Pharmacological Reports 67 (2015) 914–920

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original research article

Pretreatment with magnesium ameliorates lipopolysaccharide-induced liver injury in mice

Dalia M. El-Tanbouly *, Rania M. Abdelsalam, Amina S. Attia, Mohamed T. Abdel-Aziz

Pharmacology & Toxicology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 7 August 2014
Received in revised form 11 February 2015
Accepted 12 February 2015
Available online 26 February 2015

Keywords:
LPS
Liver damage
Magnesium
Inflammation
Septicemia

ABSTRACT

Background: Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, is involved in the pathogenesis of sepsis. LPS administration induces systemic inflammation that mimics many of the initial clinical features of sepsis and has deleterious effects on several organs including the liver and eventually leading to septic shock and death. The present study aimed to investigate the protective effect of magnesium (Mg), a well known cofactor in many enzymatic reactions and a critical component of the antioxidant system, on hepatic damage associated with LPS-induced endotoxima in mice.

Methods: Mg (20 and 40 mg/kg, *po*) was administered for 7 consecutive days. Systemic inflammation was induced 1 h after the last dose of Mg by a single dose of LPS (2 mg/kg, *ip*) and 3 h thereafter plasma was separated, animals were sacrificed and their livers were isolated.

Results: LPS-treated mice suffered from hepatic dysfunction revealed by histological observation, elevation in plasma transaminases activities, C-reactive protein content and caspase-3, a critical marker of apoptosis. Liver inflammation was evident by elevation in liver cytokines contents (TNF-α and IL-10) and MPO activity. Additionally, oxidative stress was manifested by increased liver lipoperoxidation, glutathione depletion, elevated total nitrate/nitrite (NO*x*) content and glutathione peroxidase (GPx) activity. Pretreatment with Mg largely mitigated these alternations.

Conclusion: Pretreatment with Mg protects the liver from the acute injury which occurs shortly after septicemia.

Introduction

Excessive systemic inflammation in sepsis is the most common cause of death in intensive care units [1], ultimately resulting in multiple organ dysfunction syndrome [2,3]. The liver plays an important role in the pathogenesis of sepsis both as a source of inflammatory mediators as well as the target organ for the effects of these mediators [4,5].

Lipopolysaccharide (LPS) has been extensively studied as a major factor contributing to the pathogenesis of Gram-negative bacterial infection through eliciting a systemic inflammatory response which is characterized by liver failure, accompanied by severe hepatic injury [6].

Mg deficiency is associated with inflammatory responses [7] and aggravates endotoxin lethality [8], suggesting that inadequate Mg stores may exacerbate host inflammatory responses during infection.

Similarly, deficiency of Mg exerts pro-oxidant effects on various tissues including cardiac tissue [9], brain, kidney [10], liver [11] and testis [12]. The deleterious effects of Mg deficiency are attributed to increased production of oxygen free radicals [13] and depletion of reduced glutathione [14].

Mg therapy has proven to be beneficial for numerous inflammatory conditions. For example, aerosolized magnesium sulfate is used for the clinical treatment of acute severe asthma [15], Mg supplementation *in vivo* preserves the integrity of the blood–brain barrier during experimental sepsis [16] and reduced the intrauterine growth restriction and suppresses inflammation in pregnant rats [17]. The present study was therefore, carried out to evaluate the hepatoprotective effect of Mg against liver injury in experimentally induced endotoxemia in mice.

* Corresponding author.
E-mail address: dalia.eltanbouly@pharma.cu.edu.eg (D.M. El-Tanbouly).

http://dx.doi.org/10.1016/j.pharep.2015.02.004
1734-1140/

Materials and methods

Animals

Male albino Swiss mice weighing 25–30 g were purchased from the animal facility of Faculty of Pharmacy, Cairo University. Mice were housed under the appropriate conditions of controlled humidity, temperature and constant light cycle and allowed free access to a standard rodent chow diet and water. The investigation complies with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University (Permit Number: PT 1216).

Chemicals

Mg aspartate hydrochloride, lipopolysaccharide (LPS from *E. coli* serotype 0111:B4), all biochemical reagents and co-enzymes were obtained from Sigma–Aldrich Chemicals, St. Louis, MO, USA. Mg aspartate hydrochloride was obtained from Sekem pharmaceutical company, Egypt. All other chemicals were of analytical grade.

