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Exposure to bisphenols and parabens during pregnancy and relations to steroid changes

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ABSTRACT

Background: The harmful effects of endocrine disrupting compounds (EDCs) on human health are generally well-known, and exposure during fetal development may have lasting effects. Fetal exposure to bisphenol A (BPA) has been recently relatively well-studied; however, less is known about alternatives such as bisphenol S (BPS), bisphenol F (BPF) and bisphenol AF (BPAF), which have started to appear in consumer products. Parabens are another widespread group of EDCs, with confirmed transplacental passage. The usage of many cosmetic, pharmaceutical and consumer products during the pregnancy that may contain parabens and bisphenols has led to the need for investigation.

Objectives: To shed more light into the transplacental transport of BPA, its alternatives, and parabens, and to study their relation to fetal steroidogenesis.

Methods: BPA, BPS, BPF, BPAF, methylparaben, ethylparaben, propylparaben, butylparaben, benzylparaben and 15 steroids including estrogens, corticoids, androgens and immunomodulatory ones were determined in 27 maternal (37th week of pregnancy) and cord plasma samples using liquid chromatography - tandem mass spectrometry methods.

Results: In cord blood, significantly higher BPA levels ($p = 0.0455$) were observed compared to maternal plasma. The results from multiple regression models showed that in cord blood, methylparaben ($\beta = -0.027$, $p = 0.027$), propylparaben ($\beta = -0.025$, $p = 0.03$) and the sum of all measured parabens ($\beta = -0.037$, $p = 0.015$) were inversely associated with testosterone levels.

Conclusion: To the best of our knowledge, this is the first study reporting the simultaneous detection of BPA, alternative bisphenols, parabens and steroids in maternal and cord plasma. Our study confirmed the transplacental transport of BPA, with likely accumulation in the fetal compartment. The negative association of cord blood parabens and testosterone levels points to possible risks with respect to importance of testosterone for prenatal male development.

1. Introduction

Throughout our lives, we are exposed to multiple natural and man-made chemicals which can act as endocrine disrupting compounds (EDCs). Of the anthropogenic ones, bisphenol A (BPA) is one of the most widely known and has been detected among many populations in various body fluids. BPA is used in the manufacture of polycarbonate and other plastics and epoxy resins, and is known to be released from various types of consumer plastic products such as food packaging, plastic containers for food and drinks, metal can linings, dental fillings, medical equipment or thermal receipts (Vandenberg et al., 2007). Besides estrogenic effects, BPA also interacts with other receptors such as the androgen, glucocorticoid or thyroid receptors (Teng et al., 2013; Sargis et al., 2010; Moriyama et al., 2002). It is also known to affect cellular antioxidant mechanisms and disrupts cell differentiation (Hotchkiss et al., 2008).

The major route of BPA exposure is the ingestion of contaminated food and drinks. BPA is rapidly absorbed from the gastrointestinal tract and then metabolized in the liver and intestine. At least 98% is conjugated to form BPA-glucuronides and BPA-sulfates, which are excreted via urine. In dermal and inhalation exposure, however, the first-pass liver effect is circumvented (Vandenberg et al., 2007). In the

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circulation, a maximum of 10% of BPA is free (active), while the majority is bound to various plasma proteins; however, in comparison with estrogens, this unbound fraction is quite large (Vandenberg et al., 2007; Vinas and Watson, 2013). Moreover, BPA is also known to possess lipophilic properties and therefore has the potential to accumulate in the fat stores (Vandenberg et al., 2007). There is also evidence that BPA undergoes transplacental transport (Balakrishnan et al., 2010; Pinney et al., 2017). BPA exposure has been associated with various adverse health effects in adults as well as in children. Higher BPA in children has been associated with obesity, insulin resistance, increased BMI, respiratory disorders and even impaired neurobehavioral effects (Mikolajewska et al., 2015). In connection to female reproductive health and pregnancy, the presence of BPA has been linked with reduced oocyte maturation, miscarriages, increased risk of preterm delivery, preeclampsia, shortened gestation length, infant anthropometric measures at birth, and a shorter anogenital distance in male offspring (Pinney et al., 2017; Giulivo et al., 2016; Snijder et al., 2013; Cantonwine et al., 2015; Leclerc et al., 2014; Miao et al., 2011; Kolatorova et al., 2017).

In the European Union (EU), the main regulating organ concerning the usage of EDCs is the European Chemical Agency (ECHA) establishing the REACH. The directive REACH represents the Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals and constitutes the framework legislation on chemicals in the EU (Regulation (EC) No 1907/2006). The legislation is still developing. In context with the actual scientific knowledge, the European Commission amending the annexes to the Council concerning REACH as well as the regulations of the European Parliament (https://echa.europa.eu, 2018). Another important agency of the EU is the European Food Safety Authority (EFSA) that provides independent scientific advice and communicates on existing and emerging risks associated with the food chain. EFSA supports the European Commission, the European Parliament and EU member states in taking effective and timely risk management decisions that ensure the protection of the health and food safety. Both authorities (ECHA and EFSA) are developing the scientific guidance to enable EDCs to be identified (https://www.efsa.europa.eu, 2018).

