INFLUENCE OF A LOW-DOSE COX-2 INHIBITOR DRUG ON EXERCISE-INDUCED INFLAMMATION, MUSCLE DAMAGE AND LIPID PEROXIDATION

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ABSTRACT: The purpose of this study was to examine the effect of acute low-dose celecoxib administration on exercise-induced inflammation, muscle damage and lipid peroxidation. Twenty healthy untrained males (age: 25.5±4.5 yrs, weight: 72.7±7.9 kg, height: 177.3±7.2 cm) were randomly assigned to treatment (T) or placebo (P) groups. Blood samples were obtained before, immediately after, 3 h after and 24 h after exercise. Subjects ran for 30 min at 75% VO2max on a treadmill. Participants consumed 100 mg celecoxib or a placebo immediately after and 12 h after the immediately post-exercise blood sample. Total leukocytes, neutrophils, creatine kinase (CK), C-reactive protein (CRP) and malondialdehyde (MDA) were assessed at each time point. Significant increases in total leukocytes and neutrophils were observed 3 h after exercise in both groups (P<0.05). CK and CRP levels were significantly increased immediately, 3 h and 24 h after exercise in both groups (P<0.05). A significant increase in MDA was observed immediately after exercise in both groups (P<0.05); however, no significant group differences were observed for MDA or CK. These findings suggest that inhibition of cyclo-oxygenase activity with low-dose celecoxib does not affect exercise-induced inflammation, muscle damage, or lipid peroxidation.

KEY WORDS: celecoxib; inflammation; muscle damage; lipid peroxidation

INTRODUCTION

Routine exercise training of moderate intensity is known to enhance immune function [27] and reduce risk of mortality [11,23], cardiovascular diseases, obesity, and type 2 diabetes [17]. However, an acute bout of prolonged (>1.5 h) and/or strenuous exercise (55-75% maximal O2 uptake) has also been shown to provoke muscle damage/soreness [4], and elicit an acute inflammatory [15], oxidative stress [7,9], and immunosuppressive response [25] during the post-exercise recovery period. In order to alleviate these potential adverse responses following prolonged/strenuous exercise, the consumption of non-steroidal anti-inflammatory drugs (NSAIDs) has been strategically used to reduce soreness, muscle damage, and inflammation following exercise [13].

The original NSAIDs (e.g. ibuprofen) inhibited both the constitutive cyclooxygenase (COX)-1 and the more inducible COX-2 enzyme, which both catalyze the generation of prostanoids (prostaglandins (PGE2 and PGF2α), prostacyclins and thromboxanes); which are involved in numerous inflammatory, anaphylactic, and cardiovascular physiological processes [14]. However, it has been postulated that COX-2 is the key enzyme which regulates the amount and duration of inflammatory cell accumulation [29] and lipid peroxidation [1] following exercise-induced muscle damage. Therefore, it seems plausible that the more recently developed selective COX-2 inhibitors may be more appropriate and specific to reduce excessive inflammatory reactions following exercise than the non-selective (COX-1 and COX-2) inhibitors [28].

Celecoxib, a sulfonamide, is a selective COX-2 inhibitor that has anti-inflammatory and analgesic effects comparable to non selective NSAIDs, and presents with significantly less gastrointestinal toxicity [28]. To date, several studies have demonstrated a lack of efficacy of NSAIDs treatment on muscle inflammation after exercise [12, 18,26], while others have reported enhanced recovery and reduction of inflammatory markers following exercise [8]. Therefore, given the equivocal results regarding the effect of non-selective anti-inflammatory drugs on exercise-induced inflammation, muscle damage, and lipid peroxidation, this study was designed to investigate the influence of an acute low dose of the selective COX-2 inhibitor celecoxib on inflammation, muscle damage, and lipid peroxidation following an intense bout of aerobic exercise.
TABLE 1. SUBJECTS’ CHARACTERISTICS IN TREATMENT (T) AND PLACEBO (P) GROUPS (N=10)

