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UGT1A1*28 polymorphism influences glucuronidation of bazedoxifene

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Bazedoxifene is used for the prevention and treatment of osteoporosis. After peroral application, bazedoxifene is metabolized by UDP-glucuronosyltransferases (UGTs) to bazedoxifene-4'-glucuronide (M4) and bazedoxifene-5-glucuronide (M5). It has already been shown that a relatively common *UGT1A1*28* polymorphism can considerably affect raloxifene pharmacokinetics and pharmacodynamics. As pharmacokinetics of bazedoxifene and raloxifene are very similar, the influence of *UGT1A1**28 polymorphism on metabolism of bazedoxifene was investigated by genotyped microsomes. Our results indicate an influence of *UGT1A1*28* allele on the formation clearance of both bazedoxifene metabolites. The decreased metabolic clearance was most pronounced in microsomes from polymorphic homozygote (*28/*28) where a 7 to 10-fold lower metabolic clearance was observed for both metabolites compared to other genotypes. In conclusion, the significant *UGT1A1*28* genotype effect on bazedoxifene intrinsic metabolic clearance indicates that this subject is worth further exploration *in vivo* and provides valuable information research in this field.

1. Introduction

Bazedoxifene is a third generation selective estrogen receptor modulator (SERM) which has been recently approved in Europe for the prevention and treatment of osteoporosis, and has been approved in the United States in combination with conjugated estrogens for the treatment of moderate-to-severe vasomotor symptoms associated with menopause and the prevention of postmenopausal osteoporosis. Bazedoxifene is a promising new SERM due to its favorable safety and tolerability profile and the recently discovered potential to inhibit tamoxifen resistant cancer cells (Wardell et al. 2013). The most important metabolic pathway after peroral application of bazedoxifene is glucuronidation by UGTs to bazedoxifene-4'-glucuronide (M4) and bazedoxifene-5-glucuronides (M5) (Chandrasekaran et al. 2009). Among UGTs, the highest activity of bazedoxifene glucuronidation was found with UGT1A1, UGT1A8 and UGT1A10 (Shen et al. 2010). Our research group has already shown that a relatively common *UGT1A1*28* polymorphism can considerably affect raloxifene pharmacokinetics and pharmacodynamics (Trontelj et al. 2009). Our previous extensive research experience with raloxifene metabolism, together with the results from a bazedoxifene metabolism study (Shen et al. 2010) has led us to hypothesise that bazedoxifene may be another drug with pharmacokinetics sensitive to the presence of *UGT1A1*28* polymorphism, such as irinotecan, ezetimibe, raltegravir, raloxifene and possibly etoposide (Wenning et al. 2009; Marques and Ikediobi 2010; Bae et al. 2011).

The aim of our study was to investigate the influence of *UGT1A1**28 polymorphism on the metabolism of bazedoxifene to M4 and M5. For this purpose, human liver microsomes genotyped for the *UGT1A1*28* polymorphism were used as a model which was previously used quite successfully (Yoder Graber et al. 2007; Zhang et al. 2007; Trdan Lusin et al. 2011).

2. Investigations, results and discussion

As expected, our results show that K_m values for the formation of M4 and M5 in either microsome group, did not differ significantly between the groups (p values from 0.362 to 0.881). The V_{max} value for M4 determined on microsomes from the wild-type homozygote (*1/*1) did not differ from the V_{max} values determined on microsomes from the heterozygote (*1/*28) ($p_{*1/*1vs.*1/*28} = 0.509$), however both were significantly higher than the V_{max} value from polymorphic homozygote *28/*28 ($p_{*1/*1vs.*28/*28} < 0.001$, $p_{*1/*28vs.*28/*28} = 0.002$). A similar situation with V_{max} was found in the case of M5 with the following p-values ($p_{*1/*1vs.*1/*28} = 0.498$, $p_{*1/*1vs.*28/*28} < 0.001$, $p_{*1/*28vs.*28/*28} < 0.001$). Similar to the results for V_{max}, a significant genotype influence was observed for the values of intrinsic clearances for formation of each metabolite. In case of M4, the Cl_{int} was significantly higher in polymorphic homozygote than in heterozygote and in wild-type homozygote ($p_{*1/*1vs.*28/*28} = 0.032$, $p_{*1/*28vs.*28/*28} = 0.049$), and again, there was no significant difference between the latter two microsomes ($p_{*1/*1vs.*1/*28} = 0.489$). A similar situation was found for Cl_{int} of M5 with the following p-values ($p_{*1/*1vs.*1/*28} = 0.252$, $p_{*1/*1vs.*28/*28} < 0.001$, $p_{*1/*28vs.*28/*28} = 0.003$).

