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Mono-(2-ethylhexyl) phthalate (MEHP) induces testicular alterations in male guinea pigs at prepubertal stage

M.A. Awal [a,b,*], M. Kurohmaru [b], B.B. Andriana [b], Y. Kanai [b], Y. Hayashi [c]

[a] *Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*
[b] *Department of Veterinary Anatomy, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan*
[c] *Department of Global Animal Resource Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan*

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Abstract

We have recently shown that MEHP induces spermatogenic cell apoptosis in guinea pigs at prepubertal stage in vitro. To evaluate the effects of MEHP on the testicular tissues of guinea pigs in vivo, we conducted this research work. Five weeks old male guinea pigs were used in this experiment. They received a single oral dose of 2000 mg/ml of MEHP in corn oil by gavage at a volume equal to 4 ml/kg. Control group received a similar volume of corn oil vehicle. Vehicle- and MEHP-treated guinea pigs were sacrificed at the interval of 3, 6, and 9 h, and the testicular tissues were processed for histopathological studies. Distinct histopathological changes were recognized in testes. Detachment and displacement of spermatogenic cells, thin seminiferous epithelia, vacuolization of Sertoli cells were prominent at 6 h after MEHP treatment. The lumina of the efferent ductules were frequently occupied with sloughed seminiferous epithelia from 6 to 9 h after MEHP treatment. Apoptotic spermatogenic cells appeared at 3 h in the control group. The incidence of apoptotic spermatogenic cells significantly increased ($^{*}p < 0.05$) from 3 to 9 h, and the maximal increase of apoptotic spermatogenic cells were observed at 9 h after MEHP treatment. Time-dependent increases of apoptotic spermatogenic cells was recognized throughout the experimental period. It may be suggested here that MEHP also induces spermatogenic cell apoptosis in guinea pigs in vivo and guinea pigs may be considered as a useful animal model for sensitivity test of the reproductive toxicity to some phthalate esters at their earlier stage in vivo.

Keywords: Mono-(2-ethylhexyl) phthalate (MEHP); Testicular toxicity; Apoptosis; Guinea pig; Prepubertal stage

1. Introduction

Phthalic acid esters are widespread in the environment due to their use as plasticizers in food packaging and biomedical devices. It has been reported that phthalic acid esters have been shown to reduce fertility and induce testicular atrophy in laboratory animals (Thomas and Thomas, 1984; Albro, 1987). Among phthalic acid esters, di-(2-ethylhexyl) phthalate (DEHP) is widely dispersed throughout the environment due to its increased commercial use in the production of plastic products. Recently, this phthalate is one of the most widely studied male reproductive toxicants. It has been demonstrated that phthalate esters, when administered orally, are rapidly hydrolyzed in the gut and other tissues to produce the corresponding monoesters. Mono-(2-ethylhexyl) phthalate (MEHP), one of the metabolites of DEHP, showed the most potent testicular toxicity (Albro, 1987; Richburg and Boekelheide, 1996).

The Sertoli cell is the primary target of phthalates. Recent investigations revealed that MEHP is a well-characterized Sertoli cell toxicant and induces testicular toxicity at the earlier stage of life (Gray and Butterworth, 1980; Gray and Beamand, 1984; Dalgaard et al., 2001; Suominen et al., 2003). In a wide range of laboratory animals, oral administration of MEHP induced testicular toxicity characterized with detachment and sloughing of spermatogenic cells, atrophy in seminiferous tubules, and reduction in seminal vesicle

* Corresponding author. Tel.: +81 3 5841 5384; fax: +81 3 5841 8181.
E-mail address: awall963@yahoo.com (M.A. Awal).

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and prostate weights (Gray and Gangolli, 1986; Kasahara et al., 2002).

A marked species difference in sensitivity to the testicular toxicity of phthalic acid esters has been reported (Gray et al., 1982; Richburg et al., 2000). Moreover, most of the studies using phthalic acid esters were limited to rats and mice. No phthalate esters-related investigation using male guinea pigs has been carried out during the past two decades. Therefore, the present study was designed to elucidate the effects of MEHP on testes of guinea pigs at prepubertal stage in vivo.

