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Changes of the Oxidant/Antioxidant Equilibrium in Liver, Brain and Kidney Tissues of Pregnant Rats Exposed to Aroclor1254 (2mg/kg/day) During Pregnancy

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SUMMARY

This study was conducted in order to investigate both oxidant and antioxidant parameters in liver, kidney, and brain tissues of pregnant rats exposed to Aroclor 1254. In the study, rats were divided into two groups, each of which consisted of 10 rats. The first group was the pregnant control group, and the second group was the pregnant Aroclor 1254 (2 mg/kg/day dose of Aroclor 1254 was administered by subcutaneous injection for 20 days). The pregnant rats in each group were anesthetized by using ether anaesthesia method, and their liver, brain, and kidney tissues were resected. Parameter of lipid peroxidation malondialdehyde (MDA), glutathione (GSH) levels as well as glutathione peroxydase (GSH-Px), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione S- transferase (GST) activities were measured in these tissues. As a result of the study, no statistically significant difference was determined between two groups in terms of parameters analysed in liver, kidney, and brain tissues. This study concluded that since a part of Aroclor 1254 (2 mg/kg), which was administered during pregnancy, was transmitted to the infant via placenta, the amount of Aroclor 1254, left in the body of pregnant rat, was not so effective to create oxidative damage in the pregnant rat. It is thought that the oxidative damage which could occur in the pregnant rats could be determined particularly through an advanced study in which a high dosage of Aroclor 1254 is administered to the pregnant rats.

Key Words: *Polychlorinated biphenyls, Oxidative stress, Pregnancy, Antioxidant, Aroclor*

ÖZET

Gebelik Süresince Aroclor 1254 (2 mg/kg/gün)'e Maruz Kalan Gebe Ratların Böbrek Beyin ve Karaciğer Dokularında Oksidant/Antioksidant Dengedeki Değişimler

Bu çalışma, Aroclor 1254'e maruz kalan gebe ratların beyin, böbrek ve karaciğer dokularında hem oksidant hem de antioksidant parametreleri araştırmak amacıyla yapılmıştır. Çalışmada ratlar iki gruba ayrılmıştır ve her bir grupta 10 rat bulunmaktadır. Birinci grup, gebe kontrol grubu ve ikinci grup, gebe Aroclor 1254 (2 mg/kg/gün, Aroclor 1254'ün bu dozu 20 gün boyunca subkutan olarak uygulandı). Her grupta yer alan gebe ratlar eter anestezi yöntemi ile uyutulmuş ve karaciğer, beyin ve böbrek dokuları çıkarılmıştır. Bu dokularda lipit peroksidasyon parametresi malondialdehid (MDA), glutatyon (GSH) seviyeleri ile glutatyon peroksidaz (GSH-Px), katalaz (CAT), süperoksit dismutaz (SOD), glutatyon redüktaz (GR), glutatyon S transferaz (GST) aktiviteleri ölçülmüştür. Çalışma sonucunda karaciğer, böbrek ve beyin dokularında analizi yapılan parametreler açısından iki grup arasında istatistiksel açıdan bir anlamlı bir fark tespit edilememiştir. Yapılan çalışma ile gebelik süresince uygulanan Aroclor 1254'ün (2 mg/kg) bir kısmının plasenta yoluyla yavruya geçmesi nedeniyle gebe ratın vücudunda kalan Aroclor 1254'ün miktarının gebe ratda oksidatif hasar oluşturabilecek kadar etkili olmadığı sonucuna varılmıştır. Özellikle yüksek dozda Aroclor 1254'ün gebe ratlara uygulandığı ileri bir çalışma ile; gebe ratlarda oluşabilecek oksidatif hasarın tespitinin yapılabileceği düşünülmektedir.

