

In vitro evidence for endocrine-disrupting chemical (EDC)'s inhibition of drug metabolism

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Abstract

Background: Humans can be frequently exposed to Bisphenol A (BPA) via multiple sources, and babies are considered to be the most sensitive group to exposure of BPA.

Aims: To investigate the inhibition potential of BPA towards human liver microsomes (HLMs)-catalyzed zidovudine (AZT) glucuronidation.

Materials and Methods: *In vitro* HLMs incubation system was used to investigate the inhibition potential of BPA towards AZT glucuronidation. Both Dixon and Lineweaver-Burk plots were employed to determine the inhibition kinetic type, and nonlinear repression was utilized to calculate the inhibition kinetic parameters (K_i).

Results: Concentration-dependent inhibition of BPA towards AZT glucuronidation was observed. Both Dixon and Lineweaver-Burk plots showed that BPA exerted competitive inhibition towards the glucuronidation of AZT, and nonlinear repression with competitive equation was used to calculate the K_i value to be 3.2 μ M.

Conclusion: Potential BPA-AZT interaction might occur when the patients administered with AZT is also exposed to BPA.

Key Words: Bisphenol A (BPA), zidovudine (AZT), human liver microsomes (HLMs)

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Introduction

Endocrine disrupting chemicals (EDCs) are natural and man-made chemicals acting via mimicking or blocking natural hormone functions in the body¹. EDCs can exhibit significant influence towards various hormonal systems, such as estrogen, androgen, progestagen, corticosteroid and thyroid signaling systems¹. Bisphenol A (BPA) has been widely used as monomer for the production of polycarbonate and a precursor of epoxy resins². Human can be frequently exposed to BPA via multiple sources, and babies are considered to be the most sensitive group to exposure of BPA³. For example, babies that are not breast-fed, might drink powdered or liquid milk formula, and the cans of milk formula might release BPA into milk formula. BPA is a well-known EDC, and the exposure

of BPA has been closely correlated with a variety of diseases, including cardiovascular disease and diabetes^{4,5}. Additionally, the increased exposure of BPA is closely correlated with increased inflammation and oxidative stress⁶.

Drug-metabolizing enzymes-catalyzed biotransformation process is one of the most important elimination pathways. BPA has been demonstrated to mainly undergo UDP-glucuronosyltransferases (UGTs) catalyzed glucuronidation reaction, and UGT2B15 was demonstrated to be the major UGT isoform^{7,8}, indicating the good interaction between BPA and UGT2B isoforms. Given that UGT2B isoforms are involved in the metabolic elimination of many clinical drugs, the potential influence of BPA administration towards the drug metabolism was speculated. Zidovudine (AZT), the first U.S. government approved drug for antiretroviral treatment (ART), has been clinically used and prescribed under the name Retrovir. AZT has a narrow therapeutic index, and is mainly metabolized through UGT2B isoforms-catalyzed biotransformation reaction⁹. In the present study, the inhibition capability of BPA towards the typical substrate of UGT2B zidovudine (AZT) was

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evaluated, trying to indicate the possible influence endocrine-disrupting chemical (EDC)'s inhibition of drug metabolism.

Materials and Methods

Chemicals and reagents

Bisphenol A (purity $\geq 99\%$), 3'-azido-3'-deoxythymidine (AZT), Tris-HCl, alamethicin and uridine 5'-diphosphoglucuronic acid (UDPGA) (trisodium salt) were obtained from Sigma-Aldrich (St Louis, MO). Mixed pooled human liver microsomes (HLMs) from 25 donors were purchased from Research Institute for Liver Diseases (RILD, Shang Hai, China).

AZT glucuronidation assays and analysis method

500 μM of AZT was used to incubate in the human liver microsomal incubation system (0.5 mg/ml of HLM, 5 mM UDPGA, 5 mM MgCl_2 , 50 mM Tris-HCl buffer (pH 7.4), 50 $\mu\text{g}/\text{mg}$ protein alamethicin). The incubation time was 20 min, and the analysis of AZT glucuronidation was performed on HPLC system. The HPLC column was eluted at 1 ml/min with a mobile phase of acetonitrile:aqueous containing 0.2% formic acid (v:v=12:88). The detection wavelength was set at 267 nm. The standard curve of AZT was employed to quantify the AZT metabolite due to the lack of the standard of AZT glucuronide.

