CYP2C8 is a Previously Unreported Important Contributor to Olanzapine Oxidative Metabolism

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Introduction

Olanzapine (OLZ) is an atypical antipsychotic commonly prescribed for the management of schizophrenia and related psychoses. There is wide inter-individual variability in OLZ pharmacokinetics and response and up to 80% of patients discontinue antipsychotic therapy by 5 years due to lack of efficacy and drug-related adverse effects. The metabolism of OLZ is complex and relatively poorly understood. UDP-glucuronosyltransferase 1A4 (UGT1A4), cytochrome P450 1A2 (CYP1A2), and flavin-containing mono-oxygenase 3 (FMO3) have been shown to contribute to glucuronidation and oxidative metabolic pathways, respectively (Fig. 1). Most studies have focused on the role of CYP1A2 (and its polymorphic variants) in determining variability in OLZ response. CYP3A4 is also known to contribute to the oxidative metabolism of OLZ. This enzyme shares significant overlap substrate selectivity with CYP2C8, but no study has reported the contribution of the latter enzyme to OLZ oxidative metabolism. In addition, CYP2C8 polymorphisms are known to result in variability in substrate pharmacokinetics for many clinically used drugs (e.g. rosiglitazone, pioglitazone, and repaglinide).

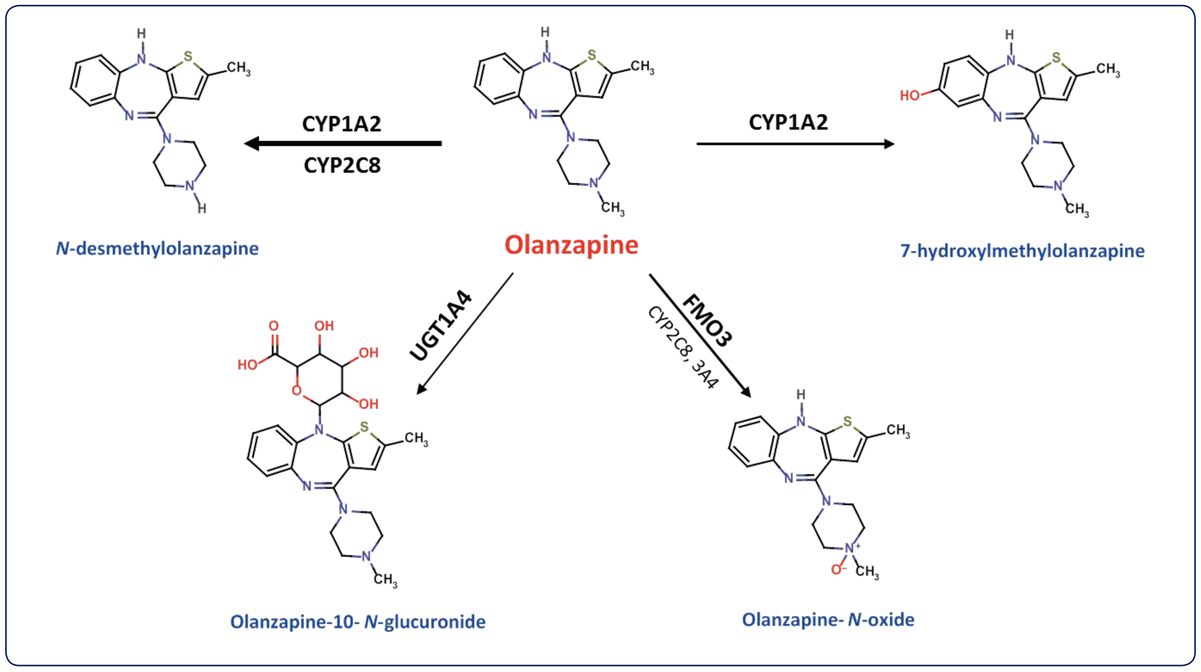

Figure 1 Major metabolic pathways of olanzapine (from HLM data). Enzymes responsible for the metabolism of olanzapine are indicated, with the most active enzymes indicated with a larger fonts.

Objective

To screen a full panel of drug metabolising enzymes (CYPs, UGTs, and FMO3), with a particular focus on elucidating the contribution of CYP2C8 to OLZ oxidative metabolism.

Methods

Expression of recombinant UGTs and CYPs:

cDNAs encoding human UGT 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B10, 2B15, and 2B17 were stably expressed in human embryonic kidney cell lines (HEK293T) as described previously (Uchaipichat et al., 2004). Recombinant human CYP 1A1, 1A2, 2B6, 2C8, 2C9, 2E1, 3A4, and NADPH cytochrome P450 oxidoreductase (CPR) were co-expressed in *Escherichia coli* according to the general procedure of Boye et al., (2004). The CYP/NADPH cytochrome P450 oxidoreductase ratios were unity. FMO3 was purchased from BD Biosciences.

