Available online at www.sciencedirect.com
ScienceDirect
Life Sciences 79 (2006) 2010–2016
Life Sciences
www.elsevier.com/locate/lifescie

Post-treatment with *N*-acetylcysteine ameliorates endotoxin shock-induced organ damage in conscious rats

Bang-Gee Hsu [a,b,e], Ru-Ping Lee [g], Fwu-Lin Yang [b,c,f], Horng-Jyh Harn [d], Hsing I. Chen [h,*]

[a] *Department of Nephrology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan*
[b] *Department of Neuro-Medical Scientific Center, Buddhist Tzu Chi General Hospital, Hualien, Taiwan*
[c] *Division of Surgical Critical Care Unit, Buddhist Tzu Chi General Hospital, Hualien, Taiwan*
[d] *Department of Pathology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan*
[e] *Division of Medicine, Tzu Chi University, Hualien, Taiwan*
[f] *Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan*
[g] *Department of Nursing, Tzu Chi University, Hualien, Taiwan*
[h] *Institute of Integrative Physiology and Clinical Sciences, Tzu Chi University, Hualien, Taiwan*

Received 14 February 2006; accepted 24 June 2006

Abstract

N-acetylcysteine (NAC) is an antioxidant and cytoprotective agent with scavenging action against reactive oxygen species and inhibitory effects on pro-inflammatory cytokines. In a previous study, we found that pretreatment with NAC attenuated organ dysfunction and damage, reduced the production of free radicals, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) following endotoxemia elicited by administration of lipopolysaccharide (LPS). In the present study, we tested the effects of post-treatment with NAC on the sepsis-induced change. Post-treatment imitates clinical therapeutic regimen with administration of drug after endotoxemia. Endotoxin shock was induced by intravenous injection of *Klebsiella pneumoniae* LPS (10 mg/kg) in conscious rats. Mean arterial pressure (MAP) and heart rate (HR) were continuously monitored for 48 h after LPS administration. NAC was given 20 min after LPS. Measurements of biochemical substances were taken to reflect organ functions. Biochemical factors included blood urea nitrogen (BUN), creatinine (Cre), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), aspartate transferase (GOT), alanine transferase (GPT), TNF-α, interleukin-6 (IL-6), and interleukin-10 (IL-10). LPS significantly increased blood BUN, Cre, LDH, CPK, GOT, GPT, TNF-α, IL-6, IL-10 levels and HR, and decreased MAP. Post-treatment with NAC diminished the decrease in MAP, increased the HR, and decreased the markers of organ injury (BUN, Cre, LDH, CPK, GOT, GPT) and inflammatory biomarkers (TNF-α, IL-6, IL-10) after LPS. We conclude that post-treatment with NAC suppresses the release of plasma TNF-α, IL-6, and IL-10 in endotoxin shock, and decreases the markers of organ injury. These beneficial effects protect against LPS-induced kidney, heart and liver damage in conscious rats. The beneficial effects may suggest a potential chemopreventive effect of this compound after sepsis.

Keywords: *N*-acetylcysteine; Lipopolysaccharide; Conscious rats

Introduction

Sepsis remains the leading cause of death among patients in intensive care units. Despite intensive care and adequate antibiotic treatment, the mortality among patients with sepsis remains high (Angus et al., 2001). Lipopolysaccharide (LPS) from the outer membrane of Gram-negative bacteria is strongly associated with septic shock (Mayeux, 1997). Binding of LPS to the CD14 receptor on the cell membrane results in activation of inflammatory cells and excessive production of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and leads to tissue injury, multiple organ failure, and death. The concomitant production of anti-inflammatory cytokines such as IL-10 counterbalances the actions of pro-inflammatory cytokines after LPS (Haveman et al., 1999). Accordingly, sepsis reflects a combined pro-inflammatory and anti-inflammatory state (Pinsky, 2001; Riedemann et al., 2003).

* Corresponding author. Institute of Integrative Physiology and Clinical Sciences, Tzu Chi University, No. 701, Section 3, Chung Yan Road, 97004 Hualien, Taiwan. Tel.: +886 3 8560824; fax: +886 3 8573053.
E-mail address: chenhi@mail.tcu.edu.tw (H.I. Chen).

