Original Article

Effects of Acetic Acid Bacteria Supplementation on Muscle Damage After Moderate-Intensity Exercise

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Abstract

Objective: Acetic acid bacteria were traditionally used to produce fermented food. Furthermore, acetic acid bacteria contain unique membrane lipids that would be expected to attenuate inflammation. This study examined the effects of oral intake of acetic acid bacteria isolated from fermented milk on muscle damage after moderate-intensity exercise.

Methods: In a double-blind placebo-controlled crossover trial, 40 untrained subjects (16 men and 24 women; age, 46.4 ± 4.3 yr; height, 162.8 ± 10.8 cm; weight, 60.2 ± 9.4 kg; body mass index, 22.6 ± 2.9 kg/m²) took 111 mg of dried acetic acid bacteria per day (supplemented group) or 111 mg of cornstarch per day (placebo group) for 1 week and walked for 60 min on the last day of administration.

Results: Leukocyte, lymphocyte and neutrophil counts, IL-6 and creatine kinase (CK) activity, myoglobin (Mb) concentration and perceived pain in parts of the body were measured pre and post exercise. All values, except for IL-6, were significantly increased post-exercise compared with pre-exercise in both groups. However, neutrophil counts and ankle pain were significantly lower in the supplemented group. In addition, the increase of CK activity in the supplemented group was significantly attenuated at 24 h after exercise (supplemented group, 114 ± 54 U/l; placebo group, 126 ± 68 U/l). The supplemented group also demonstrated a trend toward a lower level of CK activity after exercise (p = 0.06). Other values did not differ between groups.

Conclusion: These results suggested that acetic acid bacteria supplementation was useful to attenuate muscle damage after moderate-intensity exercise.

KEY WORDS: acetic acid bacteria, dietary supplement, walking, neutrophil, creatine kinase

Introduction

Regular exercise prevents and ameliorates lifestyle-related diseases, such as type II diabetes, hypertension and ischemic heart disease 1-3). Regular exercise of moderate intensity is sufficient to be of benefit with regard to those diseases 4). On the other hand, exercise, especially unaccustomed exercise, induces muscle damage related to inflammation after exercise 5), and even moderate-intensity exercise is not an exception 6-8). One factor in failure to continue regular exercise may be exercise-induced muscle damage.

Exercise-induced muscle damage is initiated by mechanical muscular contraction, which induces production and release of inflammatory mediators, such as cytokines and chemokines, resulting in neutrophil mobilization into the circulation. Circulating neutrophils infiltrate muscle tissue and cause muscle damage due to phagocytosis. Damaged muscle releases myocellular proteins such as creatine kinase (CK) and myoglobin (Mb) into the circulation 9,10). Thus, exercise-induced muscle damage is caused by inflammation after exercise. Oral intake of α-tocopherol and allicine were reported to reduce exercise-induced muscle damage through attenuation of inflammation 11,12). In addition to these compounds, some lipids, such as phosphatidylcholine (PC), terpenoids and sphingolipids, have been reported to inhibit production of inflammatory mediators 13-15) and would be expected to reduce muscle damage through attenuation of inflammation. In general, they are abundant in plants, but scarce in bacteria. However, acetic acid bacteria have unique membrane lipid components compared with other bacteria, and their membrane lipids consist of PC, terpenoids and sphingolipids 16). Acetic acid bacteria are traditionally used to produce fermented food, such as vinegar, and can be obtained as viable cells, especially from fermented milk 17,18). Although acetic acid bacteria contain unique membrane lipids and have a history of being ingested, the effects of ingestion of acetic acid bacteria on physiological function have not been studied.

With this background, in this study we focused on acetic acid bacteria isolated from fermented milk to clarify whether acetic acid bacteria supplementation attenuates inflammation and can reduce exercise-induced muscle damage after moderate-intensity exercise in untrained humans.
Methods

Subjects
Forty healthy volunteers (16 men and 24 women; 46.4 ± 4.3 yr; height, 162.8 ± 10.8 cm; weight, 60.2 ± 9.4 kg; body mass index, 22.6 ± 2.9 kg/m²) participated in this study. Subjects were recruited by an advertisement through a contract research organization, HUMA R&D Co., Ltd. (Minato-ku, Tokyo). None of the subjects had an exercise habit. Excluded from the study were current smokers; those who walked > 7000 steps per day; had a history of medical illness; took chronic medication or supplements, such as vitamin E and coenzyme Q10; had a food allergy; or had donated blood within 3 months prior to the study. Before obtaining written consent, we informed the subjects of the purpose of this study as well as possible risks and discomfort. The study protocol was approved by the ethics committee of two separate groups, the Mizkan Group Corporation and the HUMA R&D Co., Ltd., and was performed in accordance with the Declaration of Helsinki. The experiment was conducted under the management of medical doctors.

