Ecotoxicology and Environmental Safety 82 (2012) 96–103

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

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

Mitochondrial energy metabolism dysfunction involved in reproductive toxicity of mice caused by endosulfan and protective effects of vitamin E

Na Wang [a,b,1], Hong-Yan Qian [a,b,1,2], Xian-Qing Zhou [b,*], Yan-Bo Li [b], Zhi-Wei Sun [b]

[a] Department of Laboratory Animal Science, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, PR China
[b] Department of Health Toxicology and Health Chemistry, School of Public Health and Family Medicine, Capital Medical University, Beijing 100069, PR China

ARTICLE INFO

Article history:
Received 12 March 2012
Received in revised form
21 May 2012
Accepted 22 May 2012
Available online 15 June 2012

Keywords:
Endosulfan
Vitamin E
Reproductive toxicity
Oxidative stress
Energy metabolism

ABSTRACT

The experiment was designed to study the mechanism of reproductive toxicity caused by endosulfan in mice and protective effects of vitamin E. The experiment was composed of three groups: the control group did not receive any endosulfan and vitamin E; the endosulfan exposed group received 0.8 mg/kg/d endosulfan and 0 mg/kg/d vitamin E; and the endosulfan+vitamin E group received 0.8 mg/kg/d endosulfan and 100 mg/kg/d vitamin E. The results showed that vitamin E significantly reversed the decline of the concentration and motility rate of sperm, and inhibited the increase of sperm abnormality rate caused by endosulfan. The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), and lactate dehydrogenase-C4 (LDH-C4) and the level of adenosine triphosphate (ATP) in the endosulfan+vitamin E group were higher while the malondialdehyde (MDA) content was significantly lower than those of the endosulfan exposed group. The results from pathology and electron microscope observed showed vitamin E decreased the cavities formation by desquamating of spermatogenic cells, stopped the ruptures and disappearances of mitochondrial cristaes in spermatogenic cells, and prevented the breakages and partial dissolvings of sperm tails induced by endosulfan. It is likely that endosulfan could directly damage sperm structures by oxidative stress, leading to a decrease in sperm quantity and quality. It also could indirectly cause a decline in reproductive function by damaging the structure of mitochondria, resulting in energy metabolism dysfunction, which could be one of the mechanisms behind the reproductive toxicity induced by endosulfan. It was inferred that vitamin E helps maintain the structural integrities of sperm architecture and prevent mitochondrial dysfunction through inhibiting oxidative stress, and thereby prevent the reproductive dysfunctions caused by endosulfan.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methylenedioxy bridge-2,4,3-hept-3-benzo sulfur dioxide-oxide) is a highly toxic broad-spectrum organochlorine insecticide and miticide. It is an environmental endocrine-disrupting chemical and is included in the list of 129 controlled priority pollutants by the U.S. Environmental Protection Agency (Bisson and Hontela, 2002; Sinha et al., 2002). In aquatic and terrestrial ecosystems, the bioaccumulation of endosulfan has posed a threat to animal and human health and survival. Soto et al. (1994) reported that endosulfan had estrogenic activity in breast cancer MCF-7 cell proliferation in vitro experiments. It was confirmed that male genital abnormalities and sperm production were affected by embryonic endosulfan exposure as well as early postnatal exposure to exogenous estrogen (Sharpe et al., 1995; Sharpe and Skakkebaek, 1993; Fisher et al., 1999). Zhu et al. (2000, 2002) found that endosulfan reduced sperm numbers and caused abnormal morphology in adult male rats. Endosulfan also reduced the amount of testicular sperm and sperm motility in *Microtus oeconomus* and increased the sperm abnormality rate (Sun et al., 2010).

