

Preventive Effects of Antioxidants and Exercise on Muscle Atrophy Induced by Ischemic Reperfusion

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Abstract. [Purpose] This study aimed to determine whether muscle atrophy induced by ischemic reperfusion injury in rats can be prevented by the administration of antioxidants and exercise. [Subjects] Rats were randomly divided into five groups: non-treated, ischemic, exercise, ascorbic acid and exercise, and tocopherol and exercise. [Methods] The relative weight ratio of the soleus muscle and the length of the soleus muscle fiber cross-section minor axis were used for the evaluation of muscle atrophy. Pain was assessed as the weight-bearing ratio of the ischemic side. A multiple comparison test and the paired t-test were used for the statistical analyses. [Results] Compared with the non-treated group, the relative weight ratios of the soleus muscle and the lengths of the soleus muscle fiber cross-section minor axis significantly decreased in the other groups. Excluding the non-treated group, the relative weight ratios of the soleus muscle were heaviest in the tocopherol and exercise group. Excluding the non-treated group, the lengths of the soleus muscle fiber cross-section minor axis were longest in the tocopherol and exercise group, followed by the ischemic, exercise, and ascorbic acid and exercise groups. The amount of antioxidant substances did not decrease on the weight-bearing ratio of the ischemic side. [Conclusion] In this study, using an experimental rat model, we confirmed that antioxidants and exercise effect muscle atrophy induced by ischemic reperfusion. The results show that muscle regeneration was facilitated by phagocytosis in the tocopherol and exercise group.

Key words: Ischemic reperfusion, Exercise, Antioxidant

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INTRODUCTION

The use of tourniquets on the limbs in cosmetic surgery can produce ischemia-related changes. Ischemic reperfusion is known to induce several disorders such as inflammation around blood vessels after blood flow is reinstated¹⁾.

Ischemia followed by reperfusion raises the permeability in vascular edema and leads to loss of function of the skeletal muscle. It also increases edema and pain; i.e., pain may occur after ischemic reperfusion²⁻⁵⁾.

Previous studies have confirmed that inflammation and edema occur after ischemic reperfusion. Also, ischemic reperfusion decreases the weight-bearing ratio of the ischemic side. Pain is caused by inflammation and edema, which result in reduced weight-bearing on the ischemic side. Inflammation and edema can be prevented by antioxidants. Previ-

ous work has confirmed in an experimental rat model that antioxidant treatment can prevent muscle atrophy induced by ischemic reperfusion⁶⁻¹⁰⁾.

This study aimed to determine whether muscle atrophy induced by ischemic reperfusion injury in rats can be prevented by the administration of antioxidants and exercise.

MATERIALS AND METHODS

Rats

Twenty female Wistar rats (eight weeks old) were used. The rats were randomly divided into five groups of five rats each: non-treated group (left hind limb), ischemic group, exercise group, ascorbic acid and exercise group, and tocopherol and exercise group. The experiments were conducted in accordance with Prefectural University of Hiroshima Guidelines for Animal Experimentation and the U.S. National Institute of Health Guidelines.

Methods

The rats were allowed free access to a standard diet and water in their cages. Ischemia was induced with a DC1.6

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tourniquet finger cuff, and a Rapid Cuff Inflator with an AG101 air source (D.E. Hokanson, USA) was used to apply pressure. The rats were anaesthetized with sodium pentobarbital (40 mg/kg b.w.), and ischemia was induced in the right lower limb at a pressure of 300 mmHg for 90 minutes.

The ascorbic acid and exercise group was intraperitoneally injected with 40 mg/kg b.w. ascorbic acid (Fuso, Osaka, Japan). The tocopherol and exercise group was intramuscularly injected with 30 mg/kg b.w. tocopherol acetate (Eisai, Tokyo, Japan). All injections were performed six times (once every 12 hours) from just prior to the ischemia procedure until 60 hours of reperfusion had elapsed. The dosages were derived from Kondo's¹¹⁾ manual and research.