Acetic acid bacteria supplement
Acetic acid bacteria, Acetobacter malorum NCI 1683 (S24), was isolated from fermented milk by the method described by Entani and Masai. Similarity of the 16S rRNA sequence was 100% between the strain Acetobacter malorum NCI 1683 (S24) and Acetobacter malorum LMG 17467T (DSM 14337T). As the result of DNA-DNA hybridizations, DNA-DNA relatedness values between that strain and Acetobacter malorum LMG 17467T (DSM 14337T) was 76%. Thus, the strain was confirmed to be Acetobacter malorum NCI 1683 (S24). Bacteria were homogenized at high pressure and powdered by spray drying. This powder was enclosed in a capsule with soybean oil and beeswax. Acetobacter malorum NCI 1683 (S24) capsules were comprised of 7.4% dried acetic acid bacteria, 73% soybean oil and 17% beeswax. Placebo capsules contained these ingredients at the same ratios, with cornstarch replacing dried acetic acid bacteria.

Supplementation
A double-blind placebo-controlled crossover trial with randomization was used. Subjects in the supplemented group received oral supplementation with dried acetic acid bacteria capsules for 1 week, while subjects in the placebo group received placebo capsules for 1 week. Both groups performed the exercise experiment on the last day of that week. Capsules were ingested 3 times a day and total intake of acetic acid bacteria or cornstarch was 111 mg per day. Subjects recorded the ingested capsule count every day, and compliance was assessed by capsule count. The interval between administration of the supplement and administration of the placebo was 2 weeks. No side effects were observed by oral administration of the supplement or the placebo in any subject.

Experimental protocol
A flow diagram of the experimental protocol and procedure is shown in Figure 1. Briefly, the exercise consisted of walking for 60 min with the aim of achieving a Heart Rate (HR) of 120 - 130 beats per min and a Rating of Perceived Exertion (RPE) of 12 - 13. Subjects performed exercise on a flat surface in a gymnasium. During exercise, subjects recorded their HR using the HR monitor, POLAR F11 (Polar Electro Oy, Kenpele, Finland), and the RPE every 15 min. They maintained their exercise intensity throughout the 60-min period. Only ingestion of water at room temperature was allowed during the experiment. The contents of dinner before the experimental day and the menu and portions for breakfast, lunch and dinner on the experimental day were the same among subjects. The day before each experimental day, subjects finished dinner before 2000 h, and then fasted overnight.

Visual analog scale (VAS)
Subjects were asked to record perceived pain on a 100 mm VAS from 0 (Most severe pain) to 100 (No pain) before (Pre), immediately after (Post), 7-h post (Post 7 h) and 24-h post exercise (Post 24 h). The VAS consisted of 6 scales, which evaluated body, leg, thigh, calf, hip and ankle pain.

Blood sampling
Peripheral venous blood samples were collected by antecubital venipuncture before (Pre), immediately after (Post), 2-h post (Post 2 h) and 24-h post exercise (Post 24 h).

Total and differential leukocyte counts
Total leukocyte count in EDTA-treated blood was measured by a Sysmex microcell counter K-4500 (TOA Medical Electronics, Kobe-city, Hyogo). The different leukocyte types were classified using the Olympus BH2 (Olympus Corporation, Shinjuku-ku, Tokyo). The absolute number of each cell type was calculated from results of the total leukocyte count based on the percentage of each cell type.

![Fig. 1. Experimental protocol](image-url)
Serum biochemistry

Serum samples were separated from whole blood in Vacutainer blood-collection tubes by centrifugation at 1,000 g for 10 min after blood was left to clot at room temperature for 30 min. These samples were stored frozen at –80 degrees until assayed. Serum CK activity was measured using a biochemical assay kit and Mb concentration was measured by a radioimmunoassay kit. IL-6 concentration was measured by a chemiluminescent enzyme immunoassay.

