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RESEARCH ARTICLE

Impact of selenium status on Aroclor 1254-induced DNA damage in sperm and different tissues of rats

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ABSTRACT

Aroclor 1254 is a commercial mixture of polychlorinated biphenyls (PCBs), which are widespread environmental pollutants. It is used as non-flammable heat transfer agent and plasticizer. Animal studies have reported that Aroclor 1254 exerted toxic effects in different organs and systems. Although the evidences are limited, it seems reasonable that Aroclor 1254 may have a potential for similar adverse effects in humans. Selenium (Se) is a trace element and an important component of cellular antioxidant defense. This study was designed to investigate the effects of different Se status on the genotoxicity of Aroclor 1254 in sperm and different organs of Sprague-Dawley rats using Comet assay. Se deficiency (SeD) was generated by feeding 3-week old Sprague-Dawley rats with <0.05 Se mg/kg diet for 5 weeks. Se supplementation groups (SeS) were fed with 1 mg Se/kg diet. Aroclor 1254-treated rats received 10 mg/kg dose by gavage during the last 15 d of feeding period. SeD increased DNA damage in all of the organs as well as in lymphocytes and sperm. Aroclor 1254 treatment caused pronounced changes in liver, kidney and brain cells along with marked increases in lymphocytes and sperm. Se supplementation provided full or partial protection decreases in Aroclor 1254-induced DNA damage in sperm and all of tissues. Se deficiency aggravated the toxicity by increasing DNA damage caused by Aroclor 1254. Further studies should be performed to clarify the mechanism(s) underlying the protective role of Se status against Aroclor 1254 genotoxicity.

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Aroclor 1254; Comet assay; DNA damage; genotoxicity; polychlorinated biphenyls (PCBs); selenium

Introduction

Humans are widely exposed to polychlorinated biphenyls (PCBs), which are abundant environmental chemicals. Though these chemicals were banned in 1979, they can still cause important environmental pollution and high human exposure due to their high persistence in the environment and their ability to bio-accumulate (Faniband et al. 2014; Vested et al. 2014; Lauby-Secretan et al. 2016). It has been reported that PCBs might induce formation of reactive oxygen species (ROS) and genotoxic effects *via* different pathways. They are known to produce mainly hepatic and reproductive toxicity (Oakley et al. 1996; Carpenter 1998; Krishnamoorthy et al. 2005; Aly and Domènech 2009; Vested et al. 2014). In addition, they are suggested to cause developmental, neurological and behavioral disturbances (Gladen et al. 1988; Brevini et al. 2005; Rogan and Gladen 1992). On the basis of sufficient evidence of carcinogenicity in humans and experimental animals, the International Agency for Research on Cancer (IARC) classified PCBs as 'carcinogenic to humans (Group 1)' (IARC 2016; Lauby-Secretan et al. 2016).

Aroclor 1254, an important PCB congener was commonly used in some countries as a non-flammable heat transfer agent in electric capacitors, power transformers, vacuum pumps and gas-transmission turbines (Shields 2006; Erickson and Kaley 2011). Aroclor 1254 has been shown to increase ROS production in various organs (i.e. liver, brain and spleen) and the toxicity of Aroclor 1254 may be attributable to free radical generation (Banudevi et al. 2006; Aly and Domènech 2009). Aroclor 1254 was shown to significantly reduce renal antioxidant enzyme activities, hepatic and renal glutathione (GSH) levels and increase lipid peroxidation, H_2O_2 and hydroxyl radical levels in rat liver, kidney and lung (Banudevi et al. 2006). Moreover, studies have also reported that Aroclor 1254 caused changes in antioxidant systems of the ventral prostate, seminal vesicle and testis in rats. These alterations, in turn, disrupted sperm parameters (Venkataraman et al. 2004a; Venkatarama et al. 2004b; Aly et al. 2009).

Oxidative DNA lesions, such as DNA base damages, can arise from exposure to Aroclor 1254 (Buha et al. 2015). In gonads of male mice, Aroclor 1254 was shown to induce oxidative DNA damage and inhibition of DNA repair gene expressions. The incidence of DNA strand breaks in sperm of mice treated with 4 mg/kg/d Aroclor 1254 for 5 weeks were found to be increased, while there was no significant difference in mice treated with 1 and 2 mg/kg/d Aroclor 1254, indicating the dose-dependent response (Attia et al. 2014).

Selenium (Se) is an essential element with great importance for human biology. Se is a 'cellular redox homeostasis regulator' that is involved in antioxidant defense. Se plays substantial roles in physiological processes (such as ROS elimination), due to its presence in glutathione peroxidases (GPxs) and thioredoxin reductases (TrxRs) (Zeng and Combs

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2008; Jackson and Combs 2008). Se also has anti-proliferative, anti-inflammatory and immunological effects (Huang et al. 2012; Mocchegiani et al. 2014). In humans, low dietary Se intake can cause many health problems related to oxidative stress, such as reproductive system disorders, cardiovascular diseases, decreased immunity and increased risk of cancer (Huang et al. 2012; Mocchegiani et al. 2014; Benstoem et al. 2015; Duntas and Benvenga 2015; Lance et al. 2017).

Although there is evidence that antioxidants can reduce the toxicity of PCBs (Krishnamoorthy et al. 2007; Attia et al. 2014), there is insufficient data on the modulatory effects of Se status on Aroclor 1254 toxicity. Taking into account all the available knowledge, this study was designed to investigate the effects of Se status on Aroclor 1254-induced genotoxicity in different organs and sperm, by using the alkaline single gel electrophoresis (comet assay).

