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RESEARCH ARTICLE

Di(2-ethylhexyl)phthalate-induced renal oxidative stress in rats and protective effect of selenium

Pınar Erkekoglu[1], Belma Kocer Giray[1], Murat Kızılgün[2], Walid Rachidi[3], Isabelle Hininger-Favier[4], Anne-Marie Roussel[4], Alain Favier[3], and Filiz Hincal[1]

[1]Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara Turkey, [2]Department of Biochemistry, Diskapi Children's Health and Diseases, Hematology, Oncology Training and Research Hospital, Ankara, Turkey, [3]CEA Grenoble, INAC/SCIB/LAN,Grenoble Cedex, France, and [4]LBFA, Universite Joseph Fourier, Grenoble, France

Abstract

This study was designed to examine the oxidative stress potential of di(2-ethylhexyl)phthalate (DEHP) on rat kidney and to evaluate possible protective effect of selenium (Se) status. Se deficiency (SeD) was produced in 3-week old Sprague–Dawley rats by feeding them ≤0.05 Se mg/kg diet for 5 weeks; Se supplementation group (SeS) was on 1 mg Se/kg diet. DEHP treated groups received 1000 mg/kg dose by gavage during the last 10 days of the feeding period. Activities of antioxidant selenoenzymes [glutathione peroxidase 1 (GPx1), glutathione peroxidase 4 (GPx4), thioredoxin reductase (TrxR)], catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST); concentrations of total glutathione (GSH), thiols and thiobarbituric acid reactive substance (TBARS) levels were measured. DEHP treatment was found to induce oxidative stress in rat kidney, as evidenced by significant decreases in GPx1 (~20%) and SOD (~30%) activities and GSH levels (~20%), along with marked decrease in thiol content (~40%) and increase in TBARS (~30%) levels. The effects of DEHP was more pronounced in SeD rats, whereas Se supplementation was protective by providing substantial elevations of GPx1 and GPx4 activities and GSH levels. These findings emphasized the critical role of Se as an effective redox regulator and the importance of Se status in protecting renal tissue from the oxidant stressor activity of DEHP.

Keywords: Di(2-ethylhexyl)phthalate (DEHP), selenium deficiency (SeD), selenium supplementation (SeS), antioxidant enzymes, kidney, lipid peroxidation, oxidative stress

Introduction

Phthalates are phthalic acid derivative synthetic chemicals with a wide spectrum of industrial and commercial applications. Being used mostly as plasticizers to impart flexibility, transparency and durability to plastic materials, they are produced in high volume and therefore considered as the most abundant contaminants in the environment (Chou and Wright 2006; Latini 2005). Phthalates are also used to stabilize color and scent in a variety of personal care products, as solvents in insect repellant sprays, paint, and glue (Koniecki et al. 2011), as components of some drug coatings, and as solubilizers in a wide variety of plastics, including food wraps, water bottles, milk containers, and medical products (Latini 2005; Sathyanarayana 2008; Halden 2010). The widespread use of phthalates results in exposure of humans through a number of routes, including ingestion, dermal absorption, and inhalation (Latini 2005). In fact, phthalate metabolites were detected in virtually all human urine samples tested (Heudorf et al. 2007). Most studies have concluded that the diet is the major route of exposure (Fromme et al. 2007; National Toxicology Program, 2006). However, consumer products make a substantial contribution to overall exposure levels for phthalates *via* inhalation and dermal routes (Fromme et al. 2007; Duty et al. 2005). Some unique exposure pathways for phthalates include medical equipment such as iv bags and tubings

Address for Correspondence: Filiz Hincal, Hacettepe University, Faculty of Pharmacy, Department of Toxicology, 06100 Ankara Turkey. Fax: +903123092958. E-mail: fhincal@tr.net

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(U.S. Food and Drug Administration, 2001), and some pharmaceutical products (Fromme et al. 2007; Hauser et al. 2004; Heudorf et al. 2007; National Toxicology Program, 2006).