Statistical analysis

Data were presented as means (M) and standard deviation (SD). Mixed-model one-way repeated measures analysis of variance (ANOVA) was used to compare all values from the pre-exercise value to each time point within each group. Mixed-model two-way repeated measures analysis of covariance (ANCOVA) across groups and time points, with each pre-exercise value as a covariate, was used to detect the main effect for groups after exercise. Student’s paired t-test was used for between group comparisons at each time point. Significance was evaluated for all statistics at \( p < 0.05 \). The statistical analysis was performed using a statistical package (SPSS 11.5 for Windows, Chicago, IL, USA).

Results

Exercise intensity

HR and RPE data during exercise are summarized in Table 1. These data show that the physical task level in this study, which was maintenance of an HR of 120 - 130 beats per min and RPE of 12 - 13 during moderate intensity exercise, was achieved. There were no significant differences between groups in HR and RPE at each time point.

Table 1  Heart rate and Rating of Perceived Exertion data during moderate-intensity exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>Average during exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>S</td>
<td>121 ± 5</td>
<td>122 ± 4</td>
<td>123 ± 4</td>
<td>123 ± 4</td>
<td>122 ± 3</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>120 ± 6</td>
<td>122 ± 4</td>
<td>123 ± 3</td>
<td>123 ± 3</td>
<td>122 ± 4</td>
</tr>
<tr>
<td>RPE</td>
<td>S</td>
<td>11.9 ± 2.2</td>
<td>12.5 ± 2.2</td>
<td>12.9 ± 2.4</td>
<td>13.3 ± 2.6</td>
<td>12.6 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>11.7 ± 1.6</td>
<td>12.3 ± 2.1</td>
<td>12.6 ± 2.2</td>
<td>13.1 ± 2.6</td>
<td>12.4 ± 2.6</td>
</tr>
</tbody>
</table>

Note. Values are \( M ± S.D. \). S, supplemented group (n=40); P, placebo group (n=40).

Table 2  Effects of moderate-intensity exercise on total and differential leukocyte counts in supplemented and placebo groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Pre</th>
<th>Post</th>
<th>Post 2 h</th>
<th>Post 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes</td>
<td>S</td>
<td>5300 ± 1109</td>
<td>5893 ± 1339**</td>
<td>6125 ± 1250**</td>
<td>5395 ± 1330</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>5464 ± 1391</td>
<td>5970 ± 1564**</td>
<td>6163 ± 1508**</td>
<td>5533 ± 1702</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>S</td>
<td>1841 ± 583</td>
<td>2054 ± 638</td>
<td>2188 ± 576**</td>
<td>1815 ± 447</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1888 ± 628</td>
<td>1969 ± 652</td>
<td>2146 ± 697*</td>
<td>1766 ± 491</td>
</tr>
</tbody>
</table>

Note. Values are \( M ± S.D. \). Blood samples were collected before (Pre), immediately after (Post), 2-h post exercise (Post 2 h) and 24-h post exercise (Post 24 h). *Significant difference in comparison of the mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; \( p < 0.05 \). **Highly significant difference in comparison of the mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; \( p < 0.01 \). S, supplemented group; P, placebo group.

Total and differential leukocyte counts

In the placebo group, the circulating neutrophil counts from Post to Post 24 h was significantly increased compared with the Pre value, while it was significantly increased from Post to Post 2 h in the supplemented group (Figure 2). A significant effect in both groups on circulating neutrophil counts was observed after exercise. However, the neutrophil counts were lower in the supplemented group than in the placebo group (Figure 2). Circulating leukocyte and lymphocyte counts increased after exercise in both groups, with no significant difference between groups (Table 2).
Serum CK activity

In both groups, in comparison with the Pre value, serum CK activity was significantly increased at all time points from Post to Post 24 h. In the supplemented group, the increase in serum CK activity was significantly attenuated in comparison with that in the placebo group at Post 24 h (Figure 3). Although it was not significant, the effect in both groups on serum CK activity was observed after exercise, with the change less in the supplemented group than in the placebo group ($p = 0.06$) (Figure 3).

Mb concentration

Mb concentration was significantly increased only at Post exercise compared with the Pre value in both groups, but was significantly lower in the supplemented than in the placebo group (Table 3). However, the difference between groups was nearly the same both Pre and Post exercise, suggesting that the effect of acetic acid bacteria supplementation on Mb concentration was minor.

