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Dialkyl phosphate urinary metabolites and chromosomal abnormalities in human sperm

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ABSTRACT

Background: The past decade has seen numerous human health studies seeking to characterize the impacts of environmental exposures, such as organophosphate (OP) insecticides, on male reproduction. Despite an extensive literature on OP toxicology, many hormone-mediated effects on the testes are not well understood.
Objectives: This study investigated environmental exposures to OPs and their association with the frequency of sperm chromosomal abnormalities (i.e., disomy) among adult men.
Methods: Men ($n=159$) from a study assessing the impact of environmental exposures on male reproductive health were included in this investigation. Multi-probe fluorescence in situ hybridization (FISH) for chromosomes X, Y, and 18 was used to determine XX18, YY18, XY18 and total disomy in sperm nuclei. Urine was analyzed using gas chromatography coupled with mass spectrometry for concentrations of dialkyl phosphate (DAP) metabolites of OPs [dimethylphosphate (DMP); dimethylthiophosphate (DMTP); dimethyldithiophosphate (DMDTP); diethylphosphate (DEP); diethylthiophosphate (DETP); and diethyldithiophosphate (DEDTP)]. Poisson regression was used to model the association between OP exposures and disomy measures. Incidence rate ratios (IRRs) were calculated for each disomy type by exposure quartiles for most metabolites, controlling for age, race, BMI, smoking, specific gravity, total sperm concentration, motility, and morphology.
Results: A significant positive trend was seen for increasing IRRs by exposure quartiles of DMTP, DMDTP, DEP and DETP in XX18, YY18, XY18 and total disomy. A significant inverse association was observed between DMP and total disomy. Findings for total sum of DAP metabolites concealed individual associations as those results differed from the patterns observed for each individual metabolite. Dose-response relationships appeared nonmonotonic, with most of the increase in disomy rates occurring between the second and third exposure quartiles and without additional increases between the third and fourth exposure quartiles.
Conclusions: This is the first epidemiologic study of this size to examine the relationship between environmental OP exposures and human sperm disomy outcomes. Our findings suggest that increased disomy rates were associated with specific DAP metabolites, suggesting that the impacts of OPs on testis function need further characterization in epidemiologic studies.

1. Introduction

The impacts of environmental endocrine disruptors on male reproductive health has received heightened research attention in recent years (Diamanti-Kandarakis et al., 2009; Woodruff, 2011; Zoeller et al., 2012; World Health Organization, 2013). Each year more than 2 million couples in the US who want to have children

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are infertile, and over 2 million conceptions are lost before the twentieth week of gestation (McFadden and Friedman, 1997; ACOG, 2002; CDC, 2013). Most aneuploid conceptuses perish in utero; up to 50% of all spontaneous abortions are thought to be related to pre-existing chromosomal abnormalities (Jacobs, 1992; Lebedev et al., 2004). Because many chromosomal abnormalities come from the father's sperm, particularly for the sex chromosomes (X and Y), researchers have attempted to understand the paternal role in sex chromosome aneuploidy (Hassold and Hunt, 2001; Martin et al., 1991). Aneuploidy occurs when chromosome pairs fail to separate properly during cell division. In germ cells, errors in chromosome segregation during meiosis (I or II) result in imbalances in chromosome numbers; however, the exact causes of non-disjunction are unknown. Disomy is the most frequent aneuploidy observed in human sperm.

Children with sex chromosomal abnormalities (e.g., characterized in Kleinfelter and Turner syndrome), may have reproductive disorders, behavioral and/or intellectual difficulties when compared to their siblings (Martin, 2006; Boyd et al., 2011). Evidence from European birth defect registries suggests that the prevalence of chromosomal abnormalities in infants (during the first 28 days after birth) increased between 1967 and 1988 (Morris et al., 2008). Because there was no observed increase in maternally-derived chromosomal abnormalities, underlying environmental causes affecting spermatogenesis are suspected (Morris et al., 2008). Comparable birth defect data for the US are not available.

Much concern has been raised about pesticides being potential endocrine disrupting chemicals (EDCs). EDCs can modulate the endocrine system and potentially cause adverse effects (Sharpe, 2009; Woodruff, 2011; Zoeller et al., 2012; World Health Organization, 2013; NAS, 2014). Humans are exposed to EDCs through multiple routes of exposure (oral, dermal and inhalation) and pathways, including their diet (direct, indirect), environment (water, soil, air), and occupation (Tyler et al., 2000; Jørgensen et al., 2006; McKinlay et al., 2008a, 2008b; Mnif et al., 2011). Because organophosphate (OPs) insecticides accounted for a large share of all US insecticide use, they were the first group of pesticides to be reviewed under the Food Quality Protection Act (FQPA) of 1996. In 1999, the US EPA determined a common mechanism of action based on their ability to bind to and phosphorylate the enzyme acetylcholinesterase in both the central (brain) and peripheral nervous systems (US EPA, 1999). OPs are used in agriculture, recreational and commercial areas, and public pest control programs, accounting for 35% of the total US insecticide usage (US EPA, 2011). Urinary metabolites of OPs, such as dialkyl phosphates (DAPs), have been measured in a substantial proportion of the general population (Barr et al., 2004; CDC, 2009). OPs have been associated with effects on thyroid hormone levels (Lacasaña et al., 2010), decreased semen volume and sperm count (Yucra et al., 2008; Recio-Vega et al., 2008), lower sperm concentration (Perry et al., 2007b), abnormal morphology and decreased sperm motility (Hossain et al., 2010), DNA damage/fragmentation in sperm (Meeker et al., 2004; Muniz et al., 2008; Atherton et al., 2009), and sperm chromatin structure alteration (Sanchez-Pena et al., 2004). However, limited information has been published about associations between OPs and sperm abnormalities (Padungtod et al., 1999; Recio et al., 2001).

Toxicants may adversely affect germ cell DNA integrity (Mruk and Cheng, 2011); however, the exact causes of aneuploidy and the specific windows in which exposures impact the spermatogenic cycle are not well known (Herrera et al., 2008; Axelsson et al., 2010; Ashton Acton, 2013). This study investigated environmental exposures to OPs and their association with altered frequency of disomy among adult men.

2. Materials and methods

2.1. Study subjects

Study subjects were men from a parent study assessing the impact of environmental exposures on semen quality. The parent study has been described elsewhere (Hauser et al., 2003). Briefly, eligible participants were men aged 20–54 from couples seeking infertility evaluation at Massachusetts General Hospital (MGH) Fertility Center between January 2000 and May 2003. Sixty-five percent of eligible men agreed to participate; those declining participation cited lack of time during their clinic visit. Exclusion criteria included men who were at the center for post-vasectomy semen analysis and/or receiving treatment for infertility. None of the men reported occupational exposure to pesticides or other agents. All men completed a self-administered questionnaire that collected demographic, lifestyle factors, medical and fertility history information, and provided urine and semen samples. Eligibility for this analysis was based on the availability of both a urine and semen sample from the biorepository. Of the men enrolled in the parent study ($n=341$), a semen sample was available for 159 men (47%). Informed consent forms were signed by all subjects prior to participation. The parent study was approved by the Harvard School of Public Health, the Massachusetts General Hospital Human Subjects Committees, and by the Office of Human Research at the George Washington University.

2.2. Semen analysis

Measurement of the semen parameters have been previously described (Hauser et al., 2003). Researchers asked the participants to abstain from ejaculation for 48 h prior to providing a semen sample at the clinic via masturbation. Samples were liquefied at 37 °C for 20 min before analysis. Analysis of the samples took place at the MGH Andrology Laboratory. Andrologists were blind as to exposure status. The volume, pH, color, and viscosity were also determined for each semen sample. Sperm counts and percent motility were determined manually and then measured by computer-aided sperm analysis (CASA) using the Hamilton-Thorn Motility Analyzer (10HTM-IVO). A minimum of 200 sperm from 4 different fields were analyzed. A Nikon microscope with an oil immersion 100× objective was used for this analysis (Nikon Company, Tokyo, Japan). Sperm were scored normal or abnormal using the strict criteria reported by Kruger et al. (1988).

2.3. Disomy analysis

Semen samples were stored in −80 °C without cryoprotectant until FISH analysis was performed. The procedures for the detection of sex chromosome disomy have been described elsewhere (McAuliffe et al., 2012). A single investigator, blinded to exposure status, performed Fluorescence in situ hybridization (FISH) analysis for the detection of sex chromosome disomy, as the primary outcome of interest. Sex chromosome disomy is the most frequent form of sperm aneuploidy, occurring twice as frequently as disomy in the autosomes and resulting in viable offspring (i.e., disomic sperm for X or Y are capable of fertilization). The FISH procedure was carried out for three chromosomes of interest: X, Y and 18 (autosomal control) to determine XX18, YY18, XY18 and total sex chromosome disomy in sperm nuclei. A series of non-overlapping field images were taken for each FISH slide using a fluorescence microscope and scored using custom MATLAB (Mathworks Inc., Natick, MA) software. The software was designed to utilize scoring algorithms based on criteria for size and shape as reported by Baumgartner et al. (1999). Details of the sperm FISH control procedures and validation of the semi-automated scoring method