Phthalates are known as endocrine disrupting chemicals (EDC) (Lyche et al. 2009). EDCs can cause reproductive and developmental toxicity targeting fetal and pubertal testis, and possess hepatocarcinogenic activity in rodents (Rusyn et al. 2006). Most animal studies show these effects occurring at higher exposure levels than are observed in the general human population. However, certain medical procedures such as dialysis can result in much higher levels of exposure, therefore adverse effects from these exposures are a serious concern (European Union 2008; National Toxicology Program 2006). As the only phthalate derivative used in biomedical devices, di(2-ethylhexyl)phthalate (DEHP) is the major constituent of both blood bags and continuous ambulatory peritoneal dialysis (CAPD) bags (Heudorf et al. 2007; Blount et al. 2000). Patients receiving haemodialysis (HD), peritoneal dialysis or blood transfusion can, therefore, be exposed to high levels of DEHP as it can easily leach out of the plastic matrix (Mettang et al. 1999).

Multiple mechanisms of action were suggested for the hepatic and testicular effects of phthalates including peroxisome proliferator (PP)-activated or estrogen receptor-mediated mechanisms (Gazouli et al. 2002), and dysregulation of gene-expression pattern (Borch et al. 2006; Fan et al. 2010). An ever increasing possibility in this regard is the induction of oxidative stress, as recent *in vivo* and *in vitro* studies indicate the oxidant stress inducing potential of DEHP (Erkekoglu et al. 2010a; Erkekoglu et al. 2010b; Erkekoglu et al. 2011a; Erkekoglu et al. 2011b; Erkekoglu et al. 2011c). Whereas the data on renal effects of DEHP is very limited and the mechanism is not clear. Significantly higher incidence of focal cysts and decreased kidney function have been reported in DEHP-exposed rats (Crocker et al. 1988). The prevalence of acquired cystic kidney disease (ACKD) in HD patients lies between 35% and 79%. It was initially believed that ACKD occurs in HD patients exclusively; however, ACKD was later found equally often in association with CAPD (Ishikawa, 1991). On the other hand, HD and CAPD patients were reported to have high level of oxidative stress and low level of selenium (Se) (Fujishima et al. 2011; Tonelli et al. 2009, Zima et al. 1998).

Se is one of the key essential trace elements against oxidative stress (Behne et al. 1996). It is the important component of cellular antioxidant defense and involved in the modulation of intracellular redox equilibrium. Se-dependent glutathione peroxidases (GPxs) and thioredoxin reductases (TrxRs) protect the body from the endogenous products of oxidative cellular metabolism that have been implicated in DNA damage, mutagenesis, and carcinogenesis (Ganther 1999; Jablonska et al. 2009). Kidneys play an important role in the homeostasis of Se (Lockitch 1989; Nève 1991). When Se is consumed at rates close to the human nutritional requirement, its highest level is found in kidneys and then in the liver (Combs and Combs 1984). Se concentrations may be a good indicator of the progression of kidney diseases. Although whole blood and plasma Se levels did not change in early stages of chronic kidney disease patients, along with the progression of the kidney impairment Se concentration was found to be decreased (Tsukamoto et al. 1980; Lockitch 1989; Milly et al. 1992). This was particularly evident in the end-stage of the disease (Zachara et al., 2000). In patients on HD, plasma Se concentrations were also significantly lower than in healthy subjects (Kallistratos et al. 1985; Dworkin et al. 1987; Foote et al. 1987; Ceballos-Picot et al. 1996; Zachara et al. 2001).

Based on this background and taking into account the frequency of inadequate Se intakes, this study was designed to investigate the effects of DEHP on oxidant/antioxidant parameters in the rat kidney and to evaluate the possible changes by different Se status.

Materials and methods

Chemicals and reagents

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Colorimetric assay kits for TrxR and total glutathione (GSH) were also from Sigma-Aldrich. Sep-Pak C18 cartridge was obtained from Waters (Milford, MA, USA). All animal feed (A03/R03 base) were supplied by Scientific Animal Food and Engineering (SAFE) Laboratories (Augy, France).

Animals and treatment

Male Sprague Dawley (SD) rats, 3-weeks old, supplied from Hacettepe University Experimental Animals Laboratory, were used in the experiments. Animals were divided randomly in six groups of six of each, and each group was housed in plastic cages with stainless-steel grid tops. The cages were placed in a room with controlled temperature (23°C), humidity (50%) and a 12-hour light-dark cycle. Body weights (bw) were monitored weekly, including before the first dose of DEHP treatment. Feeding period was 5 weeks. The animals were treated humanely and with regard for alleviation of suffering, and the study was approved by Hacettepe University Ethical Committee.

