National Population and Family Planning Key Laboratory of Contraceptives Drugs & Devices[1], Shanghai Institute of Planned Parenthood Research, Shanghai; Laboratory of Pharmaceutical Resource Discovery[2], Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian; Graduate School of Chinese Academy of Sciences[3], Beijing; School of Traditional Chinese Medicine[4], Shenyang Pharmaceutical University, Shenyang, China

Inhibitory potential of chlormadinone acetate (CMA) on five important UDP-glucuronosyltransferases in human liver

TING HUANG[1,*], ZHONG-ZE FANG[2,3,*], YAN-YAN ZHANG[2,3], LIANG-LIANG ZHU[2,3], LING-LIN FENG[1], WEI ZHENG[1], YUN-FENG CAO[4], DONG-XUE SUN[4], LING YANG[2]

Received August 19, 2010, accepted September 29, 2010

Ling Yang, Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 116023, Dalian, China
yling@dicp.ac.cn
**These two authors contribute equally to this work.*

Pharmazie 66: 212–215 (2011) *doi: 10.1691/ph.2011.0745*

Chlormadinone acetate (CMA), a derivative of 17-a-hydroxyprogesterone, has been widely used as an orally effective progestogen in hormone replacement therapy (HRT). Glucuronidation catalyzed by UDP-glucuronosyltransferases (UGTs) is one of the major steps responsible for the metabolism of many drugs, environmental chemicals and endogenous compounds. Pharmacokinetic behaviours 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. In the present study, inhibition of five important UGT isoforms in human liver (UGT1A1, 1A3, 1A6, 1A9 and 2B7) by CMA was investigated using 4-MU as nonspecific substrate and recombinant UGT isoforms as enzyme sources. The results showed that CMA exhibited inhibitory effects on UGT1A3 ($IC_{50} = 8.6 \pm 1.4$ μM) and UGT2B7 ($IC_{50} = 14.2 \pm 3.8$ μM), with other UGT isoforms negligibly influenced. Lineweaver-Burk and Dixon plots showed that CMA noncompetitively inhibited UGT1A3 and UGT2B7. The K_i value was calculated to be 36.9 μM and 4.1 μM for UGT1A3 and UGT2B7, respectively. Considering that UGT1A3 and UGT2B7 are involved in the metabolism of many drugs, special attentions should be paid when CMA was co-administered with the drugs which mainly underwent UGT1A3, 2B7-mediated metabolism.

1. Introduction

Chlormadinone acetate (CMA), a derivative of 17-a-hydroxyprogesterone, has been demonstrated to exhibit distinct antiandrogenic activity without anabolic or androgenic activity (Terouanne et al. 2002). In 1998, the combined monophasic low-dose oral contraceptive ethinyl estradiol (EE) 0.03 mg and chlormadinone acetate (CMA) 2 mg was approved in Germany (Zahradnik et al. 1998). The efficacy of CMA in an oral contraceptive to treat acne in women has been demonstrated in a comparative study (Worret et al. 2001).

Glucuronidation catalyzed by UDP-glucuronosyltransferases (UGTs) is one of the major steps responsible for the metabolism of many drugs, environmental chemicals and endogenous compounds (Kiang et al. 2005). This conjugation reaction renders lipophilic compounds more water-soluble and stimulates their urinary and biliary excretion (Tukey and Strassburg 2000). The human UGT superfamily is comprised of 2 families (UGT1 and UGT2) and 3 subfamilies (UGT1A, UGT2A and UGT2B). The following UGT enzymes are expressed in the human liver: UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17 and UGT2B28. Among them, UGT1A1, UGT1A3, UGT1A6, UGT1A9 and UGT2B7 are regarded as the most important UGT isoforms in human liver (Fisher et al. 2001). 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 (Miners et al. 2010). Many drugs have been demonstrated to strongly inhibit the activity of these UGT isoforms, and some examples exhibited clinical significance. For example, the nonsteroidal anti-inflammatory drugs are a class of medications that are extensively glucuronidated and susceptible to UGT-mediated drug interactions (Mano et al. 2007). Plasma concentration of 3′-azido-3′-deoxythymidine (AZT) was elevated with concomitant administration of valproic and fluconazole (Lertora et al. 1994; Sahai et al. 1994). Recent experimental results showed that the elevated plasma concentrations of aldosterone in patients treated with spironolactone might be due to an inhibition of UGT2B7 by spironolactone and canrenone (Knights et al. 2000).