Anahtar Kelimeler: *Poliklorin bifenil, Oksidatif stres, Gebelik, Antioksidan, Aroclor*

INTRODUCTION

Polychlorinated biphenyls (PCBs) were used in industry as inflammable coolants and lubricants and as components of paints and plastics. They were banned in 1977 in response to emerging public awareness about their estrogenic and potentially toxic effects on humans and wildlife. Polychlorinated biphenyls continue to leach into soil, air,

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and groundwater due to idle industrial equipment, old factories, and buildings. Polychlorinated biphenyls may have various impacts depending on involved congeners or congener mixtures. Effects of polychlorinated biphenyls can be eliminated based on the organism's age at exposure, the sex of the individual, the degree of exposure and the availability of balanced diet or social support. An accurate evaluation to be made regarding ecologically relevant xenobiotic exposure depends on the close examination of all PCBs administered at various low doses (Battershill 1994; Brouwer et. al. 1999).

The neuroendocrine system acts as an interface between the central nervous system and peripheral endocrine organs and thus represents a prime target for PCB-induced endocrinedistruption (Patisaul et. al. 2006). Polychlorinated biphenyls and their metabolites can act at multiple nodes of the neuroendocrine axis: they may serve as hormone mimics (Connor et. al. 1997), alter circulating hormone levels (Desaulniers et. al. 1999), change patterns of estrous cyclicity (Meerts et. al. 2004; Brouwer 2004), disrupt hormone metabolism (Yamane et.al. 1975; Kuiper et.al. 2000; Gregoraszczuk et. al. 2005;), affect endocrine-related and hypothalamic gene expression (Salama et. al. 2003; Aluru et. al. 2004; Bansal et. al. 2005;), interfere with hormone binding proteins (Brouwer et. al. 1986; Kodavanti et. al. 2000), alter neuronal signaling to endocrine regions of the brain (Seegal et. al. 1985; Seegal et.al. 1990; Morse et. al. 1996;Khan et. al. 2001;) or indirectly affect steroid receptor availability via molecular crosstalk (Brunnberg et. al. 2003; Brouwer 2004)

Commercial PCB mixtures such as Aroclor 1254 may cause induction of CYP1A and CYP2B enzymes to laboratory animals (Ngui et. al. 1999). The induced CYP isoenzymes may be the cause for formation of reactive oxygen species (ROS) or oxidations of endogenous and exogenous substances (Twaroski et. al. 2001). Cytochrome P450 system catalyses the oxidation of low chlorinated biphenyls to mono- and dihydroxy metabolites. These subsequent hydroxy-metabolites may be converted to semiquinones and/or quinones throughout enzymatic reactions or spontaneously (Song et. al. 2008a; Song et. al. 2008b). Furthermore, some PCB-quinones may enter the redox cycle and cause ROS formation. Therefore, PCBs constitute a cause for the oxidative stress (MC Lean et. al. 2000). The placenta is a major source of oxidative stress during pregnancy (Lewandowski et. al. 2014).Since the placenta is rich in polyunsaturated fatty acids which are highly susceptible to attack by reactive oxygen species (ROS), an increase in lipid peroxidation (LPO) is expected during pregnancy. This assumption has been already clinically proven (Öztürk et. al. 2010).

After free oxygen radicals are formed in the tissues, they may damage DNA, proteins, carbohydrates, and lipids (Bassaga 1990; Poli 1993; Rangan et. al. 1993). These reactions that cause potential hazards are controlled by enzymatic and non-enzymatic antioxidant systems that remove pro-oxidants and hold free radicals. The oxidative stress reflects a shear towards pro-oxidants in the pro-oxidant-antioxidant equilibrium (Bassaga 1990; Poli 1993; Rangan et. al. 1993).

According to the literature information, the application of different PCB types and their combinations cause oxidative stress in numerous animal species (Basaga 1990; Poli 1993).

Although the effect of PCB on the pathological, histopathological and routine blood parameters in pregnant ones (Basaga 1990; Poli 1993) has been examined, sufficient number of studies conducted on the oxidative damage of PCB in pregnant individuals has not been found. The conducted studies have revealed the oxidative damage caused by PCB on normal individuals (Basaga 1990; Poli 1993). In accordance with this information, we aimed to determine the damage that may arise from exposition to PCB in pregnant individuals with weaker defence mechanisms compared to normal ones.

