European Journal of Pharmacology 786 (2016) 204–211

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and inflammation

Prophylactic acetylsalicylic acid attenuates the inflammatory response but fails to protect exercise-induced liver damage in exercised rats

Kuo-Chin Huang [a], Yi-Han Chiu [b], Kuang-Wen Liao [c], Chun-Yen Ke [c], Chung-Jen Lee [d], Yann-Fen C. Chao [e], Ru-Ping Lee [f,*]

[a] Holistic Education Center, Mackay Medical College, New Taipei City, Taiwan, ROC
[b] Department of Nursing, St. Mary's Medicine, Nursing and Management College, Yi-Lan, Taiwan, ROC
[c] Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan, ROC
[d] Department of Nursing, Tzu Chi University of Science and Technology, Hualien, Taiwan, ROC
[e] Department of Nursing, Mackay Medical College, New Taipei City, Taiwan, ROC
[f] Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan, ROC

ARTICLE INFO

Article history:
Received 13 January 2016
Received in revised form
27 May 2016
Accepted 30 May 2016
Available online 1 June 2016

Keywords:
Nonsteroidal antiinflammatory drug
Aspirin
Exhaustion exercise
Liver injury

ABSTRACT

This study evaluated the effects of acetylsalicylic acid (ASA) on exercise-induced inflammatory response, muscle damage, and liver injury in rats. Wistar-Kyoto (WKY) rats were divided into six groups: control (C), exercise (E), C+20 mg ASA, E+20 mg ASA, C+100 mg/kg ASA, and E+100 mg ASA groups. ASA or a vehicle was orally administered through gavage 1 h before a treadmill test. Upon trial completion, blood was drawn at 1, 12, and 24 h for biochemical analysis, and livers were excised at 24 h for a histological assessment. Our results revealed that 100 mg/kg ASA significantly reduced interleukin (IL)-6 and tumor necrosis factor (TNF)-α levels in the E groups; however, the IL-10 level was considerably increased. Moreover, aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels and histological hepatic damage increased significantly in the E+100 mg ASA group compared with the corresponding changes in the E group. These results suggest that the prophylactic administration of particularly high-dose ASA alleviates exercise-induced inflammatory response but exacerbates liver injury.

1. Introduction

Moderately intense activity is effective in reducing the risk of cardiovascular disease, type 2 diabetes mellitus, hypertension, and metabolic syndrome (Li et al., 2003). However, previous studies have hypothesized that acute exhaustive exercise induces inflammation and muscle damage in humans (Peake et al., 2007) and liver injury in rats (C.C. Huang et al., 2013; K.C. Huang et al. 2013). Strenuous exercise increases proinflammatory and antiinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, IL-10, and IL-8 (Sugama et al., 2013). Moreover, endurance exercises, such as marathon running, provoke marked spikes in circulating IL-6 and TNF-α levels (Bernecker et al., 2013). IL-6 is the first cytokine to be circulated in the blood during exercise and may regulate immunological and metabolic responses. Several sources of IL-6 have been identified; however, contracting skeletal muscles conduces most of the IL-6 synthesis and release into the systemic circulation in response to exercise (Fischer, 2006). Furthermore, TNF-α is a proinflammatory cytokine that plays a critical role in the immune response to prolonged running. Elevated TNF-α is balanced by the levels of TNF receptors and IL-10 (Ostrowski et al., 1999). A recent report indicated that an acute muscle damage induced by endurance running causes inflammation and deteriorates muscle power; these inflammatory responses, particularly those involving IL-6, TNF-α, and IL-10 are associated with muscle repair and regeneration (Tidball and Villalta, 2010).

Acetylsalicylic acid (ASA; or aspirin), a nonsteroidal antiinflammatory drug (NSAID), is extensively used for its analgesic, antipyretic, and anti-inflammatory properties (Dinarello, 2010). ASA blocks the production of prostaglandins (PG) and thromboxanes by inhibiting the cyclooxygenase (COX) enzymes COX-1 and COX-2 (Vane and Botting, 2003). PG is a critical class of proinflammatory factors that can induce swelling, pain, and fever. An accumulating body of evidence indicates that ASA is used as an analgesic agent in exercise-induced muscle soreness (Francis and

* Corresponding author.
E-mail addresses: kchsports@mmc.edu.tw (K.-C. Huang), chiuyiham@hotmail.com (Y.-H. Chiu), liaonms@mail.nctu.edu.tw (K.-W. Liao), brant77123@hotmail.com (C.-Y. Ke), guggilee@msn.com (C.-J. Lee), yfchaomk@mmc.edu.tw (Y.-F. Chao), fish@gms.tcu.edu.tw (R.-P. Lee).

http://dx.doi.org/10.1016/j.ejphar.2016.05.043
0014-2999/

Hoobler, 1987); however, ASA administration induces gastrointestinal mucosal damage with an increased permeability during and following moderate treadmill running (Ryan et al., 1996). In addition, several evidences showed ASA ingestion has no effect on the performance of exercises of various types and intensities (Hudson et al., 2008; Roi et al., 1994). Despite its long and widespread use, ASA has recently been linked to the etiology of liver damage (Jain et al., 2012). Salicylic acid, the active metabolite of ASA, was reported to occasionally provoke serious liver damage by triggering mitochondrial dysfunction, causing ATP deficiency (Doi and Horie, 2010). A number of studies demonstrate the effectiveness of NSAIDs on exercise-induced inflammation and muscle damage (Cobos et al., 2012; Khoshkhahesh et al., 2013). However, there currently remains a paucity of research into the effect of ASA on an acute bout of exhaustive exercise. We hypothesize that pretreated ASA has a positive effect on inflammatory response and muscle damage, but has a deterrent effect on liver function in a dose manner. Our results revealed that high dose ASA significantly reduced exercise-induced inflammatory response, but failed to protect exercise-induced liver damage in exercised rats.

2. Materials and methods

2.1. Animal preparation and grouping

We purchased 8-week-old normotensive Wistar–Kyoto (WKY) rats (n=48; weight, 240–260 g) from the National Laboratory Animal Center (Taipei, Taiwan). The rats were housed in the university animal center under a controlled temperature (22 ± 1 °C) and 12-h light/dark cycle. The rats were fed a diet of standard rat chow and water ad libitum. Furthermore, all animal experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Tzu Chi College of Technology, and the study conformed to the guidelines of protocol IACUC-2009007 approved by the IACUC Ethics Committee. All rats were randomly assigned to six groups (n=8 per group) as control (C), exercise (E), C+20 mg ASA, E +20 mg ASA, C+100 mg ASA, and E+100 mg ASA. The ASA groups were administered either a single dose of 20 mg/kg (low dose) or 100 mg/kg (high dose) ASA (Bayer AG, Leverkusen, Germany). The C and E groups were also administered equal volumes of normal saline. ASA or normal saline was orally administered through gavage 1 h before the exercise trial.

2.2. Exercise protocol

The exercise protocol was performed as described previously with slight modifications (K.C. Huang et al., 2013) and treadmill characteristics of each study group are given in Table 1. No significant differences were observed in initial and final body weight. To accustom the rats of the E groups to the treadmill, they were trained on a treadmill operating at 15–30 m/min for 15–20 min on alternate days for six successive days before the exhaustive test exercise. To encourage the rats to run continuously, electrical stimulation was applied using a small electrically charged grid placed on the end of each treadmill channel. Rats that refused to run even with electrical stimulation were excluded from the study. Furthermore, on the day of the exhaustive test exercise, the E groups were made to run until exhaustion on a six-lane inclined (10°) treadmill at a final speed of 30 m/min, which was approximately 70–75% of their VO_2 max (Brooks and White, 1978). The point of exhaustion was defined as the time when the rats no longer maintained pace with the treadmill speed (Carmichael et al., 2005). To eliminate diurnal effects, all trials were executed simultaneously (09:00 A.M.–12:00 P.M.). All rats were exposed to similar handling and noise to control for extraneous stresses that may be associated with treadmill running.

2.3. Experimental procedures

To monitor the rats in an unrestrained conscious state to enable intermittent blood sampling (1, 6, 12, 18, and 24 h after the exhaustive exercise), we adopted a procedure previously developed in our laboratory (K.C. Huang et al., 2013). Each rat was anesthetized using ether inhalation for approximately 10 min to insert a polyethylene catheter (PE-50) into the right femoral artery under sterile conditions for blood sampling. Furthermore, a sterilized stainless steel cover was used to protect the catheter from biting and dislocation, and each animal was kept awake thereafter with its tail fixed on a cage by using a piece of adhesive tape that allowed free access to food and water.

2.4. Blood sample analysis

Blood samples (0.8 ml) were drawn from the femoral artery at various time points (1, 6, 12, 18, and 24 h) following the exhaustive exercise, and an equal volume of normal saline was used for fluid resuscitation. The samples were immediately collected into heparinized tubes and centrifuged at 3000*g* for 10 min. Plasma was decanted and separated into two portions; one portion was stored at 4 °C within 1 h after collection for determining biochemical parameters. Plasma levels of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were analyzed using an autoanalyzer (COBAS Integra C111; Roche Diagnostics, Basel, Switzerland). The second portion of the separated plasma was stored at −20 °C for estimating IL-6, TNF-α, and IL-10 levels; these levels were measured using antibody enzyme-linked immunosorbent assays (ELISAs) with commercial antibody pairs, recombinant standards, and a biotin streptavidin–horseradish peroxidase detection system

Table 1
Characteristics of treadmill protocol and body weight among groups.

	Treadmill characteristics					Body weight		
	Accustom to the treadmill		Exercise trail				Initial	Final
Group	Duration (min)	Speed (m/min)	Grade (°)	Speed (m/min)	Grade (°)	Status	Mean ± S.E.M. (g)	Mean ± S.E.M. (g)
C	15–20	15–30	0	–	–	Resting	246.67 ± 2.10	247.50 ± 3.33
C+20 mg ASA	15–20	15–30	0	–	–	Resting	253.33 ± 2.35	256.25 ± 3.30
C+100 mg ASA	15–20	15–30	0	–	–	Resting	248.66 ± 2.60	251.25 ± 4.40
E	15–20	15–30	0	30	10	Exhaustion	248.33 ± 2.79	251.25 ± 4.41
E+20 mg ASA	15–20	15–30	0	30	10	Exhaustion	243.33 ± 2.11	247.50 ± 3.34
E+100 mg ASA	15–20	15–30	0	30	10	Exhaustion	250.66 ± 0.67	252.50 ± 6.67

ASA: acetylsalicylic acid; Groups: Control (C), C+20 mg ASA, C+100 mg ASA, Exercise (E), E+20 mg ASA and E+100 mg ASA (n=8 for group). Data are expressed as mean ± S.E.M. from independent groups. Results of ANOVA for body weight showed no significant difference between groups.