly ergogenic effect.

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