Material and methods

Chemicals

Aroclor 1254, Se, Hanks' Balanced Salt solution (HBSS), normal melting point agarose (NMA), low melting point agarose (LMA), sodium chloride (NaCl), sodium hydroxide (NaOH), dimethyl sulfoxide (DMSO), ethidium bromide (EtBr), Triton X-100, phosphate buffered saline (PBS) tablets, ethylenediamine tetra acetic acid disodium salt dihydrate (EDTA-Na_2), N-lauroyl sarcosinate and Tris were purchased from Sigma–Aldrich (Saint Louis, MO). All animal feed (A03/R03 base) were supplied by Scientific Animal Food and Engineering (SAFE) Laboratories (Augy, France).

Animals

Male Sprague-Dawley rats (3 weeks old, 50–70 g) were obtained from Hacettepe University Experimental Animal Laboratory. The animals were divided randomly and each group was housed in plastic cages with stainless-steel grid tops. The cages were maintained in a room with controlled temperature (23 °C), humidity (50%) and a 12-h light-dark cycle. Body weights (b.w.) were monitored daily. Animals were fed *ad libitum*, handled humanely and the study was approved by Hacettepe University Animal Ethical Committee. Animal feed supplied from SAFE laboratories contained sodium selenite as a source of selenium.

Experimental design

The study consisted of six groups of six animals each:

1. Control Group (C) received 0.15 mg/kg/d Se for 5 weeks and 1 ml of corn oil by intragastric gavage (i.g.) for the last 15 d of feeding period.
2. Se supplemented group (SeS) received 1 mg/kg/d Se for 5 weeks and 1 ml of corn oil by i.g. for the last 15 d of feeding period.
3. Se deficient group (SeD) received <0.05 mg/kg/d Se for 5 weeks and 1 ml of corn oil by i.g. for the last 15 d of feeding period.
4. Aroclor 1254 treated group (A1254) received 0.15 mg/kg/d Se for 5 weeks and 10 mg/kg/d Aroclor 1254 in 1 ml of corn oil by i.g. for the last 15 d of feeding period.
5. Aroclor 1254 treated and Se supplemented group (A1254 + SeS) received 1 mg/kg/d Se for 5 weeks and 10 mg/kg/d Aroclor 1254 in 1 ml of corn oil by i.g. for the last 15 d of feeding period.
6. Aroclor 1254 treated and Se deficient group (A1254 + SeD) received <0.05 mg/kg/d Se for 5 weeks and 10 mg/kg/d Aroclor 1254 in 1 ml of corn oil by i.g. for the last 15 d of feeding period.

The dose of Aroclor 1254 (10 mg/kg/d) was about 1% of lethal dose 50 (LD_{50}; LD_{50} of Aroclor 1254 in rats by oral route was found to be 1010 mg/kg) (ATSDR 2000). The doses of Se (1 mg/kg/d for Se supplementation and <0.05 mg/kg/d for Se deficiency) were selected on the basis of studies performed by Erkekoglu, Rachidi, Yuzugullu, et al. (2010), Erkekoglu et al. (2012), Erkekoglu, Giray, et al. (2014) and Panev et al. (2013). As also indicated by Sundae (2003), selenium supplementation at 0.05 mg/kg by diet only provides 50% of GPx1 activity whereas at 0.15 mg/kg, GPx activity reaches to plateau. Over 0.15 μg/g by diet, selenium supplementation provides supra-physiological selenium concentrations; however, due to our observations, it is not yet toxic and provides protection against oxidative stress (Erkekoglu, Rachidi, De Rosa, et al. 2010; Erkekoglu et al. 2012; Erkekoglu, Giray, et al. 2014).

Throughout the experiments, all animals appeared to remain healthy. No significant difference was observed in food intake between the groups. Twenty–four hours after the last treatments, animals were weighed, and sacrificed under thiopental (100 mg/kg) anesthesia. Animals weighed 150–200 g at the day of decapitation.

Venous blood samples were taken in heparinized tubes. For the sperm DNA damage, epididymal sperm were used. Sperm were obtained from the *cauda* of epididymis. Epididymis, liver, kidney and brain tissues were carefully dissected from their attachments, totally excised and then placed in a Petri-plate containing 2 ml of HBSS medium. The heparinized blood, sperm and organ samples were kept in the dark at 4 °C and processed within 6 h.

Detection of DNA damage using singe cell gel electrophoresis (comet assay)

The comet assay was performed to assess DNA damage. The basic alkaline technique of Singh et al. (1988) was used for the detection of DNA damage in the lymphocytes, hepatic and renal cells with minor modifications as described in detailed by Collins et al. (1993) and Speit and Hartmann (2006). The neutral comet assay, applied by Haines et al. (2002) and further described by Trivedi et al. (2010), was performed for the detection of DNA damage in the sperm.

Briefly, lymphocytes from whole heparinized blood were separated by Ficoll-Hypaque density gradient and centrifugation and later washed with PBS (Bøyum 1976). A small piece of liver, kidney or brain tissue was placed in cold HBSS (1 ml, with 20 mmol/l EDTA/10% DMSO) and was minced into fine

pieces. The tissue suspensions were pressed through 200-gauge sterile stainless steel mesh to separate the tissue fragments from the larger pieces. After they were settled for 15 min, the supernatants were used. The *cauda* of epididymis was cut into small portions in order to allow the sperm to swim out. This solution was centrifuged at 1000 rpm for 5 min and supernatant (1 ml) was used for the experiments.