Experimental design

Mice were divided into four groups, 12 animals each. Group 1 and 2 received saline. Group 3 and 4 received Mg (20 mg/kg) and (40 mg/kg), respectively. Drug was freshly prepared in saline and orally administered for 7 consecutive days [18]. All mice received a single dose of LPS: (2 mg/kg; *ip*) [19] 1 h after the last dose of drug to ensure complete oral absorption except for group 1 which served as negative-control group.

Sampling procedures

3 h after LPS injection, the time of maximal liver injury as evident from a pilot study, blood samples were withdrawn in heparinized tubes from the retro-orbital sinus of all mice. Plasma was separated and divided into small aliquots that were stored at −80 °C to be used later for estimation of the chosen parameters. Livers were rapidly excised, washed with saline, weighed and homogenized in ice-cold saline to prepare 10% homogenates that were again divided into several aliquots and stored at −80 °C. Parts of the livers from each group were preserved in 10% formalin, prepared in saline, to be used for histopathological examination.

Biochemical measurement

Evaluation of liver injury

The prepared plasma was used to for estimation of AST and ALT activities using commercially available kit (Química Clínica Aplicada S.A, Spain).

C-reactive protein assay

Plasma level of C-reactive protein (CRP) was determined using mouse CRP-ELISA kit (DRG, Germany).

Measurement of secreted cytokines

Liver homogenate was used for estimation of tumor necrosis factor (TNF)-α and interleukin-10 (IL-10) using enzyme linked immunosorbent assay (ELISA) kit (R&D system, USA).

Myeloperoxidase activity in the liver

Liver meyloperoxidase (MPO) activity as an index of neutrophil infilteration was estimated according to the method described by Bradley et al. [20]. The method is based on measuring the hydrogen peroxide-dependent oxidation of o-dianisidine, catalyzed by MPO. This results in the formation of a compound exhibiting an increased absorbance at 460 nm. One unit of MPO activity is defined as the amount of enzyme that degrades 1 μmol peroxide per min at 25 °C.

NO metabolites measurement

Liver NO was measured by quantification of NO metabolites nitrate/nitrite according to the method of Miranda et al. [21]. The assay determines NO*x* level based on the reduction of any nitrate to nitrite by vanadium followed by the detection of total nitrite by Griess reagent. The formed chromophoric azo derivative can be measured colorimetrically at 540 nm.

Lipid peroxidation measurement

Liver lipid peroxidation products were estimated by determination of the level of thiobarbituric acid reactive substances according to the described by Mihara and Uchiyama which depends on a colorimetric determination of a pink pigment product resulting from the reaction of TBARS with thiobarbituric acid in acidic medium, at high temperature. The resultant color product is extracted in n-butanol and measured at two wavelengths, namely 535 and 520 nm [22].

Antioxidant enzyme activity assay

Glutathione peroxidase (GPx) activity was measured in the liver homogenate using a specific kit obtained from OxisResearch (USA).

Determination of liver reduced glutathione content

The content of reduced glutathione (GSH) was determined in tissue homogenate according to the method of Beutler et al. [23]. The method depends on the fact that GSH (the most abundant non-protein thiol) reduces Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid)] (DTNB) to form a stable yellow product (5-mercapto-2-nitrobenzoic acid), which can be measured colorimetrically at 412 nm.

Determination of caspase-3 activity

The hepatic caspase-3 activity as a marker of apoptosis was carried out using ApoAlert caspase-3 colorimetric assay kit (USA). Caspase-3 activity was expressed as (nmol pNA/h/mg protein). Protein content was measured according to the method of Bradford [24].

Histopathological evaluation

Liver samples preserved for histopathology were fixed in 10% formalin and used to prepare paraffin blocks. Sections of 5 μm were obtained and stained with Hematoxylin and Eosin (H&E). Images were captured and processed using Adobe Photoshop (version 8).

Statistical analysis

Data were expressed as means ± standard error (SE). Results were analyzed using one-way-analysis of variance test (ANOVA) followed by Tukey Kramer multiple comparison's test. For all statistical tests, the level of significance was set at $p < 0.05$. GraphPad Prism® software package, version 5 (GraphPad Software, Inc., USA) was used to carry out all statistical tests.

Results

Assessment of liver function

LPS injection significantly increased plasma AST (Fig. 1A) and ALT (Fig. 1B). Mg only in the dose of (40 mg/kg) significantly reduced the elevated plasma AST and ALT activity.