Effects of Aroclor 1254 and vitamin E on bone oxidant / antioxidant status in adult, pregnant rats and their pups

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SUMMARY

The aim of the study was to analyze the oxidant and antioxidant parameters in bone tissue of offspring from pregnant rats, pregnant rats and not pregnant female rats exposed to Aroclor 1254 alone or associated to the vitamin E. A total of 30 pregnant Sprague-Dawley rats and 30 adult rats were divided into 3 equal groups according to the treatments instituted during the pregnancy period (20 days) with subcutaneous daily injections of Aroclor 1254 (2 mg/kg/day) alone or associated with vitamin E (50 mg/kg/day) or normal saline in controls. The oxidant / antioxidant balance in bone was investigated by determining the malondialdehyde (MDA) and glutathione (GSH) contents as well as the superoxide dismutase (SOD), catalase (CAT), the glutathione peroxidase (GSH-Px) and glutathione reductase (GR) activities in females and in their progeny (10 pups per groups). Treatment with Aroclor alone has induced significant decline in GSH content in pregnant females compared to the not treated ones and significant increases in GR and GSH-Px activities in pups whereas GSH content and the CAT activity were significantly depressed in pregnant females cotreated with Aroclor and vitamin E compared to the pregnant controls and GSH content, the GR and CAT activities were markedly enhanced in pups. Although alterations were noticed in the antioxidant systems, no significant variations in MDA contents were evidenced, suggesting that the period of pregnancy for Aroclor and/or Vitamin E treatments was too short for inducing ROS accumulation in rats.

Keywords: Rat, polychlorinated biphenyls, Aroclor 1254, vitamin E, pregnancy, progeny, oxidative stress, antioxidants, bone.

RÉSUMÉ

Effets de l’Aroclor 1254 et de la vitamine E sur le statut oxydant / antioxydant du tissus osseux chez les rata couples gestantes ou non et leur progéniture

Le but de cette étude a été de déterminer le statut oxydant / antioxydant dans le tissus osseux chez des couples gestantes ou non traitées par l’Aroclor 1254 seul ou associé à la vitamine E ainsi que chez leur progéniture. Pour cela, 30 raths Sprague-Dawley gestantes et 30 femelles non gestantes ont été réparties en 3 groupes égaux en fonction des traitements mis en place durant la gestation (20 jours), soit des injections sous-cutanées quotidiennes d’Aroclor 1254 (2 mg/kg/jour) ou/et de vitamine E (50 mg/kg/jour) soit de soluté salé chez les témoins. Le statut oxydant / antioxydant a été déterminé en mesurant les teneurs osseuses en malondialdéhyde (MDA), glutathion réduit (GSH) ainsi que les activités de la superoxyde dismutase (SOD), de la catalase (CAT), de la glutathion peroxydase (GSH-Px) et de la glutathion réductase (GR) chez les femelles et leur progéniture (10 par groupe). Le traitement par l’Aroclor seul a induit une diminution significative de la teneur en GSH chez les raths gestantes par rapport à celles non traitées et une augmentation significative des activités GR et GSH-Px chez les nouvea-nés alors que la teneur en GSH et l’activité de la CAT ont été significativement diminuées chez les femelles gestantes cotratées par l’Aroclor et la vitamine E et que la teneur osseuse en GSH et les activités GR et CAT ont été significativement augmentées chez les petits. Bien que des altérations dans les systèmes antioxydants aient été observées, aucune variation significative des teneurs en MDA n’a pu être mise en évidence, ce qui suggère que la période de gestation soit une période de traitement trop courte par l’Aroclor et/ou la vitamine E pour induire une accumulation des radicaux oxygénés chez les rats.

Mots clés : Rat, biphénols polychlorés, Aroclor 1254, vitamine E, gestation, progéniture, stress oxydatif, antioxydants, os.

Introduction

Polychlorinated biphenyls (PCBs) are highly toxic persistent environmental pollutants. They were produced commercially by catalytic chlorination of biphenyls producing complex mixtures of multiple isomers. They are used as diluents, fluids for capacitors, transformers and flame retardants [10]. Aroclor 1254 is a commercial mixture of PCBs contains about 54% of chlorine by weight, resistant to degradation and traverses the food chain [4].