Serum IL-6 concentration

In comparison with the Pre value, the only significant increase in serum IL-6 was at Post 2 h in the supplemented group. No significant changes from the Pre value were observed at any time point in the placebo group (Table 3).

VAS

Perceived body and leg pain as well as perceived thigh, calf and hip pain were significantly increased after exercise in both groups, showing that moderate-intensity exercise induced perceived pain (Table 4). As shown in Figure 4, perceived ankle pain was also significantly increased after exercise in both groups, with the VAS score higher in the supplemented group than in the placebo group. This indicated that acetic acid bacteria supplementation reduced perceived ankle pain after exercise although pain in other parts of the body did not differ significantly between groups.

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**Table 3**  
Effects of moderate-intensity exercise on biochemical markers in supplemented and placebo groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Pre</th>
<th>Post</th>
<th>Post 2 h</th>
<th>Post 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mb (ng/ml)</td>
<td>S</td>
<td>35 ± 11</td>
<td>45 ± 3 $^{+++}$</td>
<td>36 ± 22</td>
<td>36 ± 12</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>38 ± 18</td>
<td>48 ± 3 $^{**}$</td>
<td>40 ± 20</td>
<td>34 ± 11</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>S</td>
<td>1.3 ± 2.0</td>
<td>1.5 ± 1.9</td>
<td>1.7 ± 2.5 $^{**}$</td>
<td>1.4 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1.7 ± 4.5</td>
<td>1.4 ± 1.6</td>
<td>1.4 ± 1.5</td>
<td>1.7 ± 3.1</td>
</tr>
</tbody>
</table>

Note. Values are $M ± SD$. Blood samples were collected before (Pre), immediately after (Post), 2-h post exercise (Post 2 h) and 24-h post exercise (Post 24 h). *Significant difference in comparison of the mean difference between groups detected by paired Student’s $t$-test; $p < 0.05$. **Highly significant difference in comparison of the mean difference between groups detected by paired Student’s $t$-test; $p < 0.01$. †Significant difference in comparison of the mean difference between groups detected by mixed-model repeated measures ANCOVA with pre-exercise value as a covariate; $p < 0.05$.
Table 4  Effect of moderate-intensity exercise on perceived pain as evaluated by a visual analog scale (VAS) in supplemented and placebo groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Pre</th>
<th>Post</th>
<th>Post 7 h</th>
<th>Post 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS for body pain</td>
<td>S</td>
<td>62 ± 25</td>
<td>52 ± 27*</td>
<td>47 ± 25**</td>
<td>60 ± 28</td>
</tr>
<tr>
<td>(mm)</td>
<td>P</td>
<td>67 ± 22</td>
<td>50 ± 22**</td>
<td>47 ± 25**</td>
<td>62 ± 27</td>
</tr>
<tr>
<td>VAS for leg pain</td>
<td>S</td>
<td>69 ± 25</td>
<td>47 ± 29**</td>
<td>46 ± 28**</td>
<td>58 ± 28*</td>
</tr>
<tr>
<td>(mm)</td>
<td>P</td>
<td>74 ± 18</td>
<td>42 ± 24**</td>
<td>47 ± 25**</td>
<td>59 ± 27**</td>
</tr>
<tr>
<td>VAS for thigh pain</td>
<td>S</td>
<td>78 ± 21</td>
<td>57 ± 29**</td>
<td>55 ± 27**</td>
<td>63 ± 26**</td>
</tr>
<tr>
<td>(mm)</td>
<td>P</td>
<td>80 ± 19</td>
<td>54 ± 29**</td>
<td>56 ± 27**</td>
<td>63 ± 27**</td>
</tr>
<tr>
<td>VAS for calf pain</td>
<td>S</td>
<td>74 ± 28</td>
<td>52 ± 28**</td>
<td>51 ± 29**</td>
<td>61 ± 29**</td>
</tr>
<tr>
<td>(mm)</td>
<td>P</td>
<td>80 ± 19</td>
<td>48 ± 30**</td>
<td>50 ± 27**</td>
<td>61 ± 30**</td>
</tr>
<tr>
<td>VAS for hip pain</td>
<td>S</td>
<td>79 ± 22</td>
<td>62 ± 26**</td>
<td>59 ± 28**</td>
<td>68 ± 27*</td>
</tr>
<tr>
<td>(mm)</td>
<td>P</td>
<td>80 ± 22</td>
<td>61 ± 28**</td>
<td>60 ± 27**</td>
<td>69 ± 26**</td>
</tr>
</tbody>
</table>

Note. Values are M ± SD. Subjects were asked to evaluate pain on a 100 mm VAS from 0 (Most severe pain) to 100 (No pain) before (Pre), immediately after (Post), 7-h post exercise (Post 7 h) and 24-h post exercise (Post 24 h). *Significant difference in comparison of the mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; p < 0.05. **Highly significant difference in comparison of the mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; p < 0.01. S, supplemented group; P, placebo group.

