IBUPROFEN ADMINISTRATION DURING ENDURANCE TRAINING CANCELS RUNNING-DISTANCE-DEPENDENT ADAPTATIONS OF SKELETAL MUSCLE IN MICE

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Exercise training induces many adaptations in skeletal muscle, representative examples of which include an increase in the IIa myofibre and an increase in the capillary-to-fibre ratio (C:F ratio). Moreover, these phenomena are thought to be dependent on running distance. Ibuprofen is one non-steroidal anti-inflammatory drug that is often used as an analgesic, but its effect on skeletal muscle adaptation during endurance training is unclear. In the present study, therefore, we administered ibuprofen to mice during running wheel exercise for four weeks, and examined its effects on the increase in the IIa myofibre and the C:F ratio in skeletal muscle. We observed a significant increase of the IIa myofibre and C:F ratio even in the presence of ibuprofen. Moreover, in untreated mice, there was a significant positive and strong correlation between these parameters and running distance. These results indicate that the increase in the IIa myofibre and the C:F ratio in skeletal muscle usually depend on running distance. Interestingly, we observed no significant correlation between these parameters and running distance in ibuprofen-administered mice. Moreover, we found no significant increase of these parameters when the running distance was significantly increased, in comparison with untreated mice. These results indicate that ibuprofen administration during endurance training cancels running-distance-dependent adaptations in skeletal muscle. This suggests that even if ibuprofen administration facilitates longer-distance running, no further effects of training on skeletal muscle can be expected.

Key words: endurance exercise, ibuprofen, skeletal muscle, body weight, muscle adaptation, cyclooxygenase

INTRODUCTION

Exercise training is thought to be a beneficial method for improving physiological parameters such as skeletal muscle mass, percentage body fat, and body weight. Skeletal muscle is a mosaic organ composed of several types of myofibre. Fast myofibre has an ability to contract faster than slow one, conversely slow myofibre has an ability to contract for a longer time than fast one. Slow myofibre is composed by myosin heavy-chain (MHC), type I. And, fast myofibres are composed by three (IIa, IId/x, and IIb) MHCs (1). Especially, fast myofibre composed by IIa is thought to be able to contract for a longer time than other fast myofibres composed by IId/x or IIb. Endurance training, such as periodic voluntary wheel-running or treadmill running, is thought to increase the percentage of IIa myofibre in plantaris muscle (2, 3).

The capillary is a basic vascular network component that permits diffusive exchange between the inside and outside of the vascular wall. Thus, an increase in the capillary-to-fibre ratio (C:F ratio) improves the efficiency of diffusive exchange between the vascular space and the intracellular volume of myofibres (4). Periodic voluntary wheel-running also induces an increase of the C:F ratio in mouse skeletal muscle (2). Therefore, these increases in the percentage of IIa myofibre and C:F ratio are thought to be representative adaptations to endurance exercise.

Ibuprofen is a non-steroidal anti-inflammatory drug that is often used as an analgesic and antipyretic (5). Previous studies have demonstrated an inhibitory effect of ibuprofen on skeletal muscle hypertrophy induced by functional overload, suggesting that ibuprofen administration during resistance training inhibits training-induced skeletal muscle hypertrophy (6, 7). On the other hand, the effect of ibuprofen on skeletal muscle adaptations induced by periodic endurance training is still unclear. In order to elucidate the effect of ibuprofen administration on endurance training-induced skeletal muscle adaptations, we housed mice in a cage with a running wheel for four weeks, and subsequently examined the effects of ibuprofen administration on the change of the percentage of I and IIa myofiber and C:F ratio.

MATERIALS AND METHODS

Experimental approval

Animal experiments were carried out in a human manner after receiving approval from the Institutional Animal Experiment Committee of the University of Tsukuba, and in accordance with the Regulations for Animal Experiments at the University, and the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology of Japan.
**Animals and animal care**

Eight-week-old (body weight 35-40 g) male ICR mice (Kiwa Laboratory Animals Co., Ltd., Japan) were housed in the animal facility under a 12-12 hour light-dark cycle at room temperature (21±2°C) and 50±5% humidity, and fed a rodent chow with free access to water. These mice were divided into four groups (each n=8): sedentary-D.W. (SED-D.W.), sedentary-ibuprofen (SED-IBU), exercise-D.W. (EXE-D.W.) and exercise-ibuprofen (EXE-IBU). The mice in the EXE-D.W. or EXE-IBU group were housed individually in a cage with a running wheel. The mice in the SED-IBU or EXE-IBU group were given ibuprofen (50 mg/l) in drinking water. The running distance of each mouse was monitored using a device we have reported previously (8).

