Effects of estrogen supplementation on PCB 126-induced effects on vertebral bone, Vitamin D and thyroxin levels in serum of rats.

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Introduction

Environmental factors might be of importance for the dramatically increasing incidence of osteoporotic fractures in the western world since World war II. The reasons for this rapid increase in incidence is only partly understood. Results presented during recent decades have shown that the endocrine system, are affected by a number of globally-distributed, persistent organochlorines (POCs). Since estrogens to a significant degree govern the metabolism of bone tissues, exposure to POCs could theoretically induce impairments in bone tissue. From results presented over the last five years it is obvious that the bone tissue of both laboratory[1-4] and wild animals[5,6] is negatively affected when exposed to POCs. The mechanisms for POCs toxicity, however, are still unclear.

Own and others experimental studies in rat have demonstrated that high affinity Aryl hydrocarbon Receptor (AhR) ligands such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the dioxin-like PCB congener, 3,3',4,4',5-pentachlorobiphenyl (PCB126), impair normal bone metabolism and result in increased bone fragility[1-4]. No experimental study have, up to now, investigated effects of POCs on vertebra in bone-toxicological studies. Recently a Swedish epidemiological study showed that Swedish east-coast fishermen's wives have a significantly increased incidence for hospitalized vertebral fractures when compared with west-coast fishermen's wives[7]. The results give some indirect support for the notion that a high dietary intake of POCs-through fatty fish might be a risk factor for vertebral fractures. The levels of POCs are much higher in the fish from the Baltic Sea compared with fish from the sea on the Swedish West coast.

Vertebral bone consists to a larger extent than e.g. the long bones of trabecular bone which compared with cortical bone has a much higher metabolism and a more rapid bone turnover. It is therefore more likely to find more obvious effects of endocrine disruption in trabecular bone than in cortical bone. As an extension of our previous work, the goals of this study are therefore to 1) investigate interactive effects between PCB126 exposure, estrogen depletion (OVX) and estrogen supplementation 2) investigate the effects of PCB126 exposure of the trabecular rich vertebral bone 3) analyse serum levels 25OH- vitamin D and thyroxin as these are both important for bone tissue homeostasis and as biomarkers for organochlorines exposure.

Material and Methods

Forty rats exposed to 3,3',4,4',5-pentachlorobiphenyl (PCB126, ip) for 3 months (total dose 384 µg/kg bw) were randomized into OVX/sham operation or 17β-estradiol supplementation (ip, 23µg/kg, 3days weekly)/vehicle (corn oil) groups in a 2x2 factorial design. The PCB dose was planned to give maximal induction of CYP1A but not to cause any overt toxic effects.

Sham operated rats were injected with vehicle, PCB or PCB plus E2 (sham, sham+PCB and sham+PCB+E2, n = 10 per group) whereas ovariectomized were injected with vehicle, PCB or PCB plus E2(OVX, OVX + PCB and OVX + PCB + E2, n = 10 per group). As control groups served OVX or sham, and OVX + E2 (n = 10 in each group). Rats were sacrificed at 6 months of age. Serum were stored in -70ºC until analysis of 25OH-Vitamin D and thyroxin. 25OH-Vitamin D and total and free thyroxin (TT4 and FT4) were analyzed by standard RIA kits. The 4th lumbar vertebra was dissected and stored in buffer (Ringer solution pH 7.4, -20ºC) pending peripheral quantitative tomography (pQCT). The vertebra was mounted in a Stratec XCT 960A densitometer. A scan was taken at the center of the vertebra, at 4.5 mm from the endplate, using voxel size 0.148 × 0.148 × 1 mm. In this scan, the area of the vertebral body was manually selected as the region of interest and trabecular and cortical bone mineral density (BMD) was calculated. The data were evaluated by one and two-way ANOVA, followed by a post-hoc Fisher´s PLSD. Differences were considered significant when $p<0.05$.

Results and discussion

Trabecular but not cortical BMD of the lumbar vertebra, as measured by pQCT, was significantly increased by PCB126 exposure in sham operated rats but not in OVX rats (figure 1). This difference in response between sham-operated and OVX rats to PCB126 exposure and E2 supplementation is also reflected by the interaction term between OVX and E2 supplementation being significant for both cortical and trabecular BMD in the PCB126-exposed rats($P<0.05$). Thus, in ovariectomized rats PCB126 exposure in combination with E2 supplementation increased both the trabecular and cortical BMD ($p < 0.01$). On the other hand, the quality of the bone tissue in these rats are probably impaired as we have previously shown a reduced bone strength associated with a decreased collagen content in PCB126 exposed rats[3].

The serum levels of and total thyroxin (TT4) and free thyroxin (FT4) were decreased by PCB126 exposure in sham-operated as well as in OVX rats ($p < 0.001$, figure 2). Additionally an interaction between OVX and estrogen-supplementation was observed for both TT4 and FT4. Thus, in ovariectomized rats PCB126 exposure in combination with E2 supplementation decreased the TT4 levels but increased the FT4 levels ($p < 0.05$). 25OH-Vitamin D levels where decreased only in the sham-operated animals exposed to PCB126 ($p < 0.01$, figure 3).

Conclusion: Estrogen modulates PCB126 induced effects on the bone tissue of the vertebra as well as on the serum levels of thyroxin. Our findings further support an important role for resident estrogen status on the toxicity of PCB126 in bone. In extrapolation, PCB126 may, therefore, induce different effects in pre- and post-menopausal women and estrogen supplementation may interfere with the systemic effects of PCB126 exposure. Based on the findings in this study it would be of interest to study if E2 therapy could improve BMD in POC exposed postmenopausal women. The PCB126 dose used in this study is rather high but the study was designed to pick up any potential effects. In future studies it is important to investigate effects of much lower exposure levels.

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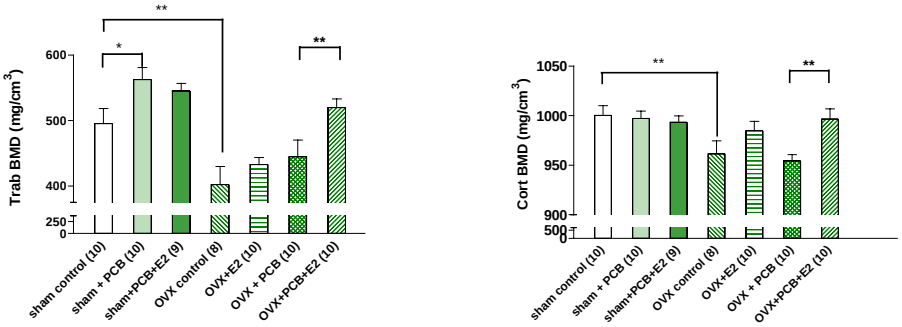

Fig 1. Effects of concomitant 17 β -estradiol supplementation and PCB126 exposure on trabecular BMD and cortical BMD in vertebral bone of rats

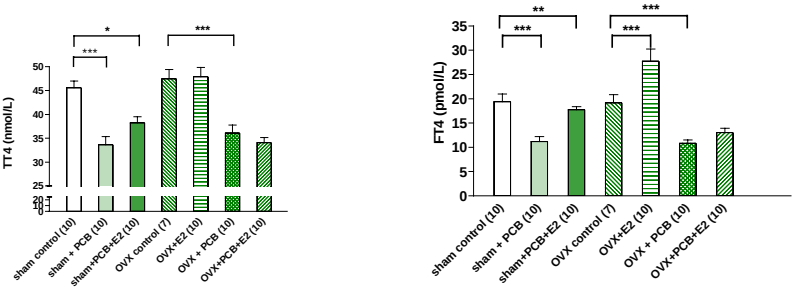

Fig 2. Effects of concomitant 17 β -estradiol supplementation and PCB126 exposure on thyroxin levels in serum of rat.

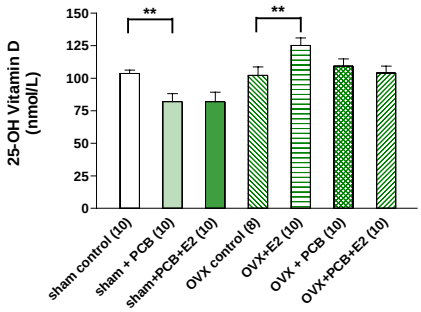

Fig 3. Effects of concomitant 17 β -estradiol supplementation and PCB126 exposure on 25-OH Vitamin D levels in serum of rat.

References:

1. Jämsa, T., M. Viluksela, J.T. Tuomisto, J. Tuomisto, and J. Tuukkanen, Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on bone in two rat strains with different aryl hydrocarbon receptor structures. J Bone Miner Res, 2001. 16(10): p. 1812-20.
2. Lind, P.M., E.F. Eriksen, L. Sahlin, M. Edlund, and J. Örberg, Effects of the antiestrogenic environmental pollutant 3,3',4,4', 5- pentachlorobiphenyl (PCB #126) in rat bone and uterus: diverging effects in ovariectomized and intact animals. Toxicol Appl Pharmacol, 1999. 154(3): p. 236-244.
3. Lind, P.M., S. Larsson, H. Oxlund, H. Håkansson, K. Nyberg, T. Eklund, and J. Örberg, Change of bone tissue composition and impaired bone strength in rats exposed to 3,3´, 4,4´, 5-pentachloro-biphenyl (PCB126). Toxicology, 2000. 150(1-2): p. 43-53.
4. Lind, P.M., E.F. Eriksen, L. Lind, J. Örberg, and L. Sahlin, Estrogen Supplementation Modulates Effects of the Endocrine Disrupting Pollutant PCB126 in Rat Bone and Uterus. Diverging Effects in Ovariectomized and Intact Animals. Toxicology, in press.
5. Lind, P.M., M.R. Milnes, R. Lundberg, D. Bermudez, J. Örberg, and L.J. Guillette, Jr., Abnormal Bone Composition in Female Juvenile American Alligators from a Pesticide Polluted Lake (Lake Apopka, FL, USA). Environmental Health Perspectives, 2004. 112 .(3): p. 359-362.
6. Lind, P.M., A. Bergman, M. Olsson, and J. Örberg, Bone mineral density in Baltic grey seal (Halichoerus grypus) in relation to pollution impact. Ambio, 2003. 32(6): p. 385-8.
7. Alveblom, A.K., L. Rylander, O. Johnell, and L. Hagmar, Incidence of hospitalized osteoporotic fractures in cohorts with high dietary intake of persistent organochlorine compounds. Int Arch Occup Environ Health, 2003. 76(3): p. 246-8.