The concentrations of the lymphocytes, the liver, renal and brain cells and sperm were adjusted to $\sim 2 \times 10^5$ cells/ml; suspended in 5% LMA and later were embedded on slides precoated with a layer of 1% NMA. Slides were allowed to solidify on ice for 5 min. Cover slips were then removed. All slides were immersed in cold lysing solution (pH 10), for a minimum of 1 h at 4 °C. For sperm samples, the slides were then incubated for additional 30 min at 4 °C with dithiothreitol followed by 90 min incubation at 20 °C with lithium diiodosalicylate (Donnelly et al. 2001; Robbins et al. 2010).

The slides, containing lymphocytes, the liver, renal and brain cells, were removed from the lysing solution, drained and then placed in a horizontal gel electrophoresis with freshly prepared alkaline electrophoresis solution (pH 13.0) while freshly prepared neutral electrophoresis solution (pH 9.0) was used for sperm to allow unwinding of the DNA and expression of DNA damage at 4 °C for 20 min at 24 V/300 mA. The slides were neutralized at room temperature by washing three times in neutralization buffer (pH 7.5) for 5 min and later the slides were incubated in graded alcohols. The dried slides were stained with EtBr (20 μg/ml), covered with a cover-glass prior to analysis with a fluorescence microscope (Leica DM1000, Wetzlar, Germany), connected to a charge-coupled device camera and a personal computer-based analysis system Comet Analysis Software version 3.0 (Kinetic Imaging Ltd., Liverpool, United Kingdom) to determine the extent of DNA damage (40X for the lymphocytes, hepatic, renal and brain cells and 63X for sperm). For each condition, 100 randomly selected comets from each of two replicate slides were scored (without the knowledge of the group codes). DNA damage parameters were expressed as DNA tail intensity, DNA tail moment (the product of the tail length and % DNA) and DNA tail migration.

Statistical analysis

Statistical analysis was performed by the computer program SPSS Software version 17.0 (SPSS Inc., Chicago, IL). The normality of the distributions was checked with Shapiro–Wilk test. Differences between the means were compared by the one way variance analysis (ANOVA) test and post-hoc analysis of differences between the groups was performed by least significant difference (LSD) test. $p < 0.05$ was considered as statistically significant.

Results

Comet pictures for lymphocytes, liver, kidney and brain cells and sperm of the study groups are given in Figure 1.

Lymphocytes

In the lymphocytes of SeS group, there were no significant changes in all DNA damage parameters (tail intensity, tail moment and tail migration) vs. control group ($p > 0.05$).

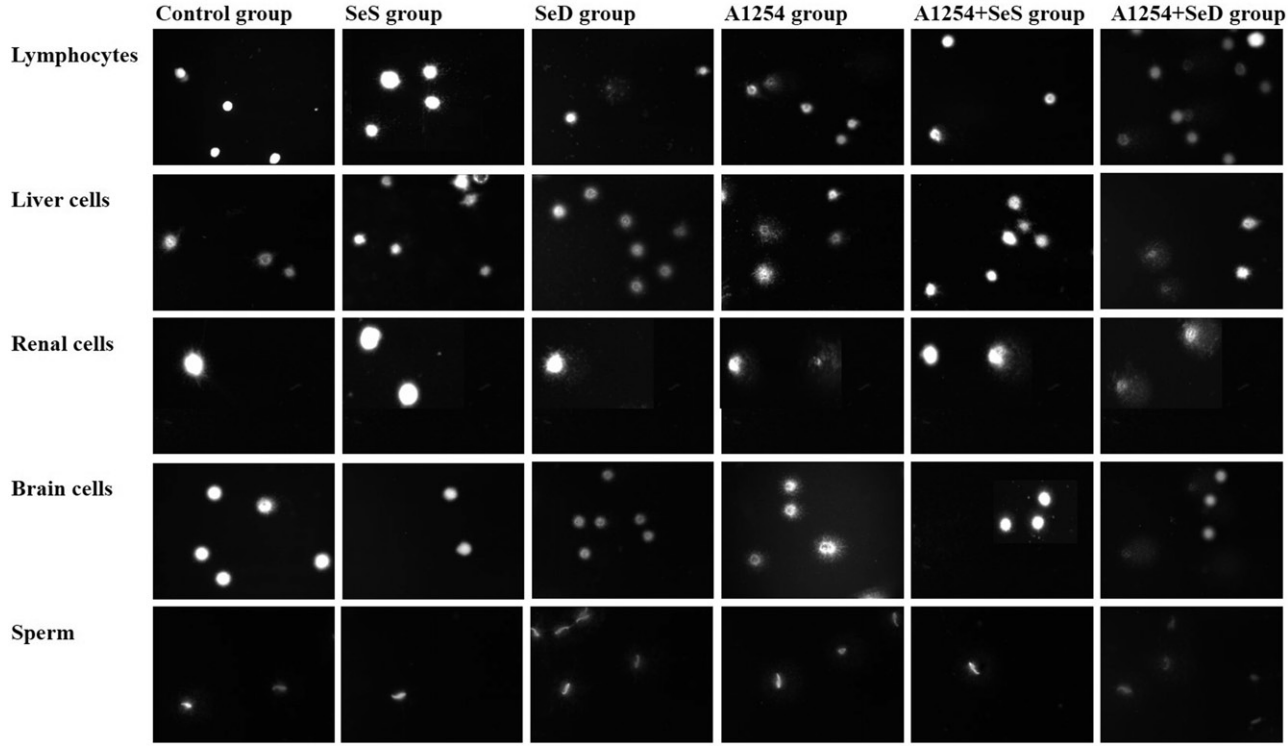

Figure 1. Comet pictures for lymphocytes, liver, kidney and brain cells and sperm of the study groups. SeS: selenium supplemented group; SeD: selenium deficient group; A1254: Aroclor 1254 treated group; A1254 + SeS: Aroclor 1254 treated and Se supplemented group; A1254 + SeD: Aroclor 1254 treated and Se deficient group.