Therefore, the main conclusion from our work is that a significantly decreased metabolic clearance could be expected in polymorphic homozygotes (*28/*28) where a 7- to 10-fold lower intrinsic metabolic clearance was observed for both metabolites compared to other genotypes. It should be mentioned that the use of individual donor *UGT1A1* genotyped microsomes instead of microsomes from several individual donors within each genotype group, does not permit the isolation of the genotype effect from other possible interindividual differences that might influence the bazedoxifene intrinsic clearance. Nevertheless, it clearly demonstrates the significant

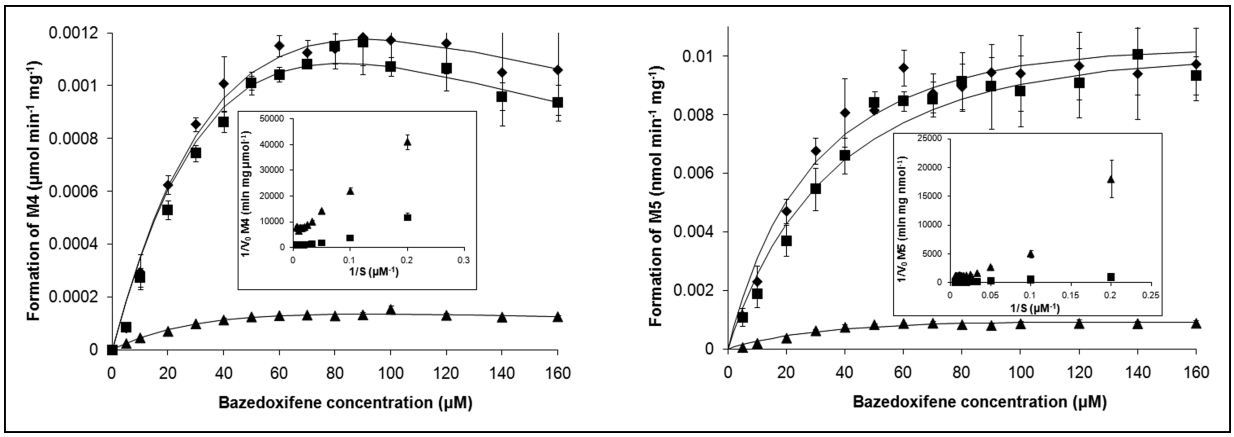

Fig. 1: Kinetic analyses of bazedoxifene glucuronidation. M4 and M5 formation rates were fitted to the substrate inhibition model (solid line). Also, Lineweaver-Burk plots are shown in insets. ♦ UGT1A1*1/*1, ■ UGT1A1*1/*28, ▲ UGT1A1*28/*28. Each data point represents the mean ± SE of triplicate determinations.

(7-10 fold) difference in bazedoxifene intrinsic metabolic clearance.

As previously reported, M4 was found to be the predominant metabolite in the liver microsomal incubations compared to M5 which was formed only in lesser amounts (Trdan Lusin et al. 2011). Unfortunately, the discrepancy between *in vitro* and *in vivo* results regarding the M4/M5 ratio still remains unexplained. However, the pronounced difference between M4 and M5 in terms of their relative abundance helped us to confirm the identification of both metabolites, which could be questionable based solely on their MRMs and retention times. Furthermore, our developed LC-MS/MS method was sensitive enough to determine the kinetic parameters also for M5 formation with liver microsomes. This aspect may be important, because it was the M5 which was recognized as the major metabolite circulating in humans. In fact, the greatest impact of *28 polymorphism was observed on the formation clearance of M5, which is also the most important metabolite found *in vivo*. This means that the probability of an observable *in vivo* genotype effect is even higher and a prospective clinical study is highly recommended where bazedoxifene pharmacokinetics and its therapeutic endpoints as well as adverse events should be correlated to *UGT1A1* genotype over a long enough period of time.

Although there have been only a few clinical trial reports with bazedoxifene, it appears that in combination with conjugated estrogens, its safety and efficacy profile is more favorable than that of raloxifene or conjugated estrogens (Pinkerton and Goldstein 2010; Silverman et al. 2012). The combination of bazedoxifene and conjugated estrogens simultaneously prevents the postmenopausal osteoporosis and relieves the hot-flashes, which is a very important determinant for the quality of life for this population (Pinkerton and Goldstein 2010). Therefore, bazedoxifene may become a valuable treatment option in the ageing western population, where osteoporosis and breast cancer can inflict a considerable risk and burden to individuals and to society. Given the similarities in metabolism and mechanism of action between raloxifene and bazedoxifene, the observed 7 to 10-fold reduction in bazedoxifene intrinsic clearance in subjects with *UGT1A1*28/*28* genotype might significantly increase its total serum concentrations and also increase its estrogen receptor modulating effects, as it was already shown for raloxifene. Moreover, the same genetic polymorphism is implied to carry an increased susceptibility to breast cancer and the interaction of both effects is worth further exploration, especially in the light of the latest discovery of bazedoxifene strong anticancer activity even in tamoxifen resistant tumors (Wardell et al. 2013).

