ELSEVIER

Journal of Hepatology 48 (2008) 442–452

Journal of Hepatology

www.elsevier.com/locate/jhep

Differential effects of pyrrolidine dithiocarbamate on TNF-α-mediated liver injury in two different models of fulminant hepatitis☆

Jin-Wei Lu[1,‡], Hua Wang[1,‡], Ji Yan-Li[1], Cheng Zhang[1], Huan Ning[1], Xiang-Yun Li[1], Heng Zhang[1], Zi-Hao Duan[1], Lei Zhao[1], Wei Wei[2], De-Xiang Xu[1,2,*]

[1]*Department of Toxicology, Anhui Medical University, Hefei 230032, China*
[2]*Institute of Clinical Pharmacology, Anhui Medical University, Hefei 230032, China*

***Background/Aims*: Pyrrolidine dithiocarbamate (PDTC) is an inhibitor of nuclear factor kappa B (NF-κB) activation. The present study aimed to investigate the effects of PDTC on lipopolysaccharide (LPS)-induced liver injury in two different models of fulminant hepatitis.**

***Methods*: Mice infected with Bacillus Calmette Guerin (BCG) were challenged with LPS (0.2 mg/kg) to induce the model of inflammatory liver injury. Mice were injected with D-galactosamine (GalN, 600 mg/kg) and LPS (20 μg/kg) to induce the model of apoptotic liver injury. In the treatment groups, mice were pre-treated with PDTC (100 mg/kg), initiated 24 h prior to LPS.**

***Results*: PDTC pretreatment reduced the infiltration of inflammatory cells, inhibited NF-κB activation and the expression of tumor necrosis factor alpha (TNF-α), attenuated nitric oxide production, and alleviated hepatic glutathione depletion. Correspondingly, PDTC reduced serum alanine aminotransferase, improved hepatic necrosis, and prolonged the survival in the BCG/LPS model. Conversely, PDTC accelerated death and aggravated liver apoptosis in the GalN/LPS model, although it reduced nitric oxide production, attenuated glutathione depletion, and inhibited the expression of TNF-α in liver.**

***Conclusions*: PDTC protects mice against BCG/LPS-induced inflammatory liver injury through the repression of NF-κB-mediated TNF-α release, while it seems to be detrimental in GalN/LPS-induced apoptotic liver damage.**

***Keywords*: Pyrrolidine dithiocarbamate; Lipopolysaccharide; Fulminant hepatitis; Nuclear factor kappa B; Tumor necrosis factor alpha**

1. Introduction

Lipopolysaccharide (LPS) is a toxic component of the cell walls of gram-negative bacteria and is widely present in the digestive tracts of humans and animals [1]. Humans are constantly exposed to low levels of LPS through infection. Gastrointestinal distress and alcohol consumption often increase permeability of LPS from gastrointestinal tract into the blood [2]. In humans, nanograms of LPS injected into the bloodstream can result in all the physiological manifestations of septic shock [3,4]. Hepatic dysfunction after sepsis is a frequent event, characterized by loss of synthetic function and hepatocellular necrosis [5,6].

Many studies have demonstrated that mice primed with Bacillus Calmette Guerin (BCG) are highly sensitive to LPS-induced liver injury [7–9]. BCG priming induces mononuclear cell infiltration into the liver lobules and granuloma formation [10]. BCG-activated

Received 9 September 2007; received in revised form 10 October 2007; accepted 25 October 2007; available online 14 January 2008
Associate Editor: C.P. Day
☆ The authors declare that they do not have anything to disclose regarding funding from industries or conflict of interest with respect to this manuscript.
* Corresponding author. Tel.: +86 551 5167923; fax: +86 551 5161179.
E-mail address: xudex@mail.hf.ah.cn (D.-X. Xu).
‡ These authors contributed equally to this work.

0168-8278/$32.00
doi:10.1016/j.jhep.2007.10.014

macrophages (such as Kupffer cells) and T lymphocytes within granuloma are highly responsive to further stimuli such as LPS, resulting in a massive release of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6 and interferon (IFN)-γ. Thus, a subsequent LPS challenge in mice primed with BCG elicits acute and massive hepatic injury with marked mononuclear cell infiltration [11–13]. In the BCG/LPS model, pro-inflammatory cytokines are the major mediators leading to liver injury [14,15].