| Group | VO_2max (ml·kg⁻¹·min⁻¹) | BMI (kg·m⁻²) | Height (cm) | Body mass (kg) | Age (yrs) | Skin folds (mm) |
|-------|--------------------------|--------------|-------------|----------------|-----------|-----------------|
| T     | 34.5 ± 2.8               | 23.3 ± 2.7   | 176.6 ± 7.7 | 72.4 ± 8.6     | 25.7 ± 3.9| 45.1 ± 6.4      |
| P     | 35.0 ± 5.2               | 23.0 ± 1.1   | 178.2 ± 6.8 | 73.1 ± 5.7     | 25.3 ± 5.2| 40.2 ± 8.4      |

Note: Data represent mean ± SD.

MATERIALS AND METHODS

Participants. Twenty untrained male volunteers took part in this study. All subjects were informed verbally and in writing about the nature and demands of the study, and subsequently completed a health history questionnaire. Qualifying volunteers were provided with and asked to sign an institutionally approved informed consent form. In addition to meeting the health criteria, individuals engaging in the following were also excluded from the study: smoking, alcohol intake, vitamin supplementation (e.g., vitamin A, C and E) or NSAIDs. Three-day diet records were used to estimate subjects’ average daily intake of vitamin A, C, and E prior to the beginning of the study. All subjects were untrained and had no known allergies to NSAIDs. Subjects were randomly assigned to treatment (T) (100 mg celecoxib) or placebo (P) groups. Each group was matched for height, weight, BMI, %fat and cardiovascular fitness (no significant group differences) (Table 1). The protocol of the study was approved by the university ethics committee in accordance with the Helsinki Declaration.

Dietary records
Subjects were asked to keep a dietary record of their food intake 3 days prior to the exercise test session. Total daily Vitamin A, C, and E intake during the 3 days prior to testing were determined using the Dietary Manager Computer program (Food process-2). Dietary analysis revealed no statistically significant differences in vitamin A, C, and E intake between groups (Table 2).

Preliminary testing
Cardiovascular fitness was determined by performing a maximal oxygen uptake test (VO_2max test). VO_2max was measured indirectly on a treadmill using the Bruce protocol. Briefly, subjects warmed up for 4 minutes at a speed of 3 mph on a 2.5% grade. Following warm-up, subjects ran on a treadmill beginning at a moderate pace; every 3 minutes the grade and intensity were increased until exhaustion [3]. Heart rate was monitored by a Polar Vantage XL heart rate monitor (Polar Beat, Port Washington, NY, USA). This was conducted at least 2 weeks prior to the scheduled exercise session.

TABLE 2. MEAN TOTAL DIETARY INTAKE OF VITAMIN A, C, AND E OF SUBJECTS IN TREATMENT (T) AND PLACEBO (P) GROUPS

| Variable     | group T     | group P     | P    |
|--------------|-------------|-------------|------|
| Vitamin A (RE) | 529.4 ± 384.3 | 570.1 ± 320.2 | 0.58 |
| Vitamin C (mg)  | 61.6 ± 26.3  | 45.9 ± 21.7  | 0.21 |
| Vitamin E (mg)   | 4.4 ± 2.6    | 3.4 ± 1.7    | 0.41 |

Note: Data represent mean ± SD.

Experimental design and procedures
On the morning of the trial, subjects arrived at the laboratory. They were instructed to have a standard breakfast consisting of two boiled eggs 2 h prior to the test. All subjects were required to avoid any strenuous exercise for 72 h prior to participation in the study. Participants performed a 10-min warm-up consisting of running at 50%VO_2max (5 min) and stretching (5 min). Following the warm-up and light stretching, participants ran on a treadmill for 30 min at 75%VO_2max. Blood samples were taken from an antecubital forearm vein before exercise, immediately after, 3 h after, and 24 h after exercise. Each sample was taken following 15 min of standing in a resting position (except for the post-exercise sample, which was taken immediately upon cessation of exercise). Subjects consumed 100 mg of celecoxib or placebo immediately following exercise and 12 h after exercise.

