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Inhibition potential of glimepiride (gli) towards important UDP-glucuronosyltransferase (UGT) isoforms in human liver

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The aim of the present study was to investigate the inhibitory potential of glimepiride towards important UDP-glucuronosyltransferase (UGT) isoforms in human liver, which play a key role in the elimination of drugs. The recombinant UGT enzymes were used as enzyme source, and a nonspecific substrate 4-methylumbelliferone (4-MU) was utilized as substrate. The results showed that 100 μM of glimepiride inhibited UGT1A1, UGT1A3, UGT1A6, UGT1A9, UGT2B7 and UGT2B15 by 54.7%, 43.1%, 100%, 70.5%, 32.7 and 37.2%, respectively. Given that glimepiride exhibited strong inhibition towards UGT1A6, further inhibitory kinetic behaviour was determined. Glimepiride exerted concentration-dependent inhibition towards UGT1A6. Both Dixon and Lineweaver-Burk plots demonstrated that inhibition of UGT1A6 was best fit for noncompetitive inhibition type, and the inhibition kinetic parameter (K_i) was calculated to be 59.8 μM. Given that UGT1A6 plays a key role in detoxification of many drugs, more attention should be given when glimepiride was co-administered with the drugs mainly undergoing UGT1A6-mediated metabolism.

1. Introduction

Glimepiride (Gli), a medium-to-long acting third-generation sulfonylurea anti-diabetic drug, has been often used with diet and exercise, and sometimes with other medications. In previous studies, many drug-drug interactions (DDIs) between glimepiride and other drugs have been reported, such as atorvastatin-glimepiride interaction (Galani and Vyas 2010), voriconazole-glimepiride interaction (Shobha and Muppidi 2010), and gemfibrozil-glimepiride interaction (Niemi et al. 2001).

Pharmacokinetics-mediated drug-drug interaction is one of the most important inducing factor for clinical drug-drug interactions. Glimepiride has a half life of 3.4 h, peaks at 2–3 h and has a duration of action of 12–24 h. Glimepiride was mainly metabolized by cytochrome P450 (CYP) 2C9, and the inhibition of CYP2C9-mediated glimepiride metabolism has been widely regarded as a main reason for DDIs between glimepiride and other drugs. However, the interaction between glimepiride and other drug-metabolizing enzymes remains unclear.

UDP-Glucuronosyltransferases (UGTs) are membrane-bound drug metabolizing enzymes which can glucuronidate various endogenous and exogenous substances. Glucuronidation has been regarded to be a powerful detoxification pathway (Kiang et al. 2005). However, UGTs also play an important role in the formation of bioactive and toxic compounds. The pharmacokinetic behaviour of drugs could be altered by inhibition of UGT isoforms, and many drugs and herbal components have been reported to inhibit the activity of UGTs (Huang et al. 2010, 2011) to potentially induce the drug-drug interaction or herb-drug interaction. For example, plasma concentration of 3'-azido-3'-deoxythymidine (AZT) was elevated after concomitant administration of valproic and fluconazole (Lertora et al. 1994; Sahai et al. 1994). Increased plasma concentrations of aldosterone in patients treated with spironolactone might be due to the inhibition of UGT2B7 by spironolactone and canrenone (Knights et al. 2010).

The aim of the present study was to investigate the inhibitory potential of glimepiride towards major UGT isoforms in human liver. The recombinant UGT isoforms were used as enzyme sources, and a nonspecific substrate 4-methylumbelliferone (4-MU) was utilized as substrate.

2. Investigations, results and discussion

As shown in Fig. 1, the residual activity of 4-MU glucuronidation was 45.3 ± 1.6% (UGT1A1), 56.9 ± 3.6% (UGT1A3), 0.0 ± 0.0% (UGT1A6), 29.5 ± 0.7% (UGT1A9), 67.3 ± 0.3% (UGT2B7), and 62.8 ± 5.1% (UGT2B15) of the control activity at 100 μM of glimepiride, respectively. Further inhibition kinetic determination showed that glimepiride exerted concentration-dependent inhibition towards UGT1A6. Dixon and Lineweaver-Burk plots showed that the inhibition of UGT1A6 was all best fit to noncompetitive inhibition type. The inhibition kinetic parameter (K_i) was determined to be 59.8 μM for UGT1A6.