Therefore, the purpose of this study was to investigate both oxidant and antioxidant parameters in liver, kidney, and brain tissues of pregnant rats exposed to Aroclor 1254.

MATERIAL and METHODS

Chemicals

All chemical substances used in this study were in analytical grade and they were purchased from some companies such as Merck, Sigma, Supelco, and Carlo Erba.

Experimental Design

Twenty Sprague-Dawley female rats, which had a weight of 150-180 g and were 4 weeks old, were used in this study. The rats were kept in cages, each of which contained 5 rats, in a room, which had standard room temperature (21±1ºC), for 12 hours during daylight and at night. Whether sexual cycle periods and pregnancy of the rats, bred in laboratory conditions in the study, developed or not was determined by using the vaginal smear method. The pregnant rats were divided into 2 groups as 10 rats in each group.

1st group: It was the pregnant control group (10 rats)

2nd group: It was Pregnant + Aroclor 1254 group (10 rats). Beginning from the first day of pregnancy, 2 mg/kg/day of Aroclor 1254 was administered to them by subcutaneous injection (for 20 days).

In order to ensure equality with the other group, normal saline was injected to the first group, the Pregnant Control group, at the same amount and duration. After the last injection, the pregnant rats in each group were anesthetized with ether, and their liver, brain, and kidney were resected. All tissues were kept at -80^{o}C until the analyses were conducted. Approval of Ethics Committee on Animal Experiments was received for this study. (Date: 29.08.2007, Number: 7, Decisions No: 3)

Preparation of Tissue Homogenates

After the tissue samples were dried between two filter papers, they were diluted in 1.15% KCl with 7.4 pH at 1/10 ratio (weight/volume), and they were homogenized in fragmented ice with a Potter-Elvehjem glass-glass homogenizer and some of the homogenates were centrifuged at 3500 rpm for 15 minutes and malondialdehyde (MDA), reduced glutathione (GSH) levels, catalase (CAT), superoxide dismutase (SOD) activities and protein determination were made on the supernatants. The remaining homogenate was put in 1.5 ml eppendorf tubes and it was centrifuged at a refrigerated centrifuge (Nüve NF 800R) at 11.000 rpm for 20 minutes. Glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione–S-transferase (GST) activities and protein determination were made on the supernatants at the end of the centrifugation process.

Lipid Peroxidation (LPO or MDA)

Determination of MDA in supernatants was conducted based on the method modified by Placer et al. (1966). The MDA formed a pink complex with thiobarbituric acid

(TBA) and the grade of LPO was determined by measuring spectrophotometrically the absorbance of this solution at 532 nm. The level of MDA in the tissue was calculated as nmol/g tissue.

Glutathione peroxidase Activity

The Beutler (1975) method was used to determine GSH-Px activity. Glutathione peroxidase activity was spectrophotometrically calculated by considering the decrease in the optic density of the system at 340 nm after the NADPH oxidation. Glutathione peroxidase activity was calculated as U/g protein.

Catalase Activity

The Aebi (1984) method was used to measure CAT activity. The degradation rate of H_2O_2 by CAT was spectrophotometrically measured by means of the fact that H_2O_2 absorbs light in the 240 nm wavelength. CAT activity was calculated as k/g protein

Superoxide Dismutase Activity

Measurement of the SOD activity was based on colouring of superoxide radical, which was produced with the xanthine-xanthine oxidase system, by degrading the nitroblue tetrazolium (NBT). The superoxide radical's degrading NBT results in formation of blue colour formazon which absorbs maximum at 560 nm (Sun et.al. 1988). Superoxide dismutase activity was calculated as U/g protein.

