Reproductive Toxicology 49 (2014) 65–73

Contents lists available at ScienceDirect

Reproductive Toxicology

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

Interactions between polymorphisms in the aryl hydrocarbon receptor signalling pathway and exposure to persistent organochlorine pollutants affect human semen quality

L.J.S. Brokken[a,*], P.J. Lundberg[a], M. Spanò[b], G.C. Manicardi[c], H.S. Pedersen[d], P. Struciński[e], K. Góralczyk[e], V. Zviezdai[f], B.A.G. Jönsson[g], J.P. Bonde[h], G. Toft[i], Y. Lundberg Giwercman[j], A. Giwercman[a,k]

[a] Department of Clinical Sciences Malmö, Molecular Reproductive Medicine, Lund University, SE-20502 Malmö, Sweden
[b] Laboratory of Toxicology, Unit of Radiation Biology and Human Health, ENEA Casaccia Research Centre, Via Anguillarese 301, IT-00123 Rome, Italy
[c] Department of Life Sciences, University of Modena and Reggio Emilia, IT-41125 Reggio Emilia, Italy
[d] Centre for Arctic Environmental Medicine, DK-3900 Nuuk, Greenland
[e] Department of Toxicology and Risk Assessment, National Institute of Public Health – National Institute of Hygiene, PL-00791 Warsaw, Poland
[f] Social Medicine and Organization of Public Health, Kharkiv National Medical University, UA-61022 Kharkiv, Ukraine
[g] Division of Occupational and Environmental Medicine, Lund University, SE-22185 Lund, Sweden
[h] Department of Occupational and Environmental Medicine, Copenhagen University Hospital, DK-2400 Copenhagen, Denmark
[i] Danish Ramazzini Centre, Department of Occupational Medicine, Aarhus University Hospital, DK-8000 Aarhus, Denmark
[j] Department of Clinical Sciences Malmö, Molecular Genetic Reproductive Medicine, Lund University, SE-20502, Malmö, Sweden
[k] Reproductive Medicine Centre, Skåne University Hospital, SE-20502, Malmö, Sweden

ARTICLE INFO

Article history:
Received 11 February 2014
Received in revised form 17 June 2014
Accepted 21 July 2014
Available online 30 July 2014

Keywords:
Cross-sectional studies
Gene–environment interaction
Genetic association studies
Single nucleotide polymorphism
Aryl hydrocarbon receptor
Aryl hydrocarbon receptor repressor
DNA damage

ABSTRACT

Persistent organic pollutants (POPs) may affect male reproductive function. Many dioxin-like POPs exert their effects by activation of the aryl hydrocarbon receptor (AHR) signalling pathway. We analysed whether gene–environment interactions between polymorphisms in AHR (R554K) and AHR repressor (AHRR P185A) and serum levels of markers of POP exposure 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene (*p,p′*-DDE) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) are associated with 21 parameters of male reproductive function in 581 proven-fertile European and Greenlandic men. In Greenlandic men, AHR variants significantly modified the association between serum levels of both *p,p′*-DDE and CB-153 and inhibin B levels, sperm chromatin integrity, and seminal zinc levels. In the total cohort, interactions between AHRR variants and serum levels of CB-153 were associated with sperm chromatin integrity and the expression of the pro-apoptotic marker protein Fas. The data indicate that susceptibility to adverse effects of POP exposure on male reproductive function is dependent on polymorphisms in genes involved in AHR signalling.

1. Introduction

Persistent organic pollutants (POPs), such as the organochlorines polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), dichloro diphenyl trichloroethane (DDT) and its major persistent metabolite 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene (*p,p′*-DDE), have been suggested to cause a variety of adverse health outcomes in wild life and humans. Whereas there is clear evidence from animal studies that exposure to POPs affects male reproductive health [1,2], the situation in humans is ambiguous [reviewed in 3]. Accidental exposure to POPs, especially during development of the reproductive system, has been shown to affect male reproduction [4,5] but data regarding lower exposure levels, as in the general population, is scarce. Interestingly, recently it was shown that even at extremely low doses, *p,p′*-DDE directly affects human sperm *in vitro* by targeting sperm-specific ion channels [6].

Dioxin-like PCDDs and PCDFs, and co-planar PCBs, exert their biological and toxicological effects by direct activation of the aryl hydrocarbon receptor (AHR) signalling pathway. Non-dioxin-like PCBs, such as CB-153, and other compounds, including DDT, DDE, as well as some polyphenols, activate AHR signalling indirectly

* Corresponding author at: Department of Clinical Sciences Malmö, Reproductive Molecular Medicine, Clinical Research Centre, Lund University, Jan Waldenströms gata 35, SE-205 02 Malmö, Sweden. Tel.: +46 40 391104; fax: +46 40 391222.
E-mail address: leon.brokken@med.lu.se (L.J.S. Brokken).

http://dx.doi.org/10.1016/j.reprotox.2014.07.073
0890-6238/

through inhibition of the metabolic turnover of the endogenous AHR ligand 6-formylindolo[3,2-*b*]carbazole (FICZ) [7–9]. Tissue levels of dioxin-like and non-dioxin-like PCBs have been shown to be highly correlated [10,11]. CB-153 is one of the most abundant PCB congeners in biological extracts and has been shown to be highly correlated with total PCB as well as with TCDD equivalents (TEQ) in plasma [11–14]. Serum levels of DDE have been shown to be highly correlated with those of CB-153 [15] and DDE decreases AHR protein expression in human placental cells [9]. Both CB-153 and DDE occur in relatively high concentrations in serum and can be determined accurately and to a reasonable cost. Therefore, in the current study, PCB153 and DDE are used as proxies for dioxin-like POPs that act through the AHR signalling pathway. The AHR, also known as the dioxin receptor, is a ligand-activated transcription factor that belongs to the family of basic helix-loop-helix Per/ARNT/Sim (bHLH/PAS) proteins [16]. Upon ligand binding at the PAS domain, the receptor migrates to the nucleus where it heterodimerises with AHR nuclear translocator (ARNT) protein [17]. Subsequently, the complex interacts with consensus dioxin or xenobiotic responsive elements in the enhancers/promoters of specific target genes including those encoding for the enzymes cytochrome P450 (CYP) 1A1, CYP1A2 and CYP1B1. AHR signalling is abrogated through a negative feedback mechanism, in which the AHR/ARNT heterodimer stimulates the expression of AHR repressor (AHRR) that competes with ARNT for binding to AHR [18]. Besides inducing the transcription of genes that activate metabolic and detoxification pathways, AHR signalling has also been shown to modulate the activity of other nuclear transcription factors, including oestrogen receptors (ERα and ERβ) [19,20] and androgen receptor [21,22].

Ethnic differences in the incidence of reproductive disorders, such as cryptorchidism, hypospadias [23], as well as testicular cancer [24], suggest that genetic components contribute to susceptibility to endocrine disruptors. Thus, genetic polymorphisms in genes involved in AHR signalling may account for differences in sensitivity for the adverse effects of POP exposure. The most widely studied single nucleotide polymorphism (SNP) in the human AHR is located in exon 10 and causes an arginine to lysine amino acid substitution at codon 554 (R554K; rs2066853) in the transactivation domain of the receptor [25]. Its functional significance is currently unclear as both upregulation (Smart and Daly, 2000) as well as loss [26] of transactivational activity have been reported for the lysine variant. Another common polymorphism has been described in the AHRR, leading to a proline-to-alanine amino acid change at codon 185 (P185A; rs2292596) [27]. This SNP has been associated with impaired semen quality [28,29] and with undermasculinisation of the external genitalia [30,31]. In this study we analysed whether these polymorphisms were associated with a broad range of parameters of male reproductive function, including sex hormone levels and markers of prostatic, epididymal, and accessory gland function, in proven fertile men. Additionally, we analysed if there was a possible gene–environment interaction between variants in the AHR signalling pathway and serum levels of *p,p′*-DDE and CB-153.

2. Materials and methods

2.1. Study populations

The present study is part of a European study on the impact of climate change on reproductive health (Climate change, environmental contaminants and reproductive health; CLEAR) using a uniform protocol for data collection in Greenland, Kharkiv in Ukraine, and Warsaw in Poland [32]. Men were included as partners of pregnant women who were consecutively listed when a pregnancy was recorded at the participating hospitals. The participation rates ranged from 29% in Warsaw, 33% in Kharkiv to 79% in Greenland. A detailed description of the study design and data collection procedures has been reported previously [33]. The men gave their informed consent and were asked to deliver a semen sample and a blood sample for CB-153 and *p,p′*-DDE analyses as well as for extraction of leucocyte DNA.

The study was approved by local ethical committees.

Background characteristics, reproductive hormone levels, semen characteristics, and levels of epididymal and accessory gland markers of the population included in the current study are presented in Tables 1 and 2. Not all analyses were available for all subjects, but earlier studies in the same cohort reported no evidence of selection bias within the total population [34,35].

2.2. Semen samples

Semen samples were obtained by masturbation at the residence or at the hospital. The participants were asked to abstain from sexual activities for at least 2 days before collection of the samples, and to report the actual abstinence time. The samples were analysed for sperm concentration, motility and morphology according to a manual for the project based on the World Health Organization manual for basic semen analysis [36] as described previously [37]. In short, sperm concentration was determined in duplicate using an improved Neubauer haemocytometer (Glaswarenfabrik Karl Hecht KG, Sondheim, Germany). Sperm motility was determined by counting the proportion of (a) fast progressive spermatozoa, (b) progressive spermatozoa, (c) nonprogressive motile spermatozoa, and (d) immotile spermatozoa. Sperm concentration and motility were determined locally, whereas sperm morphology from all centres was determined centrally at the Fertility Centre, Skåne University Hospital, Malmö on Papanicolaou-stained smears.

Sperm DNA fragmentation index (DFI) and high DNA stainability (HDS) were assessed centrally by the sperm chromatin structure assay (SCSA) at the Laboratory of Toxicology, ENEA Casaccia Research Centre, Italy, as described earlier [38] using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA). The percentage of abnormal spermatozoa with detectable DNA fragmentation was calculated from the DFI frequency histogram obtained from the ratio between red and total (red + green) fluorescence intensity. HDS was calculated based on the percentage of sperm with high levels of green fluorescence [39]. Strand breaks in DNA were determined using the terminal deoxynucleotidyl transferase-driven dUTP nick end labelling (TUNEL) assay and analysed using an Epics XL flow cytometer (Beckman Coulter, Fullerton, CA, USA) as described earlier [40].

Determination of percentage of sperm positive for the anti-apoptotic marker Bcl-xL and pro-apoptotic marker Fas was performed at the Laboratory of Toxicology, ENEA Casaccia Research Centre, Italy, and has been described in detail previously [40].

Sperm Y:X chromosome ratios were determined at Lund University by two-colour fluorescent *in situ* hybridisation using an Olympus AX-70 epifluorescence microscope equipped with single and double band pass filters as described in detail by Tiido et al. [41].

2.3. Accessory sex gland markers

Biochemical markers of epididymal function (neutral α-glucosidase, NAG), prostatic function (prostate-specific antigen (PSA) and zinc) and seminal vesicle function (fructose) were assessed as described before [42]. NAG activity was measured using a commercial assay (Episcreen; Fertipro, Beernem, Belgium) according to the manufacturer's protocol. Seminal PSA concentration was determined using the Prostatus kit (Wallac Oy, Turku,