UDP-glucuronosyltransferases (UGTs) are important drug-metabolizing enzymes from clinical perspective. For example, Gilbert's syndrome is characterized by a 70%–80% reduction

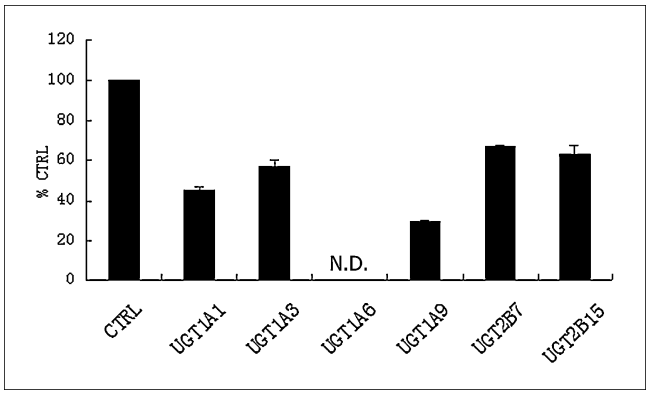

Fig. 1: Inhibitory potential of Gli towards major UGT isoforms in the liver. The recombinant UGT isoforms were used as enzyme sources, and a nonspecific substrate 4-methylumbelliferone (4-MU) was utilized as substrate

in the glucuronidation activity of UGT1A1 (Monaghan et al. 1996). UGTs also play a key role in the detoxification of some toxic drugs, including acetaminophen (APAP). Gunn rats with deficient expression of UGT1A subfamilyisoforms have been reported to exhibit 110-to 230-fold more susceptible to APAP toxicity compared with normal rats (de Morais and Wells 1988). In the past years, much attention has been paid to cytochrome P450 (CYP)-mediated DDI. The influence of inhibition of UGT isoforms on clinical DDI remains unclear. However, inhibition of UGT isoforms should draw more attention because that can not only induce clinical DDI but also aggravate some clinical deseases. For example, inhibition of UGT1A1 might further aggravate Glibert's syndrome. Tyrosine kinase inhibitors (TKIs) have been indicated to inhibit UGT-mediated APAP glucuronidation, which might be a potential cause for fatal acute liver failure induced by co-administration of these two kinds of drugs (Liu et al. 2011).

In the present study, glimepiride was demonstrated to inhibit UGT1A6 among tested important UGT isoforms in human liver. UGT1A6 is a key UGT isoform in the human liver and involved in the metabolism of many clinical drugs, including aspirin (Hutt et al. 1986) and serotonin (Krishnaswamy et al. 2003). Therefore, clinical monitoring should be given when glimepiride is co-administered with drugs which mainly undergo UGT1A6-mediated metabolism.

3. Experimental

3.1. Chemicals

Glimepiride (purity > 98%) was purchased from Aladdin Corp. (Shanghai, China). 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 supersomes (UGT1A1, UGT1A3, UGT1A6, UGT1A9, UGT2B7 and UGT2B15) 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.

3.2. Enzyme inhibition experiments

The probe substrate for all tested UGT isoforms is 4-MU which is a nonselective substrate of UGTs. Incubations with each UGT isoform were carried out as previously reported (Huang et al. 2010, 2011). The mixture (200 µl