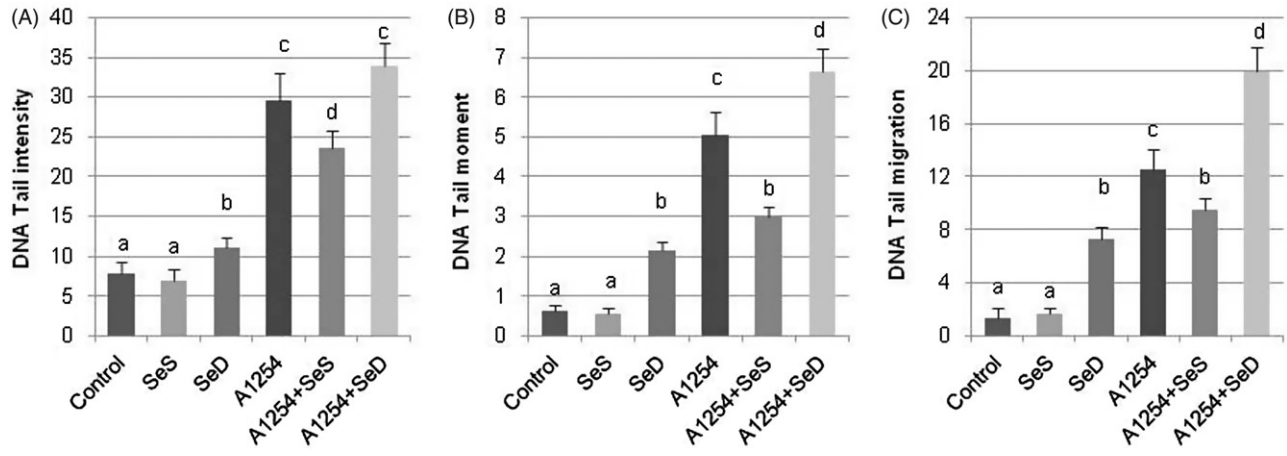

Figure 2. DNA damage in the peripheral lymphocytes of the study groups. DNA damage were expressed as (A) DNA tail intensity; (B) DNA tail moment; (C) DNA tail migration. SeS: selenium supplemented group; SeD: selenium deficient group; A1254: Aroclor 1254 treated group; A1254 + SeS: Aroclor 1254 treated and Se supplemented group; A1254 + SeD: Aroclor 1254 treated and Se deficient group. The values are given as mean ± SD for six rats in each group. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

Se deficiency caused marked increases in DNA damage parameters (42, 245, 467% in tail intensity, tail moment and tail migration, respectively, vs. control) when compared to control group ($p < 0.05$). Aroclor 1254 treatment also caused marked increases in DNA damage parameters (278, 712, 871% in tail intensity, tail moment and tail migration, respectively, vs. control; $p < 0.05$ for all) (Figure 1). Se supplementation of Aroclor 1254-treated rats supplied significant decreases in DNA damage parameters (27, 47 and 27% in the tail intensity, tail moment and tail migration, respectively) when compared to A1254 group. However, all DNA damage parameters were still higher than the control levels ($p < 0.05$). In A1254 + SeD group, DNA damage parameters, except tail intensity (20%, $p > 0.05$) increased significantly when compared to A1254-treated group (36 and 66% in tail moment, and tail migration, respectively; $p < 0.05$, both) (Figure 2).

Liver

In the hepatic cells of SeS group, there were no significant changes in DNA tail intensity and DNA tail migration vs. control group ($p > 0.05$). In SeS group, DNA tail moment decreased significantly vs. control (28%, $p < 0.05$). Se deficiency caused marked increases in DNA tail migration (84%) vs. control group ($p < 0.05$). However, there were no significant changes in DNA tail intensity and DNA tail moment vs. control group ($p > 0.05$). A1254 treatment also caused marked increases DNA damage parameters (67, 114 and 384% in tail intensity, tail moment and tail migration, respectively) vs. control. Se supplementation of Aroclor 1254-treated rats provided marked decreases in DNA damage parameters (31, 53 and 73% in the tail intensity, tail moment and tail migration, respectively) when compared to A1254 group DNA damage parameters, except DNA tail migration, were at control levels. In A1254 + SeD group, DNA tail intensity (9%) increased significantly vs. A1254-treated group ($p < 0.05$). However, in A1254 + SeD group, there were no significant changes in DNA tail moment and DNA tail migration vs. A1254-treated group (5 and 11%, respectively; $p > 0.05$) (Figure 3).

Kidney

In the renal cells of SeS group, there were no marked alterations in DNA tail intensity and DNA tail migration vs. control group; however, DNA tail moment decreased markedly (28%, $p < 0.05$ vs. control). Se deficiency caused significant increases in DNA tail migration (93%) when compared to control ($p < 0.05$). However, there were no significant changes in DNA tail intensity and DNA tail moment vs. control group ($p > 0.05$). Aroclor 1254 treatment also caused marked increases in DNA damage parameters (69, 117 and 389% in tail intensity, tail moment and tail migration, respectively) compared to control ($p < 0.05$) (Figure 1). Se supplementation provided marked decreases in DNA damage parameters (32, 53 and 73% in the tail intensity, tail moment and tail migration, respectively) when compared to A1254 group ($p < 0.05$) and all DNA damage parameters were at control levels. In A1254 + SeD group, DNA damage parameters (19 and 14% in tail intensity and tail moment, respectively) increased significantly vs. A1254-treated rats ($p < 0.05$) (Figure 4).

Brain

In the brain cells of SeS group, there were significant decreases in DNA tail moment (36%) and DNA migration (27%) vs. control group ($p < 0.05$, both). Se deficiency caused marked increases in DNA damage parameters (19, 206 and 585% in tail intensity, tail moment and tail migration, respectively) compared to control ($p < 0.05$). Aroclor 1254 treatment also caused significant increases in DNA damage parameters (93, 718 and 942% in tail intensity, tail moment and tail migration, respectively) vs. control ($p < 0.05$). Se supplementation provided marked decreases in DNA damage parameters (15, 56 and 18% in the tail intensity, tail moment and tail migration, respectively) when compared to A1254 group. In A1254 + SeD group, all DNA damage parameters increased significantly vs. control ($p < 0.05$). However, all DNA damage parameters were not still at control levels. Besides, DNA damage parameters (95, 192 and 127% in tail intensity,

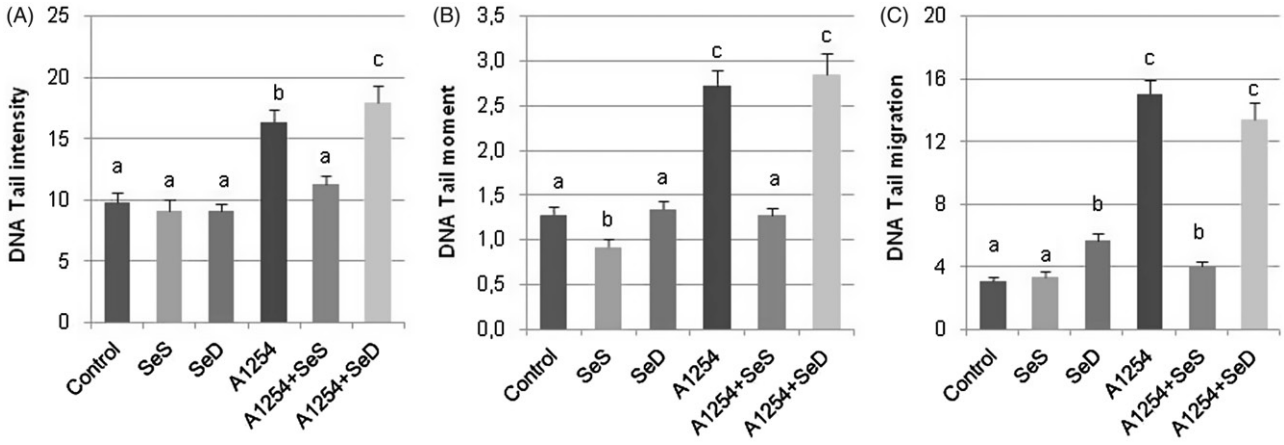

Figure 3. DNA damage in the liver cells of the study groups. DNA damage were expressed as (A) DNA tail intensity; (B) DNA tail moment; (C) DNA tail migration. SeS: selenium supplemented group; SeD: selenium deficient group; A1254: Aroclor 1254 treated group; A1254 + SeS: Aroclor 1254 treated and Se supplemented group; A1254 + SeD: Aroclor 1254 treated and Se deficient group. The values are given as mean ± SD for six rats in each group. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

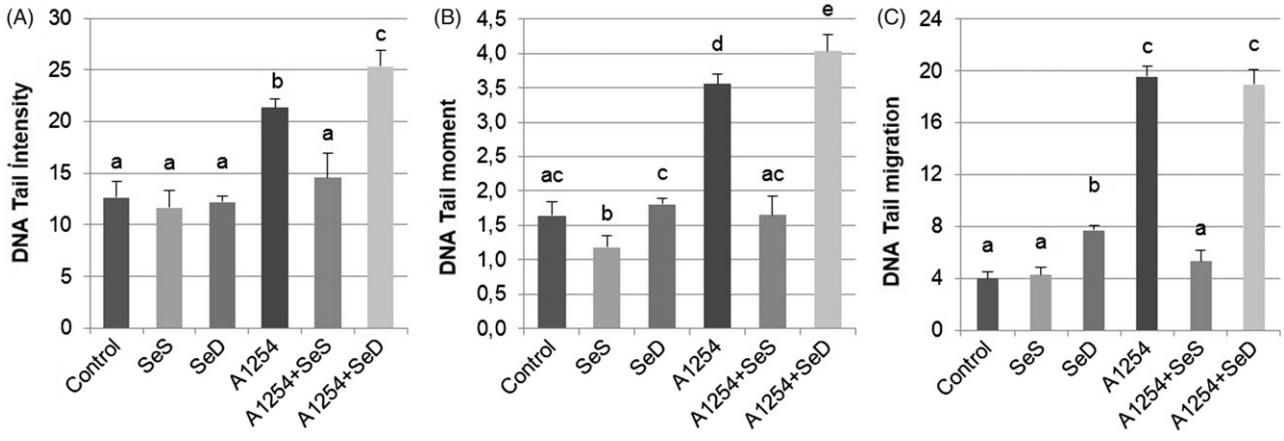

Figure 4. DNA damage in the renal cells of the study groups. DNA damage were expressed as (A) DNA tail intensity; (B) DNA tail moment; (C) DNA tail migration. SeS: selenium supplemented group; SeD: selenium deficient group; A1254: Aroclor 1254 treated group; A1254 + SeS: Aroclor 1254 treated and Se supplemented group; A1254 + SeD: Aroclor 1254 treated and Se deficient group. The values are given as mean ± SD for six rats in each group. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

tail moment and tail migration, respectively) increased significantly in A1254 + SeD group vs. A1254-treated rats (Figure 5).

