3 Joint Center for Translational Medicine[1], Dalian Institute of Chemical Physics Chinese Academy of Sciences[2], The first Affiliated Hospital of Liaoning Medical University[3], No. 457, Zhongshan Road, Dalian 116023, China

Arbidol exhibits strong inhibition towards UDP-glucuronosyltransferase (UGT) 1A9 and 2B7

Xin Liu[1], Ting Huang[2], Jian-Xing Chen[2], Jia Zeng[2], Xu-Ran Fan[3], Xu-Zhu[3], Zhen-Wen Yu[3], Xiao-Yu Sun[3], Mo Hong[3], Hong-Zhi Sun[1]

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Hong-Zhi Sun, The First Affiliated Hospital of Liaoning Medical University, Jinzhou, 121001, China
cmushz@163.com

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The aim of the present study was to investigate arbidol's inhibition towards UDP-glucuronosyltransferase (UGT) 1A9 and 2B7. The nonspecific probe substrate 4-methylumbelliferone (4-MU) and recombinant UGT enzymes (UGT1A9, UGT2B7) were firstly used to evaluate the inhibition of arbidol towards UGT1A9 and UGT2B7. Furthermore, specific substrates of UGT1A9 and UGT2B7 propofol and zidovudine (AZT) were used to determine the inhibition of arbidol towards UGT1A9 and UGT2B7. Inhibition type and inhibition kinetic parameters (K_i) were determined. *In vitro-in vivo* extrapolation (IV-IVE) was performed to predict *in vivo* DDI magnitude induced by arbidol. Arbidol was demonstrated to exhibit competitive inhibition towards UGT1A9 and UGT2B7 without substate-dependent behaviour. The inhibition kinetic parameters (K_i) were calculated to be 0.5 μM, 3.5 μM, 2.8 μM, 29.7 μM for UGT2B7-mediated 4-MU glucuronidation, UGT1A9-mediated 4-MU glucuronidation, UGT2B7-mediated AZT glucuronidation, and UGT1A9-mediated propofol glucuronidation, respectively. Using these parameters, the *in vivo* alteration of area under of concentration-time curve (AUC) was calculated to be 156%, 22%, 28% and 2.6%, respectively. Given that arbidol exhibits strong inhibition towards UGT1A9 and UGT2B7, clinical monitoring should be given when arbidol was co-administered with drugs mainly undergoing UGT1A9, UGT2B7-mediated metabolism.

1. Introduction

Knowledge about the metabolism of a new chemical entity (NCE) is important to determine the optimal window between the drug safety parameters and its therapeutic potential (Lin and Lu 1997). Metabolic reaction of drugs is mainly catalyzed by drug metabolizing enzymes (DMEs) in the liver or other organs. When the activities of these DMEs are inhibited, the metabolic and pharmacokinetic behaviour of drugs will be influenced, which might induce severe clinical drug-drug interaction (DDIs) (Fang et al. 2011a). Cytochrome P450 (CYP)-mediated DDIs have received much attention because CYP is the most important DME and involved in the metabolism of most drugs used in clinical practice. Many *in vitro* evaluation systems have been developed to predict the *in vivo* DDI situation, and many successful examples have been reported (Fang et al. 2010, 2011b; Qu et al. 2011).

The human uridine glucuronosyltransferases (UGTs) are membrane proteins of the endoplasmic reticulum and can conjugate various endogenous substances and exogenous compounds (Miners and Mackenzie 1991). Glucuronidation reactions catalyzed by UGTs account for > 35% of all phase II drug metabolism (Kiang et al. 2005). UGTs-mediated metabolisms are normally regarded as detoxifying processes, and glucuronides have greater polarity than the parent drugs. Nevertheless, some glucuronides have been reported to show pharmacological and toxicological effects (Ritter 2000). Because UGTs can conjugate a number of important endogenous substances (e.g. bilirubin, steroid hormones, thyroid hormones, bile acids, fat-soluble vitamins, etc.), inhibition of UGT by xenobiotics can induce not only serious DDIs but also metabolic disorders of endogenous substances.

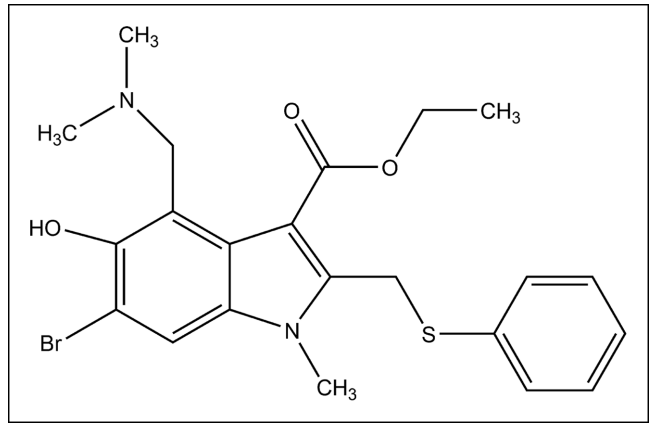

Fig. 1: The structure of arbidol.

Arbidol, ethyl-6-bromo-4-[(dimethylamino)-methyl]-5-hydroxy-1-methyl-2-[(phenylthio) methyl]-indole-3-carboxylate (Fig. 1), is an antiviral drug developed by the Chemical Drug Center of All Russian Research Institute of Pharmaceutical Chemistry together with the Institute of Medical Radiology. It has been demonstrated to exhibit inhibitory activity towards

various types of viruses, such as influenza virus, respiratory syncytial virus, adenovirus, rhinovirus, parainfluenza virus, hepatitis B virus, hepatitis C virus and coxsackie virus (Pecheu et al. 2007; Zhong et al. 2009). Therefore, arbidol is regarded as a broad-spectrum antiviral compound. Recently, arbidol has been approved for treatment of influenza in Russia, China and some European countries.

Patients often take multiple antiviral drugs and other drugs, so the DDIs associated with antiviral drugs should be considered in addition to individual drug clinical benefits and safety profiles (Taburet and Singlas 1996). The aim of the present study is to investigate the inhibitory potential of arbidol towards two important UGT isoforms: UGT1A9 and UGT2B7. Furthermore, *in vitro-in vivo* extrapolation (IV-IVE) will be carried out to predict *in vivo* DDI magnitude.

2. Investigations and results

2.1. Inhibition of UGT1A9 and UGT2B7 catalyzed 4-MU glucuronidation by arbidol

Arbidol strongly inhibited UGT2B7 and UGT1A9-mediated 4-MU glucuronidation in a concentration-dependent manner (Fig. 2A and Fig. 3A). Both Dixon and Lineweaver-Burk plots showed that both the inhibition of UGT2B7 (Fig. 2B and Fig. 3B) and UGT1A9 (Fig. 2C and Fig. 3C) were best fit to the competitive inhibition type. The reversible inhibition kinetic parameters (Ki) were calculated to be 0.5 μM and 3.5 μM for UGT2B7 and UGT1A9, respectively.

2.2. Arbidol inhibited UGT1A9-mediated propofol glucuronidation

Arbidol was demonstrated to inhibit UGT1A9-mediated glucuronidation of propofol in a concentration-dependent manner (Fig. 4A). Dixon (Fig. 4B) and Lineweaver-Burk (Fig. 4C) plots showed the inhibition behaviour was best fit to the competitive type, and the Ki value was determined to be 29.7 μM (Fig. 4D).

2.3. Investigation of the inhibitory potential of arbidol towards glucuronidation of AZT

Arbidol showed a concentration-dependent inhibition towards UGT1A9-mediated glucuronidation of AZT (Fig. 5A). Dixon (Fig. 5B) and Lineweaver-Burk (Fig. 5C) plots showed the inhibition behaviour was best fit to the competitive type, and the Ki value was determined to be 2.8 μM (Fig. 4D).

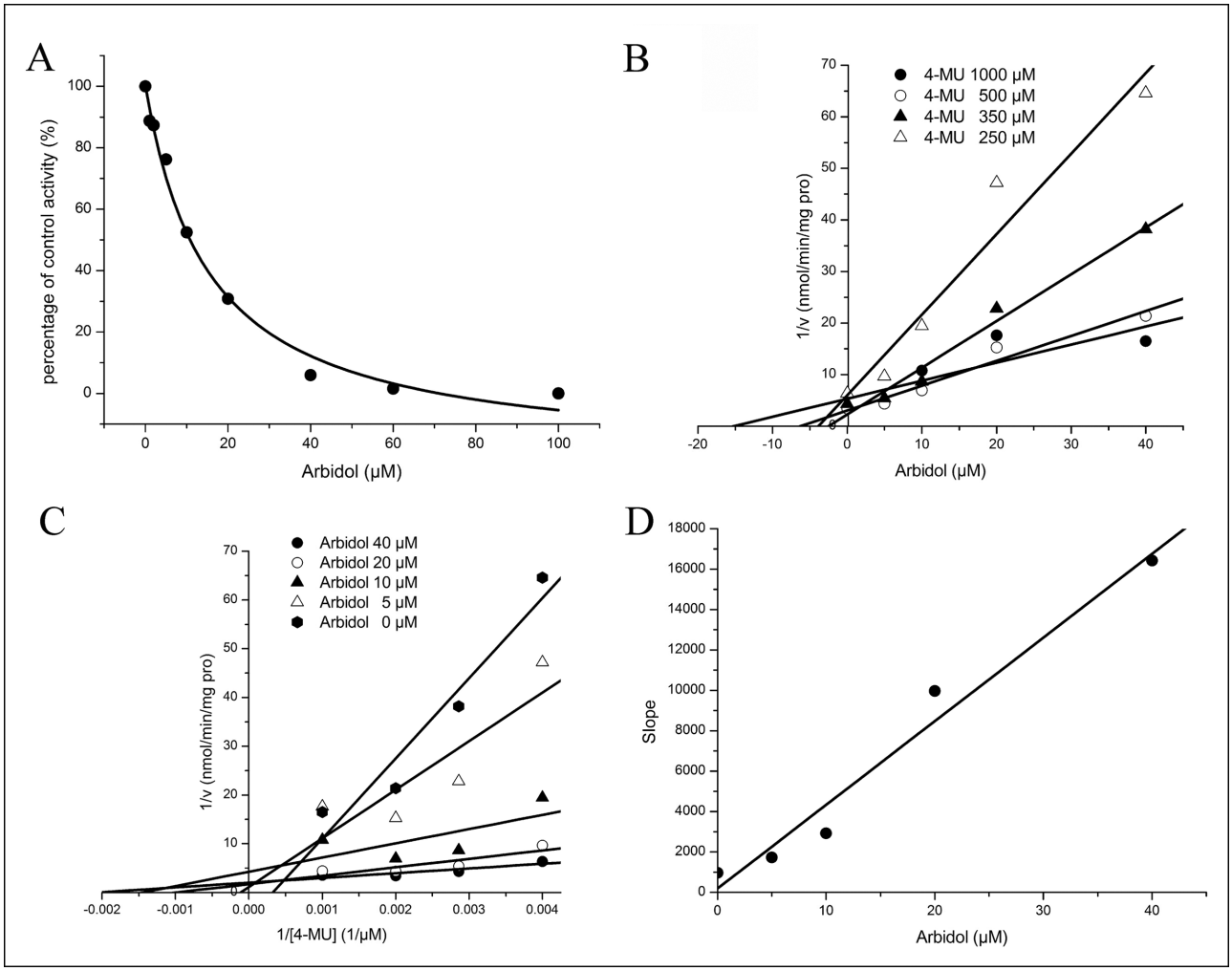

Fig. 2: Reversible inhibition of recombinant UGT2B7-mediated 4-MU glucuronidation by arbidol. (A) Inhibitory potential of arbidol on 4-MU glucuronidation activity. (C) Dixon plot of inhibitory effects of arbidol on 4-MU glucuronidation activity. (B) Lineweaver-Burk plot of inhibitory effects of arbidol on 4-MU glucuronidation activity. (D) Second plot of slopes from Lineweaver-Burk plot vs. arbidol concentrations.

