Chemico-Biological Interactions 189 (2011) 198–205

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Alpha-lipoic acid rebalances redox and immune-testicular milieu in septic rats

Abdelkader E. Ashour[a], Hala E. Abdel-Hamied[b], Hesham M. Korashy[a], Othman A. Al-Shabanah[a], Adel R.A. Abd-Allah[a,*]

[a] Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[b] Department of Pathology, College of Medicine for girls, Al-Azhar University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 21 September 2010
Received in revised form
15 December 2010
Accepted 20 December 2010
Available online 30 December 2010

Keywords:
Alpha-lipoic acid
LPS
Male infertility
Oxidative stress
Testis

ABSTRACT

In the present study, lipopolysaccharide (LPS), as an immune modulator in male adult rats and alpha-lipoic acid (ALA), as a powerful biological antioxidant and anti-inflammatory, are examined to help understanding the role of the immune and redox perturbation in testicular dysfunction with a possible protection. A total of 60 male Swiss albino rats were divided into 5 groups (10/group) respectively as follows Saline, ALA-vehicle, ALA (200 mg/kg), LPS (5 mg/kg) started with 20 rats and LPS + ALA. Obtained data from previously reported study, in our laboratory, and from the present one revealed that LPS induced marked reductions in sperm's count, motility and resulted in deterioration of the testicular histological features. In addition, LPS decreased testicular reduced glutathione (GSH) level and lactate dehydrogenase isoenzyme-x (LDH-x) activity. However, it increased testicular levels of malondialdehyde (MDA), nitric oxide (NO) and 8-hydroxydeoxyguanosine (8-HDG) in testicular DNA, along with increased serum IL-2 level. In contrast, rats pretreated with ALA showed almost complete normalization of all the tested parameters. In conclusion, LPS induced perturbation of the immune-testicular barrier as a result of redox imbalance with a subsequent testicular dysfunction. Pretreatment with ALA ameliorated all these effects by its immune-modulator and antioxidant mechanisms suggesting a protective role against male infertility in septic or severely infected patients.

Published by Elsevier Ireland Ltd.

1. Introduction

Infertility is a major medical and social problem in both magnitudes and impact on well-being. It is generally estimated that approximately 15% of couples are affected by infertility [1–3]. In approximately 50% of these infertile couples, male fertility factor is involved [4]. The cause of infertility in about half of these men is unknown. However, there is a considerable body of clinical evidence suggesting that spermatogenesis is inhibited by severe illness, infections and chronic inflammatory diseases, resulting in a temporary or permanent impairment of fertility [5–7]. Because testis is an immune-privileged organ protecting the auto-antigens of the meiotic and haploid germ cells, even minor infections can threaten organ integrity and function [8]. This under-representation of innate immune function can enable microorganisms to infect and colonize the testis, and subsequently can lead to impairment of spermatogenesis.

The inflammation associated with infection can be reproduced *in vivo* by the administration of bacterial lipopolysaccharides (LPS; endotoxins derived from the cell walls of gram-negative bacteria) [9–11]. Spermatogenesis and testicular steroidogenesis, in animals, have been reported to be inhibited by LPS [12,13], or by septic agents that generate LPS [14]. Systemic inflammation induced by LPS in rats results in the generation of reactive oxygen species (ROS) in the testis probably as a result of increased generation of lipid peroxides and impaired antioxidant defenses. ROS are known to mediate testicular damage during various pathological conditions [15]. When present in high concentration, ROS cause cellular damage by lipid peroxidation, as well as oxidation of proteins and DNA [16,17]. This, in turn, could be responsible for LPS-induced impairment of steroidogenesis and/or spermatogenesis that may lead to male infertility [15]. In that sense, our research group has reported that LPS administration markedly impaired rat testicular function through; marked reductions in sperm's count, motility and deterioration of the testicular histology [18]. In addition, LPS decreased testicular GSH level and LDH-x activity, whereas increased testicular levels of MDA, NO and 8-HDG in testicular DNA, along with increased serum IL-2 level [18]. Furthermore, it has been shown that biological compounds with antioxidant properties may protect cells and tissues against deleterious effects of ROS [19]. We also showed that L-carnitine as anti-oxidant and anti-inflammatory

* Corresponding author at: College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia. Tel.: +966 1 467 7263; fax: +966 1 467 7200.
E-mail address: arabdallah@hotmail.com (A.R.A. Abd-Allah).

0009-2797/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.cbi.2010.12.023

was able to protect against LPS-induced testicular damage [18]. This encouraged us to test the ability of alpha-lipoic acid (ALA) to protect against LPS-induced damage.

ALA, also known as thioctic acid, is a naturally occurring disulfide derivative of octanoic acid that is present in all kinds of prokaryotic and eukaryotic cells, particularly in the mitochondria serving as a coenzyme for mitochondrial dehydrogenase multi-enzyme complexes [20–22]. ALA is water and fat soluble and, therefore, is distributed in both the cellular membranes and the cytosol in plants and animals [23]. ALA contains two thiol-groups, which may be reduced rapidly in many tissues to its dithiol and dihydrolipoic acid (DHLA) form [17,24,25]. Numerous studies have demonstrated that ALA and DHLA can exert powerful antioxidant activities through several mechanisms, including quenching of free radicals in lipid and aqueous domains, chelation of transition metal ions (e.g., iron and copper), and regeneration of other antioxidants, such as ubiquinon, vitamins C and E and glutathione from their radical or inactive forms, and hence prevent toxicities associated with their loss [20,22,26–28].