Olanzapine assay:

Incubations, in a total volume 200 µL, contained rCYPs (2 - 5 pmol P450) or rUGTs (1 mg/mL), phosphate buffer (1 mM, pH 7.4), NADPH-generating system (1 mM NADP, 10 mM glucose-6-phosphate, 2 IU/mL glucose-6-phosphate dehydrogenase, and 5 mM $MgCl_2$) or UDPGA (5 mM) and OLZ with concentration range of 5 – 1500 µM. For the screening experiment, the concentrations of OLZ were 50 and 350 µM for determination the involvement of rCYPs and rUGTs, respectively. For incubations conducted in the presence of BSA (2% w/v), the effect of BSA binding (30%) was taken into account.

Physiologically Based Pharmacokinetic (PBPK) Modeling:

A substrate profile for OLZ was created in SimCYP population-based simulator based on experimental *in vitro* data generated using rCYPs and rUGT1A4, with physicochemical properties of OLZ according to reported and predicted data.

Mass Spectrometry:

Chromatography was performed using a Waters Acquity UPLC system fitted with a Waters Acquity BEH C18 (2.1 x 100 mm, 1.7 µm particle size) analytical column. OLZ and metabolites were separated from matrix components using a gradient mobile phase comprising of 10 mM ammonium formate pH 3.0 (mobile phase A) and acetonitrile (mobile phase B) at a flow rate of 0.25 mL/min. The elution gradient was increased linearly from 13% B to 70% B over 7 min. The retention times for 7-hydroxy-OLZ, N-desmethyl-OLZ, OLZ-N-oxide, and OLZ-10-N-glucuronide under these conditions were 2.5, 3.4, 3.8, and 3.5 min, respectively. Quantification was accomplished by comparison of peak area of incubation samples to those of authentic standards for each metabolite.

Results

- Consistent with previous reports, screening experiments demonstrated that UGT1A4, CYP1A2, and FMO3 were found to mainly catalyse the formation of OLZ-10-N-glucuronide, 7-hydroxy-OLZ, and OLZ-N-oxide, respectively. In addition, a previously uncharacterised contribution of CYP2C8 to OLZ N-demethylation was demonstrated.

Table 1: Derived kinetic parameters for OLZ N-demethylation by rCYP1A2 and 2C8 in the absence and presence of albumin (n = 4; mean ± SD)

rCYP	Without BSA		With BSA		Predicted *in vivo* Clearance
	K_m (µM)	V_{max} (pmol/min/pmol P450)	K_m (µM)	V_{max} (pmol/min/pmol P450)	$CL_{int.liver}$ (L/h) [a]
1A2	212 ± 69	0.7 ± 0.1	61 ± 20	1.3 ± 0.1	32.9 (10.0-76.3)
2C8	56 ± 18	1.9 ± 0.2	66 ± 7	2.3 ± 0.3	19.1 (5.15-56.1)

[*] Data represented as mean values from ten virtual trials comprising ten subjects (healthy volunteers) each, with the range given in parenthesis

- Addition of BSA (2%) resulted in a 7.7-fold increase in the intrinsic clearance for OLZ N-demethylation by rCYP1A2, due mainly to a reduction in K_m. In contrast, addition of BSA to incubations of rCYP2C8 did not alter the intrinsic clearance for OLZ N-demethylation by rCYP2C8.
- Furafylline (10 µM) and montelukast (0.2 µM) inhibited the formation of N-desmethyl-OLZ by pooled HLMs 56 and 27%, respectively, which confirmed the involvement of CYP1A2 and CYP2C8 on OLZ N-demethylation metabolic pathway.
- PBPK modeling of OLZ clearance using SimCYP population-based simulator, particularly CYP-mediated oxidative pathways, demonstrated an approximately 30% contribution of CYP2C8 to CYP-mediated OLZ oxidative metabolism.

Conclusion

This study indicates that CYP2C8 is contributed to OLZ oxidative metabolism through catalysis of OLZ N-demethylation. As significant correlation between N-desmethyl OLZ metabolic ratio with OLZ clearance has been reported mainly focusing on the significant of CYP1A2 polymorphisms, it is plausible that inter-individual variability in CYP2C8 expression and activity may contribute to the inter-individual variability in OLZ disposition and response.

References

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