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Taurine reverses endosulfan-induced oxidative stress and apoptosis in adult rat testis

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ABSTRACT

The present study was aimed to investigate the mechanistic aspect of endosulfan toxicity and its protection by taurine in rat testes. Pre-treatment with taurine (100 mg/kg/day) significantly reversed the decrease in testes weight, and the reduction in sperm count, motility, viability and daily sperm production in endosulfan (5 mg/kg/day)-treated rats. Sperm chromatin integrity and epididymal L-carnitine were markedly decreased by endosulfan treatment. Endosulfan significantly decreased the level of serum testosterone and testicular 3β-HSD, 17β-HSD, G6PDH and LDH-X. Sperm $\Delta\psi m$ and mitochondrial cytochrome c content were significantly decreased after endosulfan. Testicular caspases-3, -8 and -9 activities were significantly increased but taurine showed significant protection from endosulfan-induced apoptosis. Oxidative stress was induced by endosulfan treatment as evidenced by increased H_2O_2 level and LPO and decreased the antioxidant enzymes SOD, CAT and GPx activities and GSH content. These alterations were effectively prevented by taurine pre-treatment.

In conclusion, endosulfan decreases rat testes weight, and inhibits spermatogenesis and steroidogenesis. It induces oxidative stress and apoptosis by possible mechanisms of both mitochondria and non-mitochondria pathways. These data provide insight into the mode of action of endosulfan-induced toxicity and the beneficial role provided by taurine to counteract endosulfan-induced oxidative stress and apoptosis in rat testis.

1. Introduction

Endosulfan is a cyclodiene broad spectrum insecticide, which is widely used in many parts of the world on wide variety of crops (Mersie et al., 2003). As a result of its widespread use, it is an environmental contaminant and a public health hazard (Jaiswal et al., 2005). Endosulfan is hazardous to various organs including testes (Ozmen and Mor, 2012). Endosulfan is estrogenic and was reported to induce testicular impairment (Wade et al., 1997). The impairments included decreased sperm count, intratesticular spermatid number, and sperm morphology as well as altered activities of testicular marker enzymes (Sinha et al., 2001).

It was reported that oxidative stress is involved in endosulfan-mediated apoptosis in human peripheral blood mononuclear cells in vitro (Ahmed et al., 2008). Moreover, Takhshid et al. (2012) reported that endosulfan induced oxidative stress and sperm toxicity in rat. Apoptosis is regarded as an active and organized form of cell death, triggered in response to physiologic or pathologic stimuli (Hengartner, 2000). Cell death by apoptosis is a part of normal development and maintenance of homeostasis (Tebourbi et al., 1998; Giannattasio et al., 2002), but is also involved in pathological situations associated with sterility. In the testis, apoptosis is such a common programmed event that 75% of germ cells are reduced by spontaneous apoptosis (Allan et al., 1992). Apoptosis serves several important functions in the testis, a few of which include maintaining appropriate germ cell to Sertoli cells ratio, removing defective germ cells and maintenance of overall quality control in sperm production (Shukla et al., 2012). However, excessive or inadequate apoptosis of testicular cells will result in abnormal spermatogenesis or testicular tumors (Lin et al., 1997).

Apoptosis includes an intrinsic pathway and an extrinsic pathway. In the intrinsic apoptotic pathway, the Bcl-2 family plays a major role, which consists of two functionally distinct groups of proteins, antiapoptotic and proapoptotic proteins. After Bcl-2 separation itself from the outer mitochondrial membrane (Nakai et al., 1993), the cytochrome c is released from the mitochondria into the cytosol where it binds to apoptotic protease-activating factor-1 (Apaf-1). Cytochrome c stimulates the formation of a complex with Apaf-1 and caspase-9 (Liu et al., 1996), which subsequently

