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·*Original Article*·

Short-term effects of di-(2-ethylhexyl) phthalate on testes, liver, kidneys and pancreas in mice

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Abstract

Aim: To determine the biochemical effect of di-(2-ethylhexyl) phthalate (DEHP) on testes, liver, kidneys and pancreas on day 10 in the process of degeneration of the seminiferous epithelium. **Methods:** Diets containing 2% DEHP were given to male Crlj:CD1(ICR) mice for 10 days. The dose of DEHP was 0.90 ± 0.52 mg/mouse/day. Their testes, livers, kidneys and pancreata were examined for detection of mono-(2-ethylhexyl) phthalate (MEHP), nitrogen oxides (NOx) produced by peroxidation of nitric oxide (NO) with free radicals, and lipid peroxidation induced by the chain reaction of free radicals. **Results:** Histological observation and serum analysis showed the presence of severe spermatogenic disturbance, Leydig cell dysfunction, liver dysfunction and dehydration. Unexpectedly, the concentration of MEHP in the testes was extremely low compared with that in the liver. However, the concentration of the NOx in the testes was as high as the hepatic concentration. Furthermore, free radical-induced lipid peroxidation was histochemically detected in the testes but not in the liver. **Conclusion:** The results indicate that DEHP-induced aspermatogenesis is caused by the high sensitivity of the testicular tissues to MEHP rather than the specific accumulation or uptake of circulating MEHP into the testes. *(Asian J Androl 2007 Mar; 9: 199–205)*

Keywords: phthalate; nitrogen oxide; free radical; testis

1 Introduction

Experimentally, di-(2-ethylhexyl) phthalate (DEHP), widely used as a plasticizer for synthetic polymers, is known to induce testicular atrophy with hepatomegaly in mice and rats [1, 2]. After oral exposure, most DEHP is rapidly metabolized in the gut into mono-(2-ethylhexyl) phthalate (MEHP), the active metabolite inducing testicular atrophy [1]. Many biochemical studies have shown that the concentrations of testosterone, zinc, ascorbic acid and glutathione are decreased in both testes and sera of DEHP-treated animals [3, 4]. However, the details of mechanisms of the spermatogenic disturbance caused by DEHP remain unclear. There is speculation that the production of free-radicals might injure the seminiferous epithelium in DEHP-treated animals. Actually, Kasahara *et al.* [4] recently showed that oral administration of DEHP

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increased the generation of reactive oxygen species (ROS) (O_2- and H_2O_2) with concomitant decreases in glutathione and ascorbic acid in the rat testes. Nitric oxide (NO) is also a free radical, and is a potential biological mediator that functions at low concentration as a signal in many diverse physiological processes, but it might cause DNA damage and cell death at high concentration [5, 6]. However, so far there has been no report on nitrogen oxides (NOx) in DEHP-treated animals. Our previous study showed that feeding mice with diets containing 2% DEHP induced focal degeneration of the seminiferous epithelium from day 5 and depletion of almost all germ cells by day 15 [7]. The aim of the present study is to compare the NOx generation, the MEHP distribution and lipid peroxidation in the testis with those in the liver, kidney and pancreas in 2% DEHP-treated mice.

2 Materials and methods

2.1 Animals

Male Crlj:CD1(ICR) mice (6-week old) were purchased from Charles River (Kanagawa, Japan) and kept in the Laboratory Animal Center of Tokyo Medical University for 1 week. They were maintained at 22–24ºC and 50%–60% relative humidity with a 12 h : 12 h light : dark cycle. The approval of the Tokyo Medical University Animal Committee was obtained for the present study.