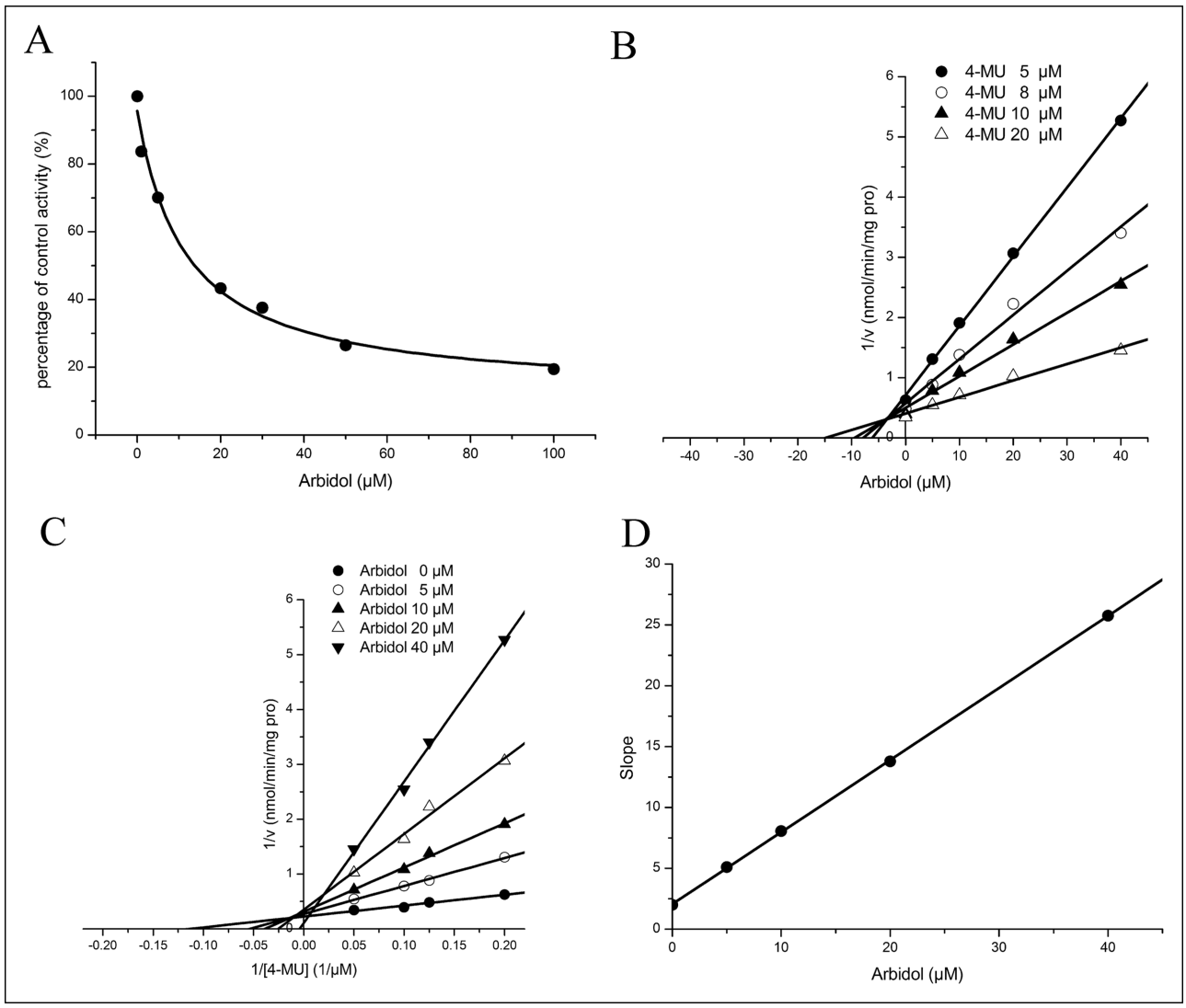

Fig. 3: Reversible inhibition of recombinant UGT1A9-mediated 4-MU glucuronidation by arbidol. (A) Inhibitory potential of arbidol on 4-MU glucuronidation activity. (C) Dixon plot of inhibitory effects of arbidol on 4-MU glucuronidation activity. (B) Lineweaver-Burk plot of inhibitory effects of arbidol on 4-MU glucuronidation activity. (D) Second plot of slopes from Lineweaver-Burk plot vs. arbidol concentrations.

2.4. Prediction of in vivo DDI magnitude

The maximum plasma drug concentration (C_{max}) of arbidol has been reported to be 414.8 ng/ml (0.78 μM) (Liu et al. 2009). Using C_{max} as the value of [I], the ratio of AUC_i/AUC was calculated to be 2.56, 1.22, 1.28, 1.026 when using the K_i value of 0.5 μM, 3.5 μM, 2.8 μM and 29.7 μM, respectively.