Sperm

In the sperm of SeS group, there were no significant changes in all DNA damage parameters vs. control group ($p > 0.05$). Se deficiency caused marked increases in DNA damage parameters (107, 37 and 49% in tail intensity, tail moment and tail migration, respectively) compared to control ($p < 0.05$). Aroclor 1254 treatment also caused marked increases in DNA damage parameters (149, 97 and 243% in tail intensity, tail moment and tail migration, respectively) vs. control ($p < 0.05$) (Figure 1). Se supplementation provided marked decreases in DNA damage parameters (55, 52 and 51% in the tail intensity, tail moment and tail migration, respectively) when compared to A1254 group ($p < 0.05$, all). In A1254 + SeD group, all DNA damage parameters increased significantly vs. control ($p < 0.05$) and DNA damage parameters (21 and 36% in tail moment and tail migration, respectively) increased significantly when compared to A1254-treated rats (Figure 6).

Discussion

Polychlorinated biphenyls consist of a family of 209 different congeners. These congeners were commercially produced as mixtures before 1980 s. They were abundantly used in industrial settings as they are stable under a wide range of chemical, thermal and electrical conditions (Safe 1994). However, due to their stability, they are very persist in the environment, despite their ban by the United States Congress in 1979 and by the Stockholm Convention on Persistent Organic Pollutants in 2001 (Porta and Zumeta 2002). Because they are lipophilic in nature, PCBs can bio-accumulate and later can bio-magnify in the food chain (Malisch and Kotz 2014).

Selenium is an essential trace element and the key component of the antioxidant defense due to its presence in GPxs and TrxRs (Stadtman 2000). These enzymes are substantial antioxidants that can reduce the intracellular levels

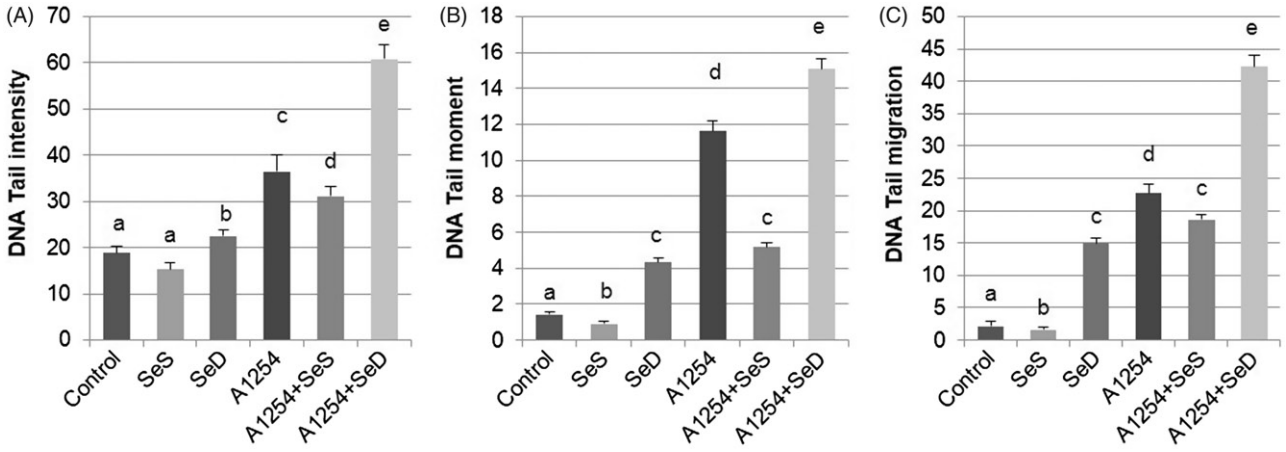

Figure 5. DNA damage in the brain cells of the study groups. DNA damage were expressed as (A) DNA tail intensity; (B) DNA tail moment; (C) DNA tail migration. SeS: selenium supplemented group; SeD: selenium deficient group; A1254: Aroclor 1254 treated group; A1254 + SeS: Aroclor 1254 treated and Se supplemented group; A1254 + SeD: Aroclor 1254 treated and Se deficient group. The values are given as mean ± SD for six rats in each group. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

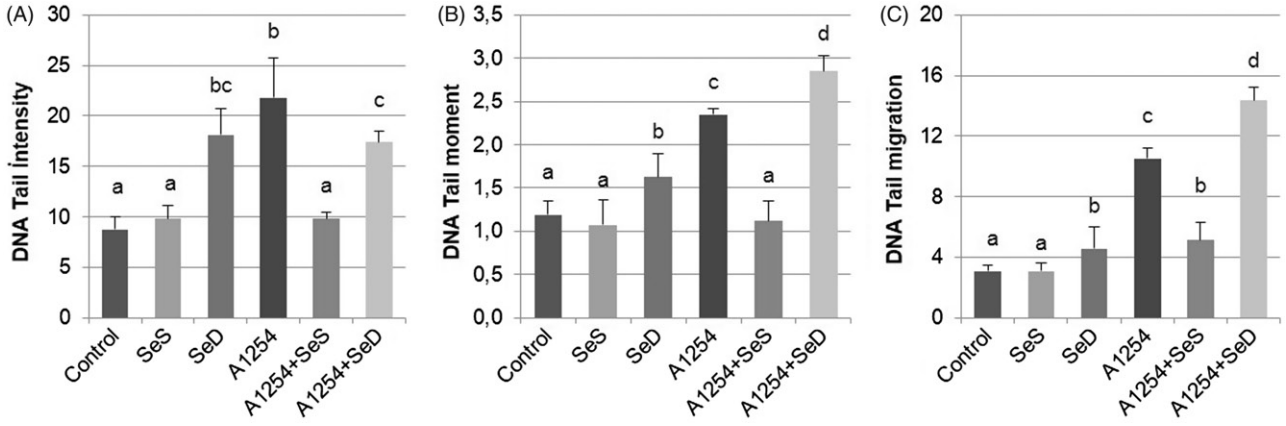

Figure 6. DNA damage in the sperm of the study groups. DNA damage were expressed as (A) DNA tail intensity; (B) DNA tail moment; (C) DNA tail migration. SeS: selenium supplemented group; SeD: selenium deficient group; A1254, Aroclor 1254: treated group; A1254 + SeS: Aroclor 1254 treated and Se supplemented group; A1254 + SeD: Aroclor 1254 treated and Se deficient group. The values are given as mean ± SD for six rats in each group. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

