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Associations of bisphenol A and polychlorinated biphenyls with spermatogenesis and steroidogenesis in two biological fluids from men attending an infertility clinic

Jana Vitku [a,*], Jiri Heracek [b,c], Lucie Sosvorova [a], Richard Hampl [a], Tereza Chlupacova [a], Martin Hill [a], Vladimir Sobotka [d], Marie Bicikova [a], Luboslav Starka [a]

[a] Department of Steroids and Proteofactors, Institute of Endocrinology, Prague, Czech Republic
[b] Department of Urology, First Faculty of Medicine, Charles University in Prague, Czech Republic
[c] Department of Urology, Military University Hospital Prague, Czech Republic
[d] Department of Urology, Third Faculty of Medicine, Charles University in Prague, Czech Republic

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ABSTRACT

Background: In the testis, steroid hormones play an important role in spermatogenesis, the production of semen, and the maintenance of secondary sex characteristics and libido. They may also play a role as a target for substances called endocrine disruptors (EDs). As yet, however, no complex study has been conducted evaluating the relationships between EDs and the steroid spectrum in the plasma and seminal plasma.
Objectives: To shed more light into mechanisms of EDs and the effects of bisphenol A (BPA) and polychlorinated biphenyls (PCBs) on human spermatogenesis and steroidogenesis.
Methods: We determined BPA and 11 steroids in the plasma and seminal plasma of 191 men with different degrees of fertility, using a newly developed liquid-chromatography mass spectrometry method. Concurrently, plasma levels of 6 congeners of PCBs, gonadotropins, selenium, zinc and homocysteine were measured. Partial correlations adjusted for age, BMI and abstinence time were performed to evaluate relationships between these analytes.
Results: Seminal BPA, but not plasma BPA, was negatively associated with sperm concentration ($r = -0.198$; $p = 0.009$), sperm count ($r = -0.178$; $p = 0.018$) and morphology ($r = -0.160$; $p = 0.044$). Divergent and sometimes opposing associations of steroids and BPA were found in both body fluids. The sum of PCB congeners was negatively associated with testosterone, free testosterone, the free androgen index and dihydrotestosterone in plasma.
Conclusion: BPA may negatively contribute to the final state of sperm quality. Moreover, our data indicate that BPA influences human gonadal and adrenal steroidogenesis at various steps. Environmental levels of PCBs negatively correlated with androgen levels, but surprisingly without negative effects on sperm quality.

1. Introduction

For nearly 30 years there has been ongoing debate regarding the potential harm of substances called endocrine disruptors (EDs). Many studies have dealt with the effects of EDs on various body organs, and despite the difficulties accompanying this research there is emerging evidence for adverse impacts on humans (for review see Diamanti-Kandarakis et al., 2009). The most often discussed EDs include polychlorinated biphenyls (PCBs), dioxins, phthalates and bisphenol A (BPA) because of their persistence in the environment and accumulation in biomass (PCBs and dioxins), as well as their ubiquity in food packaging and other materials (phthalates, BPA). People come into contact with these EDs in the environment and cannot completely avoid exposure in everyday life. The main routes of exposure are through the intake of food, water and air. A further pathway is through dermal contact (Darbre, 2015). Regarding reproduction, in 2007 it was concluded based on animal studies that there is substantial evidence of even low dose effects of BPA on reproductive health, specifically a reduction in spermatogenesis (vom Saal et al., 2007).

Human studies investigating the effects of BPA on sperm quality (Meeker et al., 2010b; Mendiola et al., 2010; Li et al., 2011; Goldstone et al., 2014; Knez et al., 2014; Lassen et al., 2014; Vitku et al., 2015b)

Abbreviations: DHEA, dehydroepiandrosterone; T, testosterone; ADIONE, androstenedione; PREG, pregnenolone; 17-OH-PREG, 17-hydroxy-pregnenolone; DHT, dihydrotestosterone; E1, estrone; E2, estradiol; E3, estriol; LH, luteinizing hormone; FSH, follicle stimulating hormone; SHBG, sex hormone-binding globulin; FT, free testosterone; FAI, free androgen index.

* Corresponding author at: Department of Steroids and Proteofactors, Institute of Endocrinology, Narodni 8, 11694 Prague 1, Czech Republic.
E-mail address: jvitku@endo.cz (J. Vitku).

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and reproductive hormone levels (Galloway et al., 2010; Kim et al., 2014; Lassen et al., 2014; Liu et al., 2015; Meeker et al., 2010a; Mendiola et al., 2010; Zhou et al., 2013; Zhuang et al., 2015) have not provided clear results. More studies have been conducted on the impact of PCBs on male reproductive functions, but have also been inconsistent (reviewed in Meeker and Hauser, 2010).

It is well known that some substances can adversely affect reproductive functions. Alternatively, other substances such as trace elements selenium (Se) and zinc (Zn) can positively influence these functions (Bleau et al., 1984; Colagar et al., 2009). Potentially beneficial agents include endogenous immunoprotective steroids with antiglucocorticoid effects as dehydroepiandrosterone (DHEA) and its 7-hydroxylated metabolites (Chmielewski et al., 2000; Hampl et al., 2003; Niro et al., 2010).

In 2015, expert panels were convened to estimate the burden and disease costs that can be ascribed to EDs, based on current evidence from the European Union (Trasande et al., 2015). According to relevant studies published by that time, low epidemiological and strong toxicological evidence for male infertility attributable to phthalate exposure were identified, resulting in a 40–69% probability of causing 618 000 additional assisted reproductive technology procedures costing € 4.71 billion annually (Hauser et al., 2015). These estimations focused on exposure to phthalates and polybrominated diphenyl ethers because their impact on reproductive functions was among the best documented in human as well as animal studies (Hauser et al., 2015).