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activates the executioner caspases-3, -6, and -7. The active caspases then cause cell apoptosis by cleaving a set of proteins, for example, poly (ADP) ribose polymerase (PARP) (Faraone Mennella, 2011). The extrinsic pathway for apoptosis involves Fas ligand (FASL) stimulation of FAS on target cells, which two recruit Fas-associated death domain (FADD) through shared death domains. The Fas/FADD complex activates initiator caspase-8 and subsequently activates executioner caspases, caspases-3 and -7, which affect apoptosis (Nagata and Golstein, 1995; Lee et al., 1997). Therefore, among the caspases, caspase-3 appears to be a key protease in both of the intrinsic and extrinsic apoptotic pathways, as the main executor which plays a vital role in the whole process of apoptosis (Yao et al., 2012).

Taurine (2-aminoethanesulfonic acid) has been identified as the major free β-amino acid in the male reproductive system (Lobo et al., 2000). Taurine has become an attractive candidate for attenuating various toxin- and drug-induced pathophysiological conditions (Ghosh et al., 2009; Das et al., 2010, 2011) through its antioxidant action (Wang et al., 2008; Zulli, 2011). It may also act as a capacitating agent (Meizel et al., 1980; Meizel, 1985) and as a sperm motility factor (Fraser, 1986; Boatman et al., 1990). It maintains the structural integrity of membrane, regulate calcium transport and modify protein phosphorylation (Zhang et al., 2010). Two sources of taurine could account for the intracellular levels of this amino acid: a biosynthetic route from cysteine and/or the specific uptake from the extracellular space. Extracellular taurine comes from dietary sources or from some tissues in which it is synthesized (e.g. liver) and released into circulation (Huxtable, 1992). However, the testicular toxicity of endosulfan and its mechanism is not fully investigated. In addition, the possible protective effect of taurine needs to be explored. The present study was aimed to investigate the mechanistic aspect of endosulfan toxicity and its protection by taurine in rat testes.

2. Materials and methods

2.1. Reagents

Endosulfan and taurine were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Other reagents were of analytical grade.

2.2. Animals

Adult male Wistar rats (90 days) weighing 170 ± 10 g were housed in clean polypropylene cages and maintained on a 12 h light/dark cycle and a temperature of 20–25 °C with *ad libitum* access to food and water. For 7 days before the experiment, rats were handled daily for 5 min to acclimatize them to human contact and minimize their physiological responses to handling for subsequent protocols (Ma and lightman, 1998). All the experiments with animals were carried out according to the guidelines of the Biochemical and Research Ethical Committee at King Abdulaziz University, Jeddah, Saudi Arabia.

2.3. Experimental protocol

The animals were randomly divided into four groups consisting of six animals each. Group I served as normal control receiving saline vehicle through the experimental period. Group II rats served as drug control group and received taurine (100 mg/kg/day dissolved in normal saline) by oral gavage. Group III treated with endosulfan suspended in olive oil (5 mg/kg/day) by oral gavage. Group IV rats received taurine 24 h prior to the administration of endosulfan. The doses of endosulfan (Uboh et al., 2011) and taurine (Das et al., 2009, 2012) in this study were selected on the basis of previous studies. The experiment was continued for 15 consecutive days.

2.4. Necropsy

Twenty four hours after the last dose, blood samples were collected from the retro-orbital sinus, under ether anesthesia. Samples were centrifuged and supernatant serum was separated from the clot as soon as possible and stored at −80 °C until testosterone and L-carnitine assay. Animals were euthanized and the testes were excised immediately, cleaned from adhering fat and connective tissues and the weights were recorded in g. The cauda epididymides from each animal were used for sperm count and motility and epididymal L-carnitine assay. Sperm from epididymal suspension were used for assay of chromatin integrity and mitochondrial membrane potential ($\Delta\psi m$).

