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Effects of p,p′-DDE on the mRNA and protein expressions of vimentin, N-cadherin and FSHR in rats testes: An in vivo and in vitro study

Maosheng Yan[a,1], *Yuqin Shi*[a,b,1], *Yuping Wang*[a,c], *Can Wang*[a], *Jun Zhou*[a], *Chao Quan*[a], *Changjiang Liu*[a], *Kedi Yang*[a,*]

[a] *MOE Key Lab of Environment and Health, Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan 430030, Hubei, PR China*
[b] *School of Public Health, Medical College, Wuhan University of Science and Technology, Wuhan 430065, PR China*
[c] *Jiangdong Center for Disease Control and Prevention, Ningbo 315010, Jiangsu, PR China*

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ABSTRACT

To elucidate the mechanism underlying the testicular effects of 1,1-dichloro-2,2bis(p-chlorophenyl)ethylene (p,p′-DDE), the expressions of vimentin, neural cadherin and follicle-stimulating hormone receptor mRNA and proteins were measured in vivo and in vitro. Sprague-Dawley rats were dosed with p,p′-DDE at 0, 20, 60 and 100 mg/kg every other day by intraperitoneal injection for 10 days, and Sertoli cells were treated with p,p′-DDE (0, 10, 30, and 50 μM) for 24 h. Results indicated that the survival rate of Sertoli cells was decreased with increasing doses of p,p′-DDE. In vitro and in vivo studies, p,p′-DDE could increase the expression of neural cadherin, follicle-stimulating hormone receptor mRNA, while decrease the levels of vimentin, neural cadherin and follicle-stimulating hormone receptor proteins. Moreover, immunohistochemistry analysis revealed that the protein expressions of vimentin, neural cadherin and follicle-stimulating hormone receptor in pubertal rat testes were disrupted by treatment with p,p′-DDE. Taken together, these results suggested that p,p′-DDE exposure could induce testicular toxicity through the changes of the mRNA and protein expressions of vimentin, neural cadherin and follicle-stimulating hormone receptor in vitro and in vivo.

1. Introduction

Organochlorine pesticides (OCPs) represent an important class of persistent organic pollutants (POPs) that have been recognized as causing adverse effects due to their bioaccumulation, recalcitrance to degradation, and potential toxicity on humans and wildlife (Jones and de Voogt, 1999; Turusov et al., 2002). 1,1-Dichloro-2,2bis(p-chlorophenyl)ethylene (p,p′-DDE), main metabolite of 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT) with the highest persistence, behaves like a potent antiandrogen in vitro and in vivo, since it blocks the union of the androgen and its receptor, and inhibits the transcriptional activity induced by the androgen and its effect on target organs

Abbreviations: p,p′-DDE, 1,1-dichloro-2,2bis(p-chlorophenyl)ethylene; N-cadherin, neural cadherin; FSHR, follicle-stimulating hormone receptor.

* *Corresponding author*. Tel.: +86 27 83693897; fax: +86 27 83657765.
E-mail address: yangkd@mails.tjmu.edu.cn (K. Yang).

[1] These authors contributed equally.

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(Sohoni and Sumpter, 1998). Also, some studies have found an association between levels of p,p′-DDE and poor seminal parameters: decreased sperm motility, decreased sperm concentration and increased damage to sperm DNA (De Jager et al., 2006), increased frequency of testicular cancer (McGlynn et al., 2008) and congenital genital malformations (Bornman et al., 2010).

The Sertoli cell is the somatic cell type found in the mammalian testicle's seminiferous tubule, and plays major roles in the maintenance and control of spermatogenesis (Griswold et al., 1988), stays spermatogenetic activity throughout the human whole life, and provides functions for the physiological health of both the seminiferous epithelium and the tubular wall (Anniballo et al., 2000). Therefore, any chemical agent that impairs the viability of Sertoli cells could cause serious effect on spermatogenesis. In previous studies, our research on the Sertoli cells exposure to p,p′-DDE revealed that p,p′-DDE can induce oxidative stress and mitochondria-mediated apoptosis in Sertoli cells in vivo (Song et al., 2008, 2011) and in vitro (Shi et al., 2010).