The PCBs find their way to humans and animals through the skin, lungs and the gastrointestinal tract. Once entering the body, they were transported through blood stream to liver, various muscles and adipose tissue where they accumulate because of their lipophilic nature [8]. PCBs are known to cause various adverse effects which include hepatic microsomal enzyme induction, increased serum activities of liver-associated enzymes indicative of possible hepatocellular damage, liver enlargement, lipid deposition, fibrosis, necrosis and carcinogenicity [36]. PCBs are known to cause various adverse effects including carcinogenicity, endocrine disruptions, neural, immune, developmental, skeletal and reproductive toxicity [14, 20, 35].

PCBs induced toxicity is mediated by increased production of reactive oxygen species (ROS). It is found to be mediated...
by the activation of microsomal monooxygenase, which catalyses the conversion of PCBs into reactive metabolites. PCBs, especially higher chlorinated PCB selectively induces cytochrome P450 [27] which serves as a potential source for ROS or alternatively the oxidation of broad range of endogenous and exogenous substances. The cytochrome P450-catalysed oxidation of lower chlorinated biphenyls produces mono and dihydroxy metabolites. The latter can auto-oxidize or be enzymatically oxidized to semi-quinone and/or quinones [22]. Some PCB-quinoines undergo redox cycling with the formation of ROS, thus, becoming another source of oxidative stress [23].

Bone is a specialized connective tissue, which forms the framework of the body. Exposure of rats to PCB (Aroclor 1254) reduced the cortical area and length of femur bone and the rats showed hypocalcaemia [3]. PCB126, which has weak estrogenic activities in ovariectomized rats, exhibits anti-estrogenic activities in ovary intact rats. With regard to its actions on bone, PCB126 increased the bone mineral density in ovariectomized rats, but impaired the process of mineralization in intact rats [17]. LIND et al. [18] found that PCB126 increased trabecula density and cortical thickness, but reduced the trabecular area in the femur of rats. Further they have shown that PCB126 induced effects are modulated by exogenous oestrogen supplementation in ovariectomized rats [16].

Recently it has been reported that Aroclor 1221 and Aroclor 1254 exert modulator effects on bone turnover markers in intact and ovariectomized rats [38]. The modulator effects are different between them and also depend upon the presence or absence of ovary in experimental rats. For example, Aroclor 1221 and Aroclor 1254 exert similar effects on serum parahormone profile in intact rats while Aroclor 1254 alone elevated the serum parahormone concentrations in ovariectomized rats. The PCB mixtures are suggested to exert their effects with oestrogenic and anti-oestrogenic mechanisms.

Oxidative stress has been linked to osteoporotic syndromes observed in relatively young males [21]. Antioxidant deficiency has negative impact on bone mass. The low level of intracellular antioxidants and the uncontrolled availability of ROS induce excess cytokines like IL-1, IL-6 and TNF-α which promote osteoclastic function and bone resorption [11, 24]. However, the Aroclor 1254 mediated oxidative stress and antioxidant effect of vitamin E in bone is not clear. Therefore, the present study was undertaken to assess the effect of Aroclor 1254 on the oxidant and antioxidant status and protective role of vitamin E in adult, pregnant rats and their pups.

Materials and methods

ANIMALS AND EXPERIMENTAL DESIGN

A total of 90 female Sprague-Dawley rats, whose weights varied between 150-180 g, were used in the study. The rats were allotted in cages (n = 5 per cage) and housed at standard temperature (21 ± 1°C) with a 12:12 light/dark cycle. After the determination of the sexual cycle periods and/or pregnancy by the vaginal smear method, rats were randomly divided into groups according to chemical treatments and physiological status. Groups 1 and 4 serve as not pregnant (n = 10) and pregnant (n = 15) controls and received normal saline subcutaneously for 20 days, respectively. In groups 2 (not pregnant females, n = 10) and 5 (pregnant females, n = 15), only Aroclor 1254 was subcutaneously administered to females at 2 mg/kg/day for 20 days (since the first day of pregnancy in pregnant rats) whereas in groups 3 (not pregnant females, n = 10) and 6 (pregnant females, n = 15), vitamin E (50 mg/kg/day) was added to the Aroclor 1254 injections. The last groups (groups 7, 8 and 9) corresponded to puppies (10 in each group) born from the control pregnant females, from females only treated with Aroclor 1254 and from females treated with Aroclor 1254 and vitamin E, respectively. On the day 20, bone tissue of 10 rats from every group was extracted after applying deep ether anaesthesia, and 5 females of each pregnant group were kept for four weeks with the babies they delivered. Deep ether anaesthesia was performed to the developed puppies (10 in each group) at the end of the 4th week in order to remove their bone tissue. Bone tissue samples were stored at -80°C until analysis. Permission was obtained from ethics committee on the animal experiments for this study.

CHEMICALS

All chemical substances used in the present study were in analytical grade and they were bought from companies such as Merck, Sigma, Supelco, and Carlo Erba.

BIOCHEMICAL ANALYSES

After drying the tissue samples between two filter papers, they were mixed with 1.15% KCl (pH 7.4) at the 1:10 ratio (weight/volume) then homogenized with a Potter-Elvehjem glass-glass homogenizer onto fragmented ice. For each homogenate, a part was centrifuged at 3 000 g at room temperature for 15 minutes and the supernatant fractions were used in order to measure the catalase (CAT) and the superoxide dismutase (SOD) activities, the malondialdehyde (MDA), the reduced glutathione (GSH) and the protein concentrations. The second half of each homogenate was centrifuged at 10 000 g at 4°C for 20 minutes and the glutathione peroxidase (GSH-Px), the glutathione reductase (GR) activities as well as the protein concentrations were determined on the obtained supernatant fractions.

The supernatant protein concentrations were measured according to the modified Lowry method [19]. The alkaline copper tartaric reagent (potassium tartrate, sodium carbonate, copper sulfate, sodium hydrochloride) reagent forms a complex with peptide bonds. When phenol reagent is added to the mixture treated with copper, a purple-blue colour is formed. The colour intensity was read at 650 nm and protein amount was given as g / g of tissue.

The MDA determination in supernatants was conducted according to the method of PLACEKR et al. [28] with slight modifications. The formed MDA created a pink complex with thioarbituric acid (TBA) and the absorbance was read at 532 nm. The tissue MDA content was expressed as nmol/g tissue.
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The GSH contents were determined according to the method of SARITA et al. [31] and were expressed as µmol/g protein.

The AEBI [1] method was used for the measurement of the CAT activity. The degradation rate of H₂O₂ by CAT is spectrophotometrically measured by means of the fact that H₂O₂ absorbs light at 240 nm wavelength. CAT activity was calculated as katal/g protein.

The SOD enzyme activity measurement is based on the nitroblue tetrazolium (NBT) degradation by the superoxide radical, which was produced with the xanthine-xanthine oxidase system. The formazan obtained at the end of the reactions exhibits a blue colour and maximally absorbs at 560 nm [34]. The SOD enzyme activity was calculated as U/g protein.

The BEUTLER method [7] was used for the GSH-Px activity determination. GSH-Px activity is spectrophotometrically calculated from the decrease in the optic density of the system at 340 nm following the NADPH oxidation. The GSH-Px activity was calculated as U/g protein.

The GR activity [32] is proportional to the amount of the 2-nitrobenzoic acid (TNB) formed by the reaction of a mole of GSH with DTNB (5,5'-dithiobis 2-nitrobenzoic acid) within 2 minutes. The absorbance was determined at 412 nm and the GR activity was calculated as U/g protein.

STATISTICAL ANALYSIS

The SPSS package program (10.0 for Windows) was used for statistical analysis. The variance analysis following by a Tukey test was performed for comparing the biochemical analyses between groups. A p value less than 0.05 was considered as statistically significant. All results were shown as mean ± standard deviation (SD).

Results

The oxidant / antioxidant status in bone tissues determined in females treated with Aroclor alone or in combination with vitamin E were summarized in Table I. In controls, bone MDA content slightly decreased during pregnancy but not significantly. The GSH-Px activity markedly decreased in control pregnant females (P < 0.05 or more) whereas the GR and SOD activity remained unchanged.

Table I: Oxidant / antioxidant balance in bone tissues from pregnant and not pregnant adult rats subcutaneously treated with Aroclor 1254 (2 mg/kg/day for 20 days) alone, with Aroclor 1254 and vitamin E (50 mg/kg/day for 20 days) or not treated (controls) during pregnancy. Results were expressed as mean ± standard deviation.

