

The Effect of a Prophylactic Dose of Ibuprofen on Plasma Level of Interleukin 1, Interleukin 6 and Tumor Necrosis Factor-Alpha in a 1500 m Running Practice

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Abstract: Problem statement: there are controversies about the effects of anti-inflammatory drugs on exercised induced changes on plasma cytokines in untrained male athletes, so the primary purpose of this study was to measure the influence of a prophylactic dose of ibuprofen use on plasma cytokines during a 1500 m running race. **Approach:** Subjects were 10 untrained male Medical University students who consumed 400 mg ibuprofen two hours before the 1500 m running practice and 10 controls that competed in the race but avoided ibuprofen and all other medications. Blood samples were collected before, 1h and 24 h following the 1500 m exercise bout to measure plasma concentrations of interleukin-1 beta (IL-1 β), interleukin-6(IL-6) and tumor necrosis factor alpha (TNF- α). **Results:** There were not any significant differences between plasma concentration of IL-1 β , IL-6 and TNF- α during the study period. Also the data showed that there were not any significant differences between plasma level of IL-1 β , IL-6 and TNF- α in ibuprofen and control group during the study period. **Conclusions/Recommendation:** Our result showed that a prophylactic dose of ibuprofen did not affect acute inflammatory cytokines levels (IL-1 β , IL-6 and TNF- α) in untrained healthy young males. So the etiology of the muscle soreness in athletes actively participating in sport races seems to be independent of inflammatory processes.

Key words: Plasma cytokines, Ibuprofen, Running race, strenuous exercise, Tumor Necrosis Factor Alpha (TNF- α), Delayed-Onset Muscle Soreness (DOMS), C-Reactive Protein (CRP), Enzyme-Linked Immunosorbent Assay (ELISA), Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), inflammatory cytokines

INTRODUCTION

It has been documented that strenuous exercise not only induces pyrogenesis but also elicits mobilization and functional augmentation of neutrophils and monocytes whereas it suppresses cellular immunity leading to increased susceptibility to infections (Shephard *et al.*, 1994). It has been documented that exhausting endurance exercise suppresses circulating lymphocyte count and cellular immune functions (Shephard *et al.*, 1994; Pedersen and Bruunsgaard, 1995; Lopez *et al.*, 1983; Trinchieri, 1989).

Stress may increase the production of Tumor Necrosis Factor Alpha (TNF- α) through the production

of norepinephrine (Gomez-Flores *et al.*, 2005). Tumor Necrosis Factor Alpha (TNF- α) level was significantly higher in a heavy-exercise in healthy young men performed bicycle ergometer exercises. The concentration of interleukin-1 alpha (IL-1 alpha) peaked at the early stage of the incremental exercise (Kimura *et al.*, 2001). Also data demonstrate that water polo practice in adolescent girls leads to profound increases in inflammatory cytokines. Significant increases were noted in circulating interleukin-6 (IL-6) and interestingly, TNF- α level decreased after the exercise. Also water polo practice caused reductions in anabolic mediators with substantial alterations in white

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blood cell subpopulations and adhesion molecules (Kimura *et al.*, 2001; Nemet *et al.*, 2003).

The increase in plasma cytokine levels has been reported in athletes competing in marathons and ultramarathons, long term bicycle exercise and athletes actively participating in sport races (Nieman, 1998; Ostrowski *et al.*, 2000; Suzuki *et al.*, 2002).

Collectively, these observations indicate that exercise increases the numbers of circulating cells positive for both pro/anti-inflammatory and growth factors, perhaps contributing to the organism's ability to effectively respond to a variety of post-exercise challenges, such as tissue injury and adaptation (Zaldivar *et al.*, 2006).

The effect of anti-inflammatory drugs in the prevention of muscle soreness and inflammatory responses is reported by investigators. Semark *et al.* (1999) reported that a prophylactic dose of flurbiprofen did not affect Delayed-Onset Muscle Soreness (DOMS), muscle soreness in trained subjects (Semark *et al.*, 1999). However, Kokkinidis *et al* reported that ibuprofen decreased muscle soreness induced after eccentric exercise (Tokmakidis *et al.*, 2003). However, Nieman *et al.* (2006) reported that ibuprofen use compared to nonuse by athletes competing in a 160 km race did not alter muscle damage or soreness but decreased inflammatory cytokines (Nieman *et al.*, 2006). Also Feng *et al.* (2000) reported that short-term aspirin therapy (81 or 325 mg/ d /7 days) in healthy male subjects doing strenuous exercise had no significant effect on C-Reactive Protein (CRP) level and suggest that aspirin may not affect the levels of inflammatory markers (Feng *et al.*, 2000).

Since there are controversies about the effects of anti-inflammatory drugs on exercised induced changes on plasma cytokines in untrained male athletes, so the primary purpose of this study was to measure the influence of a prophylactic dose of ibuprofen use on plasma cytokines during a 1500 m running race.

MATERIALS AND METHODS

Subjects were 23 untrained male Medical University students between the ages of 18 and 21 years whose characteristics were as follows (mean±SEM): height 163.1±4.0 cm, body mass 58.6±3.8 kg. They met the following selection criteria ascertained using a written questionnaire. They were non-smokers, physically healthy and had no history of recent infection or other significant health disorders. They had taken no anti-inflammatory drugs and/or medications within at least 1 week prior to the study. None of the subjects had been involved in training activities. The

Ethics Committee of the Kerman University of Medical Sciences approved the experiment procedures (K/88/53) and informed consent was obtained from the subjects.

Since the peak plasma level of Ibuprofen is reached after 1-2 h, so 12 healthy volunteers received 400 mg ibuprofen two hour before the starting of the 1500 m running race (Davies, 1998). Control group (n = 11) received placebo.

Blood sampling: Approximately 5mL peripheral blood was collected from an antecubital vein using a disposable needle and vacutainer containing heparin. Peripheral venous blood was drawn immediately before (Pre) and 1 h and 24 h (1 d) after the 1500 m running race from all the athletes in ibuprofen and control groups (Hirose *et al.*, 2004). The blood was centrifuged for 10 min to separate plasma. The samples were stored at -70°C until analyses of inflammatory mediators; IL-1β, IL-6 and TNF-α.

Cytokine assays: The heparin plasma samples were used for cytokine determination, which was performed with commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (Bender MedSystems, Austria, Europe) according to the manufacturers' instructions. They were carefully checked for specificity, sensitivity and reliability. Plasma concentrations of IL-1b, IL-6 and TNF- α were measured using a series of ELISA kits.

Statistical analysis: Data are expressed as means ± SEM. Changes from pre-race to post-race values were calculated and compared between ibuprofen and control groups using student's t tests. The difference between groups was determined by analysis of variance (ANOVA), followed by the Tukey's post hoc test with 5% level of significance (p<0.05).

RESULTS

Plasma level of cytokines in control group: Figure 1 shows the plasma concentration of TNF- α, IL-6 and IL-1β in control group before (Pre), 1h and 24 h (1d) after the 1500 m running race. As the data shows, there were not any significant differences between plasma concentration of IL-1β, IL-6 and TNF- α during the study period (Fig.1).

Plasma level of cytokines in Ibuprofen group: Figure 2 shows the plasma concentration of TNF- α, IL-6 and IL-1β in ibuprofen group before (Pre), 1h and 24 h (1d) after the 1500 m running race. As the data shows, there were not any significant differences between plasma concentration of TNF- α, IL-6 and IL-1β during the study period (Fig. 2).

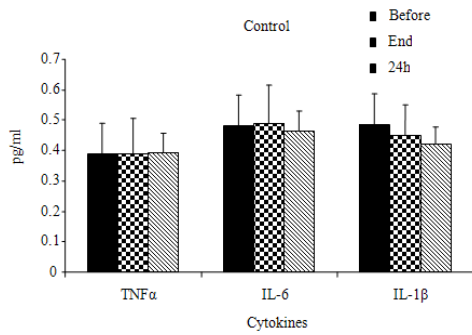


Fig. 1: Effects of exercise on plasma levels of TNF- α , IL-6 and IL-1 β before and after the 1500 m running practice. Values are means \pm SEM, n= 11

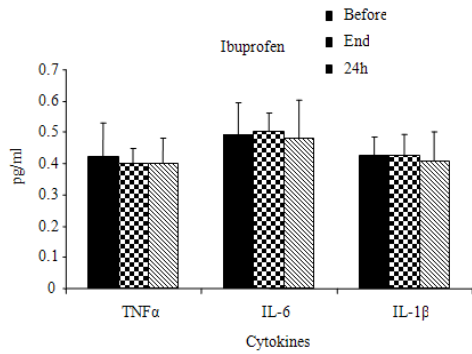


Fig. 2: Plasma cytokines level (TNF- α , IL-6 and IL-1 β) in ibuprofen users before and after the 1500 m running practice. Data are mean \pm SEM, n=12

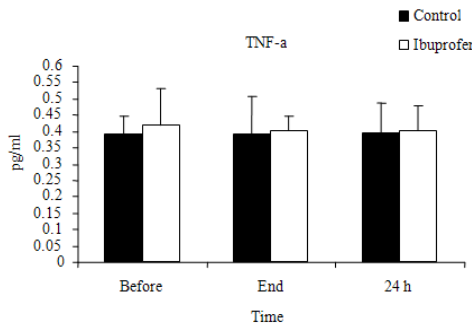


Fig. 3: Effects of exercise on plasma levels of TNF- α before and after the 1500 m running practice. Values are means \pm SEM of 11 samples in control and 12 samples in Ibuprofen group

Also the data showed that there were not any significant differences between plasma level of TNF- α , IL-6 and IL-1 β in ibuprofen and control group during the study period (Fig. 3-5).

