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Effect of verbascoside on decreasing concentration of oxygen free radicals and lipid peroxidation in skeletal muscle

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KEY WORDS free radicals; electron spin resonance spectroscopy; lipid peroxidation; exercise; skeletal muscle; malondialdehyde

ABSTRACT

AIM: To detect the effects of verbascoside on decreasing the concentration of oxygen free radicals (OFR) and lipid peroxidation in skeletal muscle resulting from exhaustive exercise. **METHODS**: Electron spin resonance (ESR) technique and thiobarbituric acid reaction (TBAR) method were used to detect the concentration of OFR in intact gastrocnemius muscle and the contents of milondialdehyde (MDA) in muscle homogenate. **RESULTS**: Verbascoside decreased the concentration of OFR ($P < 0.05$) and the level of lipid peroxidation ($P < 0.05$) in muscle caused by exercise. **CONCLUSION**: Verbascoside has the effects of reducing oxidative stress in muscle caused by exhaustive exercise by decreasing the concentration of free radicals and the level of lipid peroxidation.

INTRODUCTION

Strenuous exercise has been shown to damage the active muscle fibers of numerous animal species, including human[1]. Some forms of exercise-induced muscle damage are related to oxygen toxicity, including attacks by free radicals, lipid peroxidation, and inflammatory reaction caused by leukocytes, which in turn can be activated by free radicals. This has been documented by numerous investigations demonstrating increase in the by-products of lipid peroxidation following exercise[2-5].

Verbascoside is extracted from *Pedicularis* that is used in Chinese medicine as cardiotonics for treatments of collapse, exhaustion, spontaneous sweating, seminal emission, and senility[6], and is usually called "pseudo-ginseng" by local inhabitants of Northwestern China. Verbascoside is a phenylpropanoid glycoside that is a class of constituents of *Pedicularis*, and has been reported to have antiviral[7] and antiplatelet properties[8]. Zheng[9] reported that verbascoside exhibited antioxidant activity. Wang et al[10,11] studied the scavenging effect of verbascoside on superoxide anion and hydroxyl radical using pulse radiolysis technique and the spin trapping method. They demonstrate that verbascoside is an effective hydroxyl radical scavenger, and the number of phenolic hydroxyl group in the structure of the verbascoside is related to its scavenging activities. The present study was conducted to test the hypothesis that the higher concentration of oxygen free radicals (OFR) and the higher level of lipid peroxidation in muscle caused by strenuous exercise can be decreased by

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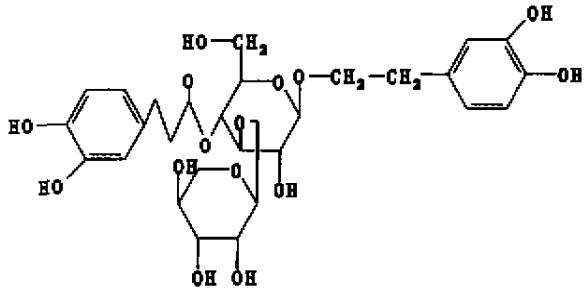
pretreatment with verbascoside.



Verbascoside

MATERIALS AND METHODS

Sprague-Dawley rats, 140 ♂, 200 – 250 g (235 g ± 13 g) were supplied by The Centre of Experimental Animals, The Chinese University of Hong Kong and were specific pathogen-free. The experiments carried out in this study comply with the current laws of Hong Kong concerning the use of animals for scientific experiments and research. The rats were housed four per cage in a room maintained at (23 ± 2) ℃ and had food and water *ad lib*. The light was on between 8:00 and 20:00.

Experimental procedures Experimental rats were randomly divided into 2 groups, exercise control and verbascoside groups. Rats in each group were randomly divided into 7 units of 10 each, 1 rest unit, and 6 exercise units. The rats were killed at half-time of running, and 5 min, 30 min, 120 min, 240 min, and 480 min after exercise. The rats in verbascoside group were administrated with 3.2 $mg \cdot kg^{-1} \cdot d^{-1}$ ig twice a day for 10 d before exercise day. Verbascoside was dissolved in water. The rats in exercise control group were administrated with the water of the same volume as verbascoside solution, ig twice a day for 10 d. Verbascoside was produced and supplied by National Laboratory of Natural and Biomimetic Drugs, Beijing Medical University, which is of the chemical reagent grade. Before the formal run, the rats were trained and acclimated to running on the treadmill. The total distance of running during training were limited to about 200 meters.