2.2 Phthalate

DEHP and MEHP (the most toxic metabolite of DEHP) were purchased from Tokyo Chemical Industries (Tokyo, Japan). The chemical purity of both DEHP and MEHP was found to be > 98% on gas-liquid chromatography. A normal CE-2 diet was purchased from Clea (Tokyo, Japan), and CE-2 diet containing 2% DEHP was prepared by Oriental Yeast Company (Chiba, Japan). We used a diet of 2% DEHP because our previous study showed that the diet containing 2%, instead of 1% DEHP, induced significant aspermatogenesis in rats when given for 2 weeks [8].

2.3 Experimental design

Seven-week-old male ICR mice were divided into control (n = 5) and DEHP-treated groups (n = 7). They were fed with normal diet and diet containing 2% DEHP for 10 days, respectively. Both the diets and tap water were freely available. Each mouse ate 4.50 ± 0.56 g and 4.50 ± 0.26 g (mean ± SE) diets in the control and the 2%-DEHP-treated group, respectively. They drank approximately 7 mL water a day. There were no significant differences in these volumes between the two groups. On days 0 and 10, the bodyweight of each mouse was determined. The dose of DEHP was 0.90 ± 0.52 mg/mouse/day. On day 10, all mice were deeply anesthetized with diethyl ether, and then blood was taken from the right atrium. Thereafter, the testis, liver, kidney and pancreas of each mouse were weighed. Organ weight/body weight (BW) × 100 is presented as the relative organ weight. The blood samples were allowed to clot and then centrifuged at 4 500 × g for 15 min at 4ºC. Each serum sample was kept at –80ºC until it was used.

2.4 Histochemical detection of lipid peroxidation

According to the method of Pompella *et al*. [9], lipid peroxidation was histochemically detected to examine the presence of free radicals-induced tissue injury. The testes, livers, kidneys and pancreata were removed from the control and DEHP-treated mice on day 10 under anesthesia with pentobarbital and then frozen at –80ºC. These frozen sections, 5 μm each, were fixed in 90% ethanol for 2 min, and then incubated at 37ºC for 5 min in 0.15 mol/L KCl and 0.05 mol/L Tris-maleate buffer, pH 7.4, containing an NADPH-Fe system (0.8 mmol/L nicotinamide adenine dinucleotide phophate [NADPH], 0.1 mmol/L $FeCl_3$ and 4.5 mmol/L Adenosine diphosphate [ADP]). The sections were incubated in 0.5 mol/L KCl and 0.05 mol/L Tris-maleate buffer containing 3 mol/L ethylenediaminetetracetic acid (EDTA). The sections were then stained for 2.5 h in the dark at room temperature with cold Schiff's reagent. After the reaction, the sections were washed with three changes of sulfide water (20 mmol/L $K_2S_2O_5$ and 0.05 mol/L NHCl) for 5 min and then counterstained with methyl green. The presence of lipid peroxide was detected as red-brown granules.

2.5 Biochemical examination of sera

Serum samples were analyzed for testosterone, total protein, alkaline phosphatase (ALP), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), creatinine (CRE), uric acid (UA), sodium (Na) and chlorine (Cl). The analyses, except that of testosterone, were carried out with a BMD/Hitachi 704/737 Chemistry Analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). The concentration of testosterone was determined by radioimmunoassay according to the method used by Verjans *et al.* [10].

2.6 Measurement of mineral components of antioxidant enzymes in the testes

Analytical grade chemicals and metal standard solutions were purchased from Wako Pure Chemical Industries (Osaka, Japan). Samples of the control and DEHP-treated testes were placed in polytetrafluoroethylene (PTFE) decontaminated decomposition vessels, and then 0.5 mL of an acid mixture, $HNO_3/HClO_4/H_2SO_4$ (50 : 50 : 1, v/v/v), was added. The vessels were kept on a heating plate at 140ºC for 4 h and then heated to 200ºC until almost dry. The residue was dissolved in 2 mL of 0.1 mol/L HCl and then properly diluted. The concentrations of Fe (a cofactor for catalase or an active center of Fe-superoxide dismutase [SOD]), Cu and Zn (active centers of Cu/Zn-SOD) were determined by "one-drop" flame atomic absorption spectrometry [11]. The analytical instrument used was a Seiko-SAS 7500 model with deuterium background correction (Seiko, Tokyo, Japan).