Glutathione Reductase Activity

Measurement of GR activity was carried out by measuring the increase of the 2-nitrobenzoic acid (TNB) formed as a result of the reaction at 412 nm for 2 minutes (Smith et. al. 1988). GR activity was calculated as U/g protein.

Glutathione–S-Transferase Activity

Glutathione–S-transferase activity was determined by spectrophotometrically measuring the 1-(S-glutathionyl)-2,4 dinitrobenzene forming as a result of the conjugation of GSH and 1-chloro -2,4-dinitro benzene (CDNB) at 340 nm. Glutathione–S-transferase activity was calculated as U/g protein (Habdous et. al. 2002).

Reduced Glutathione Level

Reduced glutathione level was determined according to the method (Sarita et. al. 2005). Reduced glutathione level was calculated as µmol/g protein.

Protein Amount

Protein amount was measured according to the modified Lowry method (By Lowry et. al. 1951). Alkaline copper tartrate reagent formed a complex with peptide bonds. When phenol reagent was added to the mixture treated with copper, a purple-blue colour was formed. This colour intensity was read at 650 nm wavelength. Protein amount was calculated as g protein.

Statistical Analysis

SPSS package program (10.0 for Windows) was used to conduct the statistical analysis. All results were shown as mean ± SEM. The value of $P \leq 0.05$ was accepted as statistically significant. The analysis of variance was performed for the biochemical analysis results and the number of rats was less than thirty nonparametric so Mann-Whitney U test was used.

RESULTS

No statistically significant difference was determined between oxidant and antioxidant parameters in liver, kidney, and brain tissues in the study (Table 1, Table 2, Table 3).

Table 1. Effect of Aroclor 1254 on oxidant and antioxidant parameters in liver tissues of rats in the Pregnant Control group and Pregnant + Aroclor 1254 (Pregnant + A1254) group

Parameters	Pregnant Control	Pregnant + A1254	P
MDA (nmol/ml)	103.83±26.65	107.71±33.99	P>0.05
GSH (µmol/gr Hb)	1.69±0.23	1.35±0.21	P>0.05
GSH-Px (U/gr Hb)	51.46±2.86	57.16±14.75	P>0.05
GR (U/gr Hb)	10.04±0.29	8.39±1.32	P>0.05
CAT (k/gr Hb)	139.74±15.13	83.78±2.45	P>0.05
GST (U/gr protein)	10.61±1.44	6.50±0.94	P>0.05
SOD (U/gr Hb)	30.80±5.44	48.35±6.50	P>0.05

Table 2. Effect of Aroclor 1254 on oxidant and antioxidant parameters in kidney tissues of rats in the Pregnant Control group and Pregnant + Aroclor 1254 (Pregnant + A1254) group

Parameters	Pregnant Control	Pregnant + A1254	P
MDA (nmol/ml)	56.04±4.91	62.75±6.22	P>0.05
GSH (µmol/gr Hb)	2.65±0.15	2.50±0.11	P>0.05
GSH-Px (U/gr Hb)	16.80±3.44	22.23±11.11	P>0.05
GR (U/gr Hb)	50.88±2.33	53.29±4.98	P>0.05
CAT (k/gr Hb)	34.20± 7.14	22.34±1.64	P>0.05
GST (U/gr protein)	0.56±0.15	0.99±0.28	P>0.05
SOD (U/gr Hb)	15.23±4.23	15.92±2.20	P>0.05

Table 3. Effect of Aroclor 1254 on oxidant and antioxidant parameters in brain tissues of rats in the Pregnant Control group and Pregnant + Aroclor 1254 (Pregnant+A1254) group

Parameters	Pregnant Control	Pregnant + A1254	P
MDA (nmol/ml)	51.55±7.59	43.08±3.50	P>0.05
GSH (µmol/gr Hb)	3.93±0.31	3.83±0.71	P>0.05
GSH-Px (U/gr Hb)	55.73±5.85	67.67±8.23	P>0.05
GR (U/gr Hb)	11.56±1.56	7.60±1.19	P>0.05
CAT (k/gr Hb)	1.13±0.22	1.87±0.32	P>0.05
GST (U/gr protein)	4.98±0.77	3.20±0.67	P>0.05
SOD (U/gr Hb)	119.02±14.67	66.56±9.10	P>0.05