3. Discussion

UGT1A9 and UGT2B7 are the most important UGT isoforms. UGT1A9 has been demonstrated to be involved in the metabolism of endogenous estrogenic and thyroid hormones, as well as a variety of drugs, such as acetaminophen (Court et al. 2001), diclofenac (King et al. 2001), propofol (Soars et al. 2003), and mycophenolic acid (Bernard and Guillemette 2004). UGT2B7 has been reported to catalyze many important clinical drugs, including the antiretroviral drug zidovudine (AZT). Inhibition of the activity of these two UGT isoforms might change the pharmacokinetic behaviour of drugs mainly undergoing UGT1A9, UGT2B7-mediated glucuronidation, which will result in serious DDIs and adverse reactions. For example, the direct inhibition of UDP-glucuronosyltransferase activities has been identified as a mechanism of potentiation of acetaminophen hepatotoxicity. Fluconazole could increase the AUC, C_{max} and $t_{1/2}$ of AZT through inhibition of AZT glucuronidation, which is demonstrated by the reduction of formation of AZT glucuronide and the urinary molar ratio of AZT glucuronide to AZT (Sahai et al. 1994).

In the present study attention was paid to the evaluation of inhibitory potential of arbidol towards UGT1A9 and UGT2B7. Different enzyme source and substrates might significantly influence the inhibition of compounds towards DMEs. The experiment carried out by Dong et al. (2011) showed that the inhibition of CYP3A by erlotinib exhited substrate-dependent behaviour. When probe substrate of CYP3A testosterone was replaced with nifedipine, the inhibition type changed from competitive inhibition to noncompetitive inhibition (Jin et al. 2011). Therefore, three different substrates were selected to evaluate the inhibitory potential of arbidol towards UGT1A9 and UGT2B7, including a non-specific substrate 4-MU, and the specific substrates of UGT1A9 and UGT2B7 propofol and AZT. The corresponding enzyme sources were recombinant UGT isoforms and human liver microsomes. Arbidol was demonstrated to exhibit competitive inhibition towards both UGT1A9 and UGT2B7.

The *in vivo* DDI magnitude was influenced by both inhibitory capacity of inhibitor and *in vivo* plasma concentration of inhibitor. Therefore, *in vitro-in vivo* extrapolation (IV-IVE) was