2.7 Measurement of MEHP concentrations in DEHP-treated mice

The MEHP levels in testes, livers, kidneys and pancreata of DEHP-treated mice were determined by high performance liquid chromatography (HPLC) with a TOSOH system (TOSOH, Tokyo, Japan). Samples of organs were homogenized in four volumes of 1 mol/L NaOH, and then 250 mg of each homogenate was mixed with 850 μL of acetonitrile. The mixtures were shaken vigorously and then kept in an ultrasonic water bath for 10 min, and then 10 μL of H_3PO_4 was added. After centrifugation for 10 min at 1 500 × *g*, 40 μL of each supernatant was injected into the HPLC system. Separation was carried out on a reversed-phase TSK gel ODS-80TM column (5 μm beads, 150.0 × 4.6 mm I.D.; TOSOH, Tokyo, Japan) at room temperature. The mobile phase comprised a mixture of acetonitrile and 0.1% H_3PO_4 (60 : 40, v/v). The flow rate was 1.0 mL/min. The ultraviolet absorbance of the effluent was monitored at 230 nm [12]. Quantification was carried out using matrix-matched standards prepared by the addition of MEHP standard solution to the reference organ homogenates. The recovery rates determined on analyses of the reference organ homogenates spiked with MEHP at the level of 100 μg/g were 92.7% for testis, 93.1% for liver, 87.6% for kidneys and 90.9% for pancreas. In control mice, no MEHP were detected in all four organs.

2.8 Measurement of NOx in DEHP-treated mice

Samples of testis, liver, kidney and pancreas tissues were homogenized in 10 volumes of 0.1 mol/L phosphate buffer (pH 7.4) containing 50% methanol. The homogenates were centrifuged 10 000 × *g* for 20 min at 4ºC, and then the supernatants were centrifuged again to remove the pellets. The final supernatants were assayed for NOx with an HPLC-UV system (ENO-10, NOD-10; EICOM, Kyoto, Japan) according to the method used by Lu *et al.* [13]. Samples were injected into the HPLC-UV system at 10-min intervals. Detection was carried out at 540 nm (absorption), and their concentrations were calculated from the area under the curve for $NaNO_2$ or $NaNO_3$ (Powerchrome; EICOM). The minimal detectable concentration for each NO metabolite was 1 pmol. NOx concentration of control mice was less than 10 pmol/10 μL in all four organs.

2.9 Statistical analysis

Statistical tests were conducted using Statveiw-J-4.5 (Abacus Concepts, Berkeley, CA, USA). Data were presented as means ± SE, and were analyzed by means of paired *t*-test or one-way ANOVA. Differences were considered significant if the $P < 0.05$.

3 Results

DEHP-treated mice had mild diarrhea for 10 days, and their bodyweights were significantly decreased (Table 1). The relative liver weights of DEHP-treated mice were significantly higher than those of control mice, showing the presence of hepatomegaly (Figure 1). The relative testis weights of DEHP-treated mice were not significantly changed from those of the controls, neither were the kidney or pancreas weights. However, histologically, it was noted that most germ cells had been deleted from the seminiferous epithelium with much fluid in the tubules of the all DEHP-treated mice (Figure 2). Histochemically, lipid peroxidation was

Table 1. Changes in body weight, data were expressed as mean ± SE. *P* values were obtained by paired *t*-test. $^bP<0.05$, $^cP<0.01$, compared with day 0.

