Environmental Research 160 (2018) 121–131

Contents lists available at ScienceDirect

Environmental Research

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

A crossover–crossback prospective study of dibutyl-phthalate exposure from mesalamine medications and serum reproductive hormones in men☆

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ARTICLE INFO

Keywords:
Phthalate
Endocrine disruptor
Crossover study
Hormones
Men

ABSTRACT

Background: Phthalates, such as dibutyl phthalate (DBP), are endocrine disruptors used in some medication coatings e.g., mesalamine to treat inflammatory bowel disease (IBD).
Objectives: Taking advantage of different mesalamine formulations with/without DBP, we assessed whether DBP from mesalamine (> 1000x background) altered serum hormones.
Methods: Men (N = 73) with IBD participated in a crossover-crossback prospective study and provided up to 6 serum samples (2:baseline, 2:crossover, 2:crossback). Men on non-DBP mesalamine (background) at baseline crossed-over for 4 months to DBP-mesalamine (high) and then crossed-back for 4 months to non-DBP mesalamine (B_1HB_2-arm) and vice versa for men on DBP-mesalamine at baseline (H_1BH_2-arm). We divided H_1BH_2-arm at the median (H_1 < 3yrs or $H_1 \geq 3$yrs). We estimated crossover and crossback % changes in serum reproductive hormones using multivariable linear mixed effect models.
Results: When B_1HB_2-arm (26 men,134 samples) crossed-over, luteinizing hormone decreased 13.9% (95% confidence interval(CI): −23.6, −3.0) and testosterone, inhibin-B, and follicle-stimulating hormone (FSH) marginally decreased; after crossback all increased 8–14%. H_1BH_2-arm, $H_1 \geq 3$yrs (25 men,107samples) had no changes at crossover or crossback whereas in H_1BH_2-arm,H_1 < 3yrs (22 men,100 samples) after crossover, inhibin-B increased 13.2% (CI: 4.2,22.9), FSH decreased 9.9% (CI: −17.9, −1.1) and after crossback, inhibin-B further increased 11.3%, and FSH marginally increased.
Conclusions: High-DBP exposure may disrupt pituitary-gonadal hormones that largely reversed after exposure removal, but only in men with no or short previous high-exposure history. Paradoxically, men with longer duration of high-DBP exposure, exposure removal did not change hormone levels, suggesting that long-term high-DBP exposure may alter the pituitary-gonadal axis and make it insensitive to exposure changes.

Abbreviations: DBP, dibutyl phthalate; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; MBP, monobutyl phthalate; NHANES, National Health and Nutrition Examination Survey; MARS, Mesalamine And Reproductive health Study; BIDMC, Beth Israel Deaconess Medical Center; BWH, Brigham and Women's Hospital; MGH, Massachusetts General Hospital; B_1HB_2, Background$_1$-High-Background$_2$ DBP exposure; H_1BH_2, High$_1$-Background-High$_2$ DBP exposure; H_1BH_2-arm, H_1 < 3 yrs, H1BH2-arm with duration on DBP-containing mesalamine medication at baseline < 3 years; H_1BH_2-arm, $H_1 \geq 3$ yrs, *H_1BH_2-arm with duration* on DBP-containing mesalamine medication at baseline ≥ *3 years*; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; LMEM, mixed effects models; N, number of men; 95% CI, 95% Confidence Interval; TT, total testosterone; FT, free testosterone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin

☆ The study was approved by the institutional review boards.

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http://dx.doi.org/10.1016/j.envres.2017.09.025
Received 14 June 2017; Received in revised form 15 September 2017; Accepted 21 September 2017

1. Introduction

Ortho-phthalates (hereto referred to as phthalates) are high production volume chemicals (Phthalates and Their Alternatives, 2011). Because of their widespread use in consumer and personal care products, phthalate exposure is ubiquitous in the general population (CDC, 2017).

In experimental studies, several phthalates, including dibutyl phthalate (DBP), have adverse impacts on male reproductive health (Foster, 2006). Although banned for several uses, DBP is still used in the enteric coatings of some medications including specific formulations of mesalamine, resulting in high-DBP exposure (Nguyen et al., 2016; Nassan et al., 2016; Jamieson and McCully, 2015; Jia et al., 2016; Wittassek et al., 2011; Kelley et al., 2012; Hauser et al., 2004). Mesalamine, the active ingredient in Asacol®, Asacol®HD, Lialda®, Pentasa®, Apriso®, and Delzicol®, is a commonly prescribed for inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD). The enteric coating of Asacol®, and Asacol®HD contains DBP as an excipient (FDA, 2017a, 2017b), whereas it is not used in other mesalamine formulations (Kelley et al., 2012). Asacol® and Asacol®HD use leads to high-DBP exposure as measured by urinary monobutyl phthalate (MBP) concentrations, the primary DBP metabolite (Hauser et al., 2004; Hait et al., 2014), that are approximately 1000 times higher than the median reported for men in the U.S. general population (National Health and Nutrition Examination Survey (NHANES)) (CDC, 2016).

