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Everolimus Drug Interactions: Application of a Classification System for Clinical Decision Making

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ABSTRACT: *Introduction*. More than half of all drugs used in medical practice are metabolized by cytochrome CYP3A. Coadministration of drugs that share this elimination pathway may lead to pharmacokinetic drug interactions. Efforts are underway by clinical, drug development and regulatory scientists to classify CYP3A-related drug interactions with the ultimate goal of improving guidance for clinical intervention. The CYP3A inhibitory classification system ranks inhibitors according to the fold-increase in area-under-the-curve (*AUC*) of a probe substrate as: strong ($\geqslant$5-fold), moderate (>2.0- to 4.9-fold), or weak ($\leqslant$2.0-fold). This classification system was applied to characterize everolimus as a CYP3A substrate.

Methods. Five open-label crossover drug interaction studies were performed in 12–16 healthy subjects each. Subjects received a single 2 mg dose of everolimus alone and again during single- or multiple-dose treatment with the probe inhibitors ketoconazole, erythromycin, verapamil, cyclosporine and atorvastatin.

Results. The fold-increase in everolimus *AUC* was: 15.0 with the strong inhibitor ketoconazole; 4.4, 3.5 and 2.7 with the moderate inhibitors erythromycin, verapamil and cyclosporine; and no change with the weak inhibitor atorvastatin. Subjects with low baseline *AUC*s when everolimus was given alone tended to have *AUC* increases of a higher magnitude (more potent interaction) in the presence of an inhibitor.

Conclusions. Strong CYP3A inhibitors should be avoided when possible during everolimus treatment as compensatory everolimus dose reductions could be difficult to manage. Everolimus therapeutic drug monitoring should be used to guide individualized dose adