ELSEVIER

Pathophysiology 18 (2011) 235–242

ISP PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Montelukast, a leukotriene receptor antagonist abrogates lipopolysaccharide-induced toxicity and oxidative stress in rat liver

Ahmed M. Mohamadin [a,*], Ahmed A. Elberry [b], Mohamed A. Elkablawy [c], Hala S. Abdel Gawad [d], Fahad A. Al-Abbasi [e]

[a] *Department of Clinical Biochemistry, College of Medicine, Taibah University, Al-Madinah Al-Munawwarah, P.O. Box 344, Saudi Arabia*
[b] *Department of Clinical Pharmacy, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia*
[c] *Department of Pathology, College of Medicine, Taibah University, Al-Madinah Al-Munawwarah, Saudi Arabia*
[d] *Department of Physiology, College of Medicine, Taibah University, Al-Madinah Al-Munawwarah, Saudi Arabia*
[e] *Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia*

Received 10 October 2010; received in revised form 21 November 2010; accepted 14 February 2011

Abstract

Endotoxemia-induced hepatotoxicity is characterized by disturbed intracellular redox balance, excessive reactive oxygen species (ROS) generation inducing DNA, proteins and membrane lipid damages. In the present study, the protective effects of montelukast (MNT) against *Escherichia coli* lipopolysaccharides (LPS)-induced oxidative stress were investigated in rat liver. LPS (10 mg/kg, i.p.) was injected and the animals were sacrificed 6 h after LPS challenge. MNT (10 mg/kg) was administered orally for seven successive days before endotoxemia induction. Blood samples were withdrawn for assessing the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and levels of serum total bilirubin, total protein, tumor necrosis factor-alpha (TNF-α) and interleukin 1β (IL-1β). Livers were dissected out and used for histological examination or stored for the determination of malondialdehyde (MDA), protein carbonyl content (PCC), reduced glutathione (GSH) levels, enzymatic activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and myeloperoxidase (MPO). Sepsis significantly increased ALT, AST, ALP, LDH, total bilirubin, TNF-α and IL-1β, MPO, MDA and PCC levels and decreased total protein, GSH and enzymatic antioxidants (CAT, SOD and GSH-Px). MNT decreased the markers of liver injury (AST, ALT, ALP, LDH, and total bilirubin), inflammatory biomarkers (TNF-alpha, IL-1β), MDA, PCC and MPO after LPS challenge. In conclusion, MNT abrogates LPS-induced markers of liver injury and suppresses the release of inflammatory and oxidative stress markers via its antioxidant properties and enhancement enzymatic antioxidant activities.

Keywords: Lipopolysaccharide; Sepsis; Montelukast; Hepatotoxicity; Oxidative stress; Male rats

1. Introduction

Liver is an important organ actively involved in many metabolic functions and a frequent target for a number of toxicants [1]. Hepatic damage is associated with distortion of its metabolic functions [2]. Liver disease is a worldwide health problem. Lipopolysaccharide (LPS) is a toxic component derived from the cell wall of Gram-negative bacteria and its administration has been commonly employed for the experimental induction of endotoxemia in laboratory animals [3]. In addition, LPS is widely present in the digestive tracts of humans and animals. Humans are constantly exposed to low levels of LPS through infection. Gastrointestinal distress and alcohol drinking often increase permeability of LPS from gastrointestinal tract into blood [4]. However, the exact mechanism of LPS-induced developmental toxicity remains unclear.

Free radicals have been implicated in the etiology of multiple organ damage and dysfunction during sepsis. Activation of macrophages and cytokines by endotoxin, and the subsequent formation of reactive oxygen species (ROS)

* Corresponding author. Permanent Address: Biochemistry Dept., Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt.
Mobile: +966 50 846 8142; fax: +966 048 470 926.
E-mail address: amohamadin@yahoo.com (A.M. Mohamadin).

0928-4680/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.pathophys.2011.02.003

and nitrogen species, are of central pathogenic importance in various inflammatory diseases including endotoxemia [2]. The release of endotoxin from bacteria is generally believed to be the initial event in the development of endotoxemia. LPS activates inflammatory cells and subsequently amplifies the inflammatory response by releasing various cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) [5]. This systemic inflammatory cascade results in polymorphonuclear leukocytes (PMNLs) sequestration in the various systemic organs. Subsequent PMNL extravasation can lead to vascular dysfunction as well as parenchymal cell dysfunction [6]. Besides their direct damaging effects on tissues, free radicals seem to trigger the accumulation of leukocytes in the tissue involved, and thus cause further injury through activated neutrophils. It has been shown that activated neutrophils secrete myeloperoxidase enzyme (MPO) and liberate oxygen radicals [2,7]. Lipid peroxidation (LPO) mediated by oxygen free radicals is believed to be an important cause of cell membrane damage.

Despite current medical and surgical advances, septic hepatic failure is still associated with a high mortality rate [8]. Hepatic damage is mainly mediated by endotoxin of Gram-negative bacteria, which is primarily a lipopolysaccharide. The liver plays a central role in this process by virtue of its dual ability not only to clear LPS, but also to respond energetically to LPS [9]. *In vivo*, LPS is implicated in the pathophysiology of acute liver injury and can induce various degrees of liver failure when co-administered with D-galactosamine (D-GalN) depending on dosage schedule [10]. Most of the toxicities of LPS, both in the liver and in the systemic circulation, have been related to the release of proinflammatory cytokines such as interleukins (IL-1β, IL-6), tumor necrosis factor alpha (TNF-α), and oxygen free radicals [6,11].

Cysteinyl leukotrienes, namely leukotriene (LT) C4, LTD4 and LTE4, are secreted mainly by eosinophils, mast cells, monocytes and macrophages, and they exert a variety of actions which emphasize their importance as pathogenic elements in the inflammatory states [12]. Anti-leukotriene agents, i.e. leukotriene receptor antagonists and synthesis inhibitors, have been shown to be effective in several inflammatory models in rats, such as in ethanol-induced gastric mucosal damage [13], burn- and sepsis-induced multiorgan damage [14] and renal ischemia/reperfusion injury [15]. Moreover, the selective LTD4 receptor antagonist, montelukast (MNT), was used to reduce eosinophilic inflammation in the airways of asthmatic patients [16].

Montelukast (MNT) is an anti-inflammatory drug used in management of bronchial asthma [17]. It is a selective and orally active leukotriene receptor antagonist that specifically inhibits the cysteinyl leukotriene ($CysLT_1$) receptor and reduces the airway eosinophilic inflammation [18]. Anti-leukotriene agents, i.e. leukotriene receptor antagonists and synthesis inhibitors, have been shown to be effective in several inflammatory rat models as burn- and sepsis-induced multi-organ damage [19] and ischemia/reperfusion injury [20]. Sener et al. [14] reported that montelukast possesses an anti-inflammatory effect on sepsis-induced hepatic and intestinal damage and protects against oxidative injury by a neutrophil-dependent mechanism. Moreover, it was used to reduce eosinophilic inflammation in the airways of asthmatic patients [21].

Based on these findings, in the present study, the hepatoprotective effects of MNT against LPS-induced hepatotoxicity and oxidative stress were examined using both biochemical and histopathological approaches, while the functional impairments were monitored by liver function tests.

2. Materials and methods

2.1. Chemicals

Montelukast (SINGULAIR®) was purchased from Merck (Whitehouse Station, NJ, USA). Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8), 2-thiobarbituric acid, tetraethoxypropane, 5,5-dithio-(2-nitrobenzoic acid) (DTNB), Tris–HCl, EDTA sodium, sucrose, trichloroacetic acid (TCA), ammonium molybdate tetrahydrate, hydrogen peroxide and other chemicals were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Diagnostic kits for determination of the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) and levels of bilirubin were purchased from Randox Chemical Co. (Antrim, UK). Tumor necrosis factor (TNF-α) and interleukin-lβ (IL-lβ) kits were purchased from R&D Systems Inc. (Minneapolis, MN, USA).

2.2. Animals

Adult male Sprague–Dawley (210–225 g) obtained from the Animal Care Centre, College of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia, were used in the present study. All the animals were fed a standard rat chow and water *ad libitum* and kept in a temperature-controlled environment (20–22 °C) with an alternating cycle of l2-h light and dark. The animals used in this study were handled and treated in accordance with the strict guiding principles of the National Institution of Health for experimental care and use of animals. The experimental design and procedures were approved by the Institutional Ethical Committee for Animal Care and Use at the King Abdulaziz University, Jeddah, Saudi Arabia.

2.3. Experimental design

At the beginning of the experiment, rats were divided into the following four groups (10 animals each):

(1) *Control group*; where the animals were treated with equivalent volume of carboxymethyl cellulose (CMC;