In the present work, the focus was given on the inhibitory potential of CMA on five major UGT isoforms in human liver using 4-methylumbelliferone as a nonselective substrate and recombinant UGT isoforms as enzyme sources.

2. Investigations and results

As shown in Fig. 1, the residual activity of 4-MU glucuronidation was 120.8 ± 5.8 % (UGT1A1), 24.7 ± 4.3 % (UGT1A3), 53.2 ± 0.8 % (UGT1A6), 70.5 ± 9.5 % (UGT1A9), 9.9 ± 1.4 %

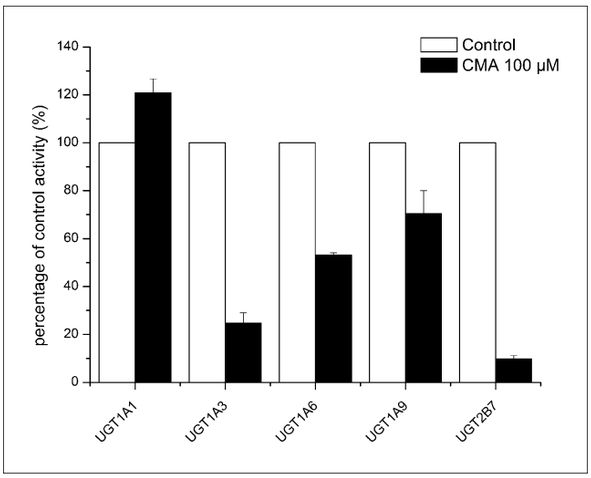

Fig. 1: Inhibition of five major UGT isoforms in human liver by CMA (100 μM). Incubation conditions were described in the Experimental section.

(UGT2B7) of the control activity at 100 μM of CMA. CMA showed inhibitory effects on the activity of UGT1A3 and UGT2B7 in a concentration-dependent manner, with IC_{50} values of 8.6 ± 1.4 μM (Fig. 2A) and 14.2 ± 3.8 μM (Fig. 3A) for UGT2B7 and UGT1A3, respectively. Furthermore, both Lineweaver-Burk and Dixon plots demonstrated that inhibition of UGT2B7 (Fig. 2B, C) and UGT1A3 (Fig. 3B, C) by CMA was all best fit for noncompetitive inhibition type. A second plot of slopes from Lineweaver-Burk plot vs. CMA concentrations was employed to calculate the K_i value, and the results showed that the K_i values were 36.9 μM and 4.1 μM for UGT1A3 (Fig. 3D) and UGT2B7 (Fig. 2D), respectively.

3. Discussion

Drug-drug interaction (DDI) is an important reason for high attrition in drug R&D, and is drawing more and more attention in recent years. Inhibition of drug-metabolizing enzymes by drugs is one of the important mechanisms inducing DDI. In the past years, abundant research has been conducted on Cytochrome P450-mediated DDI and numerous achievements in this field have been reported (Michalets 1998; Tanaka 1998; Dresser et al. 2000). In contrast, UGTs-mediated DDI have received less attention. However, UGT isoforms are very important because many drugs and their metabolites undergo UGT-mediated metabolism (Miners et al. 2004). Statistical figures show that UGTs catalyze the metabolism of approximately 35% of all drugs metabolized by Phase II enzymes, and about one-seventh of the drugs prescribed in the USA in 2002 are cleared by UGTs (Williams et al.