2. Materials and methods

2.1. Guinea pig

Twelve male guinea pigs (28-day-old) were purchased from Charles River Laboratories, Japan (Crj). The body weight of the animals ranged from 280 to 300 g. They were acclimatized for 1 week prior to use. The guinea pigs were maintained in our environmentally controlled animal facilities with free access to water and rodents chow/Pellets (Oriental Yeast Co., Ltd., Japan). The experiment was approved by the Ethical Committee of Animal Experiments (ECAE) of the University of Tokyo, Japan.

2.2. Chemical/drug

Mono-(2-ethylhexyl) phthalate was purchased from Tokyo Kasei Kogyo Co., Ltd., Japan. MEHP was dissolved in an appropriate volume of corn oil vehicle prior to administration. Apoptosis Detection Kit was purchased from Takara Chemical Co., Ltd., Japan.

2.3. Experimental design

Prepubertal male guinea pigs were divided into three groups. Each group consisted of four animals. Within each group, the animals were divided into two subgroups (Group 1: corn oil (vehicle) control, Group 2: MEHP treatment).

2.4. Experimental protocol

Guinea pigs received a single oral dose of 2000 mg/kg of MEHP in corn oil by gavage at a volume equal to 4 ml/kg. Control group received the same volume of corn oil vehicle. Vehicle- and/or MEHP-treated guinea pigs were sacrificed at 3, 6, and 9 h after treatment under sodium pentobarbital (Dainihon Seiyaku, Osaka, Japan) anesthesia. They were perfused with 0.9% physiological saline, followed by 4% paraformaldehyde and/or 5% glutaraldehyde–0.05 M cacodylate buffer (pH, 7.4). The testes were carefully excised and fixed in each fixative at room temperature for 4–6 h for paraffin embedding and/or at 4 °C for 2.5–3 h for Araldite embedding.

2.5. Light microscopy

The testes were washed with PBS (pH, 7.4), and were dehydrated in a graded series of ethanol, cleared in xylene, and embedded in paraffin. The paraffin blocks were cut at 5 μm in thickness. The sections were then stained with Meyer's hematoxylin and eosin and/or PAS–hematoxylin staining, and observed by light microscopy for general histopathological examination.

2.6. Transmission electron microscopy

For transmission electron microscopy, the specimens were fixed in 5% glutaraldehyde–0.05 M cacodylate buffer (pH, 7.4) at 4 °C for 2.5 h, and then washed three or four times with the same buffer. They were postfixed in 1% osmium tetroxide (OsO_4) for 1 h, dehydrated through a graded series of ethanol, infiltrated in propylene oxide, and embedded in Araldite. Semithin sections were cut at approximately 1 μm in thickness, stained with 1% toluidine blue, and observed using light microscopy. Ultrathin sections were cut, and stained with uranyl acetate and lead citrate, and examined with a JEM-1200 EX transmission electron microscope at 80 kV.

2.7. In situ TUNEL staining and quantitation

Terminal deoxy-nucleotidyl transferase-mediated digoxigenin-dUTP nick end-labeling (TUNEL) was performed by using the In Situ Apoptosis Detection Kit (Takara Chemical Co., Ltd.). Briefly, the tissue sections were deparaffinized and digested with 20 μg/ml proteinase K (Takara Chemical Co., Ltd.) at 37 °C for 15 min. After washing several times with phosphate-buffered saline (PBS), they were treated with terminal deoxynucleotidyl transferase (TdT) enzyme and Labeling Safe Buffer (Takara Chemical Co., Ltd.). The TdT reaction was conducted at 37 °C for 90 min. After washing several times with PBS, they were then incubated with horseradish peroxidase (HRP)-goat antibiotin (Takara Chemical Co., Ltd.) at 37 °C for 30 min. They were allowed to reincubate with 0.05% diaminobenzidine (DAB) and 0.02% H_2O_2 for visualization of HRP sites, counterstained with methyl green, and observed using a light microscope. TUNEL-positive spermatogenic cells in the seminiferous tubules were counted and tabulated.

2.8. Statistical analysis

Only intact and round seminiferous tubules were counted. The number of apoptotic spermatogenic cells was counted in 100 randomly selected round seminiferous tubules from each of three different groups. The data, calculated as a percentage of total, were expressed as the mean ± S.E.M. Significance between groups ($^{*}p < 0.05$) was determined by single factor analysis of variance (ANOVA) with a Fisher's least significant differences test comparison using Stat View software (Abacus Concepts Inc., Berkeley, USA).