Group	Body weight (g)	
	Day 0	Day 10
Controls (n = 5)	34.91 ± 0.66	37.33 ± 0.72^b
DEHP-treated mice (n = 7)	34.86 ± 0.37	28.26 ± 0.69^c

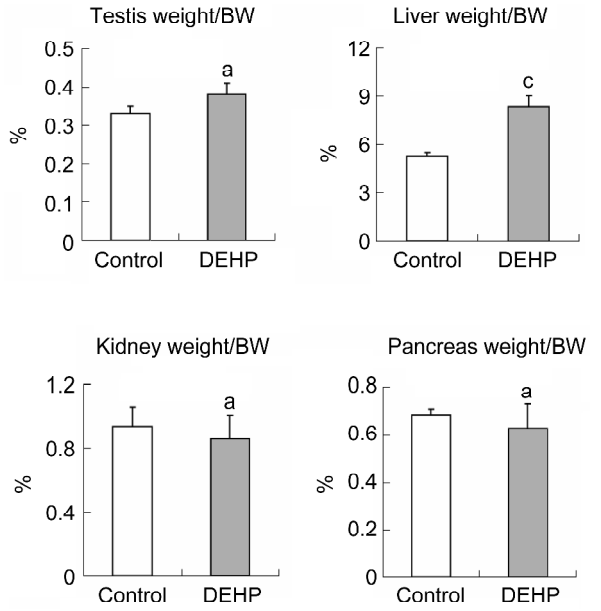

Figure 1. Changes in the relative weights of four organs in control (n = 5) and di-(2-ethylhexyl) phthalate (DEHP)-treated mice (n = 7) on day 10. The means ± SE are plotted. P-values were obtained by means of paired t-test. It was noted that testicular atrophy was not yet apparent in the DEHP-treated mice. BW, body weight. $^{a}P > 0.05$, $^{c}P < 0.01$, compaired with the corresponding controls.

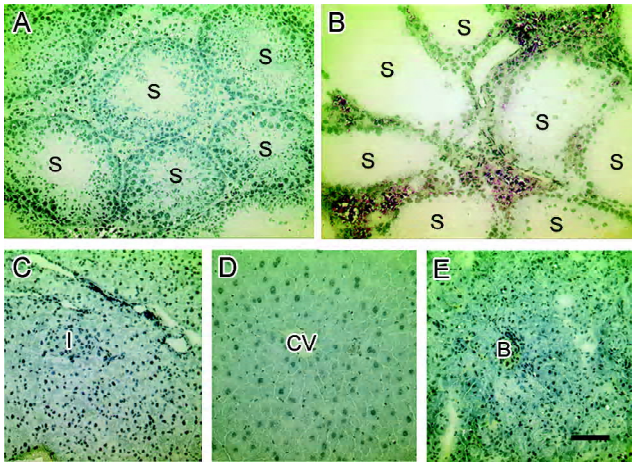

Figure 2. Histochemical detection of lipid peroxidation in a control mouse (A) and a di-(2-ethylhexyl) phthalate (DEHP)-treated mouse on day 10 (B)–(E). (A), (B): testis, (C): pancreas, (D): liver, (E): kidney. Red-brown granules were only found in the testicular tissue of the DEHP-treated mouse (B). It was noted that most germ cells had been deleted from the seminiferous epithelium with much fluid of the tubules. S, seminiferous tubules; I, islet of Langerhans; CV, central vein; B, Bowman's capsule. Bar = 100 μm.