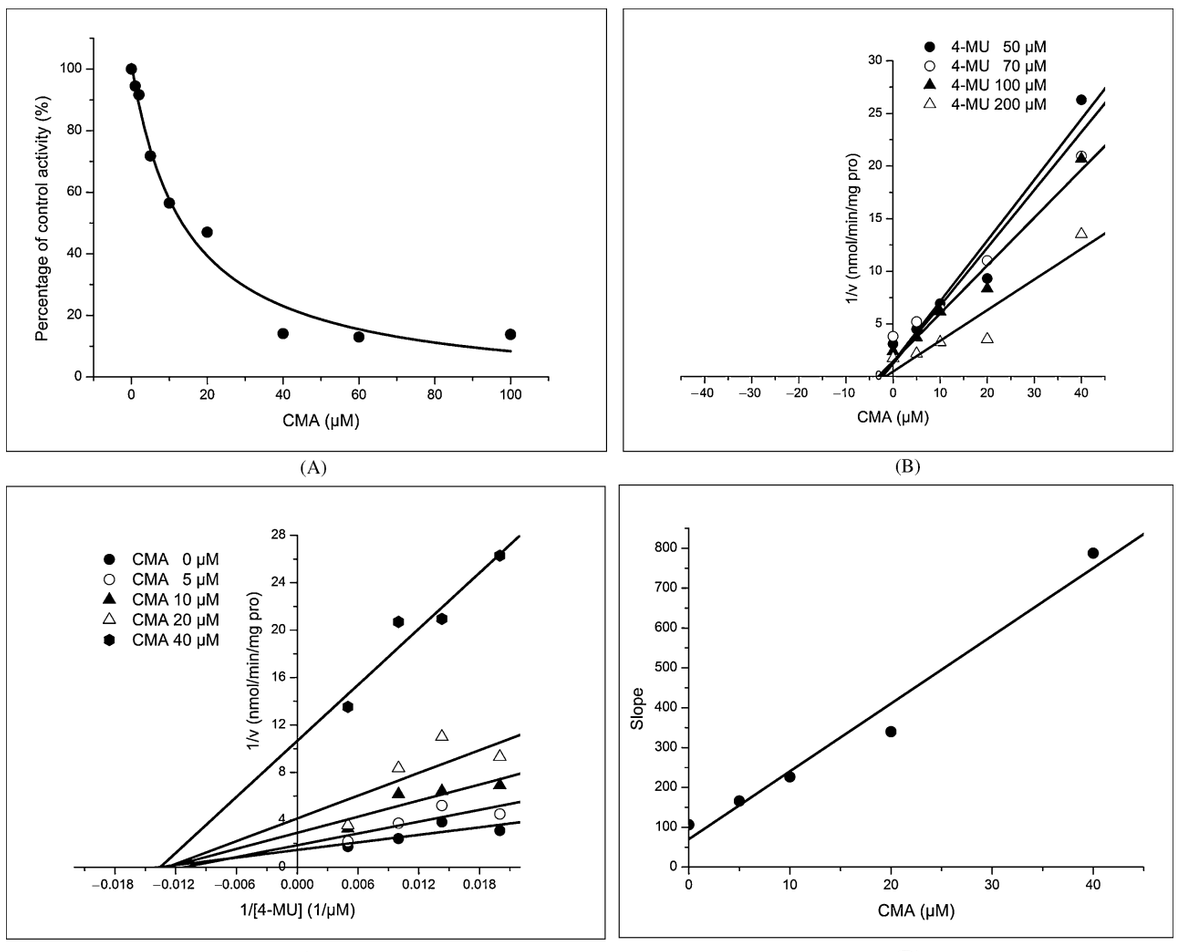

Fig. 2: A: Inhibitory effect of CMA on 4-MU glucuronidation activity (UGT2B7). B: Dixon plot of inhibitory effect of CMA on 4-MU glucuronidation activity (UGT2B7). C: Lineweaver-Burk plot of inhibitory effect of CMA on 4-MU glucuronidation activity (UGT2B7). D: Second plot of slopes from Lineweaver-Burk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