Experimental groups

(1) Control group *(C)* was fed regular diet (0.15 mg/kg Se); *(2)* Selenium supplemented group *(SeS)* was fed Se supplemented diet (1 mg/kg Se); *(3)* Selenium deficient group *(SeD)* was fed Se deficient diet (≤0.05 mg/kg Se); *(4)* DEHP treated group *(DEHP)* was fed regular diet (0.15 mg/kg Se) and received 1000 mg/kg DEHP during the last 10 days by intragastric gavage (i.g.); *(5)* Selenium supplemented DEHP group *(DEHP/SeS)* was fed Se supplemented diet (1 mg/kg Se) and received 1000 mg/kg DEHP during the last 10 days by i.g.; *(6)* Selenium deficient DEHP group *(DEHP/SeD)* was fed Se deficient diet (≤0.05 mg/kg Se) and received 1000 mg/kg DEHP

during the last 10 days by i.g. Animals were allowed to access *ad libitum* feed and drinking water.

Twenty-four hours after the last dose of DEHP treatment, overnight fasted animals were weighed, and sacrificed by decapitation under thiopental anesthesia and kidneys were removed, frozen immediately in liquid nitrogen, divided into pieces and stored at –80°C until the preparation of tissue homogenates.

Preparation of kidney homogenates

Kidney homogenates were prepared in a volume of ice-cold buffer containing Tris (10 mM), diethylenetriamine pentaacetic acid (DTPA, 1 mM), and phenylmethanesulphonyl fluoride (PMSF, 1 mM; adjusted to pH 7.4) using a Teflon pestle homogenizer to obtain 10% (w/v) whole homogenate. After centrifugation at 1500*g*, 4°C, for 10 min, thiobarbituric acid reactive substance (TBARS) concentration was measured in the supernatant. The rest of the supernatants were recentrifugated at 9500*g*, 4°C for 20 min, and the antioxidant enzyme activities and thiol groups were determined in the supernatant. For the measurement of total GSH (oxidized and reduced GSH) levels, the whole homogenate was diluted (5:1) with metaphosphoric acid (6%), centrifuged at 1500*g*, 4°C, for 10 min, and the supernatant was used.

Determination of antioxidant enzyme activities

The activity of glutathione peroxidase (GPx1) was measured in a coupled reaction with glutathione reductase (GR) as described earlier (Flohé and Günzler 1984; Günzler et al. 1974) using an automatic analyzer (Hitachi 904, B Braun Science Tec, France).

The assay of glutathione peroxidase 4 (GPx4) activity was based on the same reaction as GPx1 determination, but freshly synthesized phosphatidylcholine hydroperoxide (PCOOH) was used as a substrate and the concomitant oxidation of NADPH was monitored spectrophotometrically at 340 nm as described by Maiorino et al. (1990). One unit of enzyme was defined as the amount of GPx4 that transformed 1 µmol of NADPH to NADP per min at 37°C. PCOOH was synthesized using the method of Maiorino et al. (1990) and Weitzel et al. (1990) with some modification. Briefly, 10 mg *L*-phosphatidylcholine (Type III from soybean) was dried under nitrogen stream, dispersed in 4 mL of 3% deoxycholate and diluted with 21 mL of 0.2 M sodium borate, pH 9.0. The reaction was started by the addition of 27.7 µL (5,000,000 U) of lipoxidase (EC 1.13.11.12, Type IV), bubbling with 99% O_2 using an oximeter (Hansatech, Norfolk, UK), and stirring on a heater bath (Julabo, Allentown, PA, USA) at 37°C. The reaction mixture was then passed through a Sep-Pak C18 cartridge which was previously washed with methanol and equilibrated with water. The cartridge was washed with 20 mL water, and PCOOH was then eluted with 2 mL of methanol, aliquoted and kept at –80°C where it was stable for 4 weeks.

TrxR activity was determined colorimetrically using the Thioredoxin Reductase Assay kit. As described previously (Arnér et al. 1999), the method was based on the reduction of 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) with NADPH into 5-thio-2-nitrobenzoic acid (TNB) the concentration of which was measured at 412 nm. One unit of TrxR activity was defined as the amount of enzyme that caused an increase absorbance of 1.0 per min and per mL at pH 7.0 at 25°C.

Catalase (CAT) activity was determined according to Aebi (1983). The enzymatic decomposition of H_2O_2 was followed directly at 240 nm. One unit of CAT activity was defined as the amount of enzyme required to decompose 1 µmol H_2O_2 in 1 min.

