Chemosphere 164 (2016) 339–346

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Low-dose developmental exposure to bisphenol A alters the femoral bone geometry in wistar rats

M.H. Lejonklou [a, *], S. Christiansen [b], J. Örberg [c], L. Shen [a, 1], S. Larsson [d], J. Boberg [b], U. Hass [b], P.M. Lind [a]

[a] Department of Medical Sciences, Occupational and Environmental Medicine, Box 256, Uppsala University, SE-751 85 Uppsala, Sweden
[b] Division of Diet, Disease Prevention and Toxicology, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark
[c] Department of Environmental Toxicology, Evolutionary Biology Centre, Box 256, Uppsala University, SE-75105 Uppsala, Sweden
[d] Department of Surgical Sciences, Section of Orthopedics, Box 256, Uppsala University, SE-75185 Uppsala, Sweden

HIGHLIGHTS

- The bone is an endocrine organ and thus potentially sensitive to endocrine disruption.
- A large number of litters as well as dose groups are included in the study.
- Low-dose BPA exposure (25 μg/kg bw/day) altered the femoral geometry.
- The female femur length, and male diaphyseal cortical thickness, was affected.
- Results of the present study suggest that current risk assessments need re-evaluation.

ARTICLE INFO

Article history:
Received 1 July 2016
Received in revised form
22 August 2016
Accepted 23 August 2016
Available online 2 September 2016

Handling Editor: Shane Snyder

Keywords:
Bisphenol A (BPA)
Endocrine disrupting chemicals (EDCs)
Low dose effects
Developmental exposure
Bone geometry
Bone biomechanics

ABSTRACT

Background: Bisphenol A (BPA) is a chemical produced in large volumes for use in manufacturing of consumer products and industrial applications, and an endocrine disruptor known to affect several hormonal systems. Bone produces hormones and is additionally a sensitive hormone target tissue, and is thus potentially sensitive to low doses of endocrine disruptors such as BPA, especially during development.
Methods: 110 pregnant Wistar rats were gavaged with 0; 25 μg; 250 μg; 5000 μg or 50,000 μg BPA/kg bodyweight (bw)/day from gestational day 7 until weaning at postnatal day 22. The three-month-old offspring were sacrificed and right femurs collected for length measurements, geometrical measurements by peripheral quantitative computed tomography (pQCT), as well as for analyses of biomechanical properties using the three-point-bending method.
Results: The femur was elongated in female offspring of dams exposed to 25 or 5000 μg BPA/kg bw/day (1.8% and 2.1%, respectively), and increased cortical thickness (4.7%) was observed in male offspring of dams exposed to 25 μg BPA/kg bw/day, compared to controls ($p < 0.005$). The biomechanical properties of the bone were not significantly altered.
Conclusions: *In utero* and lactational exposure to the lowest BPA dose used in this study altered femoral geometry in both male and female offspring. This was observed at 25 μg BPA/kg bw/day, a dose lower than the Human Equivalent Dose (HED) applied by EFSA to set a temporary TDI (609 μg BPA/kg bw/day), and far lower than the No-Observed-Adverse-Effect-Level (NOAEL) (5000 μg BPA/kg bw/day) on which the US FDA TDI is based.

1. Introduction

Bisphenol A (BPA) is a chemical produced in billions of tons every year, and a known endocrine disruptor which affects several hormonal systems (Casals-Casas and Desvergne, 2011; Rubin,

* Corresponding author. Uppsala University, Department of Medical Sciences, Section of Occupational and Environmental Medicine, S-751 85 Uppsala, Sweden.
E-mail address: margareta.halin@medsci.uu.se (M.H. Lejonklou).
[1] Present address: Department of Organismal Biology, Evolutionary Biology Centre, Box 256, SE-751 05 Uppsala, Sweden.

http://dx.doi.org/10.1016/j.chemosphere.2016.08.114
0045-6535/

2011). BPA, and other bisphenols, are used in the manufacturing of numerous consumer products such as food containers, beverage bottles, can linings, dental sealants, thermal paper, and medical equipment, as well as in industrial applications and is thus widespread in the environment. When a BPA-containing product is subjected to heat, or basic or acidic solutions, the BPA polymer is hydrolyzed, and the BPA monomers can then leach out into the surrounding (Welshons et al., 2006; Vandenberg et al., 2007). BPA in thermal paper is in the free, monomeric, form (Hormann et al., 2014). As a consequence of BPA being ubiquitous, it can be detected in the urine in the great majority of the population (Calafat et al., 2008; Zhang et al., 2011; Guidry et al., 2015).

The skeleton has previously been regarded as a mainly structural organ, but research has revealed that bone is an important regulator of *e. g.* fertility and glucose metabolism (Oldknow et al., 2015). Thus, bone additionally functions as an endocrine organ, starting to form during embryogenesis. In humans this process begins with formation of cartilage around the fourth week of development, and as the fetus develops, the chondroblasts (cartilage-forming cells) are replaced with osteoblasts, and ossification begins. Bone growth is controlled by several hormones, including growth hormone, thyroid and parathyroid hormones, as well as the sex hormones testosterone and estrogen, of which the latter governs epiphyseal plate closure at the later stage of puberty, thereby ending longitudinal bone growth (Kovacs, 2014). In rats, however, despite the fact that the growth plate does not fully close, the longitudinal growth ceases with age (Roach et al., 2003).

