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Curcumin attenuates oxidative stress following downhill running-induced muscle damage

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ABSTRACT

Downhill running causes muscle damage, and induces oxidative stress and inflammatory reaction. Recently, it is shown that curcumin possesses anti-oxidant and anti-inflammatory potentials. Interestingly, curcumin reduces inflammatory cytokine concentrations in skeletal muscle after downhill running of mice. However, it is not known whether curcumin affects oxidative stress after downhill running-induced muscle damage. Therefore, the purpose of this study was to investigate the effects of curcumin on oxidative stress following downhill running induced-muscle damage. We also investigated whether curcumin affects macrophage infiltration via chemokines such as MCP-1 and CXCL14. Male C57BL/6 mice were divided into four groups; rest, rest plus curcumin, downhill running, or downhill running plus curcumin. Downhill running mice ran at 22 m/min, −15% grade on the treadmill for 150 min. Curcumin (3 mg) was administered in oral administration immediately after downhill running. Hydrogen peroxide concentration and NADPH-oxidase mRNA expression in the downhill running mice were significantly higher than those in the rest mice, but these variables were significantly attenuated by curcumin administration in downhill running mice. In addition, mRNA expression levels of MCP-1, CXCL14 and F4/80 reflecting presence of macrophages in the downhill running mice were significantly higher than those in the rest mice. However, MCP-1 and F4/80 mRNA expression levels were significantly attenuated by curcumin administration in downhill running mice. Curcumin may attenuate oxidative stress following downhill running-induced muscle damage.

1. Introduction

Downhill running including eccentric muscle contraction induces muscle damage, and generates reactive oxygen species (ROS) and inflammatory cytokines in animal and human experiments. Previous studies indicated that gene expression of inflammatory cytokines such as TNF-α and IL-1β in skeletal muscle was elevated by downhill running in human [1,2]. In addition, recent studies reported that hydrogen peroxide and inflammatory cytokine production in skeletal muscle was increased after downhill running in rodents [3,4]. Moreover, macrophages and neutrophils are present in skeletal muscle after downhill running [5–7]. Therefore, macrophages and neutrophils may play an important role in oxidative stress and inflammation following downhill running-induced muscle damage.

Although infiltration of macrophages into tissues is mediated by chemokines such as monocyte chemoattractant protein (MCP)-1 and CXCL14 [8], plasma concentration of MCP-1 was elevated by downhill running [9]. In addition, MCP-1 and MCP-1 receptor (CCR2) knockout mice attenuated macrophage infiltration in skeletal muscle after muscle damage induced by cardiotoxin injection [10]. Therefore, MCP-1 may induce macrophage infiltration, and modulate inflammation and oxidative stress after downhill running. In addition, it was shown that COX-2 modulated macrophage infiltration after muscle damage, and COX-2 inhibitor treatment attenuated infiltration of macrophages and neutrophils after lacerate-induced muscle damage [11]. COX-2 knockout mice showed less macrophage infiltration of lacerate-induced muscle damage [12]. Therefore, COX-2 may also induce macrophage infiltration,

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and modulate inflammation and oxidative stress after downhill running. Recently, it is reported that these inflammatory mediators such as TNF-α and COX-2 up-regulate expression of catabolic condition inducer such as atrogin1/MAFbx and MuRF1 in skeletal muscle, and induce muscle protein degradation [13–15]. Hydrogen peroxide also induced expression of atrogin1/MAFbx and MuRF1 in skeletal muscle myotubes [16,17]. Therefore, macrophages may be required for inflammation- and oxidative stress-induced muscle protein degradation.

Curcumin, an important constituent of *Curcuma longa*, possesses anti-oxidant and anti-inflammatory actions. It is shown that curcumin has been used to treat a variety of inflammatory conditions and chronic diseases [18]. In addition, curcumin has beneficial effects in conditions such as injured skeletal muscle. Interestingly, it is shown that curcumin reduces inflammatory cytokine concentration in skeletal muscle after downhill running [19]. These results indicate that curcumin may possess effective anti-inflammatory action after muscle damage. Downhill running-induced muscle damage also causes oxidative stress. However, it is not known whether curcumin affects oxidative stress following downhill running-induced muscle damage. Therefore, the purpose of this study was to investigate the effects of curcumin on oxidative stress following downhill running-induced muscle damage. In addition, we investigated to examine whether curcumin affects macrophage infiltration via suppression of chemokines such as MCP-1 and CXCL14 expression. We hypothesized that curcumin would suppress macrophage infiltration and oxidative stress.

2. Methods

2.1. Animals

Male C57BL/6 mice (n = 52) were purchased from Kiwa Laboratory Animals (Wakayama, Japan) at 9 weeks of age, and mice were housed together in one cage in controlled environment under a light–dark cycle (lights on at 9:00 and off at 21:00). The experimental procedures followed the Guiding Principles for the Care and Use of Animals in the Waseda University Institutional Animal Care and Use Committee and were approved (10K001). All mice were randomly divided into four groups: Rest (n = 12), Rest plus Curcumin (n = 12), Downhill running (n = 14), or Downhill running plus Curcumin (n = 14) group. All groups were allowed to eat food freely.

2.2. Downhill running protocol

All mice were initially acclimated to running on a motorized treadmill (Natsume, Kyoto, Japan) at 20 m/min, 0% grade for 20 min/day for 1 week. On the day of the experiment, the downhill running groups of mice ran at 22 m/min, −15% grade on the treadmill for 150 min.

2.3. Preparation of curcumin powder

We developed a highly absorbable curcumin formulation (THERACURMIN®) using microparticle and surface processing techniques. Curcumin powder was prepared by Theravalues (Tokyo, Japan). Curcumin powder was extracted from Indian turmeric by using alcohol. THERACURMIN® was prepared as follows; first, gum ghatti, mainly consists of polysaccharides, obtained from the exudation of ghatti trees, was dissolved in water to make gum ghatti solution. Curcumin powder was mixed into this solution, and water and glycerin was added to adjust the weight. This mixture was ground by a wet grinding mill, and then, dispersed by a high-pressure homogenizer. After this procedure, stable THERACURMIN® was obtained. THERACURMIN® consisted of 10 w/w% of curcumin, 2% of other curcuminoids such as demethoxycurcumin and isdemethoxycurcumin, 46% of glycerin, 4% of gum ghatti, and 38% of water.

2.4. Curcumin administration

Newly developed microparticle curcumin (named THERACURMIN®: 3 mg) was dissolved with normal saline (PBS) to the concentration of 15 mg/ml solution. Curcumin was administered in oral administration immediately after downhill running. PBS was administered to the control group mice. All mice were killed at 24 h after curcumin injection.

2.5. Plasma creatine kinase and lactate dehydrogenase activity measurement

Mice were anaesthetized by breathing of isoflurane (Abbott, Tokyo, Japan). Abdominal vein blood samples were collected in heparin tube, and plasma was stored at −80 °C. Plasma creatine kinase (CK) activity was measured with CK Test Wako (Wako Pure Chemical Industries, Tokyo, Japan). Plasma lactate dehydrogenase (LDH) activity was measured with LDH·J Test Wako (Wako Pure Chemical Industries).

2.6. Hydrogen peroxide assay

To examine hydrogen peroxide levels in gastrocnemius of mice, skeletal muscle was homogenized in Tissue Protein Extraction Reagent (T-PER) with Protease inhibitor (Thermo, Rockford, Illinois, USA). Protein concentration was measured using BCA Protein Assay (Thermo). Hydrogen peroxide level was measured with SensoLyte ADHP Hydrogen Peroxide Assay Kit (Fremount, California, USA)

2.7. Real-time quantitative PCR

To measure mRNA expression in gastrocnemius of mice, it was quickly immersed in RNAlater (Applied Biosystems, Carlsbad, CA) and stored at −80 °C. Total RNA was extracted using RNeasy Fibrous Tissue Mini Kit (Qiagen, Valencia, CA, USA) and RNase-Free DNase Set (Qiagen) according to the manufacturer's instructions and assessed for purity using the NanoDrop system (NanoDrop Technologies, Wilmington, DE). Total mRNA was reverse transcribed to cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed using the Fast 7500 real-time PCR system (Applied Biosystems) using Power SYBR® Green PCR Master Mix kits (Applied Biosystems). The thermal profiles consisted of 10 min at 95 °C for denaturing, followed by 40 cycles of 95 °C for 15 s, annealing at 60 °C for 1 min. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as the housekeeping gene, and all data are represented relative to its expression (i.e., using standard curve methods) as fold change from the rest group. Specific PCR primer pairs for each studied gene are shown in Table 1.

2.8. Statistical analyses

All statistical analyses were performed using SPSS V17.0. The statistical significance of differences between groups on CK and LDH in plasma, hydrogen peroxide, and mRNA expression was determined using two-way ANOVA. In any analysis, if significant interactions were observed, then comparisons with the Tukey HSD post hoc test were performed.