The total superoxide dismutase (total SOD) activity was determined by monitoring the auto-oxidation of pyrogallol at 420 nm (Marklund and Marklund 1974). One unit of total SOD activity was defined as the amount of enzyme required to inhibit the rate of pyrogallol auto-oxidation by 50%.

Glutathione S-transferase (GST) activity was determined using 1-chloro-2,4 dinitrobenzene (CDNB) as a substrate, monitoring the rate of production of 2,4-diphenyl glutathione at 340 nm and the enzyme activity was expressed in µmol/mg protein/min (Habig et al. 1974).

Determination of total glutathione levels

Kidney GSH content was assessed using a Glutathione Assay Kit based on a kinetic assay in which catalytic amounts of GSH caused a continuous reduction of DTNB to TNB at 412 nm (Akerboom and Sies 1981). Quantification was achieved by parallel measurements of a standard curve of known GSH concentrations, and results were expressed in nmol/mg protein.

Determination of lipid peroxidation

Lipid peroxidation (LP) in kidney tissue was quantified by measuring the concentration of TBARS by a spectrofluorometric assay as described by Richard et al. (1992) using a Hitachi F4500 spectrofluorometer (B Braun Science Tec, France), and the level of TBARS was expressed as µmol/g protein.

Determination of thiol groups

Thiol group concentrations in kidney tissue were determined as described by Faure and Lafond (1995). The calibration was obtained from a stock solution of 100 mM *N*-acetyl cysteine (NAC) in the range of 0.125–1 mM. Standards and samples were measured by automatic analyzer at 415 nm (Hitachi 904, B Braun Science Tec, France) in the presence of 0.05 mol/L phosphate buffer, 1 mmol/L EDTA, pH 8 and DTNB (2.5 mM).

Protein determination

Protein concentrations were determined by the standard method of Lowry et al. (1951) using an automatic analyzer (Hitachi 904, B Braun Science Tec, France).

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Statistical analysis

The results were expressed as mean ± standard error (SEM). The differences among the groups were evaluated with Kruskal-Wallis one-way analysis of variance, followed by Mann Whitney U test using a Statistical Package for Social Sciences Program (SPSS) version 17.0. p values <0.05 were considered as statistically significant.

Results

Absolute and relative kidney weights

As we previously reported (Erkekoglu et al. 2011b), all animals appeared to remain healthy throughout the experiments. Significant differences in the food intake were not observed between the groups. There was no significant alteration in body weights in any of the groups before the DEHP treatment started. After 10 days of DEHP exposure, the weight gain in all DEHP-exposed groups was found significantly lower ($p < 0.05$) than the control group, and Se supplementation was found to be partially protective. As shown in Table 1, at the termination of the experiments, both absolute and relative kidney weights in DEHP group were lower than those of control group (6% and 9%, respectively; $p < 0.05$). This effect was more evident in DEHP-exposed Se-deficient animals (7% and 16%, respectively; $p < 0.05$).

Antioxidant enzymes

Kidney selenoenzyme activities are illustrated in Figure 1. In comparison to control, kidney GPx1 activity decreased significantly (~20%, $p < 0.05$) by DEHP exposure; increased with Se supplementation (~10%, $p < 0.05$); decreased markedly in Se deficient animals (~80%, $p < 0.05$); and a much higher decrease was observed in DEHP-exposed Se deficient animals (~90%, $p < 0.05$). Se supplementation along with DEHP treatment provided an increase of ~60% in GPx1 activity when compared to DEHP group ($p < 0.05$) (Figure 1A).

Table 1. Effects of DEHP and selenium status on relative kidney weight.

Parameters	Mean kidney weight (g)	Relative kidney weight (g/100 g BW)
Control	0.82 ± 0.03[a]	0.44 ± 0.01[a]
SeS	0.86 ± 0.02[a]	0.43 ± 0.01[a]
SeD	0.82 ± 0.02[a]	0.44 ± 0.01[a]
DEHP	0.77 ± 0.01[b]	0.40 ± 0.01[b]
DEHP/SeS	0.80 ± 0.01[a]	0.44 ± 0.02[a]
DEHP/SeD	0.76 ± 0.02[b]	0.37 ± 0.03[b]

Experimental groups for 5 weeks were on: *(C)*regular diet (0.15 mg/kg Se); *(SeS)* Se supplemented diet (1 mg/kg Se); *(SeD)* Se deficient diet (≤0.05 mg/kg Se); *(DEHP)* regular diet (0.15 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; *(DEHPSeS)*Se supplemented diet (1 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; *(DEHPSeD)* Se deficient diet (≤0.05 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d.
Rows that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

Renal GPx4 activity did not change by DEHP exposure (Figure 1B), but in both SeS and DEHP/SeS groups, GPx4 activity increased (~25% and ~30% respectively; $p < 0.05$) compared to control, as well as compared to DEHP group. But there was no alteration in DEHP/SeD group.