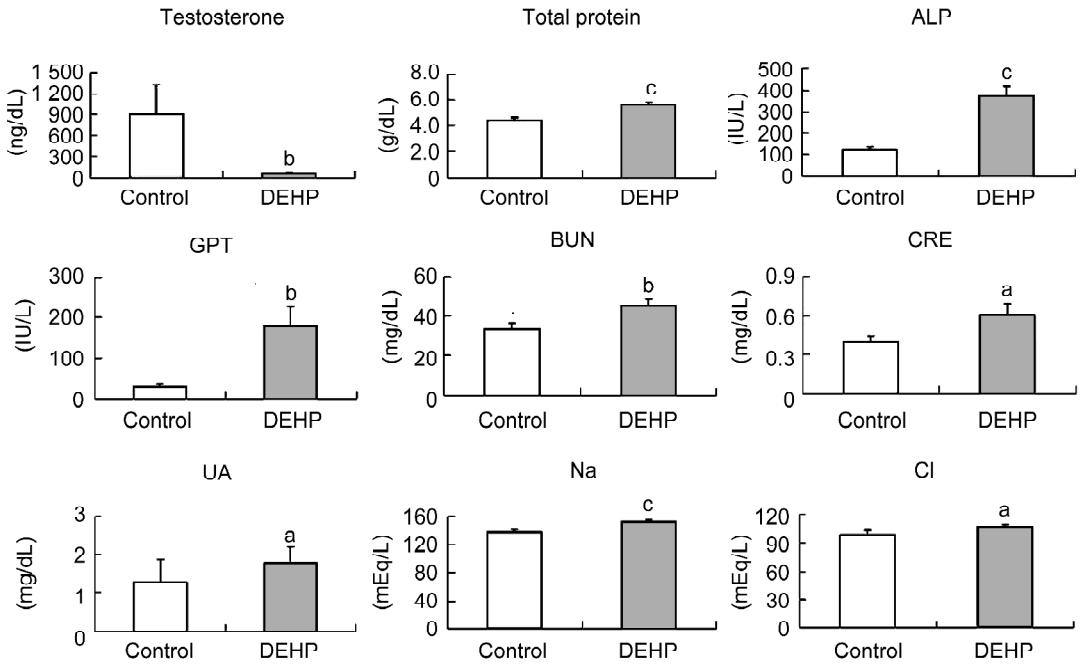

Figure 3. Serum analyses in control (n = 5) and di-(2-ethylhexyl) phthalate (DEHP)-treated mice (n = 7). The means ± SE are plotted. P values were obtained by means of paired t-test. A prominent decrease in testosterone (T) was noted in the DEHP-treated mice. ALP, alkaline phosphatase; GPT, glutamic pyruvic transaminase; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid. $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$, compaired with the corresponding controls.

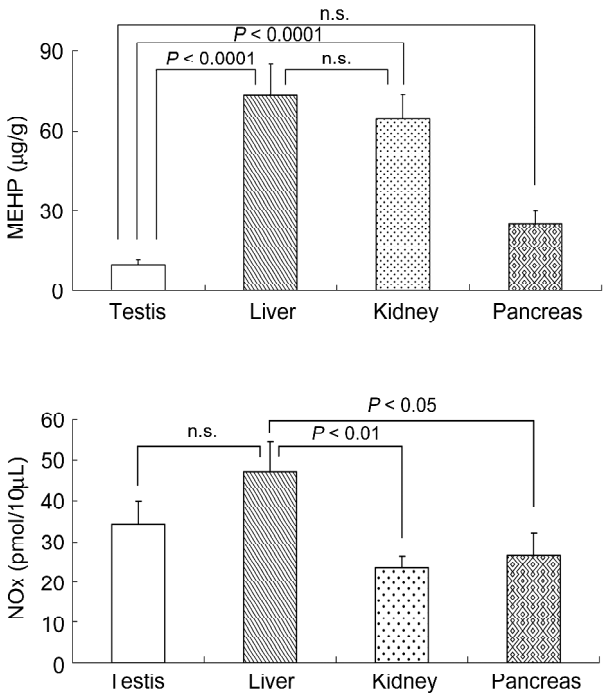

Figure 4. mono-(2-ethylhexyl) phthalate (MEHP) concentrations and NOx in the testes, livers, kidneys and pancreata of DEHP-treated mice (n = 7). The means ± SE are plotted. P-values were obtained by means of ANOVA. It was noted that little MEHP was present in the testes compared with liver and kidney, whereas testicular NOx was as high as hepatic NOx. In all four control mice, MEHP were not detected and NOx concentrations were less than 10 pmol/10 μL.

detected in the testis but not liver, kidneys or pancreas of all DEHP-treated mice (Figure 2).

