Original Article

Gemfibrozil Glucuronidation in Human Liver Microsomes is Catalyzed by UDP-Glucuronosyltransferase 2B7

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Abstract This study aimed to characterize the glucuronidation kinetics of gemfibrozil (GBZ) by human liver microsomes (HLMs) and to identify the human UDP-glucuronosyltransferase (UGT) enzymes responsible for these reactions. Using HLMs, the 2-Michaelis Menten kinetics were observed for GBZ glucuronidation with the mean high- and low-affinity apparent Km values of 5.6 ± 2.1 μM (Vmax 444 ± 176 pmol/min.mg) and 316 ± 113 μM (Vmax 1909 ± 838 pmol/min.mg), respectively. In addition, GBZ glucuronidation by HLMs was well described by a two-site model with mean derived binding affinity (Ks) and Vmax were 9.2 ± 3.2 μM and 889 ± 437 pmol/min.mg, respectively. GBZ glucuronidation by UGT 1A3 (Km 386 ± 2 μM) and 1A9 (Km 47 ± 0.3 μM) exhibited Michaelis-Menten kinetics, whereas substrate inhibition (Km 378 ± 25 μM and Ksi 2891 ± 319 μM) was observed with UGT1A6. Similar to HLMs, GBZ glucuronidation by UGT2B7 was well described by the two-site kinetic model which provided a Ks value of 7.0 ± 0.9 μM. Comparison of kinetic data suggests that UGT2B7 appears to be the enzyme responsible for the high-affinity component of human liver microsomal GBZ glucuronidation. These conclusions were confirmed using UGT2B7 (fluconazole) selective inhibitors. ©All right reserved.

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INTRODUCTION

Gemfibrozil (GBZ), 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid (Figure 1), is a fibric acid derivative which is widely used as a hypolipidaemic agent. GBZ is extensively metabolized and excreted in urine as a glucuronide metabolite.[1,2] Accumulated evidences suggested that GBZ inhibited the glucuronidation of statins and this finding has been postulated as the mechanism of interaction between both drugs.[3,4] In addition, it appears to be that GBZ glucuronide may play an important role for GBZ and statin interaction. GBZ glucuronide was found to be more potent inhibitor than its substrate to inhibit CYP2C8 and organic anion trans-porting polypeptide 1B1.[5] This mechanism appears to be the explanation for clinical drug interaction between GBZ and cerivastatin. Recent study also illustrated that the GBZ glucuronide time-dependently inhibited CYP2C8 *in vitro*, where the results described the potential mechanism of the clinical interaction reported between GBZ and CYP2C8 substrates such as cerivastatin, repaglinide, rosiglitazone and pioglitazone.[6] Given the important role of GBZ and its glucuronide metabolite involving metabolic drug interaction, we aimed to characterize the glucuronidation
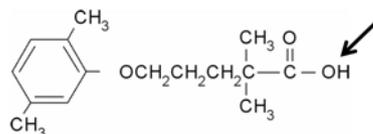

Figure 1. Gemfibrozil structure and site (arrow) of glucuronidation.

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kinetics of GBZ by human liver microsomes (HLMs) and to identify the human UDP-glucuronosyltransferase (UGT) enzymes responsible for these reactions.

MATERIALS AND METHODS

Materials

β-Glucuronidase (from *Trichoderma viride*), alamethicin (from *Escherichia coli*), gemfibrozil, and UDP-glucuronic acid (UDPGA, tri-sodium salt) were purchased from Sigma-Aldrich (St. Louis, MO). Fluconazole was obtained from Pfizer Ltd. (Sydney, Australia).