TrxR activity did not change neither by DEHP exposure nor by Se supplementation; but decreased

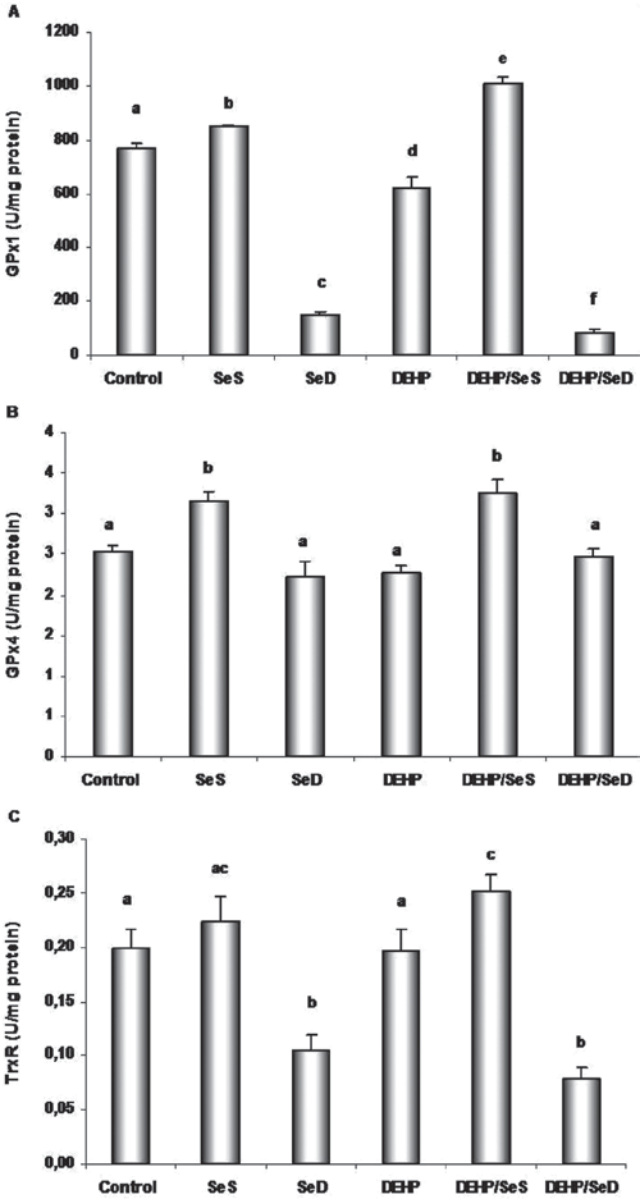

Figure 1. Effects of DEHP and/or selenium status on renal selenoenzyme activities in study groups. (A) Renal Glutathione Peroxidase 1 (GPx1) Activity (B) Renal Glutathione Peroxidase 4 (GPx4) Activity (C) Renal Thioredoxin Reductase (TrxR) Activity Experimental groups were (for 5 weeks) on: (C) regular diet (0.15 mg/kg Se); (SeS) Se supplemented diet (1 mg/kg Se); (SeD) Se deficient diet (≤0.05 mg/kg Se); (DEHP) regular diet (0.15 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHP/SeS) Se supplemented diet (1 mg/kg Se and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHP/SeD) Se deficient diet (≤0.05 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d. Bars that do not share same *letters* (*superscripts*) are *significantly different* from *each other* ($p < 0.05$).

markedly (~50%, $p < 0.05$) in SeD and DEHP/SeD groups (~60%, $p < 0.05$) compared to control animals (Figure 1C).

Other antioxidant enzyme activities in kidney are shown in Table 2. DEHP exposure caused a ~30% ($p < 0.05$) decrease in total SOD activity of kidney. But in our experimental conditions, Se nutrition had no effect in kidney SOD activity. CAT activity did not change in any of the experimental groups. Renal GST activity increased markedly in SeD group (~75%, $p < 0.05$) and in all DEHP-exposed groups (~65% in DEHP, ~50% in DEHP/SeS and ~115% in DEHP/SeD groups; $p < 0.05$) compared to control.