Although the effects of some EDs on reproductive functions are well documented, there remain uncertainties regarding the effects and mechanisms of action of other EDs. The body of literature on animal as well as human studies suggests that EDs can disrupt steroid hormone homeostasis (Clark and Cochrum, 2007). Any impairment of the delicate balance in hormone biosynthesis and metabolism can have adverse consequences in the human organism.

In this study we attempted to elucidate to what extent the most well-known environmental endocrine disruptors, namely BPA and PCBs, are associated with male reproductive function. The following issues were addressed:

(1) To what degree BPA and PCBs influence spermiologic parameters with respect to different degrees of infertility (from normospermic to azoospermic men).
(2) In connection with this, we were interested in how blood- and seminal plasma levels of the measured analytes correlate with each other.
(3) How the levels of the main reproductive hormones, steroids as well as gonadotropins correlate with the above mentioned EDs. In contrast to numerous studies reported by others, we also measured the main precursors and intermediates, enabling us to map the possible effects of EDs on biosynthetic/metabolic pathways and thus to assess effects on the activities of the responsible enzymes.
(4) To elucidate possible effects on immunity, we also measured 7-oxygenated metabolites of DHEA, which have recently been shown to be present in seminal fluid (Hampl et al., 2000).
(5) In addition, two trace elements involved in the mechanism of reproductive functions, selenium and zinc, along with homocysteine, one of the parameters of oxidative stress (Forges et al., 2007), were measured to determine whether there are any associations with EDs.

2. Experimental

2.1. Chemicals and reagents

The steroids cortisol, cortisone and DHEA were from Koch-Light Laboratories Ltd. (Colnbrook, Great Britain); 7α-hydroxy-DHEA (7α-OH-DHEA), 7β-hydroxy-DHEA (7β-OH-DHEA), 7-oxo-DHEA, testosterone (T), androstenedione (ADIONE), pregnenolone (PREG), 17-hydroxypregnenolone (17-OH-PREG), and deuterated standards of DHEA (D3-DHEA), ADIONE (D7-ADIONE), PREG (D4-PREG), 17-OH-PREG (D3-17-OH-PREG) and dihydrotestosterone (D3-DHT) were from Steraloids (Newport, RI, USA). D4-Cortisol was obtained from CDN isotopes (Ponte-Claire, Canada). D1-7α-OH-DHEA and D1-7-oxo-DHEA were obtained from Betulinines (Stribrna Skalice, Czech Republic). D1-T was synthesized by Sci-Tech (Prague, Czech Republic). DHT, D7-cortisone, 2-hydrazinopyridine, ammonium formate and trifluoroacetic acid were from Sigma-Aldrich (St. Louis, MO, USA). Methanol and water for chromatography were of HPLC grade and were from Merck (Darmstadt, Germany). Diethyl ether was obtained from Lach-Ner, s.r.o. (Neratovice, Czech Republic). The physiological solution (0.9% sodium chloride) was from B. Braun (Melsungen AG, Germany).

2.2. Study group

The studied cohort consisted of 191 Czech men attending the Pronatal Centre of Assisted Reproduction (Prague, CZ) since April 2012. All of the subjects were race homogenous group of Caucasians. Some of the patients were normospermic men, where the cause of infertility was the female factor, and the others included patients with various degrees of impaired fertility. Each patient underwent a standardized ejaculate examination (spermiogram) according to the World Health Organization (WHO) 2010 criteria. Height and weight were measured, and a basic urological and andrological examination was performed including ultrasonography of the prostate, seminal vesicles and testicles, with no pathological findings observed. Samples of plasma and seminal plasma were collected from each patient on the same day. Patients underwent sample collection between 8 and 10 am due to circadian rhythm of majority of steroids. All steps in the sample collection protocol and subsequent processing were carried out using BPA-free glass equipment and stored in glass tubes at −20 °C until analysis. For details on how we dealt with possible BPA contamination, see our previous study (Vitku et al., 2015a). Men were divided into four groups according to their spermiogram. The first group included normospermic men with a normal spermiogram (n = 89); oligospermic, asthenospermic and oligoasthenospermic men were included in the second group (n = 59); teratospermic, oligoasthenoteratospermic and oligoteratospermic men comprised the third group (n = 25); while the fourth group were azoospermic men (n = 18). We termed these groups: (1) healthy men, and (2) slightly, (3) moderately and (4) severely infertile men. Basic relevant parameters (age, BMI and length of abstinence) about the subjects in individual groups are provided in a Table 1.

The study was performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The protocol was approved by the Ethical Committee of the Institute of Endocrinology. Informed and written consent with the use of biological materials for research reasons was obtained from all subjects participating in the project.

2.3. Development and validation of a LC–MS/MS method for determining 10 unconjugated steroids in plasma and 11 unconjugated steroids in seminal plasma

2.3.1. Sample preparation

Our previously published method on selected neuro- and immunomodulatory steroids in blood plasma (Sosvorova et al., 2015) was extended to include the determination of PREG, 17-OH-PREG, cortisol, cortisone, DHEA, 7α-OH-DHEA, 7β-OH-DHEA, 7-oxo-DHEA, T and ADIONE in plasma and in seminal plasma with minor modifications. In addition, DHT was determined in seminal plasma. Briefly, a sample of plasma (500 μL) or seminal plasma (1000 μL) was spiked with 10 μL of an internal standard (IS) mixture (D4-PREG, D3-17-OH-PREG, D3-DHEA, D1-7α-OH-DHEA, D1-7-oxo-DHEA, D4-Cortisol, D7-cortisone, D1-T, D7-ADIONE, D3-DHT) and diluted with 500 μL of physiological