**Immunohistochemistry**

Immediately after a four-week experiment period, all the mice were sacrificed by cervical dislocation, and the plantaris muscle was dissected out quickly from each mouse, and then frozen in isopentane cooled with liquid nitrogen. Transverse sections several micrometers thick were made with a cryostat (CRYO-STAR HM560, Microm, Germany) cooled to less than -16°C, and then stored at -80°C until staining. The sections were dried in air for 20 minutes, and then fixed with 4% paraformaldehyde for 10 minutes in room temperature. To inhibit endogenous peroxidase, the sections were incubated in 0.3% hydrogen peroxide diluted in methanol for 10 minutes at room temperature. Anti-CD31 (Pharmingen, USA) or anti-myosin (N2.261, human neonatal slow and fast IIa fibers, Iowa University, USA) antibody was used as the primary antibody, and biotin-conjugated anti-rat or anti-mouse antibody (Pharmingen, USA) as the secondary antibody. After incubation with avidin-biotin peroxidase complex (Nakarai Tesque, Japan) for 30 minutes, visualization of antibody staining was achieved using 3,3'-diaminobenzidine tetrahydrochloride (WAKO, Japan) as the chromogen. Serial number was entered in each slide to blind the condition to investigator quantifying the number of each antibody positive stain. The stained sections were finally visualised by microscopy (BX51, Olympus, Japan) and evaluated using a specific software package (OpenLab, Improvison, USA).

**Statistical analysis**

The significance of differences in daily running distance between the EXE-D.W. and EXE-IBU groups was measured by two-way analysis of variance (ANOVA). The effects of ibuprofen or wheel-running on the percentage of I and IIa myofiber and C:F ratio were analyzed using the Tukey multiple comparison test following two-way ANOVA. Pearson correlation coefficients were used to measure associations between average daily running-distance and various parameters, including the percentage of I and IIa myofibre and C:F ratio. Differences at P<0.05 were considered to be statistically significant.

**RESULTS**

**Water intake, body weight, muscle mass and running distance**

The daily running distance in the two groups gradually increased throughout the experimental period for four weeks (Fig. 1). Two-way ANOVA revealed that there was a significant difference in the average daily running distance between the two groups (P<0.001). Table 1 shows the average daily water intake (with or without ibuprofen), body weight, and muscle mass in each group. Two-way ANOVA revealed that wheel running led to a decrease of body weight (P<0.001), whereas it increased the average daily amount of water drunk (P<0.01). However, there was no significant difference in these parameters between the groups with and without ibuprofen administration. Wet weight of plantaris muscle was not influenced by both wheel running and ibuprofen administration (Table 1).

**Lack of effect of ibuprofen administration on endurance training-induced adaptation in skeletal muscle**

Next, we measured the percentage of I and IIa myofiber and C:F ratio in each of the four groups. Tukey multiple comparison test following two-way ANOVA revealed that wheel-running for four weeks increased both the percentage of I and IIa myofiber (P<0.01) and the C:F ratio (P<0.01). However, ibuprofen administration had no effect on either of these parameters (Fig. 2).

**Abrogation of running distance-dependent adaptation to wheel training by ibuprofen administration**

It is well known that endurance training increases the percentage of I and IIa myofiber in proportion to the running

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**Table 1. Influence of ibuprofen administration during wheel running to body weight, plantaris muscle mass and daily water intake**

<table>
<thead>
<tr>
<th></th>
<th>SED-D.W.</th>
<th>SED-IBU</th>
<th>EXE-D.W.</th>
<th>EXE-IBU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>41.67 (±0.83)</td>
<td>43.69 (±0.77)</td>
<td>39.22 (±0.64)</td>
<td>39.73 (±0.41) * (SED vs EXE)</td>
</tr>
<tr>
<td>Absolute plantaris muscle mass (mg)</td>
<td>21.58 (±1.06)</td>
<td>22.23 (±1.40)</td>
<td>23.40 (±1.21)</td>
<td>25.19 (±1.45)</td>
</tr>
<tr>
<td>Average daily drink water (g)</td>
<td>6.55 (±0.26)</td>
<td>7.04 (±0.61)</td>
<td>8.42 (±0.45)</td>
<td>8.52 (±0.40) * (SED vs EXE)</td>
</tr>
</tbody>
</table>

This table shows body weight, absolute muscle mass of plantaris, and average daily water intake. SED, sedentary; EXE, exercise; D.W., Distilled Water; IBU, ibuprofen. Asterisks indicate significant difference between SED and EXE. All data are indicated as S.E.M.
Therefore, the myofibre composition and C:F ratio were each compared in terms of the average daily running distance using Pearson correlation coefficients. This revealed that without ibuprofen administration, the percentage of I and IIa myofibre and the C:F ratio were directly correlated with the average daily running distance (Fig. 3A and C), whereas this was not the case in mice administered ibuprofen (Fig. 3B and D).

**DISCUSSION**

Skeletal muscle has an ability to fit to its workload-type. For instance, it is well known that periodic resistance training induces skeletal muscle hypertrophy (9), whereas endurance training induces an increase in IIa myofibre and C:F ratio (2, 3). In fact, it was revealed that endurance training did not increase...
maximal voluntary isometric contraction, but it increased time to fatigue at 50% of maximal voluntary isometric contraction in human skeletal muscle (10). Moreover, it is also well known that exercise increased oxidative capacity and capillary density in only muscle which contracted during exercise (11). Because ibuprofen is often used as an analgesic and anti-inflammatory, we examined its effect on skeletal muscle adaptation induced by endurance training in mice. Although we had hypothesized that ibuprofen administration during endurance training would inhibit endurance training-induced adaptation, no such effect was observed. However, we observed that ibuprofen administration during voluntary wheel-running abrogated running-distance-dependent percentage of I and Ila myofibre and C:F ratio (Fig. 3).

At first, the cause of this interesting result may be that mice administered ibuprofen ran a longer distance than those without ibuprofen (Fig. 1). Ibuprofen is a non-specific inhibitor of cyclooxygenases (COX) I and II, which produce prostaglandins, known to have an analgesic effect (5). It was reported that ibuprofen reduced muscle soreness after exercise (12), which indicated that exercise-induced muscle soreness is caused by prostaglandins catalyzed by COX. Pizza et al. reported that passive stretching, isometric contraction, and lengthening contraction without severe injury in skeletal muscle increased the recruitment of leukocytes, which are known to secrete prostaglandins (13). We also observed an increase of leukocytes on a day after a single bout of endurance training without severe injury in mouse skeletal muscle (14). It was possible that voluntary running also infiltrated leukocyte-secretating prostaglandins in skeletal muscle as well as previous study (14), and muscle soreness caused by this infiltration might limit voluntary running in mice without ibuprofen administration (Fig. 1). However, in current study, we did not confirm leukocyte infiltration and some inflammatory response in skeletal muscle. In the future study, relationship between voluntary running and inflammatory response should be studied.

Inflammation is thought to have a close relationship to angiogenesis (15). Co-cultivation of cancer cells with macrophages in the presence of inflammatory stimulation synergistically enhances the migration of vascular endothelial cells in vitro, and macrophage depletion by clodronate liposomes reduces angiogenesis in vivo (16). Macrophage depletion by clodronate reduces both tumour angiogenesis and subsequent tumour growth in lung cancers (17). COX-2 is a representative mediator of inflammatory responses, and its expression is highly influenced by inflammatory stimulation. COX-2 also plays a key role in tumour angiogenesis by catalyzing the production of prostaglandins, including prostaglandin E₂ and thromboxane A₂ (18, 19). In mouse corneal angiogenesis induced by interleukin-1 beta, either administration of COX-2 inhibitors or COX-2 knockdown almost completely attenuates angiogenesis (20). There are considerable reports indicating that inflammation facilitates tumour angiogenesis (17-20). Previous studies reported that exercise increased inflammation-related cytokines such as interleukin-1 (21), interleukin-6 (22) and transforming growth factor-beta1 (23). We previously observed vascular endothelial growth factor-secretting leukocyte in mouse skeletal muscle on a day after exercise (14). These results indicated that exercise induces inflammation in skeletal muscle and suggested that exercise-induced angiogenesis also related to inflammation. In current study, we observed that running-distance dependent C:F ratio in D.W.-EXE group was abrogated in IBU-EXE group (Fig. 3). We have hypothesized the second reason of this abrogation was that exercise-induced angiogenesis was inhibited by ibuprofen administration in longer running mice in IBU-EXE group.

The percentage of I and Ila myofibre was increased by voluntary wheel running (Fig. 2). Moreover, this parameter was dependent to running distance in D.W.-EXE group (Fig. 3). Interestingly, this positive correlation between running distance and the percentage of I and Ila myofibre as well as C:F ratio was abrogated by ibuprofen administration (Fig. 3). Although the longer running induced by ibuprofen administration might be the cause of the abrogation, there is no sufficient evidence to support the effect of ibuprofen administration inhibited the increase of Ila myofibre as well as C:F ratio. However, it was reported that immuno-suppressing drug such as FK506 reduced fibre-type transition from fast to slow (24). It is possible that one immune-system factor related to inflammation has a relationship to fibre-type transition.

Soltow et al. showed that skeletal muscle hypertrophy was inhibited by ibuprofen (6), and Novak et al. demonstrated that the specific COX-2 inhibitor, NS-398, reduced skeletal muscle hypertrophy in the mouse (25). Moreover, DiPasquale et al. reported that macrophage depletion by clodronate delayed skeletal muscle hypertrophy (26). These results indicate that inflammation is an important factor for skeletal muscle hypertrophy, and also that administration of anti-inflammatory drugs (including ibuprofen) inhibits skeletal muscle hypertrophy. Taken together, the present and previous findings indicate that inhibition of inflammation may reduce the skeletal muscle adaptations induced by exercise training. In the future, however, it will be necessary to investigate the effects of administration of anti-inflammatory drugs on the I and Ila myofibre and C:F ratio using a treadmill, which will make it possible to control the running distance and intensity.

In conclusion, the present study has demonstrated that ibuprofen administration during endurance training facilitates a longer distance run, but this is not associated with effects that were originally considered to depend on running distance. On the basis of these results, we propose that administration of anti-inflammatory drugs may diminish proper effects of training that are correlated with the degree of training labor.

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Conflict of interests: None declared.

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