Total glutathione, thiol and lipid peroxidation levels

As shown in Table 3, in comparison with the control group, renal total GSH levels increased significantly (~30%, $p < 0.05$) in SeS group, and decreased in DEHP and DEHP/SeD groups (~20%, ~30%, respectively; $p < 0.05$). Whereas Se supplementation increased the GSH levels (~35%, $p < 0.05$) in DEHP-exposed rats compared to DEHP group, thus, maintained the levels as the control.

Table 2. Effects of DEHP and/or selenium status on renal antioxidant enzyme activities in the study groups.

Parameters	Total SOD (U/mg protein)	CAT (U/mg protein)	GST (μmol/mg protein/min)
Control	24.32 ± 1.90[a]	211.23 ± 2.75[a]	0.08 ± 0.01[a]
SeS	24.42 ± 0.80[a]	219.6 ± 11.46[a]	0.07 ± 0.01[a]
SeD	27.04± 0.78[a]	229.42 ± 9.77[a]	0.14 ± 0.01[b]
DEHP	17.50 ± 1.61[b]	212.96 ± 1.81[a]	0.13 ± 0.01[b]
DEHP/SeS	23.73 ± 2.04[a]	219.21 ± 5.20[a]	0.12 ± 0.01[b]
DEHP/SeD	29.96 ± 0.39[a]	222.19 ± 3.73[a]	0.17 ± 0.01[c]

Experimental groups were (for 5 weeks) on: (C) regular diet (0.15 mg/kg Se); (SeS) Se supplemented diet (1 mg/kg Se); (SeD) Se deficient diet (≤0.05 mg/kg Se); (DEHP) regular diet (0.15 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHP/SeS) Se supplemented diet (1 mg/kg Se and received 1000 mg/kg, i.g. DEHP for the last 10 d; *(DEHP/SeD)* Se deficient diet (≤0.05 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d.
Rows that do not share same *letters (superscripts)* are *significantly different* from *each other* ($p < 0.05$).

Table 3. Effects of DEHP and selenium status on renal total glutathione levels, lipid peroxidation and thiol groups.

Parameters	GSH (nmoles/mg protein)	TBARS (μmole/g protein)	SH (μmole/g protein)
Control	9.89 ± 0.65[a]	0.32 ± 0.02[a]	138.45 ± 4.56[a]
SeS	12.96 ± 0.69[b]	0.33 ± 0.01[a]	152.13 ± 6.19[a]
SeD	9.78 ± 0.62[a]	0.38 ± 0.01[bc]	89.38 ± 9.18[b]
DEHP	7.74 ± 0.45[c]	0.42 ± 0.01[c]	79.94 ± 3.23[b]
DEHP/SeS	10.63 ± 0.39[a]	0.36 ± 0.01[ab]	89.35 ± 15.30[b]
DEHP/SeD	7.09 ± 0.46[c]	0.51 ± 0.03[d]	57.35 ± 4.50[c]

Experimental groups were (for 5 weeks) on: (C) regular diet (0.15 mg/kg Se); (SeS) Se supplemented diet (1 mg/kg Se); (SeD) Se deficient diet (≤0.05 mg/kg Se); (DEHP) regular diet (0.15 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHP/SeS) Se supplemented diet (1 mg/kg Se and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHP/SeD) Se deficient diet (≤0.05 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d.
Bars that do not share same letters (superscripts) are significantly differentfromeach other ($p < 0.05$).

TBARS levels were found to be significantly higher in SeD (~20%), DEHP (~30%) and DEHP/SeD (~60%) groups than the control animals. Marked decreases in thiol groups were also found in Se deficiency (~35%, $p < 0.05$), and in all DEHP-exposed groups (~40% in DEHP, ~35% in DEHP/SeS and ~60% in DEHP/SeD, $p < 0.05$) compared to control (Table 3).

Discussion

There is a growing concern about human exposure to phthalates because they are not chemically bonded to plastic matrix and leach out from the material over time. Although phthalate esters have long been regarded as substances of low acute toxicity, they possess potential toxic effects which may be exhibited when exposed to high doses or repeated low doses. Phthalates, including DEHP, have been linked to liver and kidney damage, testicular toxicity and the under-development of reproductive organs in humans and animals (Rusyn et al. 2006; Erkekoglu et al. 2011b; Howdeshell et al. 2008; Miura et al. 2007). Hepatocarcinogenic effects of phthalates in rodents is suggested to be related with their PP activity, and ROS are suspected as one of the crucial factors in PP-induced carcinogenesis (Rusyn et al. 2006). On the other hand, several studies described oxidative stress as a major pathway in the reproductive toxicity of DEHP (Kasahara et al. 2002; Erkekoglu et al. 2010a; Erkekoglu et al. 2010b).