Reperfusion commenced when treadmill exercise reached 24 hours after 4 days. The exercise period was 20 minutes and exercise was performed twice a day. The treadmill speed began at 10 m/sec and was increased up to 20 m/sec. Walking conditions were observed during the exercises. Pain was assessed by the amount of weight distribution on the right hind limb.

All rats were weighed at the beginning and end of the experiment. The rats were anaesthetized with sodium pentobarbital (40 mg/kg b.w.) after being weighed at the end of the experiment. Then, the rats were exsanguinated by cutting the abdominal aorta. The soleus muscle of the right hind leg was extracted by cutting it between the top of the calcaneus and the bottom of the fibula head. The wet weight of the soleus muscle was measured with a precision balance immediately after the rat had been sacrificed. The relative weight ratio of the soleus muscle was calculated as the ratio of the wet weight of the soleus muscle to the body weight of the rat.

After its wet weight had been measured, the soleus muscle was divided in half and embedded in tragacanth. The excised soleus muscle was then rapidly frozen by immersing it in a liquid nitrogen bath. Sections, 10 µm thick, were prepared using a cryostat microtome and stained with hematoxylin and eosin (H&E staining). All sections were photographed using a digital camera, and the muscle fiber cross-section minor axis was measured microscopically. The images were transferred to a computer, and the fiber cross-section minor axis of the muscle was measured using image analysis software (Image ProPlus 6.2 J, USA). The fiber cross-section minor axes of more than 200 muscle fibers were measured, and the mean value was calculated.

Group data were analyzed using a multiple comparison test and the paired t-test, and the p values of <0.05 were considered to be significant.

RESULTS

The mean weight-bearing ratio before ischemia of the ischemic group was 48.3%; that of the exercise group was 51.6%; that of the ascorbic acid and exercise group was 47.0%; and that of the tocopherol and exercise group was 48.2%. The mean weight-bearing ratio of the ischemic side on the day following ischemia of the ischemic group was 33.6%; that of the exercise group was 32.0%; that of the ascorbic acid and exercise group was 28.9%; and that

Table 1. Weight-bearing ratio (ischemic lower weight/body weight %)

Group/time	Before ischemia	Day after ischemia	4 days after ischemia
Ischemic	48.3	33.6**	25.8*
Exercise	51.6	32.0**	35.0*
Ascorbic acid and exercise	47.0	28.9*	33.1*
Tocopherol and exercise	48.2	41.3	36.2

** : vs. before ischemia, $p < 0.01$, * : vs. before ischemia, $p < 0.05$

Table 2. Relative weight ratios and Fiber cross-section minor axis (mean±SD)

Group	Relative weight ratio (mg/g)	Fiber cross-section minor axis (µm)
Non-treated	0.51±0.03	46.27±3.19
Ischemic	0.41±0.03**	37.98±2.02**
Exercise	0.41±0.02**	36.43±1.17**
Ascorbic acid and exercise	0.41±0.06**	35.63±2.44**
Tocopherol and exercise	0.42±0.04**	38.53±1.83**

** : vs. normal, $p < 0.01$

of the tocopherol and exercise group was 41.3%. The mean weight-bearing ratio of the ischemic side on final day of the ischemic group was 25.8%; that of the exercise group was 35.0%; that of the ascorbic acid and exercise group was 33.1%; and that of the tocopherol and exercise group was 36.2% (Table 1).

The mean weight-bearing ratios of the ischemic side of all the ischemia groups, except for the tocopherol and exercise group, were significantly lower than their pre-intervention values on the first and last experimental days.

The mean soleus muscle relative weight ratios were 0.51 mg/g b.w. for the non-treated group, 0.41 mg/g b.w. for the ischemic group, 0.41 mg/g b.w. for the exercise group, 0.41 mg/g b.w. for the ascorbic acid and exercise group, and 0.42 mg/g b.w. for the tocopherol and exercise group.

