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Strong inhibitory effect of medroxyprogesterone acetate (MPA) on UDP-glucuronosyltransferase (UGT) 2B7 might induce drug-drug interactions

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The aim of the present study was to investigate the inhibitory effects of medroxyprogesterone acetate (MPA) on four important UGT isoforms (UGT1A1, 1A6, 1A9 and 2B7). 4-methylumbelliferone (4-MU) was used as a nonselective substrate, and recombinant UGT isoforms were utilized as an enzyme source. The results showed that MPA exhibited inhibitory effects on UGT2B7 ($IC_{50} = 29.3 \pm 1.5\ \mu M$), with a negligible influence on other UGT isoforms. The results obtained from Lineweaver-Burk and Dixon plots showed that MPA competitively inhibited UGT2B7. The K_i value was calculated to be 7.2 μM. Based on the concentration of MPA in human liver, the magnitude of *in vivo* drug-drug interaction (DDI) was predicted. The $[I]/K_i$ value was calculated to be 0.31, which suggested that DDIs might occur when MPA was co-administered with drugs which mainly undergo UGT2B7-mediated metabolism.

1. Introduction

Menopause-induced hormone loss seriously affects women's quality of life (Nappi et al. 1999). Hormone therapy (HT) is used extensively to attenuate menopause-induced symptoms, including hot flashes, urogenital atrophy and memory decline. Medroxyprogesterone acetate (MPA), a synthetic progestin, is widely used clinically. Statistics show that more than 10 million women in the United States have been prescribed MPA as the injectable contraceptive Depo Provera, and MPA could be prescribed in more than 90 countries (Ratchanon and Taneepanichskul 2000). Additionally, high-dose MPA has been employed to cure advanced breast cancer (Etienne et al. 1992; Falkson et al. 1992). Drug-drug interactions (DDIs) are an important reason for withdrawing drugs from the market and a source of high attrition in drug development. A previous report has demonstrated that MPA could exhibit inhibitory effects on the activity of CYP3A4 and CYP2C9. The results offer a possible explanation for a reported DDI case in which the serum concentration of phenytoin was elevated to four times the original level when it was co-administered with doxifluridine (5′-DFUR), MPA and cyclophosphamide (Zhang et al. 2006).
Glucuronidation catalyzed by UDP-glucuronosyltransferase (UGT) is a major step involved in the metabolism of many drugs, environmental chemicals and endogenous compounds. The pharmacokinetic behaviour of drugs could be altered by inhibition of these UGT isoforms and the search for drugs that potentially inhibit these UGT isoforms is very significant from a clinical point of view (Kiang et al. 2005). Therefore, the aim of the present study was to evaluate the inhibitory effects of MPA on four major UGT isoforms. 4-methylumbelliferone (4-MU) was used as a nonselective substrate, and recombinant UGT isoforms were utilized as an enzyme source.

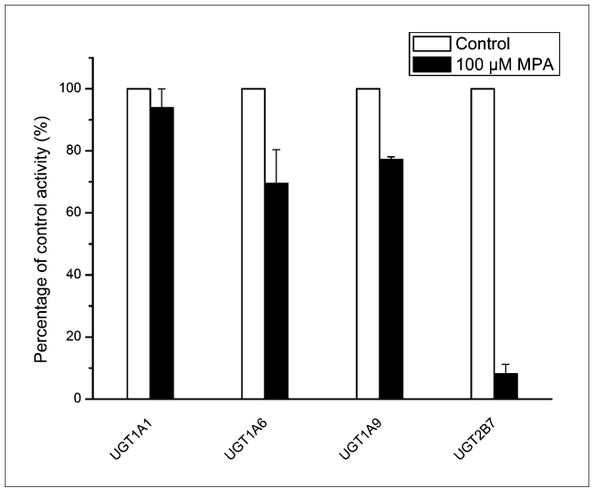

Fig. 1: Inhibition of four major UGT isoforms by MPA. Incubation conditions were described in Experimental