Experimental studies have shown that DBP is anti-androgenic and a reproductive toxicant, however, most studies have focused on *in utero* exposure (Foster, 2006; Kim et al., 2010; Motohashi et al., 2015). There is limited evidence from epidemiologic studies on the association of background DBP exposure with serum reproductive hormones (Mendiola et al., 2012, 2011; Meeker et al., 2009; Meeker and Ferguson, 2014; Joensen et al., 2012a). However, there are no studies on effects of very high-DBP exposures from medications. Therefore, we took advantage of the difference in DBP-exposure from specific mesalamine formulations and conducted a crossover-crossback prospective cohort study to examine the associations between high-DBP exposure from mesalamine medications and serum reproductive hormones in adult men.

2. Materials and methods

2.1. Participants

As previously described (Nassan et al., 2016), between 2010–2016, 73 men enrolled in the Mesalamine And Reproductive health Study (MARS) from gastroenterology clinics at three Boston hospitals, Beth Israel Deaconess Medical Center (BIDMC), Brigham and Women's Hospital (BWH) and Massachusetts General Hospital (MGH). Eligible men were 18–55 years old, with mild severity IBD, and on mesalamine for at least three months at enrollment. There were no exclusions based on history of infertility. Men with a history of steroid medication use in the last three months, vasectomy, diabetes mellitus, hepatic, or renal diseases were not eligible. MARS was approved by the institutional review boards of Harvard T.H. Chan School of Public Health, BIDMC, BWH and MGH. All men signed informed consents.

2.2. Study design

Men participated in up to six visits (v) (baseline: v1 & v2, crossover: v3 & v4 and crossback: v5 & v6). Men who started on non-DBP mesalamine (background-DBP exposure) crossed-over to high-DBP mesalamine (high-DBP exposure) then crossed-back to non-DBP mesalamine (background) (B_1HB_2-arm; $Background_1$-$High$-$Background_2$) and vice versa in men who started on DBP mesalamine (H_1BH_2-arm; $High_1$-$Background$-$High_2$) (Fig. 1). Crossover and crossback periods (between v2 & v3 and v4 & v5) were designed to be four months and durations between v1 & v2, v3 & v4 and v5 & v6 were designed to be two weeks. We a priori chose the four month periods to be longer than the spermatogenesis cycle (around 70 days) (Heller and Clermont, 1963), as one of our aims was to study semen parameters (Nassan et al., 2016). At baseline, men reported demographic, lifestyle and health information on questionnaires and height and weight were measured. At each visit, men reported fever and illness in the previous three months and gave blood, semen and urine samples. 13 out of the 47 men in the H_1BH_2-arm participated only in a short protocol consisting of up to four visits that required only crossover without crossback as previously described (Nassan et al., 2016).

2.3. Exposure assessment

High-DBP exposure was defined by medication type i.e., DBP-containing mesalamine versus non-DBP mesalamine. We relied on self-reported use of mesalamine medications as prescribed over the study period (Gifford et al., 2013).

2.4. Serum hormone analysis

Nurses collected non-fasting blood samples at each visit between 7 a.m. and 2 p.m. and noted the exact time. All samples were shipped in a single batch for analysis to the laboratories at Ansh (inhibin-B and sex hormone-binding globulin (SHBG)), National Institute of Environmental Health Sciences (NIEHS) (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)), and Mayo Clinic (total testosterone (TT) and albumin).

SHBG was measured by enzyme linked immunosorbent assay (ELISA) (quantitative two-step sandwich type immunoassay). Testosterone was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Agilent Technologies, Santa Clara, CA 95051). Albumin, used to calculate the free testosterone (FT) concentration, was measured by an enzymatic colorimetric assay on the Roche Cobas c311 chemistry analyzer (Roche Diagnostics, Indianapolis, IN 46250). Inhibin-B was measured by ELISA (quantitative three-step sandwich type immunoassay). Ansh laboratory has developed highly specific antibodies for inhibin-B with no detectable cross-reactivity to inhibin-A or activin-A and AB, and negligible cross-reactivity to activin-B (0.04% at 50 ng/mL). LH and FSH concentrations were measured by chemiluminescent microparticle immunoassay (CMIA) technology (Architect i100SR Immunoassay Analyzer, Abbott Diagnostics, Abbott Park, IL).

The intra- and inter-assay coefficients of variation (CV) were for SHBG: $< 9.0\%$ and $< 8.0\%$, testosterone: $\leq 9.0\%$ and $\leq 8.9\%$, albumin: $\leq 1.4\%$ and $\leq 2.1\%$, Inhibin-B: $< 4.4\%$ and $< 7.4\%$, LH: 2.8% and 5.7%, and FSH: 1.8% and12.7%, respectively. All laboratories were blinded to participant information. We calculated FT from the measured concentrations of TT, SHBG, and albumin using the method of Vermeulen et al. (1999). We also calculated TT/LH, FT/LH, and Inhibin-B/FSH ratios.

2.5. Statistical analysis

In primary analyses, to define exposure we used a six-level indicator variable cross-classifying each observation based on the medication type (high versus non-DBP) at each period (baseline, crossover and crossback) for the two study arms (H_1BH_2 and B_1HB_2). We modeled the hormone concentrations as natural log-transformed continuous outcomes.

We performed descriptive statistics and tested for any differences between men in the different arms using Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables.

We selected the covariates based on directed acyclic graphs and statistical considerations ($> 10\%$ change in the effect estimate). The