Discussion

In this study, 40 untrained subjects performed a 1-h walking experiment to examine the effects of acetic acid bacteria supplementation on muscle damage after moderate-intensity exercise in a double-blind placebo-controlled crossover trial. The results indicated that acetic acid bacteria supplementation attenuated circulating neutrophil counts, serum CK activity, and ankle pain that were continuously induced after moderate-intensity exercise.

Walking is a popular form of exercise in daily life, and brisk walking for 1 h has been recommended in general as moderate-intensity exercise. Therefore, we used walking for a 1-h period as a model for moderate-intensity exercise. Perceived muscle pain after exercise consisted of acute muscle pain immediately after exercise and delayed onset muscle soreness caused by inflammation. In this study, estimations by VAS could not distinguish between acute and delayed muscle pain. Perceived muscle pain persisted continuously until 24 h after exercise, and was most severe immediately after exercise in all parts of the body. In addition, neutrophils, which are induced by inflammatory mediators, and serum CK activity, which is a marker of muscle damage, were also increased up until 24 h after exercise. These results suggested that the exercise model used in this study induced muscle damage by inflammation and might be suitable to assess the effect of acetic acid bacteria supplementation.

The disruption of muscle cells due to mechanical muscular contraction and phagocytosis of neutrophils results in CK and Mb efflux into the circulation. Therefore, serum CK activity and Mb concentration are generally used markers of muscle damage. However, our results showed that time-dependent changes differed in these muscle damage markers. Mb concentration increased only immediately after exercise before returning to the pre-exercise value, while serum CK activity continued to increase until 24 h after exercise. This difference in patterns has been reported previously, with the explanation that the molecular weight of Mb was smaller than that of CK and that Mb is quickly removed from the circulation after exercise. Therefore, one possible explanation of the differences in the pattern between serum CK activity and Mb concentration after exercise in this study was that the elimination rate of Mb was higher than the accumulation rate in the circulation, resulting in the disappearance of Mb in blood soon after exercise.

We found that circulating neutrophil counts, which kept increasing up to 24 h after exercise, were attenuated by acetic acid bacteria supplementation. The result for neutrophils was similar to that for serum CK activity and suggested that acetic acid bacteria supplementation attenuated serum CK activity through the effect of neutrophil migration after exercise. Neutrophil migration was reported to be activated by cytokines and chemokines. Therefore, we measured IL-6, one of the major cytokines, but found no significant increase in the placebo group after exercise nor an attenuation in the supplemented group. This result suggested that inflammatory mediators other than IL-6 participate in neutrophil migration. This result suggested that inflammatory mediators other than IL-6 participate in neutrophil migration. The membrane lipid of acetic acid bacteria contains PC, terpenoids and sphingolipids. These components could be expected to attenuate inflammatory mediators through regulation of NF–κappaB activation. The mechanism of the anti-inflammatory effect of acetic acid bacteria supplementation and the component responsible for this effect must be clarified in the future.

Perceived ankle pain in the supplemented group was reduced in comparison with the placebo group, although perceived pain in the body, leg, thigh, calf and hip did not differ significantly between the two groups. Because the ankle supports the entire body weight during exercise, the ankle might have been more affected by the exercise load compared than the other parts examined. Therefore acetic acid bacteria supplementation might have had a marked effect on perceived ankle pain.

In conclusion, results of this study suggested that oral intake of acetic acid bacteria was useful in attenuating muscle damage by inflammation after moderate-intensity exercise.
Acknowledgement

We would like to thank Atsushi Ishikawa, Takashi Fushimi, Hiroyuki Fukami and Takahiro Oda for useful discussions, and Shin Ogawa for supplying the dry Acetobacter malorum NCI 1683 (S24) that we used in this study.

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