Serum analyses showed a significant decrease in testosterone, indicating the presence of Leydig cell dysfunction. It was also found that total protein, BUN, Na, ALP and GPT in DEHP-treated mice had been increased, indicating the presence of dehydration and hepatic insufficiency (Figure 3). The BUN / CRE ratios in control and DEHP-treated mice were 83.6 ± 3.8 (mean ± SE, n = 5) and 89.0 ± 8.6 (mean ± SE, n = 7), respectively (P < 0.6). Therefore, renal insufficiency was not apparent in DEHP-treated mice.

As expected, MEHP was specifically accumulated in the livers of DEHP-treated mice (Figure 4). It was noted that the MEHP concentration in their kidneys was as high as that in their livers, despite no significant induction of renal failure. In sharp contrast, the MEHP concentration in their injured testes was found to be extremely low. The NOx concentration in livers was significantly higher than that in kidneys or pancreata in DEHP-treated mice (Figure 4). However, the testicular NOx concentration did not significantly differ from that in the liver, although the testes contained only a small amount of MEHP.

The testicular concentrations of Zn, Fe and Cu in DEHP-treated mice were 9.27 ± 0.73 μg/g, 15.47 ± 0.84 μg/g and 1.31 ± 0.12 μg/g, respectively. These were lower than those in control testes, but the differences between control and experimental groups were not significant (P > 0.05,

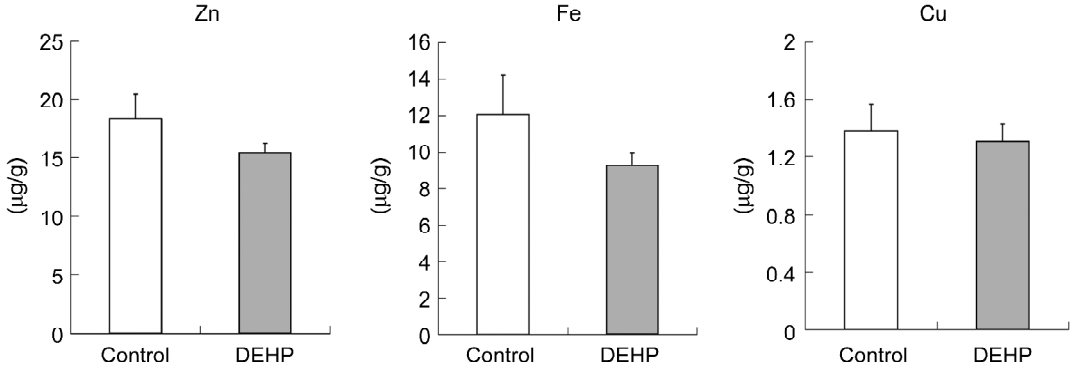

Figure 5. Mineral analyses in testes of control (n = 5) and DEHP-treated mice (n = 7). The means ± SE are plotted. P values were obtained by means of paired t-test. All three components were slightly, but not significantly, decreased in the DEHP-treated mice.

Figure 5).

4 Discussion

In the present study, we showed that the concentration of MEHP in the testes of DEHP-treated mice was significantly lower than that in the liver, however, the quantity of the testicular NOx was as high as hepatic NOx. Furthermore, free radical-induced lipid peroxidation could be histochemically detected only in the testes among the four examined organs. These results suggest that DEHP-induced aspermatogenesis might due to the high sensitivity of the testicular tissues to MEHP instead of specific accumulation or uptake of MEHP into the testis.