2. Investigations, results and discussion

As shown in Fig. 1, the residual activity of 4-MU glucuronidation was $93.9 \pm 6.1\%$ (UGT1A1), $69.5 \pm 11.0\%$ (UGT1A6), $77.3 \pm 0.8\%$ (UGT1A9), and $8.2 \pm 3.1\%$ (UGT2B7) of the

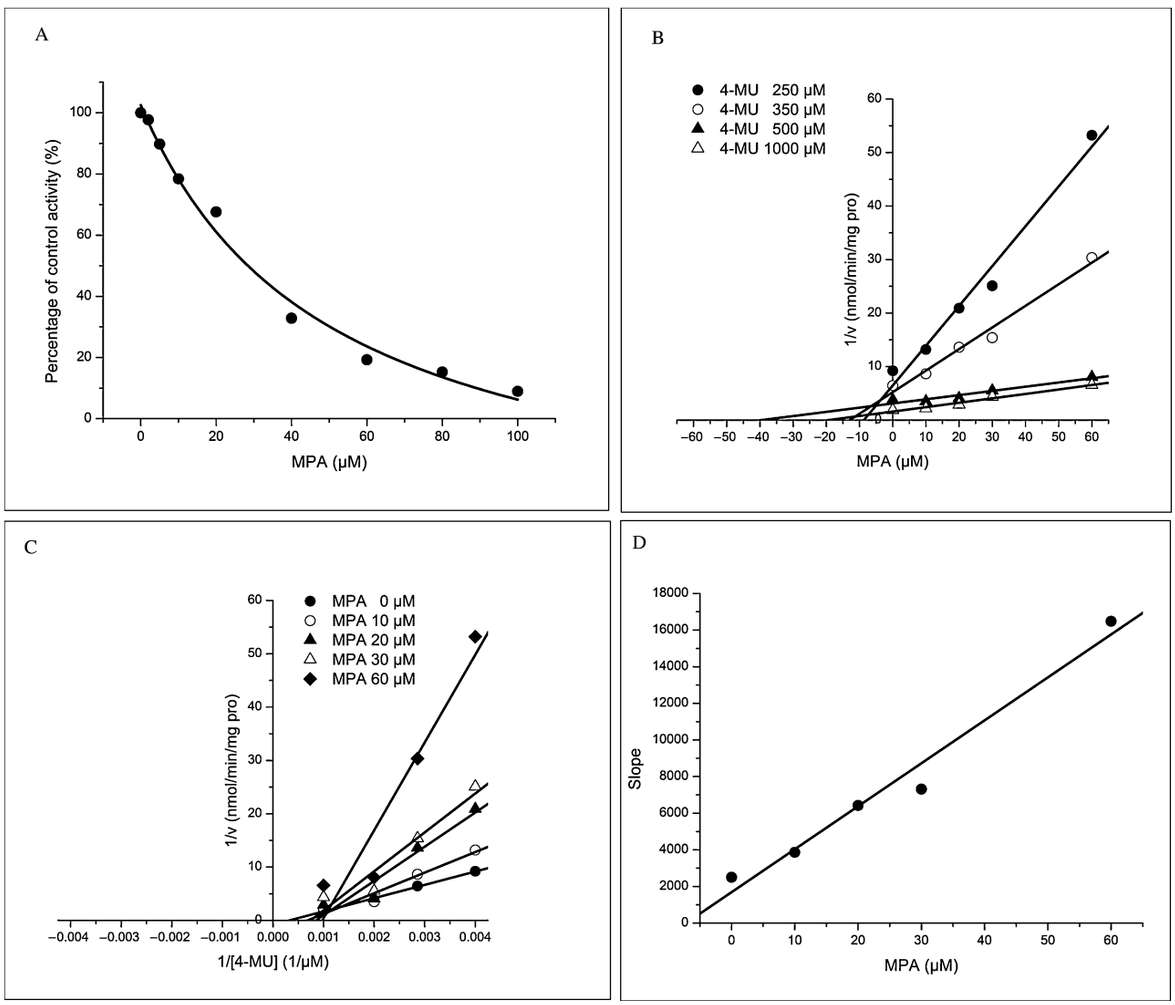

Fig. 2: A: Inhibitory effect of MPA on 4-MU glucuronidation activity (UGT2B7). B: Dixon plot of inhibitory effect of MPA on 4-MU glucuronidation activity (UGT2B7). C: Lineweaver-Burk plot of inhibitory effect of MPA on 4-MU glucuronidation activity (UGT2B7). D: Second plot of slopes from Lineweaver-Burk plot versus MPA concentrations

control activity at 100 μM of MPA. MPA showed inhibitory effects on the activity of UGT2B7 in a concentration-dependent manner, with an IC_{50} value of 29.3 ± 1.5 μM (Fig. 2A). Furthermore, both Lineweaver-Burk and Dixon plots (Figs. 2B, C) demonstrated that inhibition of UGT2B7 by MPA gave the best fit for types of competitive inhibition. A second plot of slopes from the Lineweaver-Burk plots vs. MPA concentrations was employed to calculate the K_i value, and the results showed K_i to be 7.2 μM for UGT2B7 (Fig. 2D).

UGT2B7 is arguably the most important drug metabolizing UGT isoform in human and could catalyze the glucuronidation of many drugs including nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids (Miners et al. 2010). A number of *in vitro* and *in vivo* studies have showed that inhibition of UGT2B7 might potentially induce significant drug-drug interactions (DDI). For example, an increase of 225% in the plasma concentration of AZT was reported with the coadministration of efavirenz (EFV) (Belanger et al. 2009).

The median plasma concentration of MPA was reported to be 50 ng/ml (0.13 μM) (Etienne et al. 1992). Accumulating evidence has demonstrated that orally administered MPA can accumulate in the liver during first pass metabolism in rats, and the MPA concentration in the liver was 16.9 fold more than the plasma concentration (Rautio et al. 1985). Assuming the liver to plasma concentration ratio in humans to be the same as in rats, the MPA concentration in human liver was calculated to be 2.2 μM. Using this concentration of MPA, the $[I]/K_i$ value was calculated to be 0.31 for UGT2B7, which suggested that potential for DDIs exists when MPA is co-administered with drugs which mainly undergo UGT2B7-mediated metabolism. Therefore, clinical monitoring is needed when MPA is co-administered with drugs which mainly undergo UGT2B7-mediated metabolism.

3. Experimental

3.1. Chemicals

Medroxyprogesterone acetate (MPA), 4-methylumbelliferone (4-MU), 4-methylumbelliferone-β-D-glucuronide (4-MUG), Tris-HCl, 7-hydroxycoumarin and uridine 5′-diphosphoglucu- ronic acid (UDPGA) (trisodium salt) were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant human UGT supersomes (UGT1A1, UGT1A6, UGT1A9 and UGT2B7) 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 the UGT isoforms tested was 4-MU which is a nonselective substrate of UGTs. Incubation with each UGT isoform was carried out as previously reported (Uchaipichat et al. 2004). The mixture (200 μl total volume) contained recombinant UGTs (final concentration: 0.25, 0.025,

0.05, 0.05 mg/ml for UGT1A1, UGT1A6, UGT1A9 and UGT2B7, 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 MPA. The concentrations of 4-MU were as follows: 110 μM for UGT1A1, 110 μM for UGT1A6, 30 μM for UGT1A9, and 350 μM for UGT2B7. MPA was dissolved in methanol and the final concentration of methanol was 0.5% (v/v). After 5 min pre-incubation at 37 °C, the UDPGA was added to the mixture to initiate the reaction. Incubation time was 120 min for UGT1A1 and UGT2B7, and 30 min for UGT1A6 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 20,000 × 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, and a SPD-10AVP UV detector Chromatographic separation used a C_{18} column (4.6 × 200 mm, 5 μ, Kromasil) at a flow rate of 1 ml/min with UV detection at 316 nm. The mobile phase consisted of acetonitrile (A) and H_2O containing 0.5 % (v/v) formic acid (B). The following gradient conditions were used: 0–15 min, 95–40% B; 15–20 min, 10% B; 20–30 min, 95% B.