Vimentin protein is an important element of the Sertoli cytoskeleton (Franke et al., 1982), playing a part in anchoring germ cells to the seminiferous epithelium (Amlani and Vogl, 1988) and is correlated to alterations with the lesion of structural integrity of seminiferous epithelium, as well as germ cell apoptosis after xenobiotic treatment (Richburg and Boekelheide, 1996). N-cadherin plays an important role in regulating cell–cell interactions in the seminiferous epithelium (Andersson et al., 1994; Chung et al., 1998) and has been localized in cells of the seminiferous epithelium, particularly in spermatogonia, primary spermatocytes and Sertoli cells (Tsuchiya et al., 2006). Follicle stimulation hormone receptor (FSHR) expression determines both the targets and the extent of follicle stimulation hormone (FSH) action, ultimately directing hormone response to Sertoli cells in the testis (Sang et al., 2011). However, the effects of p,p′-DDE on the expressions of vimentin, N-cadherin and FSHR, are considerably less known. Moreover, puberty is a critical period of testis development in mammalian male, and in this period exposure to environmental xenobiotics may lead to unrecoverable injury for testes (Khan et al., 1998). Therefore, in the present study, we sought to investigate whether different concentrations of p,p′-DDE would change the expressions of vimentin, N-cadherin and FSHR in Sertoli cells and the testes of pubertal rats (50-day old).

2. Materials and methods

2.1. Chemicals

p,p′-DDE (98.5% purity) were purchased from DR Co. (Augsburg, Germany). All other chemicals were of analytical grade (>99% purity) and chemicals with the same batch number were used in all in vitro and in vivo studies.

2.2. Sertoli cells culture and treatments

Sertoli cells cultures were prepared using sequential enzymatic procedures that have been described previously (Mather et al., 1990) with modifications. Briefly, testes from 18 to 20-day-old outbred Sprague-Dawley (SD) rats were collected, excised rapidly, decapsulated, cut into small fragments, and washed twice in Hanks' balanced salt solution (HBSS). The fragments were then digested sequentially in 10 ml of HBSS containing 0.25% trypsin (Amresco, Solon, OH, USA) and 0.1% collagenase (type I, Invitrogen, Grand Island, NY, USA) in a shaking water bath (37 °C, 120 cycles/min) for 30 min. The digested cells suspension was washed extensively with no-phenol red-Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Grand Island, NY, USA) to remove peritubular cells, followed by filtration through B–D Falcon cell strainers (nylon mesh size, 70 μm). The final Sertoli cells suspension was supplemented with 5% fetal bovine serum (Invitrogen, Grand Island, NY, USA) and seeded in cultured bottle in a humidified atmosphere of 95% air–5% CO_2 at 35 °C. After 24 h, these cells were extensively washed twice with HBSS solution with 20 Mm, pH 7.4, Tris–HCl for 5 min and with serum starvation for 24 h. The medium was renewed at 2-day interval.

p,p′-DDE was dissolved in dimethylsulfoxide (DMSO, Sigma–Aldrich, St. Louis, MO, USA) as stock solution and diluted with culture medium to different concentrations (0, 10, 30, 50 or 70 μM) according to the results of our preliminary experiment (Song et al., 2011) and the results of MTT assay. The final DMSO concentration in the medium was not more than 0.3% (v/v) which did not affect the viability of Sertoli cells. Control cells were cultured with 0.3% DMSO.

2.3. MTT assay

This assay is dependent on the cellular reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically. After Sertoli cells were treated with 0, 10, 30, 50 or 70 μM p,p′-DDE for 24 h, MTT (Sigma–Aldrich, St. Louis, MO, USA) was added into each well with the final concentration of 5 mg ml^{-1} for 4 h. Thereafter, 200 μl DMSO was added to dissolve the MTT formazan crystal. Then the culture plate was shaken for 10 min. The optical density (OD) of each well was measured at 490 nm with an ELISA Reader (Bio-Rad Instrument Group, Hercules, CA, USA). Cell viability (%) was calculated using the following equation: cell viability (%) = $(OD_{treatment}/OD_{control}) \times 100$.

2.4. Animals and treatments

Male outbred Sprague-Dawley (SD) rats were purchased from Tongji Medical College Animal Laboratory (Wuhan, China). All procedures on animals followed the Guide for the Care and Use of Laboratory Animals published by Ministry of Health of People's Republic of China.

Twenty healthy pubertal male rats (50-day old) were used in the study. The animals were allowed free access to standardized granular food and tap water and were maintained on a 12-h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment. The rats were randomly divided into six groups, each group containing five rats, given different doses of p,p′-DDE (20, 60, 100 mg/kg bw, respectively), and control group (corn oil (Sigma–Aldrich, St. Louis, MO, USA))