In the serum analyses, the concentration of testosterone was found to significantly decreased in DEHP-treated mice, indicating the presence of Leydig cell dysfunction. Furthermore, liver dysfunction with increased GPT and ALP concentrations was detected in the treated mice. These findings were consistent with results of previous studies [4]. It was also noted during the BUN/CRE analysis that no apparent renal dysfunction was induced in spite of the high MEHP concentration in the kidneys of DEHP-treated mice. In contrast, the amount of the testicular MEHP was found to be extremely low compared with that in other organs examined, although the MEHP-toxicity had been exerted on a number of cells including Sertoli cells, Leydig cells and germ cells [14]. This indicates that the testicular cells have a quite high sensitivity to MEHP.

To our knowledge, there has only been one previous study on free radical production in DEHP-treated animals, which showed the increased production of reactive oxygens (O_2- and H_2O_2) in the rat testes, but no other organs were examined [4]. In the present study, we compared NOx generation in testes with that in livers, kidneys and pancreata. The analysis showed that testicular NOx was as high as hepatic NOx. Therefore, NOx generation and the MEHP distribution were not correlated with each other among the four examined organs.

Additionally, we measured the testicular amounts of Fe, Cu and Zn, the important components of antioxidant enzymes such as Cu/Zn-SOD, Fe-SOD, Fe/Zn-SOD and catalase (Fe-catalase). These three elements were found to be slightly, but not significantly, decreased in DEHP-treated mice. Interestingly, a previous study showed that the activities of Cu/Zn-SOD and catalase were significantly increased in DEHP-treated testes [4]. It was also noted in another study that the Zn concentration was significantly decreased, whereas the activity of Zn-containing enzymes (alcohol dehydrogenase and aldolase) was increased in the testes of DEHP-treated rats [2]. Therefore, it is likely that Zn-related enzymes, including SOD, are activated by DEHP exposure to a certain degree in the testis, although the total amount of Zn in the organ is decreased. With regard to the non-enzymatic antioxidant system, it was shown that DEHP administration decreases glutathione, ascorbic acid (vitamin C) and vitamin E in rat testes [4, 15]. Actually, our previous study showed that the administration of vitamins C and E is effective for the prevention and treatment of DEHP-induced aspermatogenesis [7, 8]. It was also reported that antioxidant vitamins protect spermatogenesis from other toxic agents [16]. In contrast to antioxidant vitamins, the administration of Zn had failed to protect the testes from DEHP-induced injury [17]. This indicates that the testicular toxicity of DEHP is the result of the generation of free radicals rather than depletion of Zn in the testes. In regard to the effect of administration of testosterone on DEHP-treated animals, there are two studies: one showed the protection of spermatogenesis by testosterone [18], but the other displayed no significant effect on the spermatogenic disturbance [19].

Inoue *et al.* [20] reported that mild oxidative stress to the result of a nephrotoxic agent induced free radical generation accompanied by an increase in the hepatorenal glutathione levels in rats. It was also noted that DEHP administration significantly increased the glutathione concentration in both livers and kidneys but decreased it in the testis [4]. Therefore, it could be the high sensitivity of testicular tissues to DEHP that partially causes the lowered glutathione levels in testes. In contrast, the antioxidant hepatorenal glutathione activity might be relatively resistant to DEHP exposure. Experimental supplementation of glutathione is now in progress to determine whether it is useful for the prevention or curation of DEHP-induced aspermatogenesis.

