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Inhibitory effects of fifteen phthalate esters in human cDNA-expressed UDP-glucuronosyltransferase supersomes

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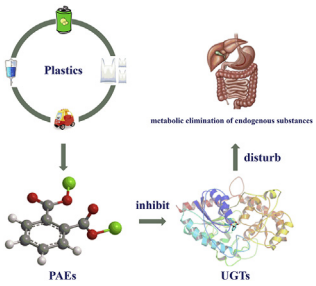
HIGHLIGHTS

- PAE showed broad inhibition on the activity of UGT1A9.
- Hydrogen bonds and hydrophobic interaction contribute to the inhibition of PAEs towards UGT1A9.
- Threshold value for PAEs' inhibition towards UGTs was obtained.

GRAPHICAL ABSTRACT



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ABSTRACT

Phthalate esters (PAEs) have been extensively used in industry as plasticizers and there remains concerns about their safety. The present study aimed to determine the inhibition of phthalate esters (PAEs) on the activity of the phase II drug-metabolizing enzymes UDP-glucuronosyltransferases (UGTs). *In vitro* recombinant UGTs-catalyzed glucuronidation of 4-methylumbelliferone was used to investigate the inhibition potentials of PAEs towards various s UGTs. PAEs exhibited no significant inhibition of UGT1A1, UGT1A3, UGT1A8, UGT1A10, UGT2B15, and UGT2B17, and limited inhibition of UGT1A6, UGT1A7 and UGT2B4. However, UGT1A9 was strongly inhibited by PAEs. *In silico* docking demonstrated a significant contribution of hydrogen bonds and hydrophobic interactions contributing to the inhibition of UGT by PAEs. The K_i values were 15.5, 52.3, 23.6, 12.2, 5.61, 2.79, 1.07, 22.8, 0.84, 73.7, 4.51, 1.74, 0.58, 6.79, 4.93, 6.73, and 7.23 μM for BBOP-UGT1A6, BBZP-UGT1A6, BBOP-UGT1A7, BBZP-UGT1A7, DiPP-UGT1A9, DiBP-UGT1A9, DCHP-UGT1A9, DBP-UGT1A9, BBZP-UGT1A9, BBOP-UGT1A9, DMEP-UGT1A9, DPP-UGT1A9, DHP-UGT1A9, DiBP-UGT2B4, DBP-UGT2B4, DAP-UGT2B4, and BBZP-UGT2B4, respectively. In conclusion, exposure to PAEs might influence the metabolic elimination of endogenous compounds and xenobiotics through inhibiting UGTs.

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1. Introduction

Phthalate esters (PAEs), composed of an rigid aromatic nucleus and two side chain esters of various lengths, have been extensively used in industry as precursors for the synthesis building materials, automobile parts, electronic products, cosmetics and medical devices, notably as plasticizers to reinforce the flexibility and stretchability of plastics (Huang et al., 2012; Net et al., 2015). Because of the hydrogen bond and van der waals force (VDW) rather than covalent bonding between PAEs and plastics, PAEs can slowly leach from plastics over time to contaminate air, soil, water and food, which can result in the exposure of humans through skin, respiratory tract, digestive tract and medical injection (Wittassek et al., 2007; Bergé et al., 2013). The abbreviations and side chains of PAEs which are generally used in industry (Kamrin, 2009) are shown in Table 1.

There have been numerous studies focusing on the carcinogenicity, teratogenicity and mutagenicity of PAEs (Gardner et al., 2016; Köksal et al., 2016; Miao et al., 2017). PAEs have an adverse impact on hormone signaling and are designated endocrine disruptors. For example, there are negative correlations between PAEs and thyroxine signaling (Huang et al., 2016). In addition, maternal PAE exposure negatively correlates with the levels of reproductive hormones in fetal blood (Araki et al., 2014). They also were demonstrated to possess estrogenic endocrine disrupting activity *in vivo* (Chen et al., 2014).

The metabolism of PAE has two steps. PAEs are hydrolyzed to phthalate monoesters and the resultant monoesters can be conjugated with uridine diphosphate glucuronic acid (UDPGA) by uridine diphosphate glucuronosyltransferases (UGTs) (Harris et al., 2016). A recent study showed that UGTs play a major role in the metabolism of DEHP by catalyzing the formation of glucuronide conjugates at the following order of potency: UGT1A9 > UGT2B7 > UGT1A7 > UGT1A8 ≥ UGT1A10 > UGT1A3 > UGT2B4 (Hanioka et al., 2017). These results possible interactions between PAEs and UGTs that many influence UGT activities toward endogenous and xenobiotic compounds.