Se, with its several forms of cellular selenoproteins, is involved in fundamental biological processes ranging from cellular antioxidant defense to the protection and repair of DNA, and apoptosis (Fischer et al. 2006). Low dietary Se intake makes the organism prone to oxidative stress-related conditions, reduced fertility and immune functions, and increased risk of cancers (Ganther 1999). Human studies have indicated that Se supplementation in a population with low basal blood Se levels decreases the incidence of several types of cancers (Duffield-Lillico et al. 2002). Thus, data from different studies have stressed the role of Se as an effective cellular redox regulator and have been in accordance with previous reports that demonstrated the protective effect of antioxidants in oxidative stress related biological effects (Hanukoglu 2006).

Current study was therefore undertaken as part of a project investigating the effects of DEHP exposure in various organ systems of rats. We hereby present the effects of DEHP on the antioxidant–oxidant status of renal system and the protection potential of Se nutrition.

The effects of DEHP on kidney

The data produced in the current study have shown alterations in the cellular antioxidant system of kidney along with concominant increase in LP. These findings appeared to reflect that subacute exposure of DEHP (1000 mg/kg, 10 days) in prepubertal SD rats. DEHP-induced oxidative stress in kidney like in the testis, which is its main target organ (Erkekoglu et al. 2011a; Erkekoglu

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et al. 2011b). Thus, in our experimental conditions, DEHP caused an imbalance between oxidant and antioxidant status of the rat kidney. This was evident with the reduction of the activity of antioxidant enzymes such as GPx1, and SOD, that detoxify active forms of oxygen; elevation in LP levels; reduction of thiol content; loss of GSH and a compensatory induction of GST activity which catalysis the transfer of GSH to reduce oxidized compounds.

Earlier data have showed that kidneys do not demonstrate a consistent response to DEHP. The most frequently reported finding has been alterations in kidney weight, yet there was no consistency among them as increased, decreased (as it is the case in the present study) or unchanged organ weights have been reported with and without changes in histology and/or renal function parameters. These discrepancies seem to be related to the study designs, including the differences in the dose and duration of exposure applied; species, age and sex of the animals used; but at the same time represent the induction of different pattern of toxic effect during the different stages of development (Arcadi et al. 1998; Cimini et al. 1994; Grande et al. 2007).

DEHP, as a PP, was reported to cause both peroxisome proliferator activated receptor α (PPAR)-dependent and -independent renal and testicular toxicities (Ward et al. 1998; Peters et al. 1997). Oxidative stress by DEHP exposure in rat kidney was suggested by Dobashi et al. (1999). They associated the decrease observed in red blood cell GPx with the oxidative stress induced in the renal tubules. Renal oxidative stress was suggested to occur through the induction of cytochrome P-450 enzymes (Kertai et al. 2000).

Considerable imbalance between oxidants and antioxidants in patients with chronic renal insufficiency have been reported (Ceballos-Picot et al. 1996; Zachara et al. 2001). Evidences suggest that ROS are not merely the consequence of treatment or progress of the disease but, one of the causal agents, and oxidative stress can take place in patients with and without HD. Profound disturbance in antioxidant systems occurs early in the course of chronic renal failure, gradually increases with its progression and is further exacerbated by dialysis (Ceballos-Picot et al. 1996; Giray et al, 2003). On the other hand, the finding of polycystic kidney disease (PKD) at autopsy in patients who had undergone long-term HD for renal failure has been linked to the effect of DEHP exposure through the dialysis process (Crocker et al. 1988). In fact, leachability of DEHP from HD tubing have been demonstrated in details (Kambia et al. 2001), and it is well documented that HD triggers a massive generation of ROS and, therefore, acts as an enhancer of oxidative stress (Witko-Sarsat et al. 1998; Loughrey et al. 1994). Supporting data also come from *in vitro* and experimental studies while MEHP was shown to have a marked nephrotoxic effect in cultured kidney epithelial cells (Rothenbacher et al. 1998), chronic progressive nephropathy and renal tubule pigmentation was reported in rats with chronic DEHP exposure (David et al. 2000). Diminished creatinine clearance and focal inflammatory changes accompained by cystic dilation of the tubules of rats fed with low dose of DEHP (0.92 mg/kg/d) for 1 year was also reported (Crocker et al. 1988).