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References

1 Gray TJ, Butterworth KR, Gaunt IF, Grasso GP, Gangolli SD. Short-term toxicity study of di-(2-ethylhexyl) phthalate in rats. Food Cosmet Toxicol 1977; 15: 389–99.
2 Oishi S. Testicular atrophy induced by di(2-ethyhexlyl) phthalate: changes in histology, cell specific enzyme activities and zinc concentrations in rat testis. Arch Toxicol 1986; 59: 290–5.
3 Oishi S, Hiraga K. Effect of phthalic acid esters on mouse testes. Toxicol Lett 1980; 5: 413–6.
4 Kasahara E, Sato EF, Miyoshi M, Konaka R, Hiramoto K, Sasaki J, *et al*. Role of oxidative stress in germ cell apoptosis induced by di (2-ethylhexyl) phthalate. Biochem J 2002; 365: 849–56.
5 Said TM, Aziz N, Sharma RK, Lewis-Jones I, Thomas AJ Jr, Agarwal A. Novel association between sperm deformity index and oxidative stress-induced DNA damage in infertile male patients. Asian J Androl 2005; 7: 121–6.
6 Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993; 329: 2002–12.
7 Ablake M, Itoh M, Terayama H, Hayashi S, Shoji S, Naito M, *et al.* Di-(2-ethylhexyl) phthalate induces severe aspermatogenesis in mice, however, subsequent antioxidant vitamins supplementation accelerates regeneration of the seminiferous epithelium. Int J Androl 2004; 27: 274–81.
8 Ishihara M, Itoh M, Miyamoto K, Suna S, Takeuchi Y, Takenaka I, *et al.* Spermatogenic disturbance induced by di-(2-ethylhexyl) phthalate is significantly prevented by treatment with antioxidant vitamins in the rat. Int J Androl 2000; 23: 85–94.
9 Pompella A, Maellaro E, Casini AF, Comporti M. Histochemical detection of lipid peroxidation in the liver of bromobenzene-poisoned mice. Am J Pathol 1987; 129: 295–301.
10 Verjans HL, Cooke BA, de Jong FH, de Jong CM, Vander der Molen HJ. Evaluation of a radioimmunoassay for testosterone estimation. J Steroid Biochem 1973; 4: 665–76.
11 Kojima I, Kato A, Lida C. Microwave digestion of biological samples with an acid mixture in a closed double PTFE vessel for metal determination by "one-drop" flame atomic absorption spectrometry. Anal Chim Acta 1992; 264: 101–6.
12 Cobellis L, Latini G, De Felice C, Razzi S, Paris I, Ruggieri F, *et al.* High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. Hum Reprod 2003; 18: 1512–5.
13 Lu X, Kassab GS. Nitric oxide is significantly reduced in *ex vivo* porcine arteries during reverse flow because of increased superoxide production. J Physiol 2004; 561: 575–82.
14 Ono H, Saito Y, Imai K, Kato M. Subcellular distribution of di-(2-ethylhexyl) phthalate in rat testis. J Toxicol Sci 2004; 29: 113–24.
15 Manojkumar V, Padmakumaran Nair KG, Santhosh A, Deepadevi KV, Arun P, Lakshmi LR, *et al.* Decrease in the concentration of vitamin E in blood and tissues caused by di-(2-ethylhexyl) phthalate, a commonly used plasticizer in blood storage bags and medical tubing. Vox Sang 1998; 75: 139–44.
16 Bustos-Obregon E, Yucra S, Gonzales GF. *Lepidium meyenii* (Maca) reduces spermatogenic damage induced by a single dose of malathion in mice. Asian J Androl 2005; 7: 71–6.
17 Oishi S, Hiraga K. Testicular atrophy induced by di-2-ethylhexyl phthalate: effect of zinc supplement. Toxicol Appl Pharmacol 1983; 70: 43–8.
18 Parmar D, Srivastava SP, Singh GB, Seth PK. Effect of testosterone on the testicular atrophy caused by di (2-ethylhexyl) phthalate (DEHP). Toxicol Lett 1987; 36: 297–308.
19 Oishi S. Effects of co-administration of di-(2-ethylhexyl) phthalate and testosterone on several parameters in the testis and pharmacokinetics of its mono-de-esterified metabolite. Arch Toxicol 1989; 63: 289–95.
20 Inoue M, Nobukuni Y, Ando Y, Hirota M, Hirata E, Morino Y. Interorgan metabolism of glutathione as the defence mechanism against oxidative stress. Dev Toxicol Environ Sci 1986; 14: 51–60.