of ROS. Se is found to be protective against a variety of toxic substances (e.g. phthalates, alkylanilines and PCBs) *in vivo* (Erkekoglu, Rachidi, Yuzugullu, et al. 2010; Erkekoğlu, Rachidi, De Rosa, et al. 2010; Erkekoglu, Chao, et al. 2014). Even modest deficiency in Se may increase the risk of diseases (McCann and Ames 2011). Se deficiency can also aggravate the oxidative stress caused by different environmental chemicals, including pesticides (Giray and Hincal 2011). Moreover, supplemental Se was shown to reduce oxidative stress in animals (Ozkol et al. 2017) and in humans (Bardia et al. 2008). Se status was suggested to be important in different types of cancers, including cancers of prostate, liver, kidney and brain (Rooprai et al. 2007; Hughes et al. 2016; Chan et al. 2016; Golabek et al. 2016; Wrobel et al. 2016; Ma et al. 2017). Se supplementation is suggested to be promising agent against cancer in animals (Wrobel et al. 2013; Nakken et al. 2016) and humans; however, high dietary Se intake (beyond the therapeutic range) can also be toxic (Oldfield 1987; Stranges et al. 2007; Bardia et al. 2008; Stemm et al. 2008).

PCBs exert a wide variety of toxicity mechanisms. Their chlorination pattern is effective in their metabolic activation and toxicity. Exposure to PCBs can induce mixed function oxidases, leading to oxidative stress which in turn can lead to cytotoxicity and DNA damage. In addition, PCBs and particularly dioxins can increase DNA binding activity of the oxidative stress-induced transcription factors, nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1), causing oxidative DNA damage (Oakley et al. 1996; Carpenter 1998; Lu et al. 2003; Brevini et al. 2005; Krishnamoorthy et al. 2005; Aly et al. 2009; Aly and Domènech 2009; Vested et al. 2014). Moreover, PCBs (Aroclor 1254 and 3,3′,4,4′-tetrachlorobiphenyl) were suggested to increase DNA adducts and cause cytochrome P450 induction in human, rat and avian liver cells (Dubois et al. 1995).

Exposure to PCBs, including Aroclor 1254, were suggested to enhance lipid peroxidation in hepatocytes of rats (Fadhel et al. 2002; Banudevi et al. 2006) and Aroclor 1254 exposure was shown to decrease antioxidant enzyme activities (superoxide dismutase, catalase, glutathione S-transferase, glutathione reductase and GPx) in liver but not in kidney of male Wistar rats (Banudevi et al. 2006). PCB-induced oxidative stress has been shown to promote carcinogenesis in rat liver (Banudevi et al. 2006; NTP 2010). Dioxin-like PCBs have been shown to decrease the hepatic mRNA levels and activity of

the selenoenzyme, GPx1 (Twaroski et al. 2001). On the other hand, Borlak et al. (2003) showed mixtures of PCBs (Aroclor 1016, Aroclor 1254) or a single isomer increased DNA adduct formation in cultures of human hepatocytes. Moreover, dioxins and dioxin-like PCBs have been shown to disrupt the homeostasis of antioxidant enzymes, including the Se-dependent GPx (Twaroski et al. 2001). Severe liver injury due to increased oxidative stress can occur if GPxs and other antioxidant enzymes cannot sufficient remove the toxic intermediates of different environmental chemicals (Polavarapu et al. 1998).

Oxidative stress is suggested to induce oxidative DNA damage, including DNA base damages which can be detected by comet assay. Comet assay is a useful, versatile, easy, sensitive and low cost technique in detecting the DNA damage. It can be applied to wide range of cells, including sperm (Balasubramanyam et al. 2010). To our knowledge, this is the first study that uses Comet assay to show the effects of selenium status in Aroclor 1254-induced multi-organ toxicity. Currently, we have observed that Se deficiency alone can induce DNA damage, particularly in brain and sperm. Aroclor 1254 increased DNA damage in blood, liver, kidney, brain and sperm as evidenced by significant increases in DNA tail intensity, DNA tail moment and DNA tail migration (Figures 1–5). Se supplementation ameliorated the toxic effects of Aroclor 1254 in blood, liver, kidney and brain (Erkekoglu et al. 2012; Erkekoglu et al. 2015). Moreover, Se protected the sperm against the DNA damage-induced by this particular PCB congener. Se deficiency, on the other hand, aggravated Aroclor 1254's toxicity in all of the organs as well as in sperm. Previously, we have shown that Se is found in higher concentrations in kidney compared to other organs. This might be the reason why Se supplementation was highly protective in kidney against Aroclor 1254 toxicity compared to other organs. Besides, in sperm, Se supplementation was also highly protective against toxic effects of Aroclor 1254 and almost prevented all of DNA-damaging effects of this particular PCB mixture.