DISCUSSION

Oxidative stress reflects a shear toward pro-oxidant in the pro-oxidant-antioxidant balance (Basaga 1990; Poli 1993; Rangan et. al. 1993) and all organs are known to be sensitive to the oxidative stress. In this respect, PCB (Aroclor 1254), an environmental contamination agent threatening the human health, was reported to alter the activities of different antioxidant enzymes in hypothalamus of albino rats and to induce the oxidative stress (Muhtuvel et. al. 1999).

The behavioural phenotype is perhaps the most sensitive and salient measure of PCB disruption of the neuroendocrine system because reproductive success hinges upon normal complement of reproductive behaviours. Previously, PCBs and their metabolites were

shown to affect neurotransmitter and steroid hormone systems underlying reproductive function (Khan et. al. 2001; Ptak et. al. 2005; Seegal et. al. 2002).

These changes in turn are likely to have profound effects on reproductive behaviours. Moreover, the timing of exposure to PCBs is significant to the severity of the reproductive phenotype. In particular, the exposure during the critical period of brain sexual differentiation is potentially detrimental. This critical period in rats has been proposed to begin in the third trimester of pregnancy and shortly after birth, and from approximately embryonic day 16 to postnatal day 5 in rats (Becu-Villalobos et. al. 1997), even though a revisitation of brain sensitivity to steroid hormones suggest that the critical period may last longer into postnatal life than previously opinions (Romeo 2003).

Chu et al.(2005) determined in their study that as a result of exposition to organochlorine compounds during gestation and lactation periods, especially cholesterol, urea nitrogen (BUN) levels, lactate dehydrogenase (LDH) activity changed in the serum; and liver tissue was target tissue for PCB. Adaptive liver changes with this natural condition have been reported after subchronic exposure to PCB congeners (Chu et. al. 1995). In the study conducted by Doğan and Erişir (2011), impairment was determined in the oxidant-antioxidant balance in liver, kidney, brain, and heart tissues of the infants of rats which received Aroclor 1254 (2 mg/kg/day x 4 weeks) during pregnancy.

In numerous studies, various practices were performed regarding the damage caused by PCB on the tissues on pregnant rats and damages were determined (Ando et al. 1986; Aly et.al. 2009). In our study, we tried to determine the relationship between the biochemical and histopathological damages arising on these tissues on pregnant rats that were exposed to PCB and oxidative damage. We could not find sufficient and appropriate studies on the oxidative damage that may occur on pregnant ones exposed to PCB. The fact that any statistically significant difference could not be determined in liver, kidney, and brain tissues in this study may be associated with the administration of injection dose of 2 mg/kg/day and maintaining the injection period only during pregnancy. However, in the study conducted with higher doses of PCB such as 15 mg/kg , histopathological and biochemical changes were determined on pregnant rats (Chu et. al. 2005). Additionally, in another study, it was determined that PCB exposure was more harmful in the postnatal period which is specified as the critical period during gestation (Primus et. al. 1990). Moreover, it was determined that oxidative damage occurred in infants as a result of the transmission of Aroclor 1254 (2 mg/kg), which was administered to the pregnant rats during pregnancy in our previous study, to the infant via placenta (Doğan et. al. 2011).In this study, a part of Aroclor 1254 (2 mg/kg), which was administered during pregnancy, was transmitted to the infant via placenta and it was thought that the amount of Aroclor 1254 left in the body of pregnant rats due to this transmission was not so effective to create oxidative damage in the pregnant rats. We considered that the oxidative damage which could occur in the pregnant rats could be determined particularly through an advanced study in which high dosage of Aroclor 1254 is administered to the pregnant rats.

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