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Ritonavir is the best alternative to ketoconazole as an index inhibitor of cytochrome P450-3A in drug–drug interaction studies

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AIMS
The regulatory prohibition of ketoconazole as a CYP3A index inhibitor in drug–drug interaction (DDI) studies has compelled consideration of alternative inhibitors.

METHODS
The biomedical literature was searched to identify DDI studies in which oral midazolam (MDZ) was the victim, and the inhibitory perpetrator was either ketoconazole, itraconazole, clarithromycin, or ritonavir. The ratios (R_{AUC}) of total area under the curve (AUC) for MDZ with inhibitor divided by MDZ AUC in the control condition were aggregated across individual studies for each inhibitor.

RESULTS
Mean (± SE) R_{AUC} values were: ketoconazole (15 studies, 131 subjects), 11.5 (±1.2); itraconazole (five studies, 48 subjects), 7.3 (±1.0); clarithromycin (five studies, 73 subjects), 6.5 (±10.9); and ritonavir (13 studies, 159 subjects), 14.5 (±2.0). Differences among inhibitors were significant ($F = 5.31$, $P < 0.005$). R_{AUC} values were not significantly related to inhibitor dosage or to duration of inhibitor pre-exposure prior to administration of MDZ.

CONCLUSIONS
Ritonavir produces CYP3A inhibition equivalent to or greater than ketoconazole, and is the best index CYP3A inhibitor alternative to ketoconazole. Cobicistat closely resembles ritonavir in structure and function, and can also be considered. Itraconazole and clarithromycin are not suitable alternatives since they do not produce inhibition comparable with ketoconazole or ritonavir, and have other significant disadvantages as well.

Introduction

The Drug Safety Communication issued by the United States Food and Drug Administration (FDA) in July 2013, warned against and limited the use of oral ketoconazole for systemic antifungal treatment, based on what was stated to be a risk of severe liver injury which could potentially lead to liver transplantation or death [1]. The European Medicines Agency Committee on Medicinal Products for Human Use issued a similar statement at the same time [2]. In October 2013, the FDA followed with a statement recommending against the use of ketoconazole as an index inhibitor of human cytochrome P450-3A (CYP3A) isoforms in clinical drug–drug interaction (DDI) studies [3].

The FDA has not provided the outcome of what they term a comprehensive benefit–risk assessment of the safety and efficacy of oral ketoconazole. Also unavailable

are the results of an independent evaluation of data from the FDA Adverse Event Reporting System (FAERS) by a hepatology expert at the FDA. A review of published literature on ketoconazole-associated liver injury [4], and an external analysis of FAERS reports [5], led to the following conclusions: 1) liver injury associated with ketoconazole is uncommon, 2) when it happens, liver injury is nearly always evident as asymptomatic and reversible alterations in liver function tests, 3) serious liver injury is rare, 4) there is no substantive or consistent evidence that ketoconazole carries a risk of liver injury different from other azole antifungals, and 5) There is negligible evidence of a liver injury risk from ketoconazole used as an index CYP3A inhibitor in DDI studies of healthy volunteers.

Notwithstanding a lack of scientific support, the regulatory decisions still impose a liability burden on clinicians who prescribe ketoconazole for systemic antifungal treatment, and on investigators who use ketoconazole in DDI studies of CYP3A-mediated drug clearance [6]. Reasonable antifungal treatment options are available as alternatives to ketoconazole, but CYP3A inhibitor alternatives for DDI studies in the course of drug development are not so clear.

For a drug candidate suspected of being a CYP3A inhibitor ('perpetrator'), a DDI study using a sensitive CYP3A substrate drug as 'victim' can be performed to determine the candidate's quantitative inhibition potency [7]. Such studies often include a trial arm using a strong CYP3A inhibitor – usually ketoconazole – as a positive control, to compare the inhibitory effect of the candidate drug with the 'worst case scenario'. If the candidate drug itself is a CYP3A substrate, a DDI study using ketoconazole as the inhibitor would map the worst case for the substrate under conditions of maximal CYP3A inhibition.

An appropriate alternative to ketoconazole as a strong index CYP3A inhibitor for DDI studies should produce maximal inhibition of CYP3A activity. Itraconazole, clarithromycin, and ritonavir have been proposed as alternatives [3, 4, 6, 8], but their quantitative inhibitory potency *in vivo* relative to that of ketoconazole has not been established. The present review evaluates published clinical DDI studies in which ketoconazole, itraconazole, clarithromycin, or ritonavir have been used as an index CYP3A inhibitor in clinical DDI studies with oral midazolam as the index substrate.

Methods

A total of 38 studies, involving 411 subjects, were identified through standard procedures for search of published biomedical literature (Table 1). We elected to consider studies of orally administered midazolam, since clearance will reflect activity of both hepatic and enteric CYP3A [10]. The majority of studies evaluated were single dose crossover trials, in which oral midazolam was given in the control state, and again with co-administration of the index inhibitor. The principal outcome variable for this review was the ratio (R_{AUC}) of total area under the plasma concentration curve (AUC) for oral midazolam during co-administration of inhibitor (AUC_I) divided by the AUC in the control condition with no inhibitor (AUC_0), as follows [7]:

$$R_{AUC} = (AUC_I)/(AUC_0)$$

For each study, we recorded the overall mean of individual R_{AUC} values, as provided by the authors in 66 % of the studies. This was not available in 34 % of the studies, in which case we used the mean value of AUC_i divided by mean AUC_0. Also recorded were the daily doses of the inhibitor, the dosage schedule (single or divided daily doses), and the duration of pre-exposure to the inhibitor prior to administration of midazolam.

R_{AUC} values were aggregated as the arithmetic mean across all studies of each inhibitor. Analysis of variance (ANOVA) for independent groups using rank-transformed data was performed to determine the overall significance of differences in R_{AUC} among the four inhibitor categories. For each inhibitor, the relation of R_{AUC} to daily dose of inhibitor and to the duration of inhibitor pre-exposure was evaluated by multiple regression analysis.

Table 1

Summary of oral midazolam drug–drug interaction studies of each inhibitor

Inhibitor	Number of studies	Number of subjects	References	Median (with range) Daily dose (mg)	Pre-exposure duration (days)	Dose schedule: single (S) or divided (D) daily doses
Ketoconazole	15	131	[9–19]	400 (200–400)	3 (0–14)	12S, 3D
Itraconazole	5	48	[9], [20–23]	200 (100–400)	3 (0.17–5)	5S, 0D
Clarithromycin	5	73	[24–28]	1000 (500–1000)	4 (3–7)	0S, 5D
Ritonavir	13	159	[31–40]	200 (100–600)	13 (0–>30)	5S, 12D

For the purpose of this study we did not consider maximum plasma concentration (C_{max}) as an outcome variable, since C_{max} depends on the rate of absorption as well as AUC.

Results

The median duration of pre-exposure to inhibitors ranged from 3 to 13 days (Table 1), with variation of individual values from 0 (the inhibitor given as a single dose concurrently with midazolam) to more than 30 days. Median daily doses were 200 mg for itraconazole and ritonavir, 400 mg for ketoconazole and 1000 mg for clarithromycin. Multiple regression analysis indicated no apparent relationship of R_{AUC} to daily dosage or duration of pre-exposure for any of the inhibitors.

Figure 1 shows overall mean R_{AUC} values for the four inhibitor groups, without weighting of individual mean values for the number of subjects in each study. If means are weighted for sample size, the outcome is essentially identical.

The overall difference among inhibitors was significant ($F = 5.31$, $P < 0.005$). R_{AUC} values for ketoconazole and ritonavir (11.5 and 14.5, respectively) were not significantly different from each other (Student–Newman–Keuls test). Both were significantly larger than values for itraconazole and clarithromycin (7.3 and 6.5), which in turn were not different from each other.

In none of the 15 studies involving ketoconazole as inhibitor were liver function abnormalities reported.

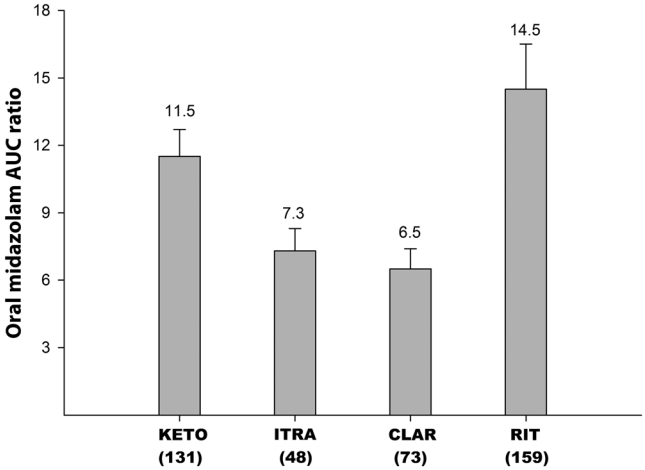

Figure 1

Ratios of total area under the curve (AUC) for oral midazolam during co-administration of each of four inhibitors divided by AUC in the control condition with no inhibitor. Each bar is the mean (±SE) value across studies for the indicated inhibitor, as described in Table 1. KETO ketoconazole, ITRA itraconazole, CLAR clarithromycin, RIT ritonavir. Numbers in parentheses are the total number of subjects participating in studies of the indicated inhibitor