In a study by Krishnamoorthy et al. (2007) investigated the possible protective effect of vitamins C and E on Aroclor 1254-induced reproductive toxicity. Adult male Wistar rats were administered Aroclor 1254 (2 mg/kg bw/d, intraperitoneally) for 30 d. Another group was treated with Aroclor 1254 along with vitamin E (50 mg/kg bw/d), while the other group were treated with Aroclor 1254 along with ascorbic acid (100 mg/kg bw/d) orally for 30 d.

The researchers determined that Aroclor 1254 significantly decreased the level of vitamin E, Vitamin C and GSH and the activities of SOD, CAT, GPx, GR and GST while elevating the levels of ROS and LPO. Besides, epididymal sperm motility and count decreased markedly vs. control in Aroclor 1254-treated rats. Simultaneous supplementation with vitamin E and vitamin C restored these parameters to that of normal range and the vitamins were found to protective against the reprotoxicity of Aroclor 1254 (Krishnamoorthy et al. 2007). In a more recent study by Attia et al. (2014), the researchers applied 1, 2 or 4 mg/kg/d of Aroclor 1254 for 5 weeks to mice. Sperm DNA damage was assessed with the standard comet assay. There were significant increases in the incidence of DNA strand breaks in sperm of the group which received 4 mg/kg/d of Aroclor 1254 while no significant difference in the DNA strand breaks was found in mice treatment with 1 and 2 mg/kg/d of Aroclor 1254. The same results were also observed with spermatocyte chromosomal analysis and obvious aberrant primary spermatocytes were noted only with the highest dose of Aroclor 1254. DNA repair gene [p53, poly(ADP-ribose) polymerase-1 (PARP-1) and Bcl-2-associated X protein (BAX)] expression were up-regulated in testes of the highest dose group (Attia et al. 2014). On the other hand, different forms of selenium (like selenium nanoparticles) were suggested to be protective against spermatoxicity of including cisplatin (Rezvanfar et al. 2013).

In our previous studies, by using Comet assay, we have observed that Se was protective against the DNA-damage inducing effects of some environmental chemicals (phthalates), alkylaniline metabolites (3,5-dimethylaminophenol, 3,5-DMAP) and natural toxins (i.e. ochratoxin A) both *in vitro* and *in vivo* (Erkekoglu, Rachidi, Yuzugullu, et al. 2010; Erkekoglu, Rachidi, De Rosa, 2010; Erkekoglu, Giray, et al. 2014). We have demonstrated that di(2-ethylhexyl)phthalate (DEHP) and its main metabolite mono(2-ethylhexyl)phthalate (MEHP) produced significant DNA damage as evidenced by increased DNA tail percentage intensity (2.9-fold and 3.2-fold, respectively), and tail moment (2.4-fold and 2.6-fold, respectively) in LNCaP cells compared to control. The overall difference between the DNA damaging effects of the parent compound and the metabolite was insignificant. Se supplementation itself did not cause any alteration in the steady-state levels of the biomarkers of DNA damage in LNCaP cells, whereas the presence of Se either in inorganic (sodium selenite, SS) or organic (selenomethionine, SM) form reduced the genotoxic effects of DEHP and MEHP as evidenced by significant decreases in tail percentage intensity (30% vs. control). These results, thus, indicated that the Se showed antigenotoxic activity against the genotoxicity of DEHP and MEHP (Erkekoğlu, Rachidi, De Rosa, et al. 2010). In another study, we have observed that both DEHP and MEHP caused DNA damage as evidenced by marked increases in tail percentage intensity (∼3.4-fold and ∼3.8-fold, respectively), and tail moment (∼4.2-fold and ∼3.8-fold, respectively) vs. control in Leydig MA-10 cells. The difference between the DNA damaging effects of the parent compound and the metabolite was insignificant. Se supplementation itself did not cause any alteration on the steady state levels of the DNA damage biomarkers of MA-10 cells. In fact, Se was highly protective against the genotoxic effects of these phthalate esters. SS supplementation decreased both tail percentage intensities and tail moments by DEHP and MEHP, whereas SM treatment also provided protection against phthalate-induced DNA damage (Erkekoglu, Rachidi, Yuzugullu, et al. 2010). It was also demonstrated that low doses of both organic (SM) and inorganic Se (SS) specifically stimulated the repair of oxidative DNA damage in p53-proficient LNCaP prostate cancer cells (de Rosa et al. 2012).

Through our recent studies, we have also determined that both SS and SM were protective against the DNA damage caused by 3,5-DMAP. 3,5-DMAP caused a significant increase in olive tail moment (299%, $p < 0.05$ vs. control). Both SS and

SM supplementations along with 3,5-DMAP treatment provided significant decreases in olive tail moment after 24 h supplementation (49 and 27% vs. DMAP group, respectively; $p < 0.05$, both). However, further treatment for another 48 h (a total of 2 h) with either of the selenocompounds did not provide further protection (Erkekoglu, Chao, et al. 2014).

Conclusion

In conclusion, this is the first study providing evidence that Se supplementation can be protective against Aroclor 1254-induced multi-organ DNA damage. Considering the DNA-damaging potential of Aroclor 1254, it can be postulated that even marginal Se deficient individuals may be more susceptible to its genotoxic effects. As Se supplementation was found to be protective against the genotoxicity of Aroclor 1254, taking adequate amounts of Se daily can protect the organism against the toxicity of this particular PCB mixture. Our results accentuate importance of Se status as an indicator of well-being. Further studies should be performed to clarify the mechanism(s) underlying the protective role of Se status against Aroclor 1254 genotoxicity. In addition, more studies are needed to show the genotoxicity of different PCB congeners and different antioxidants (in both supplementation and deficiency states) should be used to determine their interactions with PCB mixtures, particularly with Aroclor 1254.

Disclosure statement

The authors declare that there is no conflict of interest.

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