Available online at www.sciencedirect.com
SCIENCE DIRECT®
Chemico-Biological Interactions 160 (2006) 108–114
Chemico-Biological Interactions
www.elsevier.com/locate/chembioint

Modulatory role of lipoic acid on adriamycin-induced testicular injury

Chidambaram Prahalathan, Elangovan Selvakumar, Palaninathan Varalakshmi *

Department of Medical Biochemistry, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600113, India

Received 2 September 2005; received in revised form 13 December 2005; accepted 20 December 2005
Available online 24 January 2006

Abstract

The present study investigated the protective efficacy of DL-α-lipoic acid (LA) on adriamycin (ADR)-induced oxidative damage in rat testis. Adult male albino rats of Wistar strain were administered ADR (1 mg/kg body weight, i.v.), once a week for 10 weeks. ADR injected rats showed increased oxidative stress with a concomitant decrease in cellular thiols. The mRNA level for phospholipid hydroperoxide glutathione peroxidase (PHGPx) was also significantly decreased by ADR administration. Transmission electron microscopic (TEM) observations of testicular germ cells revealed abnormal ultrastructural changes in ADR treated rats. Treatment with lipoic acid (35 mg/kg body weight, i.p.) 1 day prior to ADR administration, effectively reverted these abnormal changes towards normalcy. These findings indicate a cytoprotective role of LA in this experimental model of testicular toxicity.

Keywords: Adriamycin; Lipoic acid; Oxidative stress; Cellular thiols; PHGPx; TEM; Testicular toxicity

1. Introduction

Adriamycin (ADR) is an anthracycline antibiotic that exhibits excellent antitumor activity against a variety of solid tumors. The clinical use of ADR is associated with testicular dysfunction characterized by altered sperm development, production, structural integrity and motility rates in association with increased cellular apoptosis [1–4]. Although antitumor action of ADR may be mediated by a wide number of mechanisms, oxidative stress and the generation of toxic reactive oxygen species (ROS) are the main cause of drug toxicity [5]. The oxyradicals cause damage to mitochondrial and other cytoplasmic membrane structures through peroxidation of phospholipids, proteins and nucleotides that can be detrimental to male fertility [6]. Thus, the combination of the drug delivery together with a potent antioxidant may be an appropriate approach to reduce the toxic side effects of ADR.

Phospholipid hydroperoxide glutathione peroxidase (PHGPx) is a selenium containing antioxidant that metabolizes phospholipids in membranes and protects them from oxidative damage. The mRNA for PHGPx is highly expressed in testis especially in specific spermatogenic cells during spermatogenesis and the down-regulation in PHGPx expression due to increased oxidative stress could lead to infertility [7]. Hence, it would be worthwhile to know whether PHGPx expression in the testis is influenced by ADR treatment.

Lipoic acid (LA), a naturally occurring nutraceutical, functions as an essential cofactor in metabolic

* Corresponding author. Tel.: +91 44 24480767; fax: +91 44 24926709.
E-mail address: drvlakshmi@yahoo.com (P. Varalakshmi).

0009-2797/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2005.12.007

reactions involved in energy utilization. LA and its reduced form dihydrolipoic acid are effective against conditions in which oxidative stress has a role [8]. It shows beneficial effects in oxidative stress conditions because of its synergistic action with other antioxidants [9]. LA, which is a universal antioxidant functions both in aqueous and membrane phases [10]. The present study describes the protective effect of LA; a multifunctional antioxidant on ADR-induced testicular toxicity, in correlation with testicular thiol contents and PHGPx gene expression.

2. Materials and methods

2.1. Drugs and chemicals

ADR (doxorubicin hydrochloride-Adrim) procured from Dabur Pharmaceuticals; New Delhi, India was used in the present experimental study. DL-α-lipoic acid, bovine serum albumin and 2′,7′-dichlorofluorescin diacetate (DCFH-DA) were purchased from Sigma Chemicals Co., St. Louis, USA. All other chemicals and solvents were of analytical grade.

2.2. Animal model

Adult male albino rats of Wistar strain weighing 140 ± 10 g (10–12 weeks old) were used throughout the study. The animals were maintained under standard conditions of humidity, temperature (25 ± 2 °C) and light (12 h light/12 h dark). They were fed with a standard rat pelleted diet (M/s Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and had free access to water. The animal experiments were conducted according to the guidelines of Institutional Animal Ethics Committee (IAEC).

2.3. Experimental protocol

The animals were randomly divided into four groups of six rats each as follows. Group I (control) received normal saline throughout the course of the study. Group II (ADR) received intravenous injections of ADR (1 mg/kg body weight) through the tail vein once a week for a period of 10 weeks. Group III (LA) received LA (35 mg/kg body weight) dissolved in saline at alkaline pH (7.8), intraperitoneally once a week for a period of 10 weeks. Group IV (ADR + LA) received a single injection of LA (35 mg/kg body weight) intraperitoneally; 24 h prior to the administration of intravenous injections of ADR (1 mg /kg body weight) through the tail vein once a week for a period of 10 weeks. At the end of the 10th week, the animals were killed by decapitation under anesthesia and both testes were excised immediately and used for the following analyses.

2.4. Determination of cellular oxidative stress

A 10% homogenate of the tissue was prepared in 0.01 M Tris–HCl buffer (pH 7.4) and protein content was determined by the method of Lowry et al. [11]. Oxidative stress was determined by using DCFH as a probe, according to the method of LeBel et al. [12]. In brief, the assay buffer contained 20 mM Tris–HCl, 130 mM KCl, 5 mM $MgCl_2$, 20 mM NaH_2PO_4, 30 mM glucose and 5 μM DCFH-DA. The assay medium was incubated at 37 °C for 15 min and 1 μmol of H_2O_2 was added to the mixture at the end of assay. DCF formation was measured at the excitation 488 nm and emission at 525 nm for 30 min by Shimadzu fluorescence spectrometer.

2.5. Determination of cellular thiols

Total thiol and non-protein thiols were estimated by the method of Sedlak and Lindsay [13].

2.6. RT-PCR analysis

To determine the expression of PHGPx mRNA in each group, total RNA was isolated from testes using a total RNA extraction kit (Eppendorf, Germany). The specific primers for PHGPx were used for amplification [14,15]. PCR amplification was carried out with a thermal cycler using one step RT-PCR kit (Eppendorf, Germany) according to a protocol for the initial denaturing step at 95 °C for 10 min; then 30 cycles at 95 °C for 1 min (denaturing), at 55 °C for 1 min (annealing) and 72 °C for 1.5 min (extension); and a further extension at 72 °C for 10 min. The PCR products were run on a 2% agarose gel in Tris-borate-EDTA buffer. Rat β-actin was used as an internal standard.

2.7. Electron microscopy studies

Representative electron micrographs from testis were obtained as previously [16]. Briefly, thin slices of the testis were fixed in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide. Then tissue was dehydrated in graded acetone solutions and finally embedded in epon-araldite. Ultra-thin sections were stained in uranyl acetate and lead citrate. Sections were transmitted in a Philips 201C transmission electron microscope (TEM) and photographed.