In conclusion, the significant *UGT1A1*28* genotype effect on bazedoxifene intrinsic metabolic clearance found in our study, strongly indicates that this subject is well worth further exploration *in vivo* and provides valuable information for on-going and future studies about bazedoxifene efficacy and safety.

3. Experimental

3.1. Materials

Haloperidol was purchased from Sigma Aldrich Chemie (Deisenhofen, Germany). Bazedoxifene was synthesized and characterized in our laboratory (Trdan Lusin et al. 2012). Human liver microsomes genotyped for *UGT1A1* *1/*1, *1/*28, *28/*28 variants (from single male and female Caucasian donors aged from 47-61), Solution A (25 mM uridine 5'-diphospho-glucuronic acid (UDPGA)), Solution B (250 mM Tris-HCl, 40 mM $MgCl_2$, 0.125 mg/mL alamethicin) were from BD Technologies (NJ, USA). Human serum albumin (HSA) (200 g/L) was obtained from Octapharma (Ljubljana, Slovenia).

3.2. Methods

Bazedoxifene was incubated with each of the three tested types of human liver microsomes according to the presence of *UGT1A1*28* genotype, namely **1/*1, *1/*28* and **28/*28*. The incubation mixture (final volume 150 µL) was composed as per manufacturer's instructions and contained 0.38 mg/mL of human liver microsomes, 1% HSA, and 1 - 160 µM bazedoxifene, which was dissolved in DMSO (final DMSO concentration was 1%). The reactions were terminated by adding 450 µL of ice cold methanol containing 0.5 mg/L haloperidol as an internal standard after pre-determined optimal stopping time of 7 min (assay linearity was confirmed). After centrifugation for 100 min at 4 °C and 1300 × *g*, the supernatant was subjected to LC-MS/MS analysis for quantification of bazedoxifene, M4 and M5 using haloperidol as an internal standard.

For LC-MS/MS analysis, the Agilent 1290 Infinity UHPLC system coupled to Agilent 6460 Triple Quad MS detector (Agilent Technologies, Santa Clara, USA) was applied to the analysis of incubation samples. The chromatographic separation was performed on a Kinetex C18 50 × 2.1 mm, 2.6 µm column (Phenomenex, Torrance, USA) at 50 °C. The mobile phase was delivered at a flow rate of 0.65 mL/min and consisted of 0.1% formic acid in water (mobile phase A) and 98% acetonitrile (mobile phase B) with the following linear gradient:20%-30%-50%-50%-10%-10% of mobile phase B in 0.5-1.0-1.8-2.0-2.01-2.50 min, respectively. The injection volume was 0.1 µL for the quantification of M4 and bazedoxifene, and 1 µL for M5 for greater sensitivity.

Quantification was performed in positive ESI mode using multiple reaction monitoring with the following *m/z* transitions: 647→ 471 for M4 and M5, 471→ 126 for bazedoxifene and 376→ 165 for haloperidol. The retention times of bazedoxifene, M4, M5, and haloperidol were 1.633 min, 1.296 min, 1.567 min, and 1.502 min, respectively. The method was checked for selectivity, accuracy, precision, linear range and limit of quantification. The limit of quantification for M5 was estimated to be at least 5 times lower than the lowest concentration found in any incubation (approximately 5 picomol/L, based on signal to noise ratio). However, due to the lack of analytical standards for bazedoxifene metabolites at the time of experiments, their identification was based on previously reported LC-MS/MS method (Trdan Lusin et al. 2011) and their semi-quantification in incubation samples

was performed by applying the calibration curve for bazedoxifene, thereby obtaining bazedoxifene glucuronide equivalents (Trontelj 2012).
Glucuronidation activities were determined on three replicates. The Lineweaver-Burk plots (Fig. 1) have shown curves characteristic for the substrate-inhibition kinetic model (Eq.1).