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<tr>
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<th>Not pregnant females</th>
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<th>Pregnant females</th>
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<tr>
<td></td>
<td>C</td>
<td>A₁₂₅₄</td>
<td>A₁₂₅₄ / Vit. E</td>
<td>C</td>
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<tr>
<td>MDA (nmol/g tissue)</td>
<td>53.74 ± 2.70&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>50.38 ± 7.39&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.03 ± 9.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.40 ± 1.44&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>GSH (µmol/g protein)</td>
<td>1.09 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.06 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.14 ± 0.39&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>GSH-Px (U/g protein)</td>
<td>136.86 ± 27.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.89 ± 12.74&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>52.51 ± 9.21&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10.44 ± 3.08&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>GR (U/g protein)</td>
<td>4.20 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.09 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.51 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.11 ± 0.89&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>CAT (katal/g protein)</td>
<td>9.07 ± 2.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.32 ± 1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.83 ± 0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.45 ± 5.97&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>SOD (U/g protein)</td>
<td>337.23 ± 22.77</td>
<td>284.14 ± 19.99</td>
<td>265.81 ± 17.64</td>
<td>371.55 ± 41.08</td>
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MDA: malondialdehyde; GSH: reduced glutathione; GSH-Px: glutathione peroxidase; GR: glutathione reductase; CAT: catalase; SOD: superoxide dismutase; C: control; A₁₂₅₄: Aroclor 1254; Vit. E: vitamin E.

Different superscripts a,b in the same row indicate significant differences (P < 0.05 or more) between groups.

Table II: Oxidant / antioxidant balance in bone tissues from rat puppies born from mothers subcutaneously treated with Aroclor 1254 (2 mg/kg/day for 20 days) alone, with Aroclor 1254 and vitamin E (50 mg/kg/day for 20 days) or not treated (controls) during pregnancy. Results were expressed as mean ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>A₁₂₅₄</th>
<th>A₁₂₅₄ / Vit. E</th>
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<tbody>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>31.13 ± 3.01</td>
<td>29.27 ± 1.59</td>
<td>37.52 ± 2.50</td>
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<tr>
<td>GSH (µmol/g protein)</td>
<td>5.18 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.60 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.70 ± 0.59&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>GSH-Px (U/g protein)</td>
<td>8.80 ± 1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.26 ± 2.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.70 ± 2.52&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>GR (U/g protein)</td>
<td>1.62 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.13 ± 0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.62 ± 0.84&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT (katal/g protein)</td>
<td>4.28 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.57 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.59 ± 2.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SOD (U/g protein)</td>
<td>292.58 ± 27.38</td>
<td>269.12 ± 23.55</td>
<td>341.15 ± 20.76</td>
</tr>
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</table>

MDA: malondialdehyde; GSH: reduced glutathione; GSH-Px: glutathione peroxidase; GR: glutathione reductase; CAT: catalase; SOD: superoxide dismutase; C: control; A₁₂₅₄: Aroclor 1254; Vit. E: vitamin E.

Different superscripts a,b in the same row indicate significant differences (P < 0.05 or more) between groups.
vities tended (but not significantly) to increase, the CAT activity and reduced glutathione content were dramatically enhanced ($P < 0.001$).

In the groups treated with Aroclor only, bone MDA contents as well as GR, CAT and SOD activities were similar to the control values in not pregnant and pregnant females. In addition, these parameters showed the same profiles during pregnancy as in the pregnant controls. However, the variations in GR, CAT and SOD activities were higher in the treated rodents than in the controls (GR: +21.7% in controls and +65.0% in Aroclor treated rats; CAT: +202.6% in controls and +227.5% in Aroclor treated rats; SOD: +10.2% in controls and +32.9% in Aroclor treated rats). In not pregnant rats, glutathione contents were not significantly different between the controls and the females treated with Aroclor 1254 only, but the increase observed at the end of the gestation was significantly depressed in the treated rats compared to the controls ($P < 0.001$). The GSH-Px activity appeared also diminished, but not significantly, in the not pregnant females treated with Aroclor only compared to the corresponding controls whereas the enzyme activity was quite similar in pregnant control and treated females.

Highest MDA contents were observed in the not pregnant females treated simultaneously by Aroclor and vitamin E but the gestation induced decline in MDA contents exhibited the same intensity than in the pregnant control females (-41.6% in controls and -41.3% in cotreated rats). In the same way, the SOD activities varied with the same extend at the end of the gestation in the controls and in the Aroclor/vitamin E treated females. Although the GR activities did not significantly differ between the controls and the cotreated animals, this parameter varied more stronger at the end of gestation in the treated rats (+54.5%) than in the controls (+21.7%). Surprisingly, the CAT activity, similar with the control values in the not pregnant females, did not increase during pregnancy contrary to the other 2 groups. As for females treated with Aroclor alone, the bone GSH content was similar to the control values in normal females and significantly increased during pregnancy but roughly 5 times less than in the controls. The GSH-Px activity between rats treated with Aroclor1254 alone and in combination with vitamin E were closely related in not pregnant and pregnant females; however, the lowest decrease in GSH-Px activity at the end of the pregnancy was observed in this group (-92.4% in controls, -67.9% in Aroclor group and -50.8% in Aroclor/vitamin E group).