Human Liver Microsomes and Expressed UGT Proteins

Four liver tissues (H10, H12, H29 and H40) were obtained from human liver 'bank' of the Department of Clinical Pharmacology, Flinders Medical Centre. The use of human liver tissue in xenobiotic metabolism studies was approved from the Flinders Medical Centre Research Ethics Committee and from the donor next-of-kin. Human liver microsomes (HLMs) were prepared as described by Bowalgaha *et al.*[7] Microsomes were activated by preincubation with alamethicin (50 μg/mg protein) on ice for 30 minutes before their use in incubations.[8] UGT 1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B10, 2B15, 2B17 and 2B28 cDNAs were stably expressed in a human embryonic kidney cell line (HEK293) as described previously.[9]

Gemfibrozil Glucuronidation Assay

The incubation mixture, in a total volume of 200 μl, contained 0.1 M phosphate buffer (pH 7.4), 4 mM $MgCl_2$, 5 mM UDPGA, alamethicin-activated HLMs (0.125 mg/ml), and GBZ (1.5-2000 μM). After a 5-minute preincubation, reactions were initiated by the addition of UDPGA, and incubations were performed at 37ºC in a shaking water bath for 20 minutes. Following the addition of 4% acetic acid/96% methanol (0.2 ml), samples were centrifuged at 5000 *g* for 10 minutes and a 10 μl aliquot of the supernatant fraction was injected into the HPLC column. Using pooled HLMs from 5 human livers (H7, H12, H13, H29 and H40), the rates of glucuronide

formation were optimized for linearity with respect to protein concentration and incubation time. UGTs glucuronidation activities toward GBZ were screened at HEK cell lysate protein concentrations of 0.5 mg/ml, and incubation time of 30 minutes. GBZ glucuronidation was conducted at concentrations of 5 and 1000 μM. Where activity was observed, the kinetic of individual UGT was investigated. The incubation mixture contained HEK lysate protein concentration of 0.5 mg/ml (for UGT 2B7 and 1A3) or 1 mg/ml (for UGT 1A6 and 1A9), and the incubation time was 30 minutes.

Quantification of Gemfibrozil Glucuronide Formation

HPLC was performed using an Agilent 1100 series instrument (Agilent Technologies, Sydney, Australia) fitted with a Synergi Hydro-RP 80A (3 x 150 mm, 4 μm particle size; Phenomenex Inc., CA, USA). Analytes were separated by gradient elution at a flow rate of 0.6 ml/minute and detected by UV absorption at 225 nm. The initial conditions for GBZ glucuronide assay were 65% of 2 mM triethylamine/$HClO_4$ (pH 2.7) with 10% acetonitrile (mobile phase A) and 35% of acetonitrile (mobile phase B). The condition was held for 5 minutes; then the proportion of mobile phase B was increased to 65% over 0.1 minute and held for 5 minutes, then returned to 35% and run from 10.1 minutes until 15 minutes. GBZ glucuronide and GBZ were eluted at 6.5 and 11.4 minutes, respectively. The identities of GBZ were confirmed by using β-glucuronidase hydrolysis.

Data Analysis

Kinetic constants for substrate glucuronidation by HLMs or recombinant UGTs were performed by fitting untransformed experimental data to the following kinetic models using Enzfitter version 2.0 (Biosoft, Cambridge, UK). Kinetic constants are reported as the value ± standard error of the parameter estimate. Data were fitted to Michaelis-Menten equations for a single- and two-enzyme model, substrate inhibition model, and the two-site model described by Kenworthy *et al.*[10]

RESULTS AND DISCUSSION

GBZ glucuronidation kinetics by HLMs (n = 4) exhibited the two-enzyme Michaelis-Menten kinetic. The mean ± S.D. of high- and low-affinity apparent Km values were 5.6 ± 2.1 µM (Vmax 444 ± 176 pmol/min.mg) and 316 ± 113 µM (Vmax 1909 ± 838 pmol/min.mg), respectively. The mean microsomal intrinsic clearance for high- and low-affinity components of GBZ glucuronidation were 87.6 ± 36 µl/min.mg and 6.7 ± 3.4 µl/min.mg, respectively. GBZ glucuronidation by HLMs was also well described by a two-site model with mean derived binding affinity (Ks) of 9.2 ± 3.2 µM (Figure 2). The intrinsic clearance of the high-affinity component contributed 92% of the total intrinsic clearance. The Km and the contribution of high-affinity component obtained from human liver microsomal GBZ glucuronidation from this study were consistent with the results reported recently.[11] Assuming an unbound fraction of 0.03 and the total plasma concentration ranging from 60-100 µM,[12] the approximate plasma concentration of unbound GBZ following twice daily dose of 600 mg was 1.8-3 µM. This unbound concentration was similar to the Km value for the high-affinity component, suggesting that the high-affinity UGTs may play an important role for GBZ clearance in humans.