Discussion

The findings indicate that ritonavir produces *in vivo* inhibition of CYP3A metabolic activity that is comparable with or greater than that of ketoconazole. Inhibition by itraconazole and clarithromycin were similar to each other, and both were less than the extent of inhibition produced by ketoconazole or ritonavir. Regulatory guidance classifies inhibitors as 'strong' if they produce R_{AUC} values exceeding 5.0. This qualifies itraconazole and clarithromycin as 'strong' inhibitors, but they do not produce maximal *in vivo* CYP3A inhibition comparable to ketoconazole or ritonavir. As such, itraconazole and clarithromycin are not reasonable alternatives to ketoconazole.

Ketoconazole is well recognized as a CYP3A inhibitor having high inhibitory potency [4, 6, 7, 41–47]. Ketoconazole produces reversible inhibition, with a mechanism that is mixed competitive and non-competitive [46]. Variable *in vitro* inhibition constant (K_i) values for ketoconazole have been reported among a large number of studies, but K_i typically is in the range of 0.05–0.1 μM [42, 47]. This is considerably below the usual range of plasma ketoconazole concentrations during therapeutic use (1–5 μM) [9, 48, 49], and is consistent with the high degree of inhibition observed *in vivo*. Because ketoconazole has a short elimination half-life, steady-state is reached rapidly after initiation of exposure, and no more than 24 h of pre-treatment is needed to produce maximal CYP3A inhibition [17, 50]. The time course of reversibility of ketoconazole inhibition after discontinuation is likely to be rapid as well [51].

Itraconazole also is a reversible CYP3A inhibitor. Usual *in vitro* K_i values are in the range of 0.1–0.5 μM, compared to *in vivo* plasma concentrations of 0.05–1.0 μM [4, 9, 23, 41, 42, 49, 52–55]. Itraconazole has metabolic products with CYP3A inhibitory activity that are likely to contribute to *in vivo* inhibition [23, 53, 54, 56, 57]. Because of the long elimination half-life of itraconazole and its metabolites, there is accumulation with repeated dosage [49, 57–61]. As such, the onset and offset of *in vivo* CYP3A inhibition is slower than what is established for ketoconazole [20, 22].

Clarithromycin is a macrolide derivative that produces time-dependent (mechanism-based) inhibition of CYP3A [62–66]. The inhibitory potency of clarithromycin is considerably less than ketoconazole or ritonavir. Values of the half-maximal inactivation constant or 50% inhibitory concentration (IC_{50}) for clarithromycin *in vitro* are in the range of 2–30 μM [64, 67], compared to plasma concentrations in the range of 2–6 μM [27, 68–70]. As such, the extent of *in vivo* CYP3A inhibition with clarithromycin does not approach what could be considered maximal [4, 8, 67]. As a time-dependent inhibitor [62–66, 71–74], the onset and offset of CYP3A inhibition is likely to be delayed [75]. In a study of erythromycin – also a

macrolide derivative producing time-dependent CYP3A inhibition – the apparent half-life of onset of inhibition following initiation of treatment was calculated to be 22.5 h [76].

Ritonavir is a highly potent CYP3A inhibitor *in vitro*, with a combination of reversible and time-dependent mechanisms [54, 77–80]. Values of K_i or IC_{50} generally are less that 0.2 μM, compared to plasma concentrations in the range of 1.0–10 μM [31, 32, 35, 36, 38, 81]. In clinical studies, the inhibitory potency of ritonavir is at least as great as that of ketoconazole (Fig. 1). CYP3A inhibition by ritonavir is dose- and exposure-dependent [35, 38], but in the majority of studies, daily doses in the typical 'boosting' range of 100 to 200 mg produce maximal or near-maximal inhibition [35, 38, 81–83]. The onset of CYP3A inhibition is rapid following initiation of ritonavir treatment, with maximal inhibition after 2 to 3 days of exposure [31, 35, 82, 83]. In one study of the reversal of inhibition, CYP3A activity reverted to baseline by 4 days after discontinuation of ritonavir dosage at 400 mg day^{-1} [82]. In another study, recovery from CYP3A inhibition was incomplete at 3 days after termination of ritonavir exposure at doses of 300–600 mg daily [35].