0024-3205/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2006.06.040

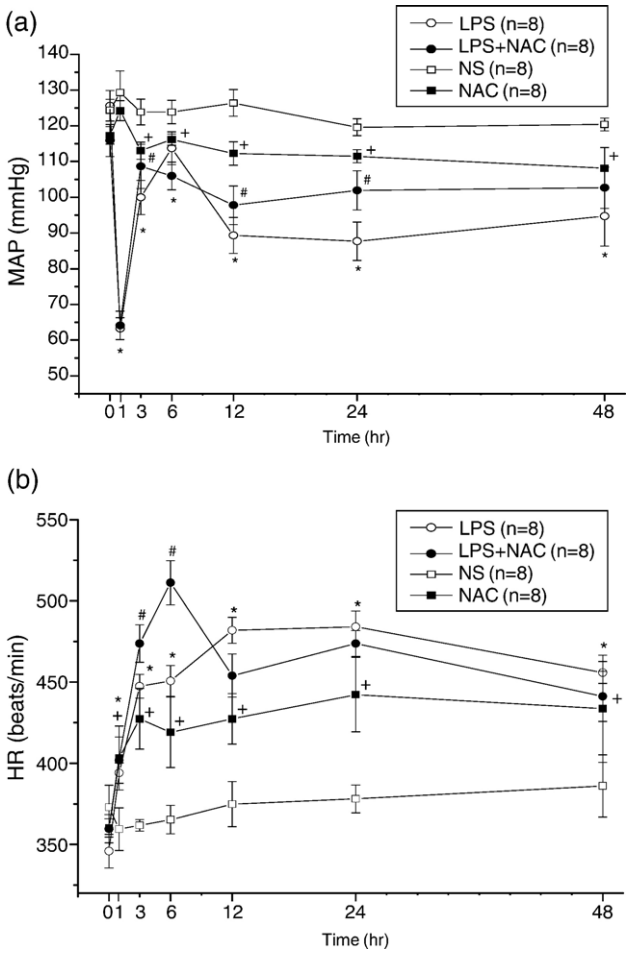

Fig. 1. Change in mean arterial pressure (MAP) (a) and heart rate (HR) (b) after endotoxin shock in conscious rats. *$p<0.05$ for the LPS group compared with the NS group. $^{\#}p<0.05$ for the LPS+NAC group compared with LPS group. $^{+}p<0.05$ for the NAC group compared with the NS group.

N-acetylcysteine (NAC) is a compound that increases the pool of glutathione (GSH). The latter is an important cellular antioxidant. It is a reactive oxygen species (ROS) scavenger and can restore the reduced cellular GSH (Aruoma et al., 1989; Bernard, 1991). It is also cytoprotective since it inhibits the activation of the nuclear transcription factor nuclear factor-κB (NF-κB) and tumor necrosis factor-α (TNF-α) production by LPS (Pahan et al., 1998). For these reasons, NAC may exert a protective effect during sepsis.

In a previous work, we observed that pretreatment with NAC suppresses the release of TNF-α, IL-1β, and hydroxyl radicals to plasma but enhances plasma NO production and protects against LPS-induced organ damage in conscious rats (Hsu et al., 2004). However, post-treatment with a therapeutic agent after induction of a disorder is more similar to clinical treatment regimen than pretreatment. There is no report with respect to the effects of post-treatment with NAC on LPS-induced shock and IL-6, IL-10 production after LPS in conscious rats. In the present study, we utilized post-treatment with NAC to investigate its effects on LPS-induced dysfunction in and damage to several organs (kidney, heart, and liver) in conscious rats. We also investigated the relationship of post-treatment with NAC on pro-inflammatory cytokines (TNF-α and IL-6), and an anti-inflammatory cytokine (IL-10) in endotoxin shock.

Materials and methods

Preparation of animals

Twenty four male Wistar–Kyoto rats weighing 260–300 g were purchased from the National Animal Center. They were housed in our animal center under a controlled environment at a temperature of 22±1 °C with a 12 h light/dark cycle. Food and water were provided ad libitum. The Animal Care and Use Committee approved the experimental protocol. The animals were anesthetized with ether inhalation for about 10 min. During the period of anesthesia, a femoral artery was cannulated and connected to a pressure transducer (Gould Instruments, Cleveland, OH, USA) to record arterial pressure (AP) and heart rate (HR) on a polygraph recorder (PowerLab, AD Instruments Co., Mountain View, CA, USA). A femoral vein was catheterized for intravenous administration of drugs or fluid. The operation was completed within 15 min, and the section wound was as small as possible (less than 0.5 cm^2). After the operation, the animal was

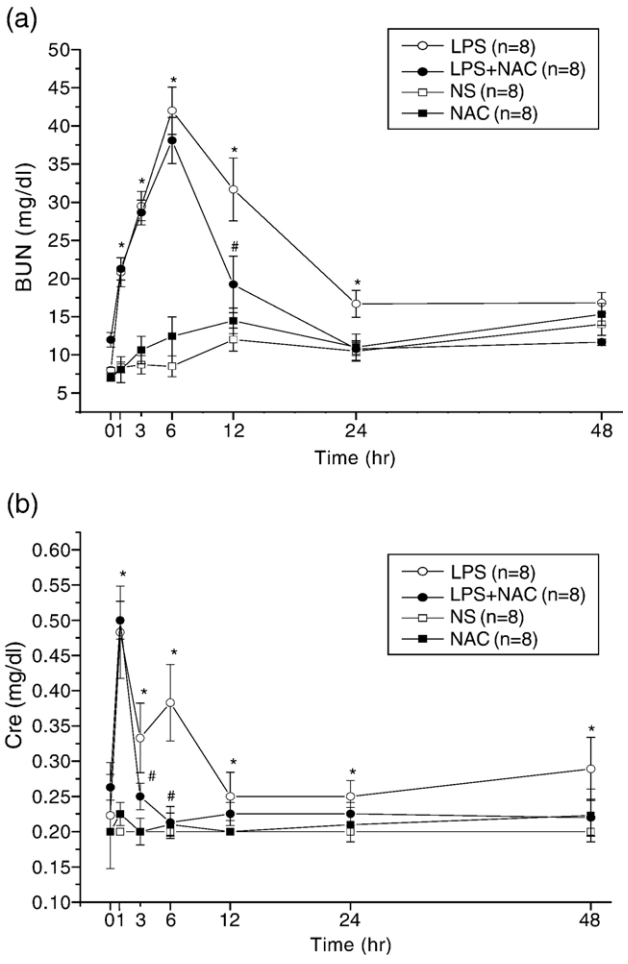

Fig. 2. Change in serum blood urea nitrogen (BUN) (a) and creatinine (Cre) (b) after endotoxin shock in conscious rats. *$p<0.05$ for the LPS group compared with the NS group. $^{\#}p<0.05$ for the LPS+NAC group compared with LPS group.