Pharmacologically, ALA has been shown to improve glycemic control and reduce symptoms of diabetic peripheral neuropathy in human [29]. ALA also possesses anti-apoptotic effects on human bone marrow stromal cells and endothelial cells [30], radio-protective properties [17], and anticancer effects [31,32]. In addition, ALA effectively mitigates toxicities associated with heavy metal poisoning [25] and minimizes the pathological consequences of cigarette smoking [17]. Most importantly, a significant number of recent studies show that ALA protects cells against a host of insults where oxidative stress is part of the underlying etiology. The powerful antioxidant activity of ALA has been implicated in the prevention or alleviation of neurodegeneration, ischemia-reperfusion injury, human immunodeficiency virus infection, hepatic diseases and insulin resistance, which is an underpinning of many cases of coronary heart disease and obesity [22,25,33–35].

Based on the aforementioned information, ALA has been chosen in the present work as a possible protective agent against LPS-induced male infertility in rats. The overall objective of the present study is to elucidate the exact underlying mechanism(s) by which ALA can protect against male infertility in severely infected or septic rats. Results from this study may shed the light on the usefulness of ALA, as a safe natural product, in such pathological situations.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (LPS) and alpha-lipoic acid (ALA) were purchased from Sigma chemical Company (St. Louis, MO, USA). Thiobarbituric acid (TBA) is a product of Fluka (Buchs, Switzerland). All the other remaining chemicals are of the highest analytical grade commercially available.

2.2. Animals

Male Swiss albino rats, weighing 200–250 g, were obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, KSA. Animals were maintained under standard conditions of temperature 24 ± 1 °C and 55 ± 5% relative humidity with regular 12 h light:12 h dark cycles and allowed free access to standard laboratory food (Purina Chow) and water.

2.3. Experimental protocol

2.3.1. Effect of LPS and/or ALA on rat survival

2.3.1.1. Assessment of the LD_{50} of LPS. The animals were divided randomly into six equal groups (12 rats/group). Each group of rats received a dose of LPS (1, 3, 5, 7 or 9 mg/kg) and the control group received saline. The percentage mortality was assessed after 24 h to determine the LD_{50} of LPS.

2.3.1.2. Dose–response curve of the possible protective effect of ALA against LPS-induced mortality. Sixty rats were divided into 6 groups. (1) Control groups received saline, the LPS-vehicle, while the second subgroup was given the vehicle of ALA (ethanol:phosphate buffer saline, pH 7.2, 6:4) 3 h prior to LPS vehicle (saline). (2) LPS-treated group, and (3) three groups were given ALA (50, 100 or 200 mg/kg) 3 h prior to LPS.

2.3.2. Protective effect of the selected dose of ALA (from the previous experiment) against the testicular damage induced by LPS

All the next parameters were subsequently assessed in four groups as follows: (1) control groups included two subgroups, one received saline and the other received ALA vehicle then saline after 3 h, (2) LPS (5 mg/kg), (3) ALA (200 mg/kg) and (4) ALA (200 mg/kg 3 h prior to LPS). The total volume of i.p. injection was 0.25 ml. Blood samples were collected from all groups after 24 h of LPS injection by direct withdrawal from the heart by means of heparinized syringes after light ether anesthesia. Blood was left to clot and then centrifuged to separate sera. The animals were then sacrificed by cervical dislocation and the testes were isolated, washed with saline, blotted dry on filter papers, and weighed. From one testis 10% (w/v) homogenates of each sample was made in ice cold saline using homogenizer (VWR Scientific, Danburg, CN, USA) and subjected for biochemical assessments.

2.3.2.1. Assessment of sperm count and motility in the cauda epididymis. Assessment of sperm count and motility was performed according to Freund and Carol [36]. Briefly, two cauda epididymis from each rat were placed in 2 ml of warmed (37 °C) saline. Small cuts were made in the two cauda epididymis where the spermatozoa were obtained and suspended in saline solution. Two hundred microliters of the suspension was diluted with 800 μl of saline. A small amount of the diluted suspension was transferred to both chambers of a Neubauer haemocytometer using a Pasteur pipette by touching the edge of the cover slip and allowing each chamber to be filled by capillary action, sperm count and motility were examined and counted under light microscope.

2.3.2.2. Assessment of LDH-x activity. Testicular LDH-x activity was evaluated according Tablado et al. [37]. One testis was homogenized in 0.25 M sucrose solution (1:3, w/v) for 1 min. The homogenate was centrifuged at 3000 rpm for 30 min and the supernatant was passed through 0.45 μm pore-size Acrodisc (German Science, Inc., Ann Arbor, MI, USA). The filtrate contains LDH-x enzyme preparation. A quartz cuvette containing 3 ml of the specific substrate (106 mM trizma + 60 mM dl-α-hydroxycaproic acid + 0.05% sodium azide + 0.9 mM NAD^+) was incubated with 20 μl of the enzyme preparation for 10 min. The temperature of the reaction mixture was kept at 30 °C and the change in absorbance was recorded at 340 nm using a Beckmann DU-640 spectrophotometer. LDH-x activity was calculated as international units per gram of tissue according to the following equation:

$$\text{LDH} - x(\text{U/g testis}) = \frac{\Delta A}{6.3 \times 10^{-3}} \times \frac{3.02}{0.02} \times \frac{3}{1000}$$

2.3.3. Determination of GSH content in testicular tissue

Tissue levels of acid soluble thiols, mainly GSH, were determined colorimetrically at 412 nm according to Ellman [38]. Briefly, 0.5 ml of previously prepared homogenate was added to 0.5 ml of 5% trichloroacetic acid, and after centrifugation at 3000 rpm for