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EFFECTS OF PFOA ON BONE MORPHOLOGY AND BONE CELL DIFFERENTIATION - RESULTS FROM IN VITRO, IN VIVO AND MASS-SPECTROMETRY STUDIES

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Introduction

Perfluorooctanoic acid (PFOA), a member of perfluoroalkylated substances (PFAS), has been widely used in surfactant and polymer industries because of its stability and surface tension-lowering properties. As a result PFAS are widely distributed in wild life, human tissues and sediments (Zareitalabad, et al. 2013). Due to toxicological findings, e.g. neonatal and reproduction mortality, in combination with the physicochemical properties, PFOA has been proposed as a substance of very high concern (SVHC) (EHCA 2013).

PFOA is an agonist of the peroxisome proliferator-activated receptor α (PPAR-α), and to some extent PPAR-γ and β/δ, which have important roles in lipid metabolism, energy homeostasis and cell differentiation (Poulsen, et al. 2012). Bone is a potential target for PPAR agonists as PPARs are expressed in osteoblasts and osteoclasts (Chan, et al. 2007, Giaginis, et al. 2007).

Disturbances in bone remodelling can lead to osteoporosis or osteopetrosis. Many environmental chemicals have been found to affect bones in vivo and bone cells in vitro (Korkalainen, et al. 2009, Koskela, et al. 2012, Tsukamoto, et al. 2004). Likewise, PFOS has been shown to accumulate in mouse bone and bone marrow (Bogdanska, et al. 2011) and PFOA has been detected in human rib bone samples (Perez, et al. 2013). These findings led us to investigate whether bone acts as a target tissue for PFOA toxicity.

Materials and methods

Samples

Six pregnant C57BL/6/Bkl mice received either PFOA mixed with food pellets at dose of 0.3 mg/kg/day throughout pregnancy (total dose 6.3 mg/kg) or pellets alone (controls). Offspring were weaned on postnatal day 21 and sampled at 13 (n=5) or 17 (n=5) months of age. Tibias and femurs were dissected free of mucle and stored in PBS in -20 °C for further investigations. All mice experiments were performed in accordance with the rules of the Swedish animal protection legislation and were approved by the local Animal Ethics Committee (Stockholms Norra Djurförsöksetiska Nämnd).

Bone bank samples

Human femoral head samples (2 females, 10 males, age 53-79 years) were obtained from the Oulu University Hospital Bone Bank to be analysed with μCT and mass-spectrometry. Special National Supervisory Authority for Welfare and Health (Valvira) granted a permission for use of aged cadaver and live donor bone collection of clinically unusable specimens for research purposes.

Bone analyses

Mouse femurs and tibias were scanned with a μCT, and biomechanical properties of diaphysis and femoral neck were measured by 3-point bending method.

For chemical analyses, the femurs and tibias were powderized in a liquid nitrogen mill. In mice, tibias and femurs of each group were pooled in order to gain enough sample. PFOA was extracted from the homogenized bone powder with ammonium acetate in methanol and analysed with LC-MS/MS. In addition to PFOA, from human bone and bone marrow samples, we analysed PFNA, PFDA, PFUnA, PFDoA, PFTrA, PFTeA, PFHxS, PFHpS, PFOS and PFDS as well.

Cell cultures

Femurs and tibias of two mice were dissected free from adhering tissue, bone ends were removed and bone marrow centrifuged out, homogenized and resuspended.

Cells directed to osteoclasts were placed on bovine bone slices in differentiation medium. The next day, cells were exposed to 0, 0.1, 1, 10, 100 or 200 μM of PFOA dissolved in DMSO. Medium was changed every 3-4 days and the cells cultured for 10 days. Osteoclasts were counted after TRACP/ hoecsth-staining, and the resorption activity assessed by quantifying the total area of WGA-lectin stained resorption pits on a bone slice.

Murine calvarial osteoprogenitor MC3T3-E1 cells were induced to differentiate into osteoblasts. The next day, the cells were exposed as described above. Cell viability was assessed with MTT-test and osteogenic differentiation with both chemical and gene expression analysis of alkaline phosphatase (ALP) and osteocalcin (OCN).

Cell experiments were also repeated with human bone marrow stromal cells and monocytes acquired from total hip arthroplasty patients, who had given a written permission for research use of the cells.

Results and discussion

In LC-MS/MS analyses, the mouse bone PFOA concentrations of controls were 0.73 and 0.64 ng/g ww at the age of 13 and 17 months, respectively, and those of PFOA exposed mice 3.0 and 3.7 ng/g ww at 13 and 17 months, respectively. Human trabecular bone and bone marrow samples contained PFOA 0.15-1.10 ng/g and PFOS 0.16-2.10 ng/g.

In mouse femurs, the bone marrow area of the exposed group was 10% higher than in controls. In tibias, the cortical bone mineral density of the exposed group was 2.5% lower than in controls. In trabecular bone, no significant differences were observed. Biomechanical properties did not differ between groups, but there was a trend for increasing maximum force in both exposed age groups. In human samples, no linear correlation was seen between uCT parameters and PFAS concentrations.

MTT-tests showed decreased mesenchymal stem cell viability at two highest concentrations. The amount of calcium peaked at 1 and 10 μM, and then drastically decreased. The same effect was observed in osteocalcin at day 7. In osteoclast cultures, the number of cells increased concentration-dependently reaching statistical significance at 10 μM and above. Low concentrations stimulated the resorption activity, which peaked at 1 μM, but higher concentrations reduced it. We also confirmed this phenomenon with human-derived osteoclasts.

This study identified bone as a target tissue of PFOA both in vivo and in vitro. Maternal exposure to PFOA during pregnancy resulted in accumulation of the compound in bones of the offspring, from which the elimination was so slow that elevated concentrations were detectable even at the age of 17 months, which is equivalent to middle-age in humans. Low concentrations of PFOA promoted the differentiation of osteoblasts and increased the number of osteoclasts and their resorption activity, but higher concentrations inhibited both cell types, as the degree of cell viability was decreased. Furthermore, mild alterations in bone morphometry and decreased bone mineral density were still detectable at the age of 17 months.

In conclusion, this study confirmed that PFOA accumulates in bone also in humans, is eliminated very slowly and can affect bone homeostasis both directly via bone cells, and indirectly via increased body weight and decreased activity. PFOA levels were also detectable in control mice, suggesting that even in controlled laboratory environment exposure is possible.

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