One testis was used for evaluation of daily sperm production (DSP). The other testis was homogenized at 4 °C in RIPA buffer containing 150 mM NaCl, 1 mM EDTA, 10 μg/ml PMSF, 1% Triton X-100 and 20 mM Tris–HCl, pH 7.4 in a glass teflon homogenizer for 10s and centrifuged at 14,000×*g* for 20 min at 4 °C. The cytosol supernatant was removed and used as enzyme source for biochemical assay (Bustamante-Marín et al., 2012). The pellet containing the mitochondria was resuspended in lysis buffer and centrifuged at 10,000×*g* for 10 min. Supernatants (mitochondrial fraction) were used for cytochrome c assay. Protein concentrations were determined using a BCA kit (Pierce, Rockford, USA) that employed bovine serum albumin as a standard.

2.5. Sperm count and motility

Cauda epididymides were dissected out, immediately minced in 5 ml of physiological saline and then incubated at 37 °C for 30 min to allow spermatozoa to leave the epididymal tubules. The percentage of motile sperms was recorded using a phase contrast microscope at a magnification of 400×. Total sperm number was determined by using a Neubauer hemocytometer as previously described (Yokoi et al., 2003). To determine sperm motility, 100 sperms each were observed in three different fields, and classified into motile and non-motile sperms, and the motility was expressed as percentage incidence.

2.6. Sperm viability

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 under the microscope using 400× magnifications. Dead sperms appear pink and live sperms were not stained (Wyrobek et al., 1983). Two hundred sperms were counted for each sample and viability percentage was calculated. Sperm viability was defined as the percentage of intact cells (Eliasson, 1977).

2.7. Daily sperm production (DSP)

Daily sperm production (DSP) was determined in adult rats as previously described (Blazak et al., 1993). The testis was decapsulated and homogenized in 50 ml of ice-cold 0.9% sodium chloride solution containing 0.01% Triton X-100 using a Polytron homogenizer (Sharpe et al., 1995). The homogenate was allowed to settle for 1 min and then was gently mixed, and a 10 ml aliquot was transferred to a glass vial and stored on ice. After thorough mixing of each sample, the number of sperm heads (step 19 spermatid head) in four chambers of Neubauer type hemocytometer was counted under a light microscope with 40× objective. To calculate DSP, the number of spermatids at stage 19 was divided by 6.1, which is the number of days of the seminiferous cycle in which these spermatids are present in the seminiferous epithelium.

2.8. Chromatin integrity

Dried smears were fixed in 96% ethanol and acetic acid (1:1) for 30 min, then hydrolyzed with 0.1 N, HCl for 5 min in 4 °C. The slides were washed with distilled water 3 times for 2 min and stained with 0.05% toluidine blue (TB) for 5 min. Sperms with light blue heads were normal with good DNA integrity, while those with dark heads (purple) were abnormal with poor DNA integrity (Erenpreisa et al., 2003; Tsarev et al., 2009).

2.9. Epididymal L-carnitine

L-Carnitine was determined by a modification of the DTNB method (Pearson et al., 1974) using deproteinized sample solution (Fourie et al., 2001). A 0.1 ml portion of the deproteinized solution was added to 1.2 ml of reaction medium containing 0.1 mol/l Tris–HCl buffer (pH 7.5), AcCOA 0.12 ml (1.2 mg/ml), DTNB 30 μl (2 mg/ml) and CAT 6 μl (1.6 mg/ml). The mixture was incubated at 37 °C for 30 min, then 2 ml of Tris–HCl buffer was added and absorbance was measured at 412 nm. L-Carnitine concentration was assayed using the standard curve method and expressed as μmol/mg protein (protein weight before deproteinization).

2.10. Serum L-carnitine

Serum L-carnitine level was determined as previously described (Hisatomi et al., 2008) after deproteinized with perchloric acid. A mixture of 500 μl of sample solution, 1000 μl of coenzyme buffer, 100 μl of enzyme suspension and 600 μl of distilled water was incubated at room temperature for 10 min and absorbance at 340 nm was measured. Reaction was started by addition of 5 μl of enzyme suspension carnitine acetyl transferase to this mixture. Absorbance at 340 nm was measured at two time points, exactly 30 and 40 min after adding the suspension. Serum L-carnitine was expressed as μM.