Recombinant UGT 1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B10, 2B15, 2B17 and 2B28 activities toward GBZ glucuronidation were screened at substrate concentrations of 5 and 1000 µM (Figure 3).
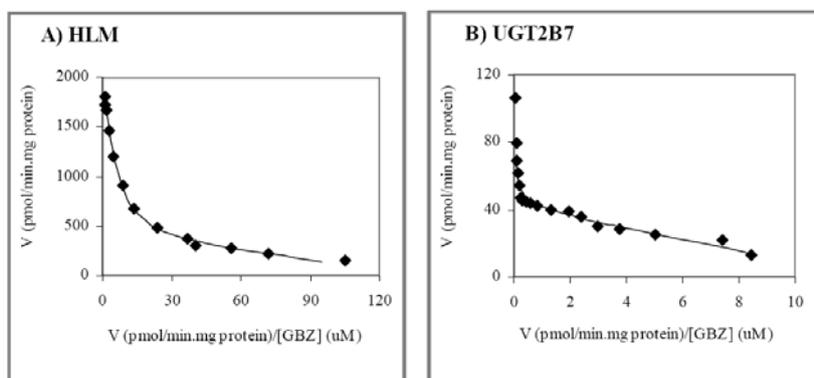

Figure 2. Representative Eadie-Hofstee plots for gemfibrozil glucuronidation by (A) human liver microsomes and (B) recombinant human UGT2B7. Points are experimentally determined values, whereas lines are from two-site model fitting.
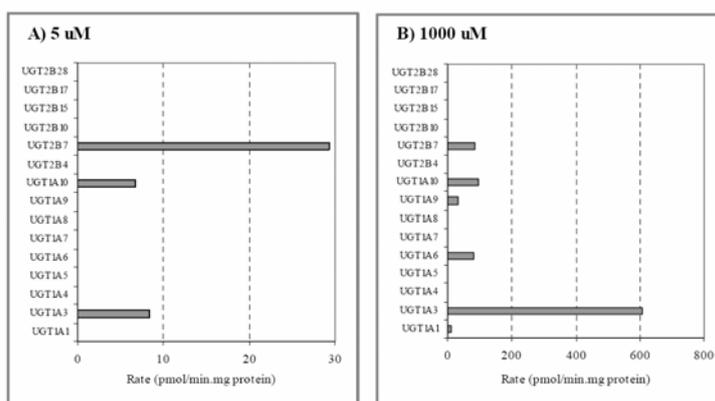

Figure 3. Formation of gemfibrozil glucuronide by recombinant human UDPglucuronosyltransferases at substrate concentrations of (A) 5 µM and (B) 1000 µM.

Only UGT 1A3, 1A10 and 2B7 glucuronidated GBZ at low substrate concentration (5 µM). UGT2B7 showed the highest activity which was approximately 4-fold higher than those of UGT 1A3 and 1A10. At high concentration of GBZ (1000 µM), the glucuronidation activities by UGT 1A1, 1A3, 1A6, 1A9, 1A10 and 2B7 were detected, where UGT1A3 showed the highest activity. The activities of UGT1A3 were about at least 6-fold higher than the others. The kinetic study of hepatic UGTs which exhibited measurable activities (*viz.* UGT 1A3, 1A6, 1A9 and 2B7) was further investigated. GBZ glucuronidation by UGT 1A3 (K_m 386 ± 2 µM) and 1A9 (K_m 47 ± 0.3 µM) exhibited Michaelis-Menten kinetics, whereas substrate inhibition (K_m 378 ± 25 µM and K_{si} 2891 ± 319 µM) was observed with UGT1A6. Similar to human liver microsomes, GBZ glucuronidation by UGT2B7 was well described by the two-site kinetic model which provided a K_s value of 7.0 ± 0.9 µM (Figure 2).