In the formal run the slope of the treadmill was set at 5°. The rats were allowed to warm up for 10 min running at a speed of 6 $m \cdot min^{-1}$. They progressed to running at 12 $m \cdot min^{-1}$, 18 $m \cdot min^{-1}$, 24 $m \cdot min^{-1}$, each for 15 min, then finally running to exhaustion at 28.2 $m \cdot min^{-1}$. The average distance covered amounted to 3500 m. The average running time to exhaustion was about 140 min. The animal model used in this study was based on the information from previous study and pilot study[12].

After running, the rats were placed back in their cages in the animal quarters with food and water *ad lib* until death. Rats were killed at different time point, half-time of running, and 5 min, 30 min, 120 min, 240 min, and 480 min after exercise under anesthesia with pentobarbital 40 $mg \cdot kg^{-1}$ ip. Muscle samples were taken from the belly of the gastrocnemius muscle.

The level of lipid peroxidation in muscle homogenate was indicated by the content of malondialdehyde (MDA) in muscle. Thiobarbituric acid reaction (TBAR) method was used to determine the MDA. 1, 1, 3, 3-Tetramethoxypropane (TMP) was used as an external standard, and the MDA content was expressed as nmol per gram of wet muscle. All reagents were purchased from Sigma Company and of analytical reagent grade. Concentration of OFR in intact muscle was detected by using Bruker ESP-300 Electron Spin Resonance (ESR). When muscle sample was taken, it was put in a special tube and frozen in liquid nitrogen. The muscle sample was placed in the ESR Dewar containing liquid nitrogen. Instrumental conditions were set at temperature 123 K; microwave frequency 9.43 GHz; microwave power 1 mW; modulation amplitude 0.235 mT; modulation frequency 100 kHz; time constant 164 ms; and scan width, 50

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mT. The concentration of OFR was represented by the peak value of OFR signals recorded in integrative spectrum of ESR. The greater the resonance peak signal, the greater the free radical production was.

The data obtained were analyzed using SPSS of Window software. The results were presented as $\bar{x} \pm s$. One-way ANOVA and multiple comparison test were applied to identify statistical significances. *Post hoc* analysis was conducted by Scheffes significant difference test to evaluate the significant mean difference.

RESULTS

The concentration of OFR increased during exercise and reached the highest level immediately after exercise in both groups. The averaged concentrations of OFR in muscle of the verbascoside treatment group were all significantly lower ($P < 0.01$ or $P < 0.05$) than those measured in the exercise control group at each time point. The highest 30 % increase of intensity of OFR signals was detected immediately after exercise in the group of exercise control, while only an increase of 12.5 % was found immediately after exercise in verbascoside group (Tab 1).

MDA contents in muscle increased ($P < 0.05$) in the exercise control group after running. MDA content in muscle was still higher than the control level ($P < 0.05$) 480 min after exercise, showing similar trend to the changes of the concentration of OFR in muscle. The determination of MDA content in muscle homogenate from verbascoside group indicated that there were lower levels of lipid peroxidation at each time point after exercise compared with those measured at the same measuring points in exercise control group. MDA contents were 223.7 nmol·g^{-1}(wet wt) and 216.15 nmol·g^{-1} (wet wt) for exercise control group, 190.40 nmol·g^{-1} (wet wt) and 183.44 nmol·g^{-1}(wet wt) for verbascoside group at 5 min and 30 min, respectively after running. The changes of MDA in muscle were matched with the measured concentration of OFR at some time point (Tab 1).

DISCUSSION

The results of the present study showed that pretreatment with verbascoside could significantly decrease the concentration of OFR and the levels of lipid peroxidation in muscle resulted from exhaustive exercise. The effects of verbascoside on decreasing concentration of OFR as well as the level of lipid peroxidation caused by exercise in muscle may be related to verbascosides antioxidant effect[9-11].