$$v = \frac{V_{\max} \times S}{K_m + S \times \left(1 + \frac{S}{K_{si}}\right)} \quad (1)$$

where v represents the reaction velocity, V_{max} is the maximum velocity, S is the substrate concentration, K_m is the Michaelis-Menten constant and K_{si} is a substrate inhibition constant. The kinetic parameters K_m, V_{max} and K_{si} for bazedoxifene glucuronidation were estimated by nonlinear regression of experimental data to Eq. (1) using PASW 18.0 software (IBM company, Illinois, Chicago). Goodness of fit to Eq. (1) was assessed by visual inspections of Michaelis-Menten plots and by determination of the relative standard errors (RSE). Intrinsic clearance was calculated as V_{max}/K_m. The difference in glucuronidation levels among *UGT1A1* genotypes were tested using the z-test with Holm's correction and significance criterion of $p < 0.05$.

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References

Bae JW, Choi CI, Lee JH, Jang CG, Chung MW, Lee SY (2011) Effects of UDP-glucuronosyltransferase polymorphisms on the pharmacokinetics of ezetimibe in healthy subjects. Eur J Clin Pharmacol 67: 39–45.

Chandrasekaran A, McKeand WE, Sullivan P, DeMaio W, Stoltz R, Scatina J (2009) Metabolic disposition of [14C]bazedoxifene in healthy postmenopausal women. Drug Metabol Dispos 37: 1219–1225.

Marques SC, Ikediobi ON (2010) The clinical application of UGT1A1 pharmacogenetic testing: gene-environment interactions. Human Genom 4: 238–249.

Pinkerton JV, Goldstein SR (2010) Endometrial safety: a key hurdle for selective estrogen receptor modulators in development. Menopause 17: 642–653.

Shen L, Ahmad S, Park S, DeMaio W, Oganesian A, Hultin T, Scatina J, Bungay P, Chandrasekaran A (2010) In vitro metabolism, permeability, and efflux of bazedoxifene in humans. Drug Metabol Dispos 38: 1471–1479.

Silverman SL, Chines AA, Kendler DL, Kung AW, Teglbjaerg CS, Felsenberg D, Mairon N, Constantine GD, Adachi JD, Bazedoxifene Study Group (2012) Sustained efficacy and safety of bazedoxifene in preventing fractures in postmenopausal women with osteoporosis: results of a 5-year, randomized, placebo-controlled study. Osteoporosis Int 23: 351–363.

Trdan Lusin T, Tomasic T, Trontelj J, Mrhar A, Peterlin-Masic L (2012) In vitro bioactivation of bazedoxifene and 2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol in human liver microsomes. Chem-Biol Interact 197: 8–15.

Trdan Lusin T, Trontelj J, Mrhar A (2011) Raloxifene glucuronidation in human intestine, kidney, and liver microsomes and in human liver microsomes genotyped for the UGT1A1*28 polymorphism. Drug Metabol Dispos 39: 2347–2354.

Trontelj J (2012) Quantification of glucuronide metabolites in biological matrices by LC-MS/MS, in: Prasain J. (ed.): Tandem Mass Spectrometry - Applications and Principles, Intech, pp 531–558.

Trontelj J, Marc J, Zavratnik A, Bogataj M, Mrhar A (2009) Effects of UGT1A1*28 polymorphism on raloxifene pharmacokinetics and pharmacodynamics. Brit J Clin Pharmacol 67: 437–444.

Wardell SE, Nelson ER, Chao CA, McDonnell DP (2013) Bazedoxifene exhibits antiestrogenic activity in animal models of tamoxifen-resistant breast cancer: implications for treatment of advanced disease. Clin Cancer Res 19: 2420–2431.

Wenning LA, Petry AS, Kost JT, Jin B, Breidinger SA, DeLepeleire I, Carlini EJ, Young S, Rushmore T, Wagner F, Lunde NM, Bieberdorf F, Greenberg H, Stone JA, Wagner JA, Iwamoto M (2009) Pharmacokinetics of raltegravir in individuals with UGT1A1 polymorphisms. Clin Pharmacol Ther 85: 623–627.

Yoder Graber AL, Ramirez J, Innocenti F, Ratain MJ (2007) UGT1A1*28 genotype affects the in-vitro glucuronidation of thyroxine in human livers. Pharmacogenet Genomics 17: 619–627.

Zhang D, Zhang D, Cui D, Gambardella J, Ma L, Barros A, Wang L, Fu Y, Rahematpura S, Nielsen J, Donegan M, Zhang H, Humphreys WG (2007) Characterization of the UDP glucuronosyltransferase activity of human liver microsomes genotyped for the UGT1A1*28 polymorphism. Drug Metabol Dispos 35: 2270–2280.