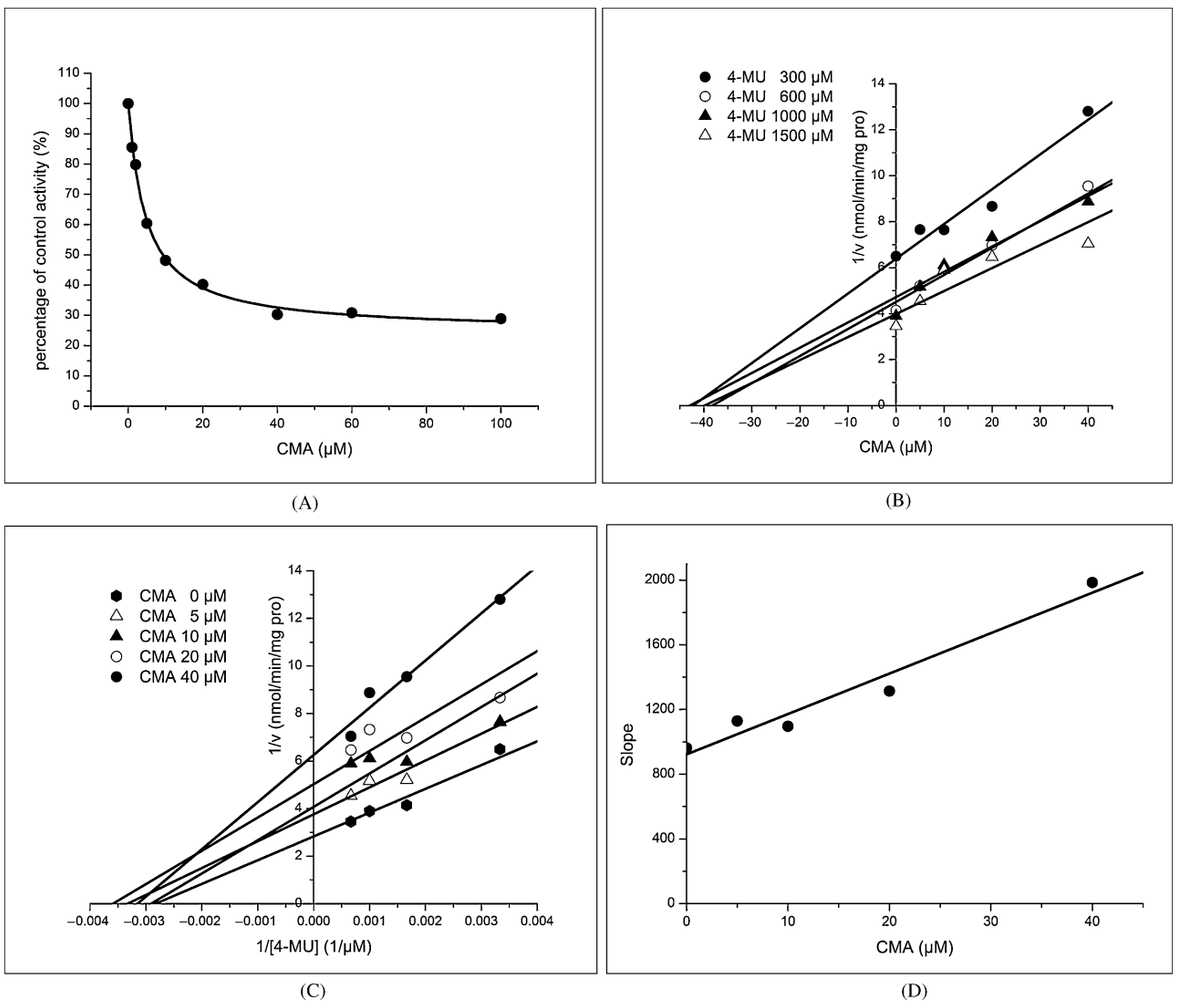

Fig. 3: A: Inhibitory effect of CMA on 4-MU glucuronidation activity (UGT1A3). B: Dixon plot of inhibitory effect of CMA on 4-MU glucuronidation activity (UGT1A3). C: Lineweaver-Burk plot of inhibitory effect of CMA on 4-MU glucuronidation activity (UGT1A3). D: Second plot of slopes from Lineweaver-Burk plot versus CMA concentrations.