Determination of BPA's inhibition towards the glucuronidation reaction of AZT

Inhibition ability of different concentrations of BPA

towards the glucuronidation of AZT was determined, and the IC_{50} value was determined as previously described¹⁰. The reaction velocity was determined at multiple concentrations of BPA and AZT. Dixon plot (1/reaction velocity versus the concentration of BPA) and Lineweaver-Burk plot (1/reaction velocity versus 1/[the concentration of AZT]) were employed to determine the inhibition kinetic type, and nonlinear regression equation was performed using the equations for competitive inhibition (1), noncompetitive inhibition (2).

$$V = V_{\text{max}} * [S] / (K_m / (1 + ([I]/K_i)) + [S]) \quad (1)$$

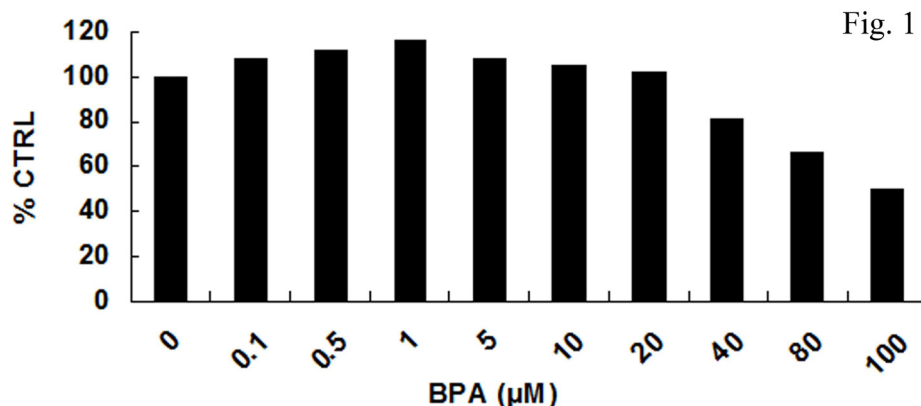
$$V = V_{\text{max}} * [S] / (K_m + [S]) * (1 + ([I]/K_i)) \quad (2)$$

Where the items are defined as followed: V is the reaction velocity, [S] and [I] are the concentrations of substrate and inhibitor, respectively. K_m value is the substrate concentration in which the velocity reached to half of the maximum velocity (V_{max}) of the reaction. K_i value is the inhibition constant.

Results

The concentration-dependent inhibition behaviour of BPA towards human liver microsomes (HLMs)-catalyzed AZT glucuronidation was firstly investigated using multiple concentrations of BPA. The results (Fig. 1) showed that the activity of AZT glucuronidation was inhibited by -7.6%, -10.9%, -16.4%, -7.9%, -5.1%, -2.1%, 19.1%, 33.7%, and 50% by 0.1, 0.5, 1, 5, 10, 20, 40, 80, and 100 μM of BPA.

Fig. 1 Concentration-dependent inhibition of BPA towards human liver microsomes (HLMs)-catalyzed zidovudine (AZT) glucuronidation. The used concentration of BPA was 0.1, 0.5, 1, 5, 10, 20, 40, 80, and 100 μM .



Dixon plot (Fig. 2A) and Lineweaver-Burk plot (Fig. 2B) have been regarded to be the most common methods to determine the inhibition type.

