Toxicology and Applied Pharmacology 292 (2016) 75–84

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Bisphenol A sulfonation is impaired in metabolic and liver disease

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ARTICLE INFO

Article history:
Received 3 June 2015
Revised 11 December 2015
Accepted 16 December 2015
Available online 19 December 2015

Keywords:
Bisphenol A
Phase-II
Sulfotransferase
Obesity
Diabetes
Steatosis
Cirrhosis

ABSTRACT

Background: Bisphenol A (BPA) is a widely used industrial chemical and suspected endocrine disruptor to which humans are ubiquitously exposed. The liver metabolizes and facilitates BPA excretion through glucuronidation and sulfonation. The sulfotransferase enzymes contributing to BPA sulfonation (detected in human and rodents) is poorly understood.
Objectives: To determine the impact of metabolic and liver disease on BPA sulfonation in human and mouse livers.
Methods: The capacity for BPA sulfonation was determined in human liver samples that were categorized into different stages of metabolic and liver disease (including obesity, diabetes, steatosis, and cirrhosis) and in livers from ob/ob mice.
Results: In human liver tissues, BPA sulfonation was substantially lower in livers from subjects with steatosis (23%), diabetes cirrhosis (16%), and cirrhosis (18%), relative to healthy individuals with non-fatty livers (100%). In livers of obese mice (ob/ob), BPA sulfonation was lower (23%) than in livers from lean wild-type controls (100%). In addition to BPA sulfonation activity, Sult1a1 protein expression decreased by 97% in obese mouse livers.
Conclusion: Taken together these findings establish a profoundly reduced capacity of BPA elimination via sulfonation in obese or diabetic individuals and in those with fatty or cirrhotic livers versus individuals with healthy livers.

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1. Introduction

Bisphenol A is an industrial chemical and suspected endocrine disruptor with a widespread exposure in humans. Urinary BPA (total, indicating free BPA plus BPA-conjugates) has been detected at a mean of 2.6 μg/L in ~96% of samples from 2011 to 2012 NHANES study conducted by the Center of Disease Control (CDC) (http://wwwn.cdc.gov/nchs/nhanes/2011-2012/EPH_G.htm), as well as fetus, adult blood and placenta (Volkel et al., 2002). According to the most recent U.S. Food and Drug Administration (FDA) update, the average dietary exposure of BPA from food is estimated to be 0.2–0.4 μg per kilogram body weight per day (μg/kg·bw/day) for infants and 0.1–0.2 μg/kg bw/day for children and adults (FDA, 2010). Although human exposure to BPA is widespread, studies report a wide range of effective concentrations for specific pathways of BPA mediated endocrine disruption such as estrogenicity, aromatase and androgen receptor inhibition (Judson et al., 2010; Reif et al., 2010).

BPA is predominantly metabolized in the liver to corresponding glucuronide and sulfate conjugates (Pritchett et al., 2002; Hanioka et al., 2008). In both humans and rodents, BPA-glucuronide is the major metabolite detected in blood and urine, whereas sulfated conjugates (mono- and di-sulfates) are minor metabolites (Nishiyama et al., 2002; Volkel et al., 2002; Teeguarden et al., 2015; Thayer et al., 2015). Glucuronide and sulfate conjugated BPA metabolites are eliminated from the body into the urine via glomerular filtration. In addition, in rodents biliary excretion of BPA-glucuronide into the feces is substantial. *In vitro* ATPase activity assays have demonstrated that BPA-glucuronide has a high affinity for rodent Mrp2 and human MRP3 (ABCC3, basolateral) but is a non-substrate for human MRP2 (ABCC2, apical) transporters (Mazur et al., 2012). In rats, conjugated and unconjugated BPA is primarily (~66%) disposed through biliary excretion and detected in feces 6 h after oral or i.v. administration (Kurebayashi et al., 2003) potentially due to high BPA-G affinity to Mrp2. In rats administered BPA, ~81% of administered dose was detected (measured as total BPA-conjugated and unconjugated) in feces, ~16% in urine while ~0.1% accumulated in tissue. However, urinary excretion is the major route of BPA elimination from the body in humans, which have higher affinity of BPA-G to basolateral MRP3 and relatively low affinity to apical MRP2 (Mazur et al., 2012). Conjugated BPA (glucuronide/sulfate) may be de-conjugated in the intestinal tract by glucuronidases/sulfatases and undergo enterohepatic recirculation that has been reported in rodents, but not humans (Ginsberg and Rice, 2009).

Abbreviations: BPA, Bisphenol A; *p*NP, *para*-Nitrophenol; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; SULT, sulfotransferase; DHEA, dehydroepiandrosterone.

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http://dx.doi.org/10.1016/j.taap.2015.12.009
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BPA-sulfate metabolites are detected in human serum and urine at a geometric mean of 0.124 ng/mL and 0.104 ng/mL, respectively (Liao and Kannan, 2012) with females having lower glucuronidated and higher sulfated BPA conjugates relative to males (Kim et al., 2003; Kurebayashi et al., 2003; Ye et al., 2005). BPA sulfonation is potentially SULT1A1-mediated, as determined using in vitro enzymatic methods (Nishiyama et al., 2002). However, the majority of studies describing BPA sulfonation utilize recombinant enzyme systems to determine BPA sulfonation by SULTs, and further studies are needed to determine and confirm BPA sulfonation in human liver.

Rodent studies and human epidemiological studies have revealed a significant correlation between BPA exposure and endocrine disruption, reproductive and developmental defects in rodents, as well as with metabolic disorders such as hypertension, diabetes and obesity (Christiansen et al., 2014; Khalil et al., 2014; Alonso-Magdalena et al., 2015). Extrapolation of observed BPA effects in rodents to humans is controversial, although building evidence suggests refinement of risk assessment towards more vulnerable populations such as fetuses, infants (Myers et al., 2009; Valentino et al., 2015) and potentially disease states with compensated liver function. Two studies have demonstrated ability of BPA to promote lipid accumulation in hepatocytes (Huc et al., 2012; Wang et al., 2013); the effect of this morphological and phenotypic change on BPA metabolism needs to be explored.

Non Alcoholic Fatty Liver Disease (NAFLD) is the accumulation of lipids exceeding 5% by weight of hepatocytes. NAFLD has also been referred to as "hepatic manifestation of insulin resistance" ranging from steatosis (fatty liver) to non-alcoholic steatohepatitis (fatty liver with liver cell damage and inflammation) to progressive hepatic fibrosis, cirrhosis and hepatocellular carcinoma (McCullough, 2011). In the United States, prevalence of NAFLD alone or in combination with increased liver enzymes in serum, as diagnosed by various techniques, was between 5–33% among adults (Lazo and Clark, 2008). Studies have shown that expression of several drug metabolism enzymes and transporters is altered in humans and rodent models of nonalcoholic fatty liver disease (steatosis) and obesity (Merrell and Cherrington, 2011). In addition, our recent studies showed that SULT1A1 expression and activity with a probe substrate was reduced in steatosis, diabetic cirrhosis, and alcoholic cirrhosis (Hardwick et al., 2013; Yalcin et al., 2013). This may result in modified metabolism and disposition of BPA, and potentially modified toxicity and adverse effects.

While sulfotransferase enzymes are an important class of Phase-II detoxification enzymes known to metabolize endogenous and xenobiotic compounds (James and Ambadapadi, 2013), sulfotransferase expression and activity for BPA has not been characterized in human livers under diseased conditions. Although secondary to glucuronidation, repression of SULT1A1 and SULT1A3 protein and activity in diseased human livers (Yalcin et al., 2013) points towards a potentially decreased ability for BPA biotransformation in the diseased liver. The purpose of this study herein was to characterize BPA sulfonation and SULT1A1 expression in human livers from individuals diagnosed with metabolic or liver disease, as well as in the ob/ob mouse under normal and fasted conditions to model metabolic-induced fatty liver disease. Herein, we describe decreased BPA sulfonation in diseased livers from both humans and mice.

2. Materials and methods

2.1. Chemicals

[35S]PAPS (1.5–2.54 Ci/mmol) and scintillation fluid (Ultima Flo-M) were purchased from PerkinElmer Life and Analytical Sciences. *p*-Nitrophenol, and BPA were purchased from Sigma Aldrich. Sult1a1 antibody was obtained from Santa Cruz Biotechnology (TX, USA) and Gapdh antibody was obtained from Cell Signaling Technologies (MA, USA).

2.2. Animal treatment and fasting

Adult male C57BL/6 (WT, n = 6–8/group) and Lep$^{-/-}$ (B6.V-Lepob/J, ob/ob, n = 6–8/group) mice (Jackson Laboratories, Bar Harbor, ME, USA) were fed Harlan TekladLM-485 Mouse/Rat sterilizable diet or food-withheld for 24 h. Mice were housed in a temperature-, light-, and humidity-controlled environment in cages with corncob bedding. All the animal experiments were carried out at the University of Rhode Island, Fogarty facility with IACUC approval. Livers were collected, snap frozen in liquid nitrogen, and stored in −80 °C until further analysis.

2.3. Cytosol isolation from human and mouse liver tissue

Human liver tissues were purchased from Liver Tissue Cell Distribution System (LTCDS), University of Minnesota, Minneapolis, MN. Details of the human liver donors are described in Yalcin et al. (2013). Liver samples were stored frozen at −80 °C until the cytosolic fractions were prepared. Method for isolating cytosolic fractions has been reported previously (Yalcin et al., 2013). Briefly, liver tissue was homogenized by sonication in buffer containing sucrose/Tris/EDTA buffer supplemented with 0.01 M EDTA and 0.5 mM BHT, and cytosolic fractions were used for sulfonation assays.

2.4. BPA sulfonation activity

Activity assays for each liver tissue were performed in duplicate, and the average of duplicate data was analyzed. Human liver cytosols were incubated with radiolabeled sulfonyl donor [^{35}S]-3′-phosphoadenosine-5′-phosphosulfate (^{35}S-PAPS, 4 μM) and 4 μM BPA in 20 mM potassium phosphate (pH 7.0). Reaction mixture was incubated for 30 min at 37 °C, stopped by heating in boiling water for 30 s, and centrifuged at 14,000 ×*g* for 1 min to pellet the protein. To separate reaction components, supernatant was injected onto Phenomenex Synergi Polar-RP column (50 × 2.00 mm, 4 μm). A linear gradient of 15–80% acetonitrile and 20 mM potassium phosphate (pH 2.7) in 8 min was used as mobile phase at 1 mL/min flow to separate excess ^{35}S-PAPS from ^{35}S-BPA. ^{35}S-labeled peaks were quantified on a flow scintillation analyzer (Packard Bioscience, 500 TR series) with Perkin-Elmer Ultima Flo-M scintillation cocktail. ^{35}S-PAPS was eluted at 0.5 min, ^{35}S-BPA-disulfate at 4.2 min, and ^{35}S-BPA-monosulfate at 4.8 min.

2.5. Probe sulfonation activity assays

Sulfonation assays for probe substrates was performed as described in (Yalcin et al., 2013). Briefly, cytosolic fraction of liver tissue was incubated with radiolabeled sulfonyl donor ^{35}S-PAPS (4 μM) and a prototype substrate. Sulfated products of p-nitrophenol (pNP, 4 μM), estradiol (20 nM), and Dehydroepiandrosterone (DHEA, 10 μM) were separated on Synergi Polar-RP column (Phenomenex, Torrance, CA) and dopamine-sulfate on a Hypersil Duet C18/SAX column (Thermo Fisher Scientific). Radiolabeling was detected and quantified on a flow scintillation analyzer (500 TR series; Packard Bioscience, Meriden, CT) with PerkinElmer Ultima Flo-M scintillation cocktail.

2.5.1. Western blotting. Cytosols (50 μg) were electrophoretically separated by SDS-PAGE and transferred onto a polyvinylidene difluoride membrane. After blocking the membrane with non-fat dry milk, blot was incubated with SULT1A1 antibody (Santa Cruz Biotechnology, TX, USA) and subsequently with corresponding HRP-labeled secondary antibody. Blots were visualized using Pierce ECL-Plus Western blot detection reagent (Thermo Fisher Scientific, Rockford, IL, USA) and quantified using ImageQuant software (Bio-Rad, Hercules, CA).

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