2004). Therefore, a better understanding of UGTs-mediated DDI is very essential.

In the present experiment, inhibition of five important UGT isoforms in human liver by CMA was investigated. The limitation of any UGT enzyme inhibition study is the lack of a relatively specific probe substrate. Therefore, recombinantly expressed UGT isoforms and the nonspecific probe substrate 4-MU were adapted in our experiment. The results showed that CMA exhibited noncompetitive inhibition towards UGT1A3 and UGT2B7. It should be noted that K_i values of reported inhibitors of UGT2B7 were from 38 μM (ethinylestradiol) to 47000 μM (sulfisoxazole) (Herber et al. 1992; Resetar et al. 1991). Additionally, due to absence of BSA in our present study, the actual K_i value of CMA should be lower than the present experimental value (Rowland et al. 2007). Based on these considerations, CMA appeared to be a strong inhibitor of UGT2B7. UGT2B7, arguably regarded as the most important UGT isoform, could metabolize various endogenous compounds (such as fatty acids and 3-hydroxy steroids) and xenobiotic compounds (such as anticonvulsants, antineoplasstics, and non-steroidal anti-inflammatory drugs) (Miners et al. 2010). Accumulating data from *in vitro* and *in vivo* studies showed that inhibition of UGT2B7 by many compounds might induce clinically significant DDI, such as fluconazole-zidovudine interaction (Sahai et al. 1994) and methadone-zidovudine interaction (McCance-Katz et al. 1998). Therefore, the strong inhibition of UGT2B7 by CMA should be paid special attention.

In conclusion, the results of the present study demonstrated that CMA is a noncompetitive inhibitor for UGT1A3 and UGT2B7. Clinical monitoring is needed when CMA was co-administered with drugs which are mainly cleared by UGT1A3 and UGT2B7. All these data are of significance for the clinical application of CMA.

4. Experimental

4.1. Chemicals

Chlormadinone acetate (CMA) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products. 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 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.

4.2. Enzyme inhibition experiments

4-MU is utilized as a nonspecific probe substrate for all tested UGT isoforms. Incubations with each UGT isoform were carried out as previously reported (Uchaipichat et al. 2004). The mixture (200 μl total volume) contained

recombinant UGTs (final concentration: 0.25, 0.05, 0.025, 0.05, 0.05 mg/ml for UGT1A1, UGT1A3, 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 CMA. The concentrations of 4-MU were as follows: 110 μM for UGT1A1, 1200 μM for UGT1A3, 110 μM for UGT1A6, 30 μM for UGT1A9, and 350 μM for UGT2B7. CMA 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 and UGT2B7, 75 min for UGT1A3, 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, a SPD-10AVP UV detector. Chromatographic separation was carried out using a C_{18} 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 H_2O 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;

4.3. Determination of inhibition kinetic parameters

For UGT1A3 and UGT2B7 whose activities were inhibited more than 50% at 100 μM CMA, various concentrations of CMA were used to determine the half inhibition concentration (IC_{50}). To evaluate the inhibitory kinetic type and calculate the inhibition parameters, various concentrations of CMA (0, 5, 10, 20, 40 μM) were added to the reaction mixture consisting of different concentrations of 4-MU (50, 70, 100, 200 μM for UGT2B7, and 300, 600, 1000, 1500 μM for UGT1A3). Dixon and Lineweaver-Burk plots were adapted to determine the inhibition type, and second plot of slopes from Lineweaver-Burk plot vs. CMA concentrations was utilized to calculate K_i value.

Acknowledgement: This work was supported by the 973 program (2009CB522808) of the Ministry of Science and Technology of China, the National Key Technology R&D Program in the 11th Five-year Plan of China (2008ZX10002-019).

References

Dresser GK, Spence JD, Bailey DG (2000) Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. Clin Pharmacokinet 38: 41–57.

Fisher MB, Paine MF, Strelevitz TJ, Wrighton SA (2001) The role of hepatic and extrahepatic.