Both developing and adult bone have earlier been reported to be affected by persistent environmental contaminants, in experimental settings (Miettinen et al., 2005; Yilmaz et al., 2006; Hermsen et al., 2008; Alvarez-Lloret et al., 2009), in wildlife (Lind et al., 2003, 2004; Sonne et al., 2004; Fox et al., 2008), domestic animals (Lundberg et al., 2006; Lind et al., 2009), and in humans (Glynn et al., 2000; Alveblom et al., 2003; Hodgson et al., 2008). Concerning bone effects of exposure to low doses of non-persistent endocrine disrupting compounds, Pelch et al. developmentally exposed C57BL/6J mice to low doses of ethinyl estradiol (EE_2), diethylstilbestrol (DES), or BPA. Following BPA exposure (10 μg BPA/kg bw/day) a significant elongation of the male femur bone was observed, whereas EE_2 and DES exposures caused a significant elongation of the female femur. Further, they reported that EE_2 and DES decreased the torsional strength of the female bone to a greater extent than BPA (Pelch et al., 2012). BPA has been shown to be a selective estrogen receptor modulator (SERM), which implies that it does not induce the same effects as estrogens or classical estrogenic substances, but rather has different modes of actions in different types of cells and tissues (Kuiper et al., 1997; Gould et al., 1998; Diel et al., 2000; Routledge et al., 2000).

The aim of the present study was to evaluate effects on bone geometry and bone strength of developmental exposure to BPA (0; 25; 250; 5000; and 50,000 μg/kg bw/day), using peripheral Quantitative Computational Tomography (pQCT) (Gasser, 1995; Lind et al., 2011) and biomechanical measurements (Turner and Burr, 1993; Miettinen et al., 2005). In the study the lowest BPA-dose corresponds to 50% of the US Food and Drug Administration (FDA) reference dose (RfD) of 50 μg/kg bw/day (US Food and Drug Administration, 2008), and is 24 times lower than the human equivalent dose (HED) of 609 μg/kg bw/day applied by the European Food Safety Authorities (EFSA) to set a temporary Tolerable Daily Intake (TDI) (European Food Safety Authority, 2015). The two highest doses (5000 and 50,000 μg/kg bw/day) correspond to regulatory No-Observed-Adverse-Effects-Levels (NOAEL) used to derive reference values (Tyl et al., 2002, 2008). The bones analyzed in the present study originate from a study on Wistar rats examined for the effects of developmental BPA exposure, from gestational day 7 until weaning at postnatal day 22, on early sexual and mammary gland development, sperm count, as well as on behavior (Christiansen et al., 2014; Hass et al., 2016; Mandrup et al., 2016).

2. Materials and methods

2.1. Materials

BPA (CAS no. 80-05-7; purity >99.5%) and corn oil were bought from Sigma-Aldrich (Brøndby, Denmark). Corn oil was delivered in glass bottles. The BPA solutions were kept at room temperature in the dark, and were continuously stirred during the period of dosing.

2.2. Animals

110 time-mated Wistar rats (HanTac: WH, SPF, Taconic Europe, Ejby, Denmark) arrived at gestational day 3 (GD 3). The day of detection of a vaginal plug was designated GD 1. The dams were housed in pairs until GD 17, and after GD17 housed individually in poly-sulfone cages (semi-transparent PSU 80-1291 HOOSU Type III, Tecniplast, Buguggiate, Italy; 15 × 27 × 43 cm) under standard conditions. Aspen wood-chip bedding (Tapvei, Gentofte, Denmark), Enviro Dri nesting material, and Tapvei Aspen wood shelters (Brogaarden, Denmark) were used. The animal room was maintained with a 12-h light-dark cycle (500 lux light intensity), 55% ± 5 humidity, temperature 21 °C ± 1 °C, and the air turnover was 10 times per hour. All rats were given standard soy and alfalfa-free ALROMIN 1314 diet (ALTROMIN GmbH, Lage, Germany). Individual body-weight was recorded daily from GD 7 for dams, and the pups were weighed on postnatal day (PD) 7 and 14. Tap water was provided *ad libitum* in poly-sulfone bottles (84-ACBTO702SU Tecniplast). The animal experiments were performed at the DTU National Food Institute facilities (Mørkhøj, Denmark).

2.3. Ethical statement

The Danish Animal Experiments Inspectorate approved the experiments (authorization number 2012-15-2934-00089 C4), and the National Food Institute's In-house Animal Welfare Committee for Animal Care and Use followed the procedures.

2.4. Experimental design

On GD 4, the 110 dams were randomly distributed into five groups (0, 25 μg, 250 μg, 5000 μg, and 50,000 μg BPA/kg bw/day), with 22 rats of similar mean body-weight per group. BPA was dissolved in corn oil, and the dams were gavaged (stainless probe 1.2 × 80 mm) once daily from GD 7 until weaning at PD 22, at a constant volume of 2 ml/kg bw. The dams in the control group received corn oil. The litters were not culled. Maximum numbers of pups from each dam included in the present bone sub-study were one female and one male (n = 14–21 per sex). The experiment was performed in three blocks, separated with one week, with dams from the same dose group equally distributed among blocks. For further details on the experimental setup, we refer to the paper by Christiansen et al. (Christiansen et al., 2014).

2.5. Preparation of bones

Pups were sacrificed at three months of age, and right femurs from the 89 male and 93 female offspring were dissected, cleaned and placed in centrifuge tubes (10 ml) with Ringer solution (pH 7.4, Tris 0.3 g/l, NaCl 9 g/l, $CaCl_2 \cdot 2H_2O$ 0.24 g/l, KCl 0.4 g/l, 2.05×10^{-3} M HCl) and frozen at −18 °C until used for peripheral quantitative computed tomography (pQCT) and biomechanical testing.