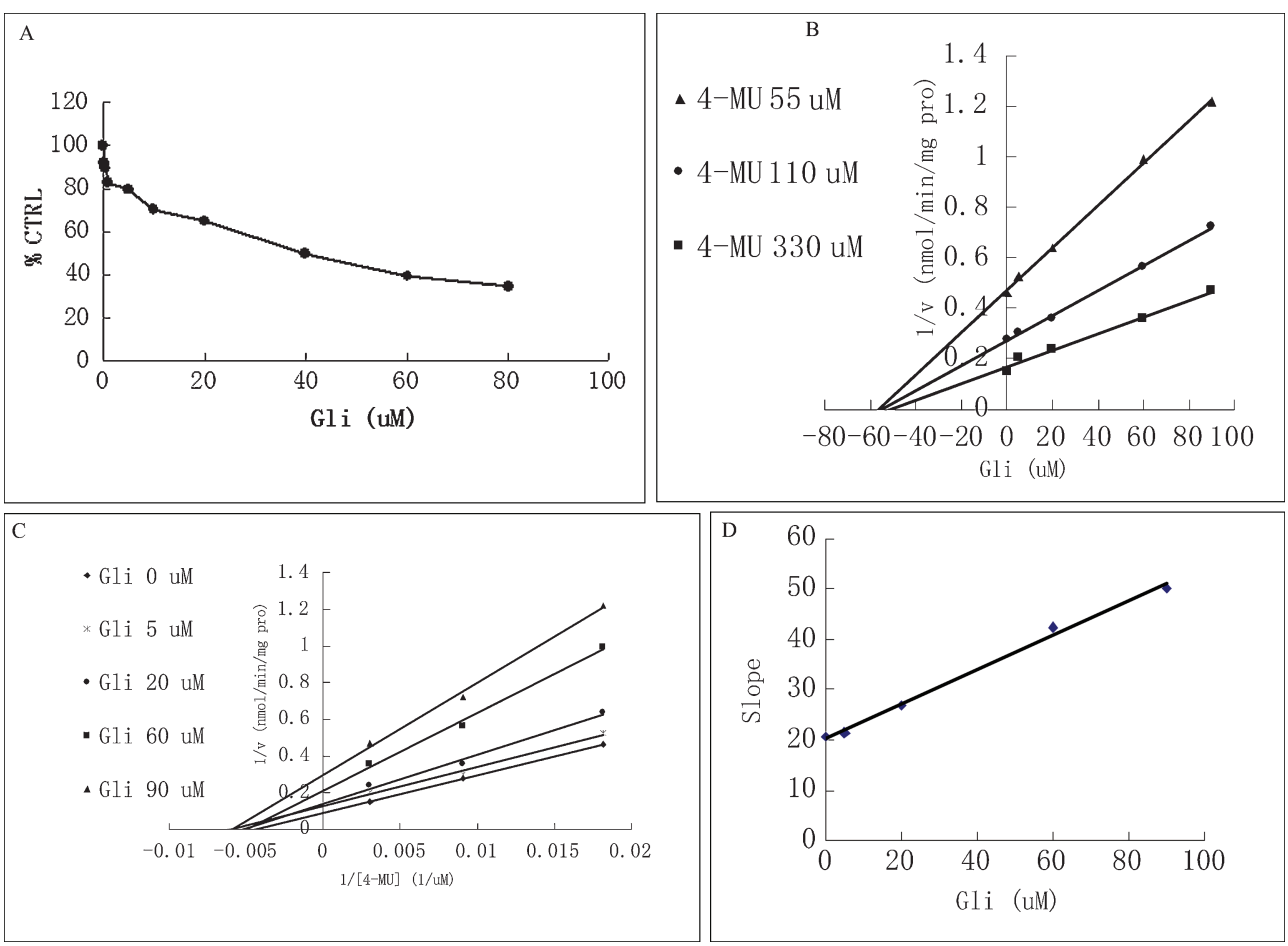

Fig. 2: Reversible inhibition of UGT1A6 by gli. A. Determination of IC50 value of UGT1A6. B. Dixon plot of inhibitory effect of tacro on UGT1A6-mediated 4-MU glucuronidation reaction. C. Lineweaver-Burk plot inhibitory effect of tacro on UGT1A6-mediated 4-MU glucuronidation reaction. D. Second plot of slope from Lineweaver-Burk plot vs. gli concentration

total volume) contained recombinant UGTs (final concentration: 0.25, 0.05, 0.025, 0.05, 0.05, 0.375 mg/ml for UGT1A1, UGT1A3, UGT1A6, UGT1A9, UGT2B7, and UGT2B15 respectively), 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 Gli. The concentrations of 4-MU are as follows: 110 μM for UGT1A1, 1200 μM for UGT1A3, 110 μM for UGT1A6, 30 μM for UGT1A9, 350 μM for UGT2B7, and 250 μM for UGT2B15. Gli was dissolved in methanol and the final concentration of of methanol was 0.5% (v/v). After 5 min pre-incubation at 37 °C, the UDPGA was added in the mixture to initiate the reaction. Incubation time was 120 min for UGT1A1, UGT2B7, and UGT2B15, 75 min for UGT1A3, 30 min for UGT1A6 and UGT1A9, reapectively. The reactions were quenched by adding 100 μl acetonitrile with 7-hydroxycoumarin (100 μM) as internal standard. The mixture was centrifuged at 20,000 × g for 10 min and an aliquot of supernatant was transferred to a 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 and UV detector at 316 nm. The mobile phase consisted of acetonitrile (A) and H2O 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.

3.3. Determination of inhibition kinetic parameters

For UGT1A6 whose activities were strongly inhibited at 100 μM of glimepiride, various concentrations of glimepiride were used to determine whether glimepiride exerted concentration-dependent inhibition towards UGT1A6. To evaluate the inhibitory kinetic type and calculate the inhibition parameters, various concentrations of glimepiride (0, 5, 20, 60, 90 μM) were added to the reaction mixture consisting of different concentrations of 4-MU (55, 110, 330 μM). Dixon and Lineweaver plots were adapted to determine the inhibition type, and second plot of slopes from Lineweaver-Burk plot vs. glimepiride concentrations was used to calculate the Ki value.

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