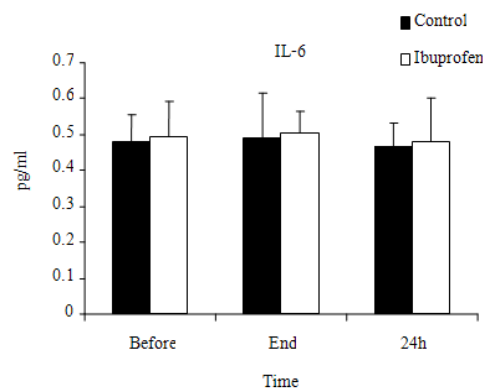


Fig. 4: Effects of exercise on plasma levels of IL-6 before and after the 1500 m running practice. Values are means \pm SEM of 11 samples in control and 12 samples in Ibuprofen group

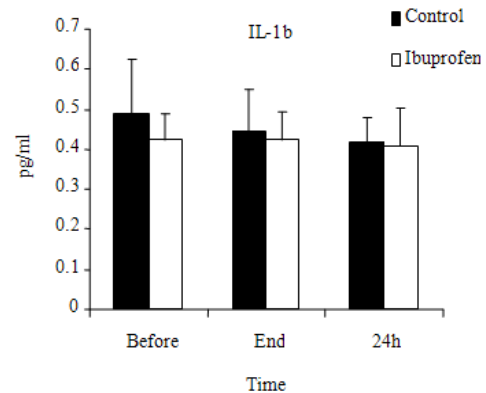


Fig. 5: Effects of exercise on plasma levels of IL-1 β before and after the 1500 m running practice. Values are means \pm SEM of 11 samples in control and 12 samples in Ibuprofen group

DISCUSSION

The results of this study showed that there were no significant differences between the plasma cytokine (TNF- α , IL-6 and IL-1 β) levels at pre and post 1500 m running race in untrained healthy young males. Our results are comparable to Suzuki *et al.* (2002) study who reported that the circulating concentration of these cytokines is either unchanged following exercise, or exhibits relatively small, delayed increments (Suzuki *et al.*, 2002). However, our results are not comparable to some other studies in which the increase in acute inflammatory cytokines levels such as IL-1 α and TNF- α has been reported in athletes competing in marathons and ultramarathons, long term bicycle exercise and athletes actively

participating in sport races (Nieman, 1998; Ostrowski *et al.*, 2000; Suzuki *et al.*, 2002; Nemet *et al.*, 2002). However, others reported the decrease in tumor TNF- α level after the exercise (Nemet *et al.*, 2003).

Also our result showed that a prophylactic dose of ibuprofen did not affect acute inflammatory cytokines levels (IL-1 β , IL-6 and TNF- α) in untrained healthy young males. Our results are not comparable to Nieman *et al.* (2006) study which showed that ibuprofen use compared to nonuse was linked to a greater increases (pre-to-post race) in serum C-Reactive Protein (CRP) and plasma cytokine levels for interleukin (IL)-6, IL-10, IL-8, IL-1 ra, but not tumor necrosis factor alpha (Nieman *et al.*, 2006). However Feng *et al.* (2000) reported that in healthy male subjects CRP levels were not significantly reduced by short-term aspirin therapy. Also the effect of a prophylactic dose of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) on muscle damage or soreness is controversial. Tokmakidis *et al.* (2003) reported that ibuprofen use compared to nonuse by athletes can decrease muscle soreness induced after eccentric exercise which is not comparable to the results of some other similar studies (Suzuki *et al.*, 20002; Semark *et al.*, 1999; Nieman *et al.*, 2006). Since ibuprofen did not affect the inflammatory cytokines in untrained athletes, so the etiology of athletes muscle soreness seems to be independent of increases in prostaglandin (Semark *et al.*, 1999).

CONCLUSION

The results of this study showed that a prophylactic dose of ibuprofen did not affect acute inflammatory cytokines levels (IL-1 β , IL-6 and TNF- α) in untrained healthy young males. So the etiology of the muscle soreness in athletes actively participating in sport races seems to be independent of inflammatory processes. Our data, taking together with other reports, suggest that ibuprofen may not affect the levels of inflammatory cytokines levels. However, further studies are needed with a longer duration of therapy, among trained and untrained athletes and using additional markers of inflammation besides IL-1 β , IL-6 and TNF- α to determine the short and long-term effects of ibuprofen use.

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