Recently, Uchaipichat *et al.*[13] demonstrated that fluconazole is a competitively selective inhibitor of UGT2B7. Employing fluconazole at concentration of 2.5 mM, we found that the inhibition of fluconazole on the high-affinity component of HLMs and UGT2B7 catalyzed GBZ glucuronidation were comparable (about 50% inhibition), whereas the inhibition was less than 20% for the low-affinity component of HLMs. Taken together, UGT2B7 is likely to be the high-affinity enzyme involved in hepatic GBZ glucuronidation. Results from this study were consistent to the data reported recently.[11]

CONCLUSION

Results presented here indicate that UGT2B7 appears to be the enzyme responsible for the high-affinity component of human liver microsomal GBZ glucuronidation. Comparing the plasma concentration of unbound GBZ and the binding affinity value obtained from *in vitro* study, it is likely that UGT2B7 may play an important role for metabolic drug-drug interaction between GBZ and other glucuronidated drugs.

REFERENCES

1. Nakagawa A, Shigeta A, Iwabuchi H, *et al.* Simultaneous determination of gemfibrozil and its metabolites in plasma and urine by a fully automated high performance liquid chromatographic system. *Biomed Chromatogr* 1991; 5: 68-73.

2. Okerholm RA, Keeley FJ, Peterson FE, *et al.* The metabolism of gemfibrozil. *Proc R Soc Med* 1976; 69(Suppl 2): 11-4.

3. Prueksaritanont T, Tang C, Qiu Y, *et al.* Effects of fibrates on metabolism of statins in human hepatocytes. *Drug Metab Dispos* 2002; 30: 1280-7.

4. Prueksaritanont T, Zhao JJ, Ma B, *et al.* Mechanistic studies on metabolic interactions between gemfibrozil and statins. *J Pharmacol Exp Ther* 2002; 301: 1042-51.

5. Shitara Y, Hirano M, Sato H, *et al.* Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. *J Pharmacol Exp Ther* 2004; 311: 228-36.

6. Ogilvie BW, Zhang D, Li W, *et al.* Glucuronidation converts gemfibrozil to a potent, metabolism-dependent inhibitor of CYP2C8: implications for drug-drug interactions. *Drug Metab Dispos* 2006; 34: 191-7.

7. Bowalgaha K, Elliot DJ, Mackenzie PI, *et al.* Naproxen and desmethylnaproxen glucuronidation by human liver microsomes and recombinant human UDP-glucuronosyltransferases (UGT): Role of UGT2B7 in the elimination of naproxen. *Br J Clin Pharmacol* 2005; 60: 423-33.

8. Boase S Miners JO. *In vitro-in vivo* correlations for drugs eliminated by glucuronidation: investigations with the model substrate zidovudine. *Br J Clin Pharmacol* 2002; 54: 493-503.

9. Sorich MJ, Smith PA, McKinnon RA, *et al.* Pharmacophore and quantitative structure activity relationship modelling of UDP-glucuronosyltransferase 1A1 (UGT1A1) substrates. *Pharmacogenetics* 2002; 12: 635-45.

10. Kenworthy KE, Clarke SE, Andrews J, *et al.* Multisite kinetic models for CYP3A4: simultaneous activation and inhibition of diazepam and testosterone metabolism. *Drug Metab Dispos* 2001; 29: 1644-51.

11. Mano Y, Usui T, Kamimura H. The UDP-glucuronosyltransferase 2B7 isozyme is responsible for gemfibrozil glucuronidation in the human liver. *Drug Metab Dispos* 2007; 35:2040-4.

12. Todd PA, Ward A. Gemfibrozil. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in dys-lipidaemia. *Drugs* 1988; 36: 314-39.

13. Uchaipichat V, Winner LK, Mackenzie PI, *et al*. Quantitative prediction of *in vivo* inhibitory interactions involving glucuronidated drugs from in vitro data: The effect of fluconazole on zidovudine glucuronidation. *Br J Clin Pharmacol* 2006; 61: 427-39.