Evaluation of Ketoconazole and its Alternative Clinical CYP3A4/5 Inhibitors as Inhibitors of Drug Transporters: The *In Vitro* Effects of Ketoconazole, Ritonavir, Clarithromycin, and Itraconazole on 13 Clinically-Relevant Drug Transporters

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Abstract

What is already known about this subject?

- The effects of the alternative clinically used CYP3A4/5 inhibitors on other CYPs are largely established.
- Several studies have investigated the effects of ketoconazole and its alternatives on one or more drug transporters, but none have comprehensively compared their inhibitory effects on the clinically relevant drug transporters in the same study.

What this study adds:

- This study systematically assesses the inhibitory effects of ketoconazole, clarithromycin, ritonavir and itraconazole (and its CYP3A-inhibitory metabolites, hydroxy-itraconazole, keto-itraconazole and *N*-desalkyl itraconazole), on OATP1B1 (SLCO1B1), OATP1B3 (SLCO1B3), OAT1 (SLC22A6), OAT3 (SLC22A8), OCT1 (SLC22A1), OCT2 (SLC22A2), MATE1 (SLC47A1), MATE2-K (SLC47A2), P-gp (ABCB1), BCRP (ABCG2), MRP2 (ABCC2), MRP3 (ABCC3) and BSEP (ABCB11).

Ketoconazole is a known CYP3A4/5 inhibitor and was previously used in this role for clinical drug-drug interaction (DDI) studies. The FDA and EMA recently recommended the suspension of ketoconazole in clinical DDI studies and suggested that clarithromycin or itraconazole be used. It is well-established that these alternatives are moderate or strong inhibitors of CYP3A4/5, but the effect on drug transporters is limited.

Aims: 1) Determine the inhibitory effects of ketoconazole, clarithromycin, ritonavir, itraconazole (and its metabolites: hydroxy-, keto-, and *N*-desalkyl-itraconazole) towards 13 clinically relevant drug transporters.
2) To aid in the selection of clinical CYP3A4/5 inhibitors when drug transporters contribute to a drug candidate's pharmacokinetics.

Methods: HEK293 cell lines or membrane vesicles expressing the transporters were used. A range of concentrations for each inhibitor was used and cells or vesicles were preincubated for 15 min in the presence of the inhibitor and then the probe substrate was added. Samples were analyzed using LSC or LC-MS/MS.

Results: Similar to ketoconazole, results indicate that ritonavir, clarithromycin, and itraconazole have differing transporter inhibition profiles. The data demonstrate that no single alternative to ketoconazole indicate a clean inhibition profile towards all 13 drug transporters tested. The results show the majority of the inhibitors tested may interact with one or more transporters in clinical studies (i.e. some values were calculated to be greater than the cutoff values specified by the FDA and EMA).

Conclusion: The results provide guidance for the selection of clinical CYP3A4/5 inhibitors when transporters are involved in a drug candidate's pharmacokinetics.

Background & Purpose

Ketoconazole is an orally available, synthetic, broad spectrum, antifungal agent. Approved in 1982 by the FDA for use in fungal infections, it is a known substrate and strong inhibitor of cytochrome P450 (CYP) 3A4 and 3A5.[1]

Previously, a high dose of ketoconazole was considered the gold standard for use in clinical drug-drug interaction (DDI) as a strong CYP3A4/5 inhibitor. By 2013, ketoconazole use in clinical studies had been banned due in part to evidence demonstrating the potential for liver injury following long dosing periods. Typically, patients would exhibit asymptomatic, reversible liver function test abnormalities. As early as 1984, Van Tyle demonstrated evidence of DILI in approximately 0.1 to 1.0% of patients, with results indicating that there was no association with the dose, but with the duration of dosing.[2] In later estimates, studies showed that ~134 per 100,00 persons, 4.9 cases per 10,000 patients, and 3.6 to 4.2% demonstrated liver abnormalities.[3]