As shown in Table II, there was no significant difference in the MDA contents and SOD activity in the bone tissues among rat puppies. Compared to puppies born from control females, the GR activity was significantly increased in puppies born from females treated with Aroclor 1254 alone or combined with vitamin E ($P < 0.01$) whereas the GSH content ($P < 0.001$) and the CAT activity ($P < 0.01$) were significantly increased only in puppies whose mothers were treated with Aroclor and vitamin E during pregnancy. By contrast, the GSH-Px activity was significantly enhanced only in pups born from mothers treated with Aroclor alone compared to the control ones ($P < 0.001$).

Discussion

Oxidative stress reflects that the pro-oxidant/antioxidant balance has shifted towards pro-oxidants [6, 29, 30]. It is a known fact that all organs are sensitive towards oxidative stress. With this in mind, PCBs (Aroclor 1254), an agent that is an environmental pollutant that threatens human health, have altered different antioxidant enzyme activities and are considered as able to induce oxidative stress [26]. Aroclor 1254 is well known to induce oxidative stress by raising the production of ROS and suppressing the components of the antioxidant system in various tissues [13]. Aroclor 1254 induced lipid peroxidation is predominant rather than its suppressive effect on the antioxidant enzymes [13]. In addition, Aroclor 1254 treatment in general registered a catabolic effect by upregulating osteoclastic activity and down regulating osteoblastic activity in the femur of rats [13]. ROS is believed to induce bone related diseases by suppressing bone formation and stimulating bone resorption. Recent studies have shown that H$_2$O$_2$ or xanthine oxidase (XXO) generated superoxide anions that are able to inhibit osteoblastic differentiation in mouse [25] and in rabbit marrow cells [5]. Other studies found that superoxide anions may stimulate osteoclast differentiation and bone resorption [9, 15, 33].

However, in the present study, MDA contents in bone tissues were not found increased in pregnant and not pregnant female rats treated with Aroclor 1254 as well as in progeny. The reduced glutathione content and the GR, CAT and SOD activities were similar between the control and treated not pregnant females and even treatment with Aroclor during pregnancy has at least moderately amplified the increases in GR, CAT and SOD activities induced by the pregnant status, whereas the variations in GSH contents were minored. Only the GSH-Px activities appeared depressed in the not pregnant females by the Aroclor treatment but differences were not statistically significant. In pups born from treated and control mothers, the bone GSH content and the CAT and SOD activities were similar while the GR and GSH-Px activities were significantly increased. The discrepancies between the present study and the previous reports may be resulted from a shorter administration period, 20 days (pregnancy duration) here instead of 30 days in the other studies. As the Aroclor treatment period was the pregnancy duration, the baby rats only received it via placenta, considerably restricting in this way the foetus exposure to PCB. It would be probable that lower cumulated doses were not sufficient for induce a durable ROS accumulation, leading to increase MDA contents in bone but sufficient for a transient ROS production which might partly stimulate antioxidant systems.

On the other hand, it is a well known fact that Vitamin E is a strong antioxidant and establishes a defence line to protect the polyunsaturated fatty acids in the cell membrane of phospholipids from the effect of free radicals [2]. Besides, vitamin E has been shown to suppress the production of cytokines such as IL-1 and IL-6 that have been shown to increase bone loss [12, 37] and adequate dietary intake reduced the risk of hip fracture [24].
In the present study, all parameters investigated have not significantly differed between the not pregnant controls and females co-treated with Aroclor and vitamin E, although bone MDA content tended to increase in this group. In pregnant females, GSH content and CAT activities were depressed compared to the corresponding controls and variations in GSH content and in the GSH-Px activity induced by pregnancy were the lowest in this group. Bone GSH content and the GR and CAT activities were significantly elevated in progeny. These observations suggest that in the present study the vitamin E treatment has exhibited only minimal antioxidant effects mainly in progeny, probably because of the short treatment period.

As a conclusion, treatment with Aroclor 1254 alone or in combination with vitamin E during pregnancy (20 days) in female rats was not sufficient for evidencing relevant alterations in oxidant / antioxidant equilibrium in adults and even in progeny. Further studies are required for studying the effects of longer exposure periods to various doses (eventually lower) of PCBs in female rats and in their puppies.

References