Li *et al*[13] investigated the antioxidant activity of verbascoside for inhibiting the lipid peroxidation induced by Fe^{2+}/ascorbic acid in mouse liver microsome. They found that verbascoside could inhibit microsomal lipid peroxidation

Tab 1. Intensity of oxygen free radical signals and MDA content of muscles during and after exercise. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* rest control. $^eP < 0.05$, $^fP < 0.01$ *vs* the same time point measured in exercise group.

	Rest	Half-time	5 min	30 min	120 min	240 min	480 min
Intensity of OFR (mm peak values) samples from 5 rats							
Exercise control	10.00 ± 0.00	12.00 ± 0.70^b	13.00 ± 1.20^b	11.70 ± 1.30^b	11.40 ± 0.50^c	10.80 ± 0.20^b	11.20 ± 0.60^b
Verbascoside group	10.00 ± 0.00	11.60 ± 0.13^e	11.25 ± 0.23^{ce}	10.06 ± 0.44^e	9.50 ± 0.61^f	9.78 ± 0.46^f	10.24 ± 0.54^e
MDA content [nmol·g^{-1}(ww)] samples from 10 rats							
Exercise control	177 ± 9	180 ± 8	224 ± 9^e	216 ± 16^e	211 ± 9^e	190 ± 11^e	200 ± 17^e
Verbascoside	178 ± 8	180 ± 5	190 ± 9^{ef}	183 ± 10^f	184 ± 8^f	180 ± 12	187 ± 13

concentration-dependently and efficiently. Its inhibitory effect was stronger than that of cistanoside D, glutathione, and gallic acid. Zheng *et al*[9] studied the activity of verbascoside and other phenylpropanoid glycosides as chain-breaking antioxidants on the autoxidation of linoleic acid in cetyl trimethylammonium bromide micelles at 37 ℃ and found that verbascoside inhibited the autoxidation effectively. Wang *et al*[10,11] investigated the scavenging effects of verbascoside using pulse radiolysis technique and the spin trapping method. The results demonstrated that verbascoside had scavenging effects to superoxide anion and hydroxyl radicals. The results obtained from present study were consistent with the findings from experiments *in vitro*[9-11,13]. Therefore, the effects of pretreatment with verbasocide on decreasing the concentration of OFR and the level of lipid peroxidation in muscle should be attributed to the scavenging effects of verbascoside.

Studies on tissue injury and oxidative stress demonstrated that the attack of free radicals could induce damage of tissue structure. Jackson[14] studied the relationship between muscle injury and the concentration of free radicals using ESR technique and enzyme analysis. Jackson examined the effects of 30 min of excessive muscle contractile activity on the amplitude of a major electron spin resonance signal with value from 0.20036 to 0.2004 mT. This signal was believed to be composed of semi-quinone type radicals in rat hind limb muscles. They reported a 70 % increase in the amplitude of the major signal in active intact muscle compared with the rest. They found that there was a linkage between the condition of muscle injury and the concentration of free radicals. Higher concentration of free radicals induced increased permeability of muscle sarcolemma, suggesting the membrane damage. In the present study, the concentration of free radicals in muscle increased as reported by the published literature[3,14]. The results of the present study *in vivo* showed that verbascoside had scavenging effect on free radicals produced during and after strenuous exercise, which was consistent with the results from the studies *in vitro*[9-11].

TBAR have been widely used to measure lipid peroxidation in cell membrane and fatty acids. The thiobarbituric acid technique has been shown to be sensitive to MDA and a good general index of oxidative stress in biological systems[15]. A lot of studies showed that there is a trend of increasing MDA content in muscle after exercise[2,3]. Results of the present study showed that MDA contents in muscle increased immediately after running and maintained high level for a longer time. The alterations of MDA in muscle showed the similar changing trend to those of concentration of free radicals in muscle, suggesting a parallel relationship between the level of lipid peroxidation and the concentration of free radicals. It is known that lipid peroxidation results from the attack of free radicals and reactive oxygen species to lipids in tissues. Pretreatment with verbascoside significantly decreased the concentration of OFR, thus the lipid peroxidation was partly inhibited.

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126-130

毛蕊花苷对骨骼肌中氧自由基和脂质过氧化物的清除和抑制效应

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关键词 自由基; 电子自旋共振谱; 脂质过氧化; 运动; 骨骼肌; 丙二醛

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