After ketoconazole was banned in clinical study use, the FDA recommended clarithromycin or itraconazole as an alternative, but indicating that other drugs may be used. Ritonavir was suggested by some as an alternative CYP3A4/5 inhibitor. Following an extensive study by Ke *et al*. where inhibitors were systematically evaluated, only itraconazole and clarithromycin were considered acceptable. Exclusion criteria included, the drug not being approved in the U.S., known non-specific inhibition of CYPs, safety issues, exclusive use with ritonavir, or only moderate information of CYP3A4/5.[4]

There is minimal drug transporter data in the literature; which includes ketoconazole and ritonavir inhibition of P-gp and OATP1B1.[5] The goal of this study was to allow for a more informed choice of a strong CYP3A4/5 inhibitor for clinical DDI studies involving a drug candidate known to be a substrate of one or more of the transporters and to help reduce confounding DDI results.

Materials & Methods

Chemicals and Reagents. Methanol, estradiol-17β-glucuronide, estrone-3-sulfate, *p*-aminohippurate, verapamil, Ko143, cyclosporin, benzbromarone, cimetidine, rifampin, probenecid, quinidine, ritonavir, clarithromycin, itraconazole, and ketoconazole were purchased from Sigma-Aldrich (St. Louis, MO). [^{3}H]-estrone-3-sulfate, [^{3}H]-estradiol-17β-glucuronide, [^{3}H]-*p*-aminohippuric acid, and [^{3}H]-taurocholic acid were purchased from Perkin Elmer (Oak Brook, IL), hydroxy-itraconazole, keto-itraconazole, and *N*-desalkyl itraconazole were purchased from Toronto Research Chemicals (Ontario, Canada). N-methylquinidine was purchased from Solvo Biotechnologies (Boston, MA), [^{14}C]-tetraethylammonium bromide was purchased from American Radiolabeled Chemicals (St. Louis, Mo.), and [^{14}C]-metformin was purchased from Moravek Biochemicals (Brea, CA). 10x Hanks balanced salt solution (HBSS) was purchased from Invitrogen (Waltham, MA).
HEK-293 inhibition assays. Inhibition of OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K in transfected and control HEK-293 cells was carried out as previously described with some modifications.[6] Briefly, cells were washed once with HBSS (pH 7.4) and then HBSS containing the inhibitor or solvent control was added for 15 min. Inhibitor solutions were removed and replaced with HBSS containing the inhibitors (or solvent control, DMSO) and probe substrate for the designated time. Substrate solutions were removed and cells were washed once with 0.2% BSA in PBS and two times with 1x PBS. After the final wash, cells were lysed with 0.1N NaOH for liquid scintillation analysis.
Vesicle inhibition assays. Inhibition of efflux of probe substrates into membrane vesicles expressing P-gp, BCRP, MRP2, MRP3, and BSEP were carried out according to manufacturer's instructions with some modifications. Briefly, vesicle membrane suspensions were added to a 96-well plate stored on ice. Incubation media containing ketoconazole, itraconazole, hydroxy-itraconzaole, keto-itraconazole, *N*-desalkyl-itraconazole, clarithromycin, or ritonavir were added to the plate and incubated for 15 min. Substrate solutions containing either MgATP or MgAMP and probe substrate were added to the plate for the designated time. The incubation was ended by the addition of ice-cold wash mix. The sample solution was transferred to a filter plate and washed five times with wash mix. Plates were allowed to dry at room temperature for approximately 1 hr, after which scintillation cocktail was added to the filter plate wells and incubated for 1 hr prior to analysis by liquid scintillation.

Results

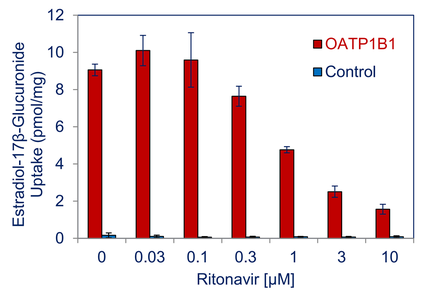
Table 1. List of inhibitors tested in HEK-293 and membrane vesicles for inhibition profiles of 13 clinically relevant drug transporters

Inhibitors	Transporters	
Ketoconazole	OATP1B1	MATE2-K
Clarithromycin	OATP1B3	P-gp
Ritonavir	OAT1	BCRP
Itraconazole	OAT3	MRP2
Hydroxyitraconazole	OCT1	MRP3
Ketoitraconazole	OCT2	BSEP
N-desalkyitraconazole	MATE1	

Table 2. IC_{50} values of ketoconazole and alternative CYP3A4/5 inhibitors for 13 clinically relevant drug transporters.

