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New insights for the risk of bisphenol A: Inhibition of UDP-glucuronosyltransferases (UGTs)

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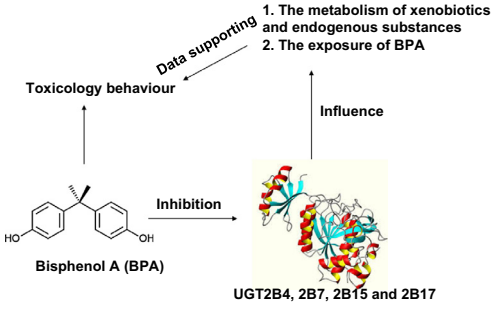
HIGHLIGHTS

- Stronger inhibition of bisphenol A towards UGT2B isoforms than UGT1A isoforms.
- Competitive inhibition for UGT2B4, noncompetitive inhibition for 2B7, 2B15, 2B17.
- *In vitro-in vivo* extrapolation (IV-IVE) was performed.

GRAPHICAL ABSTRACT



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ABSTRACT

Bisphenol A (BPA), the important endocrine-disrupting chemical (EDC), has been reported to be able to induce various toxicity. The present study aims to understand the toxicity behavior of bisphenol A through evaluating the inhibition profile of bisphenol A towards UDP-glucuronosyltransferase (UGT) isoforms. *In vitro* recombinant UGTs-catalyzed 4-methylumbelliferone (4-MU) glucuronidation reaction was employed as probe reaction for all the tested UGT isoforms. The results showed that bisphenol A exerted stronger inhibition towards UGT2B isoforms than UGT1A isoforms. Furthermore, the inhibition kinetic type and parameters (K_i) were determined for the inhibition of bisphenol A towards UGT2B4, 2B7, 2B15, and 2B17. Bisphenol A exhibited the competitive inhibition towards UGT2B4, and noncompetitive inhibition towards UGT2B7, 2B15 and 2B17. The inhibition kinetic parameters (K_i) were calculated to be 1.1, 32.6, 5.6, and 19.9 μM for UGT2B4, 2B7, 2B15 and 2B17, respectively. In combination with the *in vivo* concentration of bisphenol A, the elevation of exposure dose was predicted to increase by 29.1%, 1%, 5.7%, and 1.6% for UGT2B4, 2B7, 2B15, and 2B17, indicating the high influence of bisphenol A towards the *in vivo* UGT2B isoforms-mediated metabolism of xenobiotics and endogenous substances. All these data provide the supporting information for deeper understanding of toxicology of bisphenol A.

1. Introduction

Bisphenol A (BPA, 4,40-isopropylidenediphenol), an important endocrine-disrupting chemical (EDC), has been widely used as a key component in manufacturing polycarbonate plastics and epoxy resins. BPA can be detected in our everyday consumer products,

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such as baby bottles, toys, dental sealants, eyeglass lenses, reusable water bottles, plastic stretch films, consumer electronics, digital media (CDs, DVDs), automobiles, medical equipment, food and beverage can linings and glass jar tops (Vandenberg et al., 2007).

The risk of BPA utilization has been speculated in recent years. The exposure of humans to BPA have been correlated with a variety of diseases, including diabetes (Lang et al., 2008), cardiovascular disease (Melzer et al., 2010), recurrent miscarriages and increased numbers of premature deliveries in women (Sugiura-Ogasawara et al., 2005; Cantonwine et al., 2010), and influence of immune system (Yang et al., 2009). Based on these reported risk of BPA, more and more government agencies provide a strick regulation towards the utilization of BPA. For example, in Europe, the European food safety authority (EFSA) sets the tolerable daily intake (TDI) for BPA to be 0.05 mg kg^{-1} body weight (bw) day^{-1} (Tyl et al., 2008).

Human uridine glucuronosyltransferases (UGTs) are the membrane proteins of the endoplasmic reticulum, and involved in the conjugation metabolism of multiple xenobiotics and endogenous substances (Fang et al., 2013; Song et al., 2013; Dong et al., 2013). To date, the role of UGTs in the metabolic elimination of BPA has been detailedly studied. BPA monoglucuronide has been identified to be the predominant *in vivo* metabolite in rats, monkeys and humans (Pottenger et al., 2000; Kurebayashi et al., 2002; Volkel et al., 2002). The human UGT2B15 was demonstrated to be main UGT isoform involved in the glucuronidation of BPA (Hanioka et al., 2008a,b). Based on these results, some contradictions existed that rapid glucuronidation elimination of BPA can significantly weaken the risk of BPA (Trdan Lusin et al., 2012).

Inhibition of various UGT isoforms by various xenobiotics and endogenous substances have been widely demonstrated in the previous literatures (Schneider et al., 1993; Fang et al., 2013; Lu et al., 2013), which might result in the slower elimination of drugs and possible metabolic disorders of endogenous substances. In the present study, we tried to evaluate the risk of bisphenol A from the new insights of UGTs' inhibition. As previously reported, the *in vitro* recombinant UGTs-catalyzed 4-methylumbelliferone (4-MU) glucuronidation reaction was used in the present study.