UDP-glucuronosyltransferases in human drug metabolism. Drug Metab Rev 33: 273–297.

Herber R, Magdalou J, Haumont M, Bidault R, van Es H, Siest G (1992) Glucuronidation of 3′-azido-3′-deoxythymidine in human liver microsomes: enzyme inhibition by drugs and steroid hormones. Biochim Biophys Acta 1139: 20–24.

Kiang TKL, Ensom MHH, Chang TKH (2005) UDP-Glucuronosyltransferases and clinical drug-drug interactions. Pharmacol Ther 106: 97–132.

Knights KM, Bowalgaha K, Miners JO (2010) Spironolactone and canrenone inhibit UGT2B7-catalyzed human liver and kidney microsomal aldosterone 18β-glucuronidation: A potential drug interaction. Drug Metab Disp 36: 1056–1062.

Lertora JJL, Rege AB, Greenspan DL, Akula S, George WJ, Hyslop NE Jr, Agrawal KC (1994) Pharmacokinetic interaction between zidovudine and valproic acid in patients infected with human immunodeficiency virus. Clin Pharmacol Ther 56: 272–278.

Mano Y, Usui T, Kamimura H (2007) Inhibitory potential of nonsteroidal anti-inflammatory drugs on UDP-glucuronosyltransferase 2B7 in human liver microsomes. Eur J Clin Pharmacol 63: 211–216.

McCance-Katz EF, Rainey PM, Jatlow P, Friedland G (1998) Methadone effects on zidovudine disposition. J Acquir Defic Syndr Hum Retrovirol 18: 435–443.

Michalets EL (1998) Update: clinically significant cytochrome P450 drug interactions. Pharmacotherapy 18: 84–112.

Miners JO, Smith PA, Sorich MJ, McKinnon RA, Mackenzie PI (2004) Predicting human drug glucuronidation parameters: application of *in vitro* and *in silico* modeling approaches. Annu Rev Pharmacol Toxicol 44: 1–25.

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.

Resetar A, Minick D, Spector T (1991) Glucuronidation of 3′-azido- 3′-deoxythymidine catalyzed by human liver UDP-glucuronosyltransferase. Biochem Pharmacol 42: 559–568.

Rowland A, Gaganis P, Elliot DJ, Mackenzie PI, Knights KM, Miners JO (2007) Binding of inhibitory fatty acids is responsible for the enhancement of UDP-glucuronosyltransferase 2B7 activity by albumin: implications for *in vitro-in vivo* extrapolation. J Pharmacol Exp Ther 321: 137–147.

Sahai J, Gallicano K, Pakuts A, Cameron DW (1994) Effect of fluconazole on zidovudine pharmacokinetics in patients infected with human immunodeficiency virus. J Infect Dis 169: 1103–1107.

Tanaka E (1998) Clinically important pharmacokinetic drug-drug interactions: role of cytochrome P450 enzymes. J Clin Pharm Ther 23: 403–416.

Terouanne B, Paris F, Servant N, Georget V, Sultan C (2002) Evidence that chlormadinone acetate exhibits antiandrogenic activity in androgen-dependent cell line. Mol Cell Endocrinol 198: 143–147.

Tukey RH, Strassburg CP (2000) Human UDP-glucuronosyltransferases: metabolism, expression and disease. Annu Rev Pharmacol Toxicol 40: 581–616.

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 Disp 32: 413–423.

Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, Peterkin V, Koup JR, Ball SE (2004) Drug-drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUC_i/AUC) ratios. Drug Metab Disp 32: 1201–1208.

Worret I, Arp W, Zahradnik HP, Andreas JO, Binder N (2001) Acne resolution rates: results of a single-blind, randomized, controlled, parallel phase III trial with EE/CMA (Belara) and EE/LNG (Microgynon). Dermatology 203: 38–44.

Zahradnik HP, Goldberg J, Andreas JO (1998) Efficacy and safety of the new antiandrogenic oral contraceptive Belara. Contraception 57: 103–109.