Vitamin E, a fat-soluble vitamin, is an effective antioxidant. Vitamin E plays a very important role in protecting the integrity of cell membrane structure and promoting reproductive function. It was shown to inhibit reproductive system damage from oxidative stress, reverse reactive oxygen species (ROS) induced embryo toxicity and improve the blastocyst development rate (Wang et al., 2002). ROS generation and the oxidative damage in

Abbreviations: SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; MDA, malondialdehyde; ATP, adenosine triphosphate; LDH-C4, actate dehydrogenase-C4; SDH, succinate dehydrogenase

* Corresponding author. Tel.: +86 10 83911775; fax: +86 10 59784719.
E-mail addresses: xqzhou@ccmu.edu.cn, xianqingzhou@yahoo.com.cn (X.-Q. Zhou).

[1] Na Wang and Hong-yang Qian contributed equally to this work.

[2] Present address: Department of Immunology, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, PR China.

0147-6513/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ecoenv.2012.05.014

sperm membranes led to an increase in sperm abnormality rate and fusion barriers of sperm and egg. Vitamin E protected the damage of the sperm, mitigated biochemical changes induced by aflatoxin in testes (Awad et al.,1994), reduced oxidative stress in the epididymis and epididymal sperm and prevented abnormal testicular morphology and changes in both sperm counts and sperm mobility induced by methoxychlor (Hamm et al., 2000). Although reports on endosulfan induced reproductive toxicity are available (Zhu et al., 2000, 2002; Sun et al., 2010), little is known about the mechanisms. In the present study, we examined the effects of vitamin E on reproductive function in endosulfan-treated mice and further examined the mechanism of reproductive toxicity induced by endosulfan through oxidative stress and energy metabolism. The present study provides a scientific basis for endosulfan mediated reproductive dysfunctions in males and possible effects on fertility.

2. Materials and methods

2.1. Materials

Endosulfan (96% purity) was provided by Jiangsu Kuaida Agrochemical Co., Ltd. Vitamin E was obtained from Sigma-Aldrich Co., LLC. The corn oil was supplied by COFCO Food Sales & Distribution Co., Ltd. The kits were obtained from Nanjing Jiancheng Bioengineering Institute.

2.2. Animals

Clean male mice (Institute of Cancer Research/CD-1) obtained from the Beijing Vital River Laboratory Animal Technology Limited Corporation, China were used in the present study. Each mouse was raised in a standard cage (26 cm × 15 cm × 15 cm). They were maintained at a constant temperature of 22 ± 2 °C, humidity of 50 ± 5%, and on a 12:12 light/dark cycle. The animal feed was provided by Beijing Keao Xieli Feedstuff Co., Ltd. The pad was changed twice per week. All mice were provided food and drinking water ad libitum. The experiments were initiated after a 10-day acclimation period and conducted in accordance with institutional guidelines for animal welfare, and the protocols were reviewed by the animal experimentation committee.

2.3. Design of experiments in vivo

Five-week-old male mice were selected for the study. Mice were randomly assigned to three groups, with seven mice per group. Control group did not receive endosulfan and vitamin E; endosulfan exposed group received 0.8 mg/kg/d endosulfan and 0 mg/kg/d vitamin E; and endosulfan + vitamin E group received 0.8 mg/kg/d endosulfan and 100 mg/kg/d vitamin E. The basis of endosulfan dose selection was based on previous reports (Zhang and Zhou, 2010; Zhang et al., 2012). Vitamin E and endosulfan were dissolved in corn oil and administered via oral gavage. Endosulfan was given at approximately 8 a.m. daily, and 2 h later, vitamin E was administered. The mice in the control group received an equal volume of corn oil. The mice were sacrificed after a 3-week exposure to endosulfan, which is short term repeated-dose toxicity (Wang, 2007). The testes and epididymides were collected to begin the experiments.

2.4. Evaluation of testicular structures

Testes were rapidly removed from the mice and fixed in 10% buffered formalin for 24 h. Testicular tissue was then dehydrated and embedded in paraffin by standard procedures. Sections (4 μm thickness) were deparaffinized and rehydrated. After hematoxylin and eosin (HE) staining, the histopathology of the testes was observed under light microscopy (Leica Microsystems DM1000, USA).