Fig. 2 Determination of inhibition kinetic type for

BPA's inhibition towards HLMs-catalyzed zidovudine (AZT) glucuronidation. (A) Dixon plot for BPA's inhibition towards HLMs-catalyzed zidovudine (AZT) glucuronidation. (B) Lineweaver-Burk plot for BPA's

inhibition towards HLMs-catalyzed zidovudine (AZT) glucuronidation. Each data point represents the mean

of duplicate experiment. Fig. 3 and parameter (K_i)

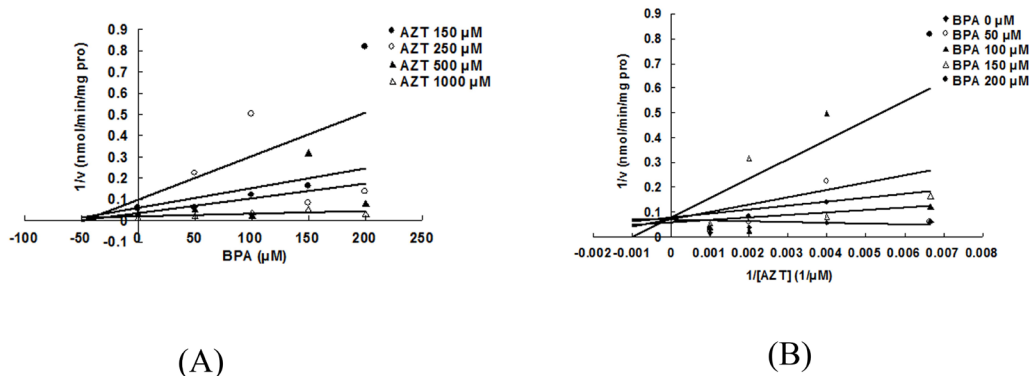


Fig. 2

The intersection point was located in the second quadrant and vertical axis in the Dixon and Lineweaver-Burk plot, respectively. The inhibition kinetic parameter (K_i) was calculated to be $3.2 \mu\text{M}$ through the data fitting with competitive inhibition equation (Fig. 3).

Discussion

Humans will be exposed to numerous xenobiotics everyday, such as food, drugs, herbs, and environmental pollutants. Therefore, the interaction among the compounds existed in these substances will occur, including drug-drug interaction and herb-drug interaction. All these interactions are mainly induced through affecting the pharmacokinetic behaviour, especially for the influence towards the metabolism of compounds. Many great efforts have been given to cytochrome P450 (CYP) which has been the most important DME involved in the metabolism of most of clinical drugs, and many compounds have been demonstrated to inhibit the activity of CYP isoforms. For example, the potential anti-tumor drug noscipine can inhibit the activity of CYP3A4 and CYP2C9 to explain the clinical noscipine-warfarin interaction¹¹. The herbal components ginsenosides have been reported to exhibit inhibition towards the activity of several CYP

isoforms¹². Halogenated aromatic compounds (HACs) have been reported to inhibit CYP 1A2-dependent MROD activity¹³.

The important role of UDP-glucuronosyltransferases (UGTs) in the metabolic elimination of xenobiotics and endogenous substances has been drawing more and more attention in recent years¹⁴, and some compounds have been observed to show inhibitory potential towards UGT isoforms. For example, ketoconazole has high potential to inhibit lorazepam clearance to a clinically significant extent through affecting the glucuronidation of R- and S-Lorazepam¹⁵. The inhibition of ginsenosides towards several UGT isoforms have been suspected to be a potential reason for ginseng-drugs interaction¹⁶. In the present study, we tried to investigate the inhibition of HLMs-catalyzed AZT glucuronidation by BPA which is an important endocrine disrupting chemical. Possible BPA-AZT interaction was indicated due to the competitive inhibition of BPA towards the glucuronidation of AZT with relatively low K_i value. Additionally, some previous literatures have indicated that the addition of bovine serum albumin (BSA) in the incubation system might significantly influence the *in vitro* inhibition parameters¹⁷. Therefore, different

inhibition kinetic parameters might be obtained if the BSA was added in the *in vitro* incubation system. It should be noted that BPA can inhibit the activity of another important UGT isoform UGT1A6 when using serotonin (5-HT) and 4-methylumbelliferone (4-MU) as the probe substrates¹⁸. All these results indicated that exposure of BPA will affect the metabolic elimination of some clinical drugs through influencing the activity of UGT isoforms.

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