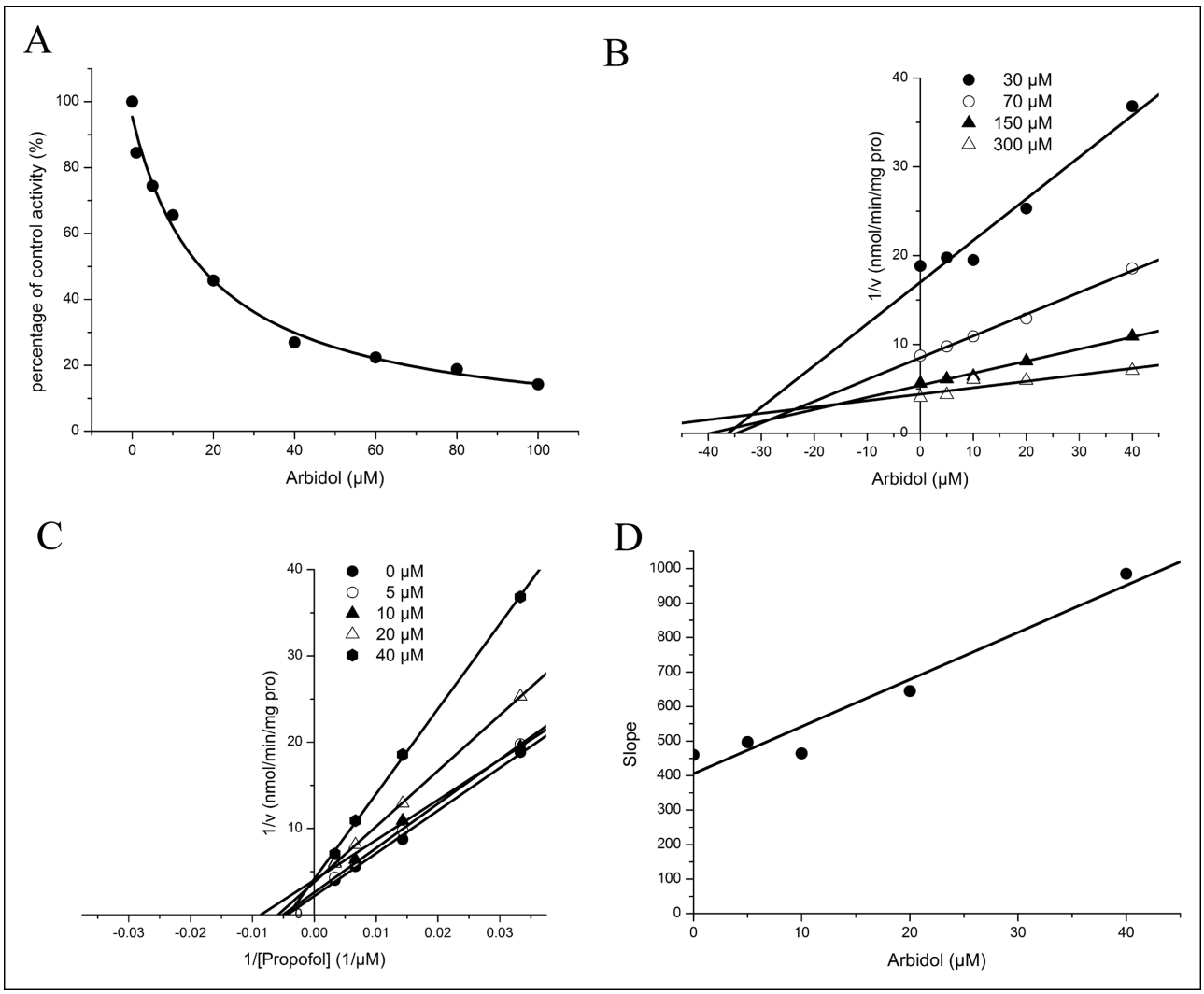

Fig. 4: Reversible inhibition of UGT1A9-mediated propofol glucuronidation reaction. (A) Inhibitory potential of arbidol on propofol glucuronidation activity. (C) Dixon plot of inhibitory effects of arbidol on propofol glucuronidation activity. (B) Lineweaver-Burk plot of inhibitory effects of arbidol on propofol glucuronidation activity. (D) Second plot of slopes from Lineweaver-Burk plot vs. arbidol concentrations.

further performed using the maximum plasma concentration of arbidol, and the AUC alteration of co-administered drugs was calculated to be 156%, 28% for UGT2B7-mediated metabolism when using 4-MU and AZT as probe substrates, respectively. The change of AUC of co-administered drugs was 22% and 2.6% for UGT1A9-catalyzed metabolism when utilizing 4-MU and propofol as probe substrates, respectively.

In conclusion, the combination of arbidol with other drugs might result in DDIs with drugs mainly undergoing UGT1A9 and UGT2B7-mediated metabolism. Therefore, clinical monitoring is needed when using arbidol.

4. Experimental

4.1. Reagents

Arbidol hydrochloride (purity > 98%) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, People's Republic of China. 7-hydroxycoumarin, propofol, zidovudine (AZT), 4-methylumbelliferone (4-MU), 4-methylumbelliferone-β-D-glucuronide (4-MUG), alamethicin, magnesium chloride ($MgCl_2$), D-saccharic acid 1, 4-lactone, UDP-glucuronic acid trisodium salt (UDPGA) were purchased from Sigma-Aldrich (St. Louris, MO). All other reagents were of HPLC grade or of the highest grade commercially available.

Human liver samples were obtained from Dalian Medical University (Dalian, Liaoning province, China) with the approval of the local ethics committee at the university. Any information on the medication history of the samples is not gained. A panel of human liver microsomes (HLMs) was prepared from twelve liver samples obtained from male and female patients by differential ultracentrifugation as described previously (Sun et al. 2010a, b). Microsomal protein concentrations were determined by the Lowry method with bovine serum albumin as standard (Lowry et al. 1951).