2.5. Determinations of the concentration, motility rate and abnormality rate of epididymal cauda sperm

The sperm concentration and sperm motility rate were determined using the method of cell counts, and the sperm abnormality rate was analyzed according to the method of Wyrobek and Bruce (1975).

The epididymides cauda were quickly placed in a Petri dish with 2 ml of saline and cut into pieces, and a small amount of sperm suspension was added to the cell count plates. The total sperm number and motile sperm number in four large cages were counted using a high-magnification microscope. Sperm concentration = the total sperm number/($4 \times 10^4 \times 2$); sperm motility rate = the motile sperm number/the total sperm number × 100%.

A small amount of sperm suspension was drawn and smeared on a slide, fixed for 10 min with methanol, stained for 1 h with 1% eosin, and then washed with water. A total sperm number of 1000 was counted, and the abnormal sperm number was counted using a high-magnification microscope. Sperm abnormality rate = the abnormal sperm number/1000 × 100%.

2.6. Determinations of protein concentration, the activities of superoxide dismutase, glutathione peroxidase and the level of malondialdehyde in testicular tissue

Testes homogenates were prepared with saline on ice according to the instructions of a kit provided by the Nanjing Jiancheng Bioengineering Institute. The protein concentration of testes tissue was assayed using a Coomassie Protein Assay Kit. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) and the level of malondialdehyde (MDA) were determined using a Multiskan Ascent microplate reader (Thermo LabSystems Inc., Holland) at 550 nm, 412 nm and 532 nm, respectively.

2.7. Determination of the activities of succinate dehydrogenase and lactic acid dehydrogenase-C4 and the level of adenosine triphosphate in testicular tissue

Testes homogenates were prepared with saline on ice according to the instructions of the kit provided by the Nanjing Jiancheng Bioengineering Institute. The activities of succinate dehydrogenase (SDH) and lactic acid dehydrogenase-C4 (LDH-C4) and the level of adenosine triphosphate (ATP) were determined using a Multiskan Ascent microplate reader (Thermo LabSystems Inc., Holland) at 600 nm, 440 nm and 636 nm, respectively.

2.8. Evaluation of testicular tissue ultrastructure

The testes were removed immediately, cut into small pieces, placed in 2.5% glutaraldehyde at 4 °C for 2 h, and washed three times for 10 min each with phosphate buffer at pH 7.2. They were then fixed with 1% osmium tetroxide at 4 °C for 2 h, washed three times for 10 min each with a phosphate buffer at pH 7.2, and dehydrated with ethanol. The dehydrated samples were embedded with EPON 812, cut with an LKB-V microtome, and then stained by 3% uranyl acetate-lead citrate. Testicular ultrastructures were observed using an H-7650 transmission electron microscope (TEM).

2.9. Statistical analyses

All data were analyzed with the statistical software package SPSS 13.0. The significance of differences between the control group vs. the endosulfan exposed group and the endosulfan exposed group vs. the endosulfan + vitamin E group were determined using *t*-tests. All values are expressed as the mean ± s.d. The results were considered significant at $P < 0.05$.

3. Results

3.1. The changes in testicular structures

In the control group, the basement membrane of the seminiferous tubules maintained its integrity and was smooth; the layers of the seminiferous epithelium were thick; the regular lumen was filled with mature sperm; and the spermatogenic cells developed normally from spermatogonia to mature sperms (Fig. 1A and B); In the endosulfan exposed group, the seminiferous epithelium was thinner (only 2–3 layers) than that of the control group; the arrangement of spermatogenic cells was irregular; the decrease of mature sperm in the lumen was significant; and a large number of spermatogenic cells desquamated to form cavities between the spermatogonia and spermatocytes (Fig. 1C and D). In the endosulfan + vitamin E group, the seminiferous epithelium was thicker than that of the endosulfan exposed group; there were more mature sperm in the regular lumen; and the cavities were smaller than those of the endosulfan exposed group (Fig. 1E and F).

Download English Version:

https://daneshyari.com/en/article/4420854

Download Persian Version:

https://daneshyari.com/article/4420854

Daneshyari.com