UGTs not only affect the metabolic stability of many drugs *in vivo*, but also metabolize many endogenous substances. For example, thyroid hormone is mainly catalyzed by UGT1A1 in liver, and by UGT1A8 and UGT1A10 in jejunum (Yamanaka et al., 2007). Estrogen and its metabolites are converted by UGTs to inactive glucuronides (Cheng et al., 1998). Similarly, the glucuronidation reaction catalyzed by UGTs is critical to the termination of androgen signaling (Bélanger et al., 2003). UGTs also participate in the metabolism of many other endogenous components such as bilirubin (Bosma et al., 1994), serotonin (Krishnaswamy et al., 2003) and bile acids (Monaghan et al., 1997). Therefore, the inhibition of UGT might seriously affect the metabolism of various endogenous substances.

The aim of the present study was to investigate the potential inhibition of PAEs towards the human UGTs. Fifteen PAEs (DAP, DBP, DCHP, DEHP, DEP, DiBP, DiOP, DiPP, DMEP, DMP, DHP, DNP, DPP, BBOP and BBZP) were tested on 11 recombinant human UGTs (UGT1A1, 1A3, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17). Furthermore, enzyme kinetics and *in silico* docking experiments were carried out to explain at the molecular level, the kinetic relationship between PAEs and UGTs.

Table 1
The abbreviations and side chains of PAEs.

Name	Abbreviation	Side chains
Diallyl phthalate	DAP	Allyl
Dibutyl phthalate	DBP	Butyl
Dicyclohexyl phthalate	DCHP	Cyclohexyl
Di-2-ethylhexyl phthalate	DEHP	2-Ethylhexyl
Diethyl phthalate	DEP	Ethyl
Diisobutyl phthalate	DiBP	Isobutyl
Diisooctyl phthalate	DiOP	Isooctyl
Diisopentyl phthalate	DiPP	Isopentyl
Dimethoxyethyl phthalate	DMEP	Methoxyethyl
Dimethyl phthalate	DMP	Methyl
Dihexyl phthalate	DHP	Hexyl
Dinonyl phthalate	DNP	Nonyl
Dipentyl phthalate	DPP	Pentyl
Bis-2-Butoxyethyl Phthalate	BBOP	2-Butoxyethyl
Butyl benzyl phthalate	BBZP	Butyl/Benzyl

2. Materials and methods

2.1. Chemicals and reagents

PAEs were purchased from J&K Chemical (Beijing, China). Recombinant human UGT isoforms (UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT-2B7, UGT2B15 and UGT2B17) expressed in baculovirus-infected insect cells were purchased from BD Gentest Corp. (Woburn, MA, US). 4-Methylumbelliferone (4-MU), UDPGA (Trisodium salt), Tris-HCl, $MgCl_2$ and 7-hydroxycoumarin were purchased from Sigma-Aldrich (St. Louis, MO, US). Millipore Elix 5 UV and Milli-Q Gradient Ultra-Pure Water System was used to make ultra-pure water. All other reagents were of high-performance liquid chromatography (HPLC) grade or of the highest grade commercially available.

2.2. Preliminary screening of PAEs using in vitro incubation

4-MU was used as a nonselective probe substrate for recombinant UGTs to determine the inhibition of PAEs (Liu et al., 2016). The incubation system (Chen et al., 2017) containing 100 μM PAEs, 50 mM Tris-HCl buffer (pH 7.4) and 5 mM $MgCl_2$ in a total volume of 200 μL, was prepared, in which different concentrations of 4-MU (110, 1200, 110, 30, 750, 30, 30, 1000, 350, 250 and 2000 μM 4-MU) and 0.125, 0.05, 0.025, 0.05, 0.025, 0.05, 0.05, 0.25, 0.05, 0.2 and 0.5 mg/ml of UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17, respectively were incubated together to ensure that the reaction rates were within the linear range. Incubations without PAEs were used as controls. After a preincubation at 37 °C for 5 min, 5 mM UDPGA was added to initiate the incubation reactions. The reactions were quenched by the addition of 200 μL acetonitrile including 100 μM 7-hydroxycoumarin as an internal standard after 30–120 min. The mixtures were centrifuged at 10,625 g for 10 min to obtain the supernatant, which was then transferred to an auto-injector vial for UPLC analysis. Chromatographic separation was carried out using a C18 column (4.6 × 200 mm, 5 μm, Kromasil) at a flow rate of 0.2 mL/min and UV detector at 316 nm. The mobile phase consisted of H_2O containing 0.5% (v/v) formic acid (A) and acetonitrile (B). The following gradient condition was applied: 0–3.50 min, 90% A and 10% B; 3.50–4.00 min, 35% A and 65% B; 4.01–7.00 min, 90% A and 10% B. The calculation curve was generated by peak area ratio (4-MUG/internal standard) and all experiments were performed in two independent experiments in duplicate. The PAEs whose inhibition ratios to UGTs were more than 75% were screened out to proceed with the subsequent experiment.

2.3. Inhibition kinetics assay

Concentration-dependent inhibition of PAEs on the activity of UGTs was determined. PAEs at 0, 0.5, 1, 5, 10, 20, 40, 60, 80 and 100 μM, selected from preliminary screening, were prepared to