The soleus muscle relative weight ratios of all groups were significantly larger than those of the non-treated group (Table 2).

The mean length of the soleus muscle fiber cross-section minor axis of the non-treated group was 46.27 µm; that of the ischemic group was 37.98 µm; that of the exercise group was 36.43 µm; that of the ascorbic acid and exercise group was 35.63 µm; and that of the tocopherol and exercise group was 38.53 µm. The soleus muscle fiber cross-section minor axis of all the ischemia groups were significantly longer than those of the non-treated group (Table 2).

DISCUSSION

This study aimed to determine whether muscle atrophy induced by ischemic reperfusion injury in rats can be prevented by the administration of antioxidants and exercise.

Nakashima reported that oxidative stress causes protein oxidation, and as a result, proteasomes, proteolytic enzyme complexes, were activated, promoting proteolysis. This was confirmed by examining the mechanism of the skeletal muscle proteolysis induced by oxidative stress in a culture experiment using skeletal muscle cells derived from chick embryos¹²). Thus, oxidative stress is responsible for the skeletal muscle atrophy that occurs after ischemia/reperfusion, and it is important to alleviate oxidative stress to prevent amyotrophy. Kondo reported that the injection of vitamin E resulted in a decrease in immobility-induced muscle atrophy, while oxidative stress increased during the muscle atrophy recovery period¹¹).

In the present study, ascorbic acid and tocopherol were used as antioxidants to remove oxidative molecules. Ascorbic acid and tocopherol are chain-reaction-abrogating type antioxidants and are able to prevent oxidative stress. Ascorbic acid is distributed in both the extracellular fluid and the cytoplasm of muscle cells. Its two hydroxyl groups at the C-2 and C-3 positions participate in the stabilization of radicals before they can attack the cellular membrane. In contrast, fat-soluble tocopherol exerts antioxidative effects on biomembranes, and it binds to peroxyl radicals, which facilitate oxidative chain reactions^{13–15}).

In the present study, the relative soleus muscle weight ratio was significantly lower, and the soleus muscle fiber cross-section minor axis was significantly shorter in the ischemic, exercise, ascorbic acid and exercise, and tocopherol and exercise groups than in the non-treated group. However in comparison with the ischemic group, the weight-bearing ratio (ischemic lower weight/body weight %) on the ischemic side was significantly higher in the exercise, ascorbic acid and exercise, and tocopherol and exercise groups.

In previous studies that were designed to cause painful edema, it was reported that when edema occurred pain or abnormal sensations were experienced, which resulted in a decrease in weight-bearing on the effected side^{4, 5}). In the present study, focal ischemic reperfusion pain caused a decrease in the amount of weight-bearing. Compared to the pre-ischemia values, the ischemic group, the exercise group, and the ascorbic acid and exercise group showed significant decreases in weight-bearing on the day after ischemia began. In addition, the pain from the ischemia lasted until the final day. However, the differences in the weight bearing ratios of the tocopherol and exercise group were not significant. Exercise at the same time as the administration of antioxidants suggests that ischemic reperfusion pain may be prevented. The administration of antioxidants should be done soon after the application of the ischemia reperfusion to prevent pain and promote early movement.

In this study, using an experimental rat model, we con-

firmed that antioxidant and exercise treatments can prevent muscle atrophy induced by ischemic reperfusion. Wistar rats produce large quantities of endogenous ascorbic acid, and its normal level is much higher than that of endogenous tocopherol. Therefore, the injection of tocopherol is an efficient method of inducing antioxidative effects. In addition, the antioxidative ability of tocopherol may be reinforced by the coexistence of ascorbic acid. Consequently, tocopherol was a more potent antioxidant than ascorbic acid in the present study.

Further studies should be carried out to establish the appropriate effective doses of ascorbic acid and tocopherol, including the combination treatments of both antioxidants and exercise.

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