Blood sampling and analysis
Approximately 6 ml of whole blood was withdrawn at each time point. 1.5 ml from each sample was added to tubes containing ethylenediaminetetra-acetic acid (EDTA) for determination of leukocyte differentials using a cell counter (K-1000 Sysmax, Japan). Haemoglobin and haematocrit concentrations from whole blood samples were used to estimate plasma volume shifts. All post-exercise samples were corrected for plasma volume change according to the methods of Dill and Costill [5]. 4.5 ml of the blood was allowed to clot at 37.5°C, and centrifuged at 5,000 g for 30 min. Serum was prepared according to standard methods. Serum creatine kinase (CK) was determined using commercially available methods (Roche Hitachi-911 Chemistry Autoanalizer, Germany and Japan). Serum CRP was measured by a nephelometric procedure using commercially available kits (Minineph, ZK044.L.R, Birmingham, UK). For malondialdehyde (MDA) measurement, 0.05 ml serum was added to 0.25 ml of 0.1M TCA and 0.7 ml distilled water, vortexed in a 1.5 ml centrifuge tube for 10 s, centrifuged at 4500 g for 5 min and used for high performance liquid chromatography (HPLC) (Jasco, Japan) analysis [10].

Statistical analysis
All data are expressed as means ± SD. A two (groups) x four (time) analysis of variance (ANOVA) with repeated measures on time was used to compare group, time, and group x time interactions for each variable. Significant interactions were further explored using a Bonferroni correction analysis. The significance level for this study was set at P<0.05 for all tests.
RESULTS

Effect of exercise on markers of inflammation. Leukocyte, neutrophil, monocyte and lymphocyte counts, and CRP concentrations before, immediately after, 3 h after, and 24 h after exercise are shown in Table 3. Total leukocyte and neutrophil counts were significantly increased 3 h after exercise in both groups and returned to pre-exercise values 24 h after exercise (P<0.05). Lymphocyte counts decreased significantly 3 h after exercise in both groups (P<0.05). There were no significant changes in monocyte counts in either group (P>0.05). Serum CRP concentration increased significantly immediately after exercise and remained elevated for 24 h after exercise in both groups (P<0.05). No significant changes were found for total leukocyte, neutrophil, monocyte and lymphocyte counts, or CRP concentration between groups (P>0.05).

Markers of Muscle Damage and Oxidative Stress Following Exercise:

Serum CK activity were significantly increased immediately after, 3 h after, and 24 h after exercise in both groups (P<0.05) (Figure 1). Serum MDA concentrations were significantly elevated immediately after exercise in both groups (P<0.05) (Figure 2). However, there were no significant differences in CK or MDA concentration between groups (P>0.05) (Figures 1 and 2).

DISCUSSION

The purpose of this study was to assess the effect of acute low-dose celecoxib administration on exercise-induced inflammation, muscle damage and lipid peroxidation markers following intensive aerobic exercise. To date, the efficacy of NSAIDs treatment on muscle damage, inflammation, and lipid peroxidation remains unclear. The equivocal results between studies, some demonstrating no effect [12,18,26], and others reporting a reduction in inflammatory markers following exercise [8], make it difficult to determine if NSAIDs provide protection against exercise-induced muscle damage and inflammation. In the present study, we used a selective COX-2 inhibitor and found no significant differences in all inflammatory markers between groups and each time period. These findings suggest that post-exercise administration of celecoxib does not prevent or alleviate exercise-induced inflammation.

There are several possible explanations for the lack of efficacy of celecoxib administration on inflammation markers in the present study compared with other studies: Celecoxib is a single-action NSAID blocking the COX-2 pathway and has no effect on COX-1 and lipoxygenase (LIPOX) pathways of arachidonic acid (AA) metabolism. Therefore, it has been proposed that celecoxib may not prevent the...
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CONCLUSIONS

In conclusion, our results indicate that celecoxib use following exercise does not alleviate inflammation, muscle damage, or oxidative stress. The relationship between selective and non-selective COX-2 inhibitors and muscle damage, inflammation, and oxidative stress should be further explored in future studies.

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