D-galactosamine (GalN) is an amino sugar selectively metabolized by the hepatocyte, which induces a depletion of the uridine triphosphate pool and thereby an inhibition of RNA synthesis [16]. When given together with a low dose of LPS, GalN highly sensitizes animals to develop lethal liver injury mimicking fulminant hepatitis [17]. Although TNF-α is the major mediator leading to liver injury [18], nitric oxide (NO) also plays an important role in GalN/LPS-induced apoptotic liver injury [19,20]. Recent studies have shown that hydrogen peroxide and glutathione (GSH) depletion sensitized hepatocytes to TNF-α-mediated apoptosis [21–23]. Conversely, antioxidants, such as rosmarinic acid and melatonin, protected against GalN/LPS-induced apoptotic liver injury [24,25].

Nuclear factor kappa B (NF-κB) activation is a common pathway that mediates LPS-induced up-regulation of gene encoding for pro-inflammatory cytokines. Pyrrolidine dithiocarbamate (PDTC) is an antioxidant and an inhibitor of NF-κB activity. An earlier study showed that PDTC protected against thioacetamide-induced fulminant liver failure [26]. The present study aimed to investigate the effects of PDTC on LPS-induced liver damage in two different models of fulminant hepatitis.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8), pyrrolidine dithiocarbamate (PDTC) and D-galactosamine (GalN) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO) if not otherwise stated.

2.2. Animals and treatments

Female CD-1 mice (6–8 weeks old, 22–24 g) were purchased from Beijing Vital River (Beijing, China). The animals were allowed free access to food and water at all times and were maintained on a 12-h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment for a period of 1 week before use.

Mice infected intravenously (i.v.) with BCG (2.5 mg, suspended in 0.2 mL saline) were intraperitoneally (i.p.) injected with LPS (0.2 mg/kg) to induce the model of inflammatory liver injury. Mice were injected with GalN (600 mg/kg, i.p.) and LPS (20 μg/kg, i.p.) to induce the model of apoptotic liver injury. In the treatment groups, mice were pre-treated with PDTC (100 mg/kg, i.p.), initiated 24 h prior to LPS. All procedures on animals followed the guidelines for human treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. Evaluation of liver injury

Serum ALT was colorimetrically measured using a commercially available kit. Liver specimen was fixed in 4% formaldehyde phosphate buffer. Liver sections were stained with hematoxylin and eosin and scored by two pathologists who were not aware of sample assignment to experimental groups. The degree of necrosis was expressed as the mean of twelve different fields within each slide classified on a scale of 0–3 (normal-0, mild-1, moderate-2, severe-3). The number of inflammatory cells was counted in twelve randomly selected fields from each slide at a magnification of ×400.

2.4. Measurement of hepatic GSH

Hepatic GSH content was measured by the method of Griffith [27]. GSH was expressed as nmol mg^{-1} protein.

2.5. Reverse transcription polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RT and PCR were performed as described previously [28]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal positive control standard. The primers were synthesized by Sangon Biological Technology (Shanghai, China), according to sequence designs previously described [28]. The number of cycles, annealing temperature and the size of the amplified fragments are given in Table 1. The amplified PCR products were electrophoresed at 75 v through 1.5% agarose gels (Sigma, St. Louis, MO) for 45 min. The pBR322 DNA digested with AluI was used for molecular markers (MBI Fermentas). Agarose gels were stained with 0.5 μg/ml ethidium bromide (Sigma, St. Louis, MO) TBE buffer.

2.6. Nitrate plus nitrite assay

Nitrate plus nitrite, the stable end products of L-arginine-dependent NO synthesis, were measured using a colorimetric method based on the Griess reaction [29].

Table 1
The number of cycles, annealing temperature and the size of the amplified fragments

Names	Denaturation (°C)	Annealing (°C)	Extension (°C)	The number of cycles (*n*)	The size of fragments (bp)
GAPDH	94	56	72	25	340
TNF-α	94	60	72	34	307
IL-1β	94	60	72	32	502
IL-6	94	60	72	35	600