Ketoconazole, itraconazole, and ritonavir have inhibitory actions against other human CYP isoforms in addition to CYP3A [41, 42, 77, 78, 84–88]. However the values of K_i or IC_{50} *vs*. isoforms other than CYP3A are at least one order of magnitude higher (lower inhibitory potency) than for CYP3A [42, 77, 78, 88]. In clinical DDI studies, ketoconazole co-administration had minimal effects on the pharmacokinetics of antipyrine, caffeine, theophylline, and chlordiazepoxide [6]. Co-administration of itraconazole with the CYP2D6 substrate drugs aripiprazole [89] and tramadol [90] increased AUC values by factors of 1.48 and 1.11, respectively. In DDI studies involving ritonavir as a CYP inhibitor, short term exposure to boosting doses of ritonavir produced only small or negligible inhibition of clearance of dextromethorphan (CYP2D6) [30], desipramine (CYP2D6) [91], bupropion (CYP2B6) [92], omeprazole (CYP2C19) [37], S-warfarin (CYP2C9) [39] and flurbiprofen (CYP2C9) [37]. In cell culture models, ritonavir produces transcriptional activation and increased expression of a number of CYP isoforms and transport proteins [34, 93–95]. In clinical studies, induction of clearance of substrate drugs such as caffeine (CYP1A2) [95], olanzapine (CYP1A2) [96] and tolbutamide (CYP2C9) [95] has been demonstrated with extended exposure to relatively high doses of ritonavir. However the lower 'boosting' doses produce only small or modest degrees of induction [29]. Taken together, the data suggest that ketoconazole, itraconazole, and ritonavir all have high relative specificity as CYP3A inhibitors. The low dosage range for ritonavir, along with the short exposure durations typical of DDI studies, minimizes concerns about induction effects.

The candidate CYP3A inhibitors all produce some degree of inhibition of transport mediated by P-glycoprotein (ABCB1). This is evident from *in vitro* and experimental studies, as well as clinical DDI studies evaluating enteric uptake, partitioning across the blood–brain barrier, or renal clearance of P-glycoprotein substrates [30, 36, 97–110]. For victim drugs that are potential substrates both for metabolism by CYP3A and transport by P-glycoprotein, the outcome of DDI studies using these candidate inhibitors is likely to reflect concurrent inhibition of both CYP3A and P-glycoprotein.

Cobicistat is closely related to ritonavir in structure and pharmacologic properties [111–113]. Cobicistat has been approved as a single entity agent for pharmacokinetic boosting in antiretroviral therapy. In a DDI study directly comparing the inhibitory effect of 200 mg cobicistat and 100 mg ritonavir on clearance of oral midazolam, the mean R_{AUC} values for midazolam were 19.0 for cobicistat and 23.9 for ritonavir [40]. Like ritonavir, cobicistat is an inhibitor of P-glycoprotein activity [114], and is a relatively, but not completely, specific inhibitor of CYP3A. Both ritonavir and cobistat inhibit CYP2D6 activity *in vitro*, with IC_{50} or K_i values in the range of 3–14 μM [78, 84, 112]. In a clinical DDI study of cobicistat with the CYP2D6 substrate desipramine (as reported in the product label, but not published), cobocistat increased desipramine AUC by a factor of 1.65. Ritonavir increased desipramine AUC by a factor of 1.26 in a similarly-designed DDI trial [91]. The available data on cobicistat suggest that it could serve as an index CYP3A inhibitor for DDI studies. The product label for cobistat indicates that the drug can decrease creatinine clearance due to inhibition of tubular secretion of creatinine without affecting glomerular function. The ritonavir label describes elevations in serum transaminases in patients receiving ritonavir alone, or in combination with other antiretroviral drugs. However there is no evidence that either of these issues is of concern for DDI studies in healthy volunteers with no renal or hepatic disease.

Conclusions

There is no established risk of liver injury when ketoconazole is used as an index CYP3A inhibitor for DDI studies in healthy volunteers. Still, the regulatory action against ketoconazole forces consideration of alternatives. Itraconazole and clarithromycin have been proposed, but neither produces *in vivo* CYP3A inhibition approaching that of ketoconazole. Itraconazole has a long half-life and active metabolites, such that the onset and offset of CYP3A inhibitory activity are delayed. Clarithromycin is a time-dependent (mechanism-based) inhibitor, and its onset and offset of activity also are likely to be delayed. Ritonavir produces rapid onset CYP3A inhibition of magnitude at least as great as ketoconazole, and is the most reasonable alternative. Cobicistat closely resembles ritonavir, and also warrants consideration.

Competing Interests

Both authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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