In the light of its negative effects, BPA has been restricted in infant feeding bottles across the EU since 2011 (Commission regulation (EU) No. 10/2011). In Belgium, Sweden and Denmark, it is also banned in other plastics coming into contact with food for infants and children under three years of age. France has banned BPA in all food packaging, containers and utensils. In the rest of the EU, BPA is permitted in plastics which are in contact with food; however, there is a maximum quantity allowed to leach out of the material (0.6 mg/kg). According to the EFSA opinion (2015), the European Commission proposed to lower this limit to 0.05 mg/kg and also introduce similar limit for varnished or coated materials of articles intended to come into contact with food (https://echa.europa.eu, 2018). Concerning BPA exposure during pregnancy, information and awareness still seem to be insufficient. In the EU, there is a current limit of 0.1 mg/L of the BPA amount that is allowed to leach out of toys for children up to three years and in toys intended to be placed in a child's mouth. In July 2016, the European Commission published a proposal to lower that limit to 0.04 mg/L that should come into force in 2018. Moreover, the European Commission resolved that by March 2018 all BPA containing products must label BPA as a substance “toxic for reproduction, category 1B” (https://echa.europa.eu/chemicals-in-our-life/hot-topics/bisphenol-a, 2017).

During the process of legislative restrictions, many “BPA-free” products were introduced to the market. These plastics are presented as being safe, but they still may contain other bisphenols substituting for BPA. The list of such bisphenol alternatives is currently long and still growing. Bisphenol S (BPS), bisphenol F (BPF) and bisphenol AF (BPAF) are among of the most discussed (Caballero-Casero et al., 2016). These alternative bisphenols may substitute for BPA, or be combined with BPA to achieve the allowed limits. The total bisphenol levels may be therefore much higher than the levels of BPA alone. Moreover this situation complicates evaluations of the BPA risk assessments, and these alternative bisphenols arouse further concern (Sartain and Hunt, 2016). Thus the potential risk for a fetus from the total bisphenol exposure during pregnancy as well as in the postnatal life is an important issue, and the general notion that “BPA-free” products should be considered safe needs to be evaluated, especially during pregnancy (Kolatorova et al., 2017).

In addition, a possible “cocktail effect” arising from the addition or multiplication of the effects of multiple EDCs needs to be investigated. Therefore, in addition to BPA and alternative bisphenols, we also analyzed parabens. These are a group of substances used as antimicrobial agents and preservatives, predominately in cosmetics and pharmaceuticals, but also in food and industrial products. Structurally they are esters of p-hydroxybenzoic acid with various alkyl substituents (Bledzka et al., 2014). The antimicrobial activity as well as the estrogen activity tends to increase with the alkyl substituent length. The most commonly used are methylparaben (MP) and propylparaben (PP), which often occur together. Ethylparaben (EP), butylparaben (BP) and benzylparaben (BenzylP) are also worth mentioning. The paraben family has been listed as EDCs, and their use is now regulated in the EU (Regulation (EC) No. 1223/2009 of the European Parliament), the USA and Canada (0.4% of content for a single paraben, 0.8% for paraben mixtures) (Bledzka et al., 2014; Buzek and Ask, 2009; Boberg et al., 2010). The use of food parabens as food additives is authorized in the EU by regulation (EC) No. 1333/2008 of the European Parliament. In 2013, the Scientific Committee on Consumer products of the European Commission stated that although MP and EP are considered to be safe, there is not sufficient data to complete a safety statement on either PP or BP (SCCS Scientific Committee on Consumer Safety, 2013). In the light of current studies, starting in March 2017 ECHA plans to perform various reproduction tests to obtain more relevant information about PP toxicity, with results updated until 17 June 2019 (European Chemicals Agency, 2017). Besides the estrogenic effect, several parabens have also been reported to bind to androgen receptors and possess anti-androgenic activity as well as causing the inhibition of testosterone-induced transcription (Bledzka et al., 2014).

Parabens may enter the human body through ingestion, skin absorption and inhalation. After ingestion, parabens are metabolized by esterases in the intestine and liver to p-hydroxybenzoic acid, which is later conjugated with glycine to form p-hydroxyhippuric acid and excreted in the urine as well as bile and feces (Boberg et al., 2010; Vela-Soria et al., 2014). When applied transdermally, parabens with shorter alkyl chains can cross the stratum corneum more easily than their longer chain analogues (Vela-Soria et al., 2014; Andersen, 2008). During pregnancy, the area of skin used for application of various cosmetic products, especially for stretch marks, increases. As a result of the daily application of paraben-containing cosmetics, parabens may accumulate in the body (Kolatorova et al., 2017; Mathiesen et al., 2013). Recent studies have also highlighted the need for awareness on paraben exposure in infants, whose detoxification system is still immature (SCCS Scientific Committee on Consumer Safety, 2013). *In vivo* studies have suggested that parabens may impair reproduction, development and homeostasis. In humans, parabens have been detected in serum, milk, amniotic fluid, placental tissue and cord blood (Vela-Soria et al., 2014; Philippat et al., 2013; Towers et al., 2015; Azzouz et al., 2016). Parabens have been found to be able to cross the human placenta and affect the fetus. Human studies have found associations between parabens and oxidative stress, sperm DNA damage, and serum thyroid hormones (Giulivo et al., 2016; Bledzka et al., 2014).

In the present work we evaluated the intrauterine exposure of selected EDCs (BPA, their alternatives, and parabens) by measuring their content in cord and maternal plasma. In all samples selected steroid hormones that may be influenced by EDCs were also determined. In addition, immunomodulatory 7-oxygenated dehydroepiandrosterone steroid metabolites, which counteract the main stress steroid cortisol,