3.3. Determination of inhibition kinetic parameters

The activity of UGT2B7 was inhibited by more than 50% at 100 μM MPA, and various concentrations of MPA were used to determine the half inhibition concentration (IC_{50}). To evaluate the order of inhibition kinetics and calculate the inhibition parameters, various concentrations of MPA (0, 10, 20, 30, 60 μM) were added to reaction mixtures containing different concentrations of 4-MU (300, 600, 1000, 1500 μM). Dixon and Lineweaver plots were adapted to determine the inhibition type, and the second plot of slopes from Lineweaver-Burk plot vs. MPA concentrations was utilized to calculate K_i value.

3.4. Prediction of in vivo DDI magnitude induced by inhibition of UGT2B7 by MPA

The following equation was used as previously reported (Fang et al. 2010):

$$\frac{AUC_I}{AUC} = 1 + \frac{[I]}{K_i}$$

Where AUC_I/AUC is the predicted ratio of *in vivo* exposure of drugs with co-administration of MPA *vs.* control exposure, [I] is the concentration of MPA in human liver and K_i is the *in vitro* inhibition parameter. Potential for DDI may be divided into three categories, based on the $[I]/K_i$ ratio: (1) likely, $[I]/K_i > 1$; (2) possible, $0.1 < [I]/K_i < 1$; (3) remote, $[I]/K_i < 0.1$.

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References

Belanger AS, Caron P, Harvey M, Zimmerman PA, Mehlotra RK, Guillemette C (2009) Glucuronidation of the antiretroviral drug efavirenz (EFV) by UGT2B7 and an *in vitro* investigation of drug-drug interaction with zidovudine (AZT). Drug Metab Dispos 37: 1793–1796.

Etienne MC, Milano G, Frenay M, Renee N, Francois E, Thyss A, Schneider M, Namer M (1992) Pharmacokinetics and pharmacodynamics of medroxyprogesterone acetate in advanced breast cancer patients. J Clin Oncol 10: 1176–1182.

Falkson CI, Falkson G, Falkson CB, Falkson HC (1992) Mitomycin C+ high-dose medroxyprogesterone versus cyclophosphamide+doxorubicin plus fluorouracil as first-line treatment for metastatic breast cancer. Oncology 49: 418–421.

Fang ZZ, Zhang YY, Ge GB, Huo H, Liang SC, Yang L (2010) Time-dependent inhibition (TDI) of CYP3A4 and CYP2C9 by noscapine potentially explains clinical noscapine-warfarin interaction. Br J Clin Pharmacol 69: 193–199.

Kiang TK, Ensom MH, Chang TK (2005) UDP-glucuronosyltransferases and clinical drug-drug interactions. Pharmacol Ther 106: 97–132.

Miners JO, Mackenzie PI, Knights KM (2010)The prediction of drug glucuronidation parameters in humans: UDP-glucuronosyltransferase enzyme-selective substrate and inhibitor probes for reaction phenotyping and *in vitro-in vivo* extrapolation of drug clearance and drug-drug interaction potential. Drug Metab Rev 42: 189–201.

Nappi RE, Sinforiani E, Mauri M, Bono G, Polatti F, Nappi G (1999) Memory functioning at menopause: Impact of age in ovariectomized women. Gynecol Obstet Invest 47: 29–36.

Ratchanon S, Taneepanichskul S (2000) Depot medroxyprogesterone acetate and basal serum prolactin levels in lactating women. Obstet Gynecol 96: 926–928.

Rautio A, Saami HU, Arranto AJ, Stengård JH, Kärki NT, Sotaniemi EA (1985) Metabolism of medroxyprogesterone acetate and hepatic drug metabolism activity. Res Commun Chem Pathol Pharmacol 47: 305–308.

Uchaipichat V, Mackenzie PI, Guo XH, Gardner-Stephen D, Galetin A, Houston JB, Miners JO (2004) Human UDP- glucuronosyltransferases: isoform selectivity and kinetics of 4-methylumbelliferone and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenecid. Drug Metab Dispos 32: 413–423.

Zhang JW, Liu Y, Li W, Hao DC, Yang L (2006) Inhibitory effect of medroxyprogesterone acetate on human liver cytochrome P450 enzymes. Eur J Clin Pharmacol 62: 497–502.