Recombinant human UGT supersomes UGT1A9 and UGT2B7 expressed in baculovirus-infected insect cells were purchased from BD Gentest Corp. (Woburn, MA, USA).

4.2. Inhibition of 4-MU glucuronidation

The probe substrate for all tested UGT isoforms was 4-MU which is a nonselective substrate of UGTs. Incubations with each UGT isoform were carried out as reported previously (Huang et al. 2010, 2011; Dong et al. 2012). The mixture (200 μL total volume) contained 0.05 mg/ml of recombinant UGT1A9 and UGT2B7, 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris-HCl buffer (pH 7.4) and 4-MU in the absence or presence of different concentrations of arbidol. The concentrations of 4-MU were 30 μM and 350 μM for UGT1A9 and UGT2B7, respectively. Arbidol was dissolved in methanol and the final concentration of methanol was 0.5% (v/v). After 5 min preincubation at 37 °C, the UDPGA was added in the mixture to initiate the reaction. The incubation time was 120 min and 30 min for UGT2B7 and UGT1A9, respectively. 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 autoinjector 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, and a SPD-10AVP UV detector. Chromatographic separation was carried out using a C18 column (4.6 × 200 mm, 5 μm, Kromasil).

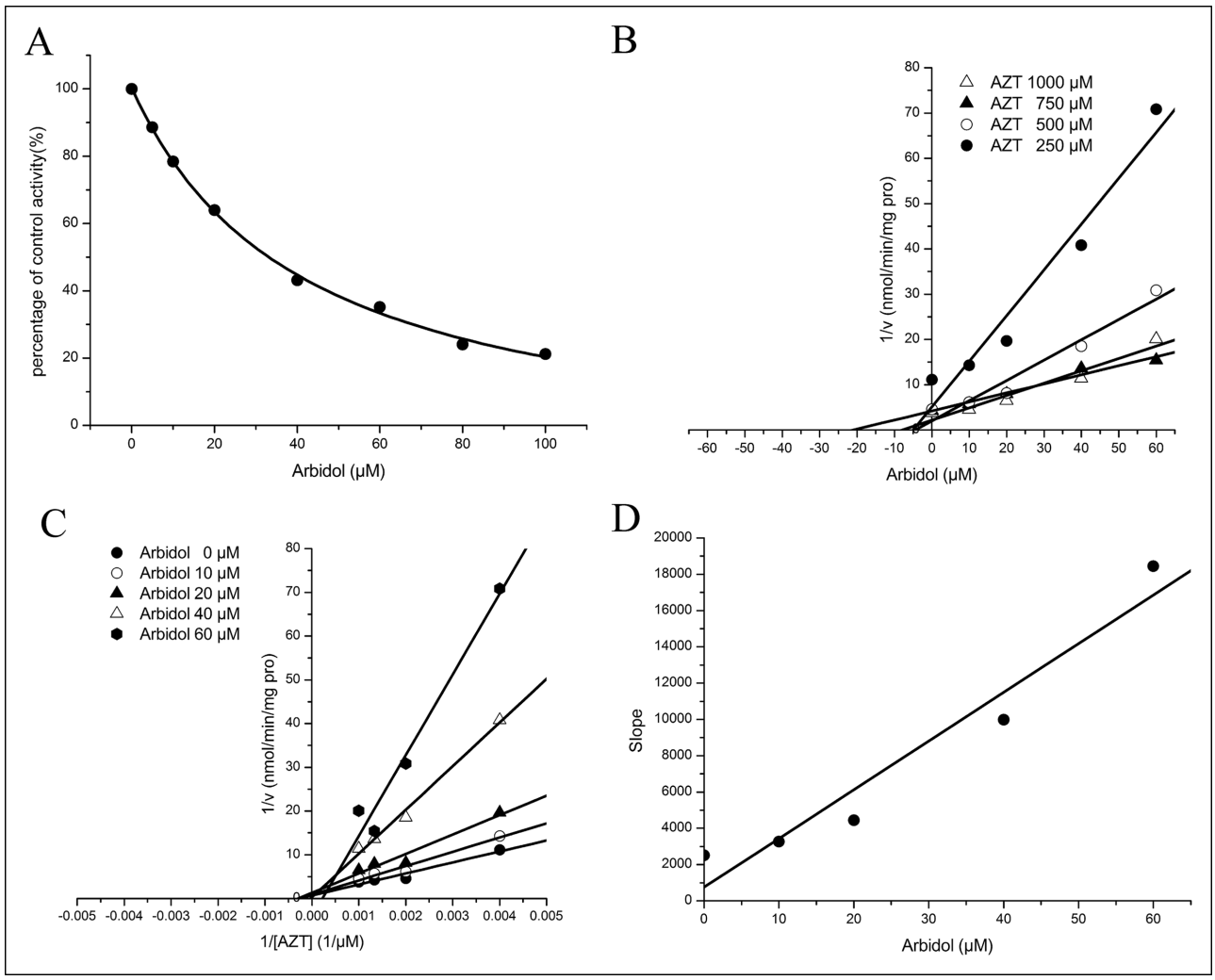

Fig. 5: Reversible inhibition of recombinant UGT2B7-mediated AZT glucuronidation by arbidol. (A) Inhibitory potential of arbidol on AZT glucuronidation activity. (C) Dixon plot of inhibitory effects of arbidol on AZT glucuronidation activity. (B) Lineweaver-Burk plot of inhibitory effects of arbidol on AZT glucuronidation activity. (D) Second plot of slopes from Lineweaver-Burk plot vs. arbidol concentrations.

4.3. Inhibition of propofol glucuronidation

Propofol, mainly metabolized by UGT1A9, could be used as a probe substrate for UGT1A9 (Liang et al. 2011). A typical incubation mixture contained 0.1 mg/mL HLM, 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris-HCl buffer (pH = 7.4), 50 μg/mg protein alamethicin and propofol (70 μM) in the absence or presence of different concentrations of arbidol. A Shimadzu LC/MS-2010EV mass spectrometer (Kyoto, Japan) was used to analyze the reaction mixture. It was equipped with two LC-20AD pumps, a