Transporter	Ketoconazole	Itraconazole	Hydroxy-itraconazole	Keto-itraconazole	N-Desalkyl-itraconazole	Clarithromycin	Ritonavir
OATP1B1	1.8 ± 0.2	>10	0.23 ± 0.03	0.29 ± 0.04	>0.2	5.3 ± 1.3	0.68 ± 0.17
OATP1B3	3.9 ± 0.6	>10	0.10 ± 0.01	0.088 ± 0.035	>0.2	14 ± 2	2.3 ± 0.4
OAT1	5.7 ± 0.5	>10	>3	>3	>0.2	>50	17 ± 3
OAT3	0.86 ± 0.68	>10	2.0 ± 0.3	>3	>0.2	>50	>30
OCT1	0.13 ± 0.03	0.74 ± 0.24	0.01 ± 0.00	0.04 ± 0.01	>0.2	>50	4.1 ± 0.6
OCT2	0.89 ± 0.35	>10	>3	>3	>0.2	>50	>30
MATE1	0.37 ± 0.03	>10	0.84 ± 0.21	1.1 ± 0.2	>0.2	>50	1.2 ± 0.2
MATE2-K	>2	>10	>3	>3	>0.2	>50	15 ± 2
P-gp	5.6 ± 0.4	0.048 ± 0.04	0.49 ± 0.14	0.12 ± 0.12	0.26 ± 0.05	8.9 ± 0.5	0.24 ± 0.02
BCRP	12 ± 9	1.9 ± 0.3	0.44 ± 0.03	0.10 ± 0.01	>0.2	>50	6.6 ± 0.5
MRP2	>20	>10	>3	>3	>0.2	>50	>30
MRP3	>20	>10	>3	>3	>0.2	>50	>30
BSEP	2.4 ± 0.6	1.8 ± 0.0	1.2 ± 0.8	0.11 ± 0.02	>0.2	59 ± 8	6.1 ± 0.9

Figure 1. Example of inhibition profile of ritonavir for OATP1B1 (HEK-293 cell system) with a calculated IC_{50} value of 0.68 µM (n = 3).



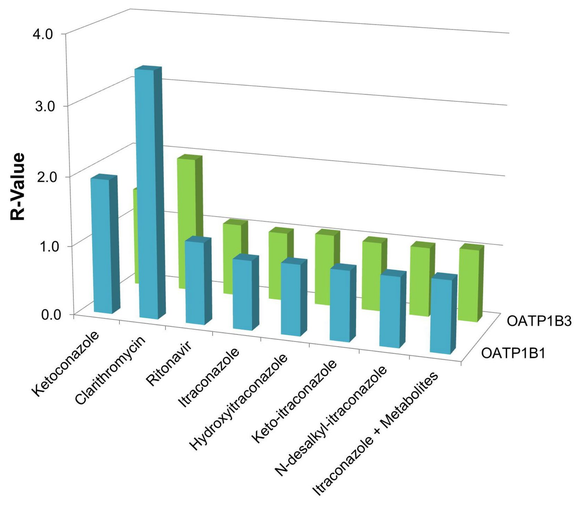


Figure 2. R-value determination for DDI prediction of OATP-mediated hepatic uptake.

$$\boldsymbol{R\ value} = 1 + (f_u * \frac{I_{in,max}}{IC_{50}})$$

Using an FDA cutoff value of 1.25, only ketoconazole and clarithromycin are predicted to affect OATP1B1 and OATP1B3. Ritonavir and itraconazole (and metabolites) demonstrated R-values<1.25.

