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Toxicology 217 (2006) 71–78
TOXICOLOGY
www.elsevier.com/locate/toxicol

Chemoprotective effect of lipoic acid against cyclophosphamide-induced changes in the rat sperm

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Received 29 July 2005; received in revised form 30 August 2005; accepted 31 August 2005
Available online 3 October 2005

Abstract

Treatment with cyclophosphamide (CP), a commonly used anticancer and immunosuppressive agent, may result in oligospermia and azoospermia. CP administration induces oxidative stress and is cytotoxic to normal cells. In this context, we have studied the effect of an established antioxidant, lipoic acid on its influence on CP-induced oxidative injury in rat sperm. In this study, we have assessed the possible protective efficacy of lipoic acid on the sperm characteristics, peroxidative damages and abnormal antioxidant levels in the epididymal sperm of CP-administered rats. Male Wistar rats of 140 ± 20 g were categorized into four groups. Two groups of rats were administered CP (15 mg/kg body weight once a week for 10 weeks by oral gavage) to induce testicular toxicity; one of these groups received lipoic acid treatment (35 mg/kg body weight intraperitoneally once a week for 10 weeks; 24 h prior to CP administration). A vehicle treated control group and a lipoic acid drug control group were also included. CP-treated rats showed a significant decrease in sperm count and motility with an increase in dead and abnormal sperms. The epididymal sperm of untreated CP-exposed rats showed 1.9-fold increase in lipid peroxidation, along with a significant increase in protein carbonyl level. These changes were associated with significant increase in DNA damage in the sperm as evidenced by increased single strand breaks in fluorimetric analysis of DNA unwinding (FADU). In rats treated with CP, abnormal changes in the activities/levels of enzymic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymic (reduced glutathione, ascorbate and α-tocopherol) antioxidants, were also observed. Pretreatment with lipoic acid improved the semen quality and reduced the oxidative stress and DNA damage induced by CP, thereby demonstrating the protection rendered by lipoic acid.

Keywords: Chemoprotection; Lipoic acid; Cyclophosphamide; Rat sperm; DNA damage; Oxidative stress

1. Introduction

Cyclophosphamide (CP), a cytotoxic alkylating agent, is extensively used as an antineoplastic agent for the treatment of various cancers, as well as an immunosuppressive agent for organ transplantation, systemic lupus erythematosus and other benign diseases (Dollery, 1999). However, despite its wide spectrum of clinical uses, CP is known to cause several adverse effects including reproductive toxicity in humans and experimental animals (Anderson et al., 1995). Adult male patients treated with CP for more than 4 months have

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doi:10.1016/j.tox.2005.08.020

demonstrated diminished sperm counts and absence of spermatogenic cycles in their testicular tissue (Howell and Shalet, 1998). Previous studies shown that male rats exposed to this drug have oligospermia and azoospermia, and manifest with biochemical and histological alterations in the testis and epididymis (Meistrich et al., 1995; Kaur et al., 1997). It has also been reported that postmeiotic germ cells are also specifically sensitive to CP exposure (Qiu et al., 1995). The cellular/biochemical mechanisms by which CP causes reproductive toxicity is poorly understood; however, numerous studies have shown that CP exposure enhances intracellular reactive oxygen species (ROS) production, suggesting that biochemical and physiological disturbances may result from oxidative stress (Das et al., 2002; Ghosh et al., 2002; Manda and Bhatia, 2003). When produced in excessive amounts, the ROS stimulate DNA fragmentation and a loss of sperm function associated with peroxidative damage to the mitochondria and plasma membrane. Further, spermatozoa are more susceptible to peroxidative damage because of high concentration of polyunsaturated fatty acids and low antioxidant capacity (Vernet et al., 2004). Thus the combination of the drug delivery together with potent antioxidant may be the appropriate approach to reduce the side effects of CP.

Lipoic acid, a dithiol, is found naturally in mitochondria as the coenzyme for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (Packer et al., 1995). Exogenous supplementation with this substance has been reported to increase unbound lipoic acid levels, which can act as a potent antioxidant and reduce oxidative stress both in vitro and in vivo (Bustamante et al., 1998). Inside cells and tissues, lipoic acid is reduced to dihydrolipoic acid which is even more potent as an antioxidant (Packer et al., 1995). Recently, we reported that some of the undesirable effects of CP treatment in adult male rats could be ameliorated by lipoic acid treatment (Selvakumar et al., 2005a, 2005b). The present study explores the biochemical efficacy of lipoic acid on CP-induced peroxidative changes and antioxidant status of the epididymal sperm of the rat.

2. Materials and methods

2.1. Drugs and chemicals

Cyclophosphamide (Endoxan®) was purchased from German Remedies Limited, Goa, India. DL-α-lipoic acid, bovine serum albumin, reduced glutathione and 1,1,3,3-tetraethoxypropane were obtained from Sigma Chemicals, St. Louis, MO, USA. All other chemicals and solvents used were of analytical grade.

2.2. Animal model

Adult male albino rats of Wistar strain (140 ± 20 g) were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The animals were maintained under standard conditions of humidity, temperature (25 ± 2 °C) and light (12 h light/12 h dark). They were fed 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. Experimental animals were handled according to the University and Institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.3. Experimental protocol

The rats were randomly divided into four groups consisting of six animals each. Group I served as control receiving saline throughout the experimental period. Group II received CP (15 mg/kg body weight) dissolved in saline, once a week for a period of 10 weeks by oral gavage. Group III rats served as drug control group and received lipoic acid (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 rats received a single injection of lipoic acid intraperitoneally 24 h prior to the administration of CP once a week for a period of 10 weeks. At the end of 10-week experimental period all animals were killed by decapitation. The epididymides were excised immediately and processed for the following analysis.

2.4. Sperm characteristics

Epididymal sperms were collected by chopping the epididymis in 5 ml of Hank's solution and incubated for 5 min at 37 °C in an atmosphere of 5% CO_2 to allow sperm to swim out of the epididymal tubules. One drop of sperm suspension was placed on a microscope slide, and a cover slip was placed over the droplet. At least 10 microscopic fields were observed at 400× magnification using a phase contrast microscope, and the percentage of motile sperm was recorded according to WHO (1999) recommendations. Sperm motility was expressed as a percentage of motile sperm of the total sperm counted.

The epididymal sperm counts were obtained by the method described in the WHO Manual (1999). Briefly, a 5 μl aliquot of epididymal sperm was diluted with 95 μl of diluent (0.35% formalin containing 5% $NaHCO_3$ and 0.25% trypan blue) and approximately 10 μl of this diluted specimen was transferred to each of the counting chambers of the hemocytometer, which was allowed to stand for 5 min in a humid chamber to prevent drying. The cells sedimented during this time and were counted with a light microscope at 400×.

A 20 μl of sperm suspension was mixed with an equal volume of 0.05% eosin-Y. After 2 min incubation at room temperature, slides were viewed by bright-field microscope with 400× magnification. Dead sperms appear pink and live