DGU-20A3 vacuum degasser, an SIL-20ACHT autosampler, a CTO-20AC column oven, an SPD-M 20A diode array detector, a CBM-20A communications bus module and a mass detector with an ESI interface. A Shim-pack XRODS column (100 mm × 2.0 mm, 2.2 μm, Shimadzu) was kept at 40 °C. The mobile phase contains acetonitrile (A) and H_2O containing 0.2% acetic acid (B). The gradient conditions were as follows: 0–2 min, 95–83% B; 2–7 min, 83–76% B; 7–9.5 min, 10% B; 9.5–12.5 min, 95% B. The flow rate was 0.3 mL/min and the injection volume was 10 μL. The MS conditions were as follows: voltage, 4 kV; interface voltage, 40 V; nebulizing gas (N_2) flow was 1.5 L/min and the drying gas (N_2) pressure was set at 0.06MPa. Negative ion of [M-H]- of propofol O-glucuronide which was 353 and [M-H]- of I.S. (4-MUG) which was 351 were employed for determination with the SIM mode.

4.4. Inhibition of zidovudine (AZT) glucuronidation

The glucuronidation of zidovudine (AZT) has been widely regarded as the probe substrate of UGT2B7, and the experiment was carried out as previously described (Barbier et al. 2000). In brief, a typical incubation mixture contained 0.1 mg/mL HLM, 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris-HCl buffer (pH = 7.4), 50 μg/mg protein alamethicin and AZT (500 μM) in the absence or presence of different concentrations of arbidol.

4.5. Determination of inhibition kinetic parameters

Inhibition kinetic parameters (Ki) were determined utilizing various concentrations of 4-MU, propofol, or AZT in the presence of different concentrations of arbidol. Dixon and Lineweaver plots were adapted to determine the inhibition type, and second plot of slopes from Lineweaver-Burk plot *vs* arbidol concentrations was utilized to calculate the Ki value.

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

The equation $AUC_i/AUC = 1 + [I]/K_i$ was employed to predict the *in vivo* DDI magnitude. The terms in the equation were defined as follows: AUC_i/AUC is the predicted ratio of *in vivo* exposure of co-administered drugs with co-administration of arbidol *vs*. that in control situation. K_i is the reversible inhibition constant. [I] is the *in vivo* concentration of arbidol.

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