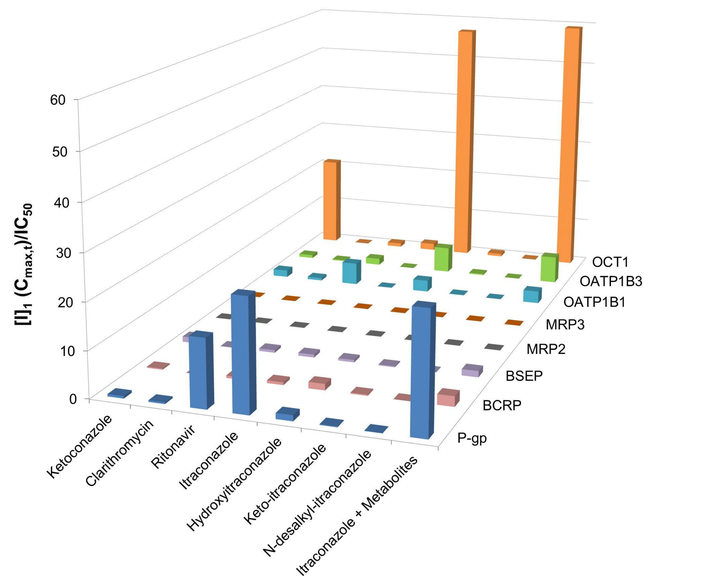


Figure 3. DDI predictions for hepatic uptake and efflux transport using FDA basic model ($[I]_1/IC_{50}$) where $[I]_1$ is the total C_{max} (free and unbound at steady state). FDA deems a value greater than 0.1 as having the potential for a clinically relevant interaction.
Ketoconazole, ritonavir, itraconazole and hydroxy-itraconazole all demonstrated values considerably greater than 0.1.

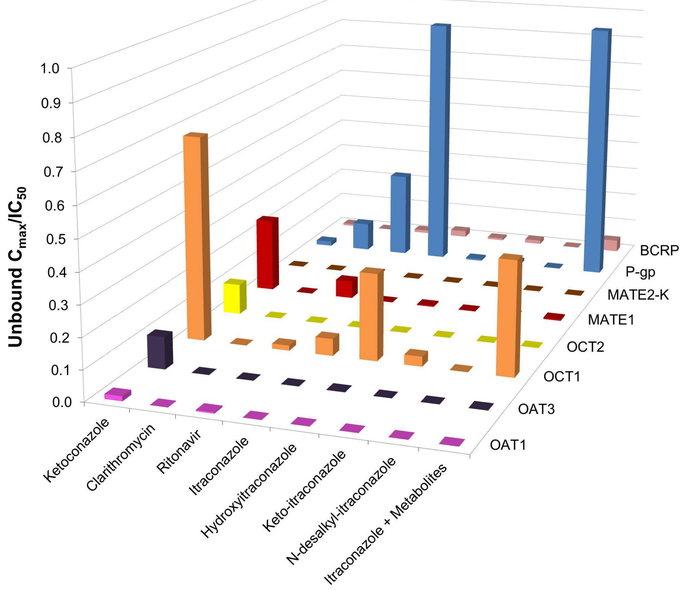


Figure 4. DDI predictions for renal or BBB (blood brain barrier) transporters based on FDA basic model (unbound C_{max}/IC_{50}). All transporters except OAT1 are predicted to be affected, with values greater than 10-fold the cut-off of 0.1. Ketoconazole and hydroxy-itraconazole had the greatest impact, while clarithromycin did not have any values greater than 0.1.

Conclusions

- None of the alternatives to ketoconazole provided a clean inhibition profile towards all 13 drug transporters evaluated.
- Each alternative to ketoconazole for potential use in a clinical DDI study has a unique transporter inhibition profile (MRP2 and MRP3 were not inhibited by any alternative inhibitors).
- Ritonavir and itraconazole may be the best alternative for CYP3A4/5 substrates which are transported by OATP1B1/1B3.
- CYP3A4/5 substrates transported by OAT1 may not be affected by any of the tested alternative inhibitors.
- Clarithromycin may be best choice for substrates of renal transporters or substrates of P-gp or BCRP (P-gp may still be affected).
- **Overall: The best choice of a strong clinical CYP3A4/5 inhibitor will depend on the unique transporter substrate profile of the drug candidate.[6]**

References

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