All these data support the findings of the current study which shows detailed evidences of renal oxidative stress induction by DEHP exposure in rats.

The effects of selenium on kidney

In our experimental conditions, neither body nor kidney weights were affected by Se nutrition and there was no difference in food intakes. These findings were in accordance with the study of Fujieda et al. (2007) where significant decreases in bw and organ weight were observed only 6 weeks after the dietary assignment. Kidneys play an important role in Se homeostasis and Se concentration in the rodent kidney is typically higher than any other tissue (Dworkin et al. 1987). In the present study, Se deficiency was evident with a significant decrease (~80%) of kidney Se concentration (0.83 ± 0.03 ng/g tissue in control group vs. 0.15 ± 0.01 ng/g tissue in SeD group), determined as previously described (Erkekoglu et al. 2011b) and with a profound reduction in the activities of two selenoenzymes: GPx1 (~80%) and TrxR (~50%). There were no changes in the specific activities of the other antioxidant enzymes, SOD and CAT, and in the activity of GPx4, but a marked increase in GST activity was observed. This latter finding was in agreement with earlier reports showing that dietary deficiency of Se results in an increased GST activity in a variety of rat tissues (Chang et al., 1990). Along with these alterations in antioxidant parameters of the renal cells, increased TBARS and decreased GSH and thiol levels were supportive evidences of renal oxidant stress induction in Se deficiency.

Se supplementation increased the tissue Se concentration by ~65% (1.37 ± 0.04 ng/g tissue); did not disturb the antioxidant/oxidant balance except causing a ~25% increase in GxP4 activity and ~30% elevation in total GSH content, thus showed an antioxidant status enhancing activity in the rat kidney.

The effects of selenium status on kidney in DEHP exposure

The role of antioxidants in reversing oxidative stress has been of long-standing interest to basic scientists and clinicians. Se was found to be protective, for instance, against cadmium, 7,12-dimethylbenz[a]anthracene and cisplatin induced renal toxicity (Messaoudi et al. 2010; Kocdor et al. 2005; Francescato et al. 2001). However, to our knowledge, studies on the effects of either a Se-deficient or supplemented diet in DEHP exposed rats' kidney have not been reported.

The data presented herein showed an augmenting effect of Se deficiency on the effects of DEHP exposure in renal tissue as demonstrated significant decreases of GPx1 and TrxR activities and GSH content in DEHP/SeD group compared to those of DEHP group. This

aggravating effect was further evident with high TBARS levels (~20%, $p < 0.05$) and marked decrease of (~30%, $p < 0.05$) thiol levels in DEHP/SeD group compared to DEHP animals, whereas Se supplementation was at least partially protective against DEHP-induced oxidative stress. This was evidenced particularly with elevated levels of renal GPx1 and SOD activities and GSH levels; enhanced GPx4 activity; and decreased level of LP as shown with low TBARS and high thiol concentrations in DEHP/SeS compared to DEHP. Thus, current results showed the influence of Se status on the renal oxidant stress inducing effects of DEHP in rats, as it was shown earlier in rat testis (Erkekoglu et al. 2011c) and emphasized once more the critical role of Se as a cellular redox status modulator.

In conclusion, the data presented herein demonstrate the renal oxidative stress inducing effect of DEHP in rats and the protection by Se. The augmenting effect of Se deficiency on the effects of DEHP exposure may also be important for human subjects with chronic kidney failure as they have lower blood Se levels at all stages of the disease compared to healthy subjects (Zachara et al. 2004). Their GPx1 activity was also reported to be significantly reduced and this was increased with the progress of the disease (Tsukamoto et al. 1980; Lockitch 1989; Milly et al. 1992). Those patients undergoing HD may particularly be vulnerable as they are exposed to DEHP through the dialysis bags and tubings. In such cases both their low Se levels and the oxidative stress potential of DEHP may contribute to the low levels of GPx1 and make the patient more prone to oxidative stress and its consequences. Our results highlight the importance of examining renal toxicity potential of other phthalate derivatives particularly diethylphthalate, and the protective effect of other antioxidants in phthalate-induced renal oxidative stress.

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Declaration of interest

The authors report no conflicts of interest.

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