2. Materials and methods

2.1. Chemicals

Bisphenol A (purity ⩾ 99%), 4-methylumbelliferone (4-MU), 4-methylumbelliferone-β-D-glucuronide (4-MUG), Tris–HCl, 7-hydroxycoumarin, and uridine-5′-diphosphoglucuronic acid (UDPGA) (trisodium salt) were purchased from Sigma–Aldrich (St Louis, MO). Recombinant human UGT isoforms (UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17) expressed in baculovirus-infected insect cells were obtained form BD Gentest Corp. (Woburn, MA, USA). All other reagents were of HPLC grade or of the highest grade commercially available.

2.2. Initial screening of bisphenol A's inhibition towards recombinant UGTs-catalyzed 4-MU glucuronidation

4-MU, the nonspecific probe substrate for all the UGT isoforms, was used in the present study to initially screen the inhibition potential of ginsenosides as previously reported (Dong et al., 2012). A typical incubation system (total volume = 200 μL) contained recombinant UGTs, 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris–HCl (pH = 7.4), and 4-MU in the absence or presence of different concentrations of bisphenol A. Bisphenol A was dissolved in DMSO, and the final concentration of DMSO was below 0.5% (v/v). The used incubation time and protein concentration were previously determined to ensure the reaction rate within the linear range. The 4-MU concentration was equally to known K_m or S_{50} values for each UGT isoform (Zhu et al., 2012). The incubation reaction was initiated through adding UDPGA to the mixture after 5-min pre-incubation at 37 °C. The reactions were quenched by adding 100 μL acetonitrile with 7-hydroxycoumarin (100 μM) as internal standard. The mixture was centrifuged at 20000 × g for 10 min, and an aliquot of supernatant was transferred to an auto-injector vial for HPLC analysis. The HPLC system (Shimadzu, Kyoto, Japan) contained a SCL-10A system controller, two LC-10AT pumps, a SIL-10A auto injector, a SPD-10AVP UV detector. Chromatographic separation was carried out using a C18 column (4.6 × 200 mm, 5 μm, Kromasil) at a flow rate of 1 mL min^{-1} and UV detector at 316 nm. The mobile phase consisted of acetonitrile (A) and H_2O containing 0.5% (v/v) formic acid (B). The following gradient condition was used: 0–15 min, 95–40% B; 15–20 min, 10% B; 20–30 min, 95% B.

2.3. Inhibition of bisphenol A towards recombinant UGT1A4-catalyzed trifluoperazine (TFP) glucuronidation

Due to the low catalytic activity of UGT1A4 towards 4-MU glucuronidation (Uchaipichat et al., 2008), the UGT1A4-catalyzed TFP glucuronidation was performed to evaluate the inhibition potential of bisphenol A towards UGT1A4 activity. TFP (40 μM, near its km value) was incubated with recombinant UGT1A4 (0.1 mg mL^{-1}) at 37 °C for 20 min in the absence or presence of bisphenol A (Uchaipichat et al., 2006).

2.4. Kinetic analysis of bisphenol A's inhibition towards UGT2B4, UGT2B7, UGT2B15, and UGT2B17 activity

The glucuronidation velocity was determined at various concentrations of 4-MU and bisphenol A. Dixon plot and Lineweaver–Burk plot were performed to determine the inhibition kinetic type, and the second plot using the slopes from the Lineweaver–Burk plot versus the concentrations of bisphenol A.

2.5. In vitro-in vivo extrapolation (IV-IVE)

In vitro-in vivo extrapolation (IV-IVE) was performed using the following equation as previously described (Fang et al., 2013)

$$AUC_i/AUC = 1 + [I]_{in\,vivo}/K_i$$

The terms are defined as follows: AUC_i/AUC is the predicted ratio of *in vivo* exposure of xenobiotics or endogenous substances with or without the co-exposure of bisphenol A. $[I]_{in\ vivo}$ is the *in vivo* exposure concentration of bisphenol A, and the K_i value was *in vitro* inhibition kinetic parameters.

3. Results

3.1. The stronger inhibition of bisphenol A towards UGT2B isoforms than UGT1A isoforms

As shown in Fig. 1, at 100 μM of bisphenol A, the activity of UGT1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, and 2B17 was inhibited by 76.7%, 79.5%, 89.1%, 73.7%, 32.4%, 55%, 76.8%, 100%, 94.3%, 100%, and 100%, respectively. From these data, we made a conclusion that bisphenol A exhibited stronger inhibition towards UGT2B isoforms than UGT1A isoforms.

3.2. Inhibition kinetic type and parameters of bisphenol A towards UGT2B isoforms

The inhibition kinetic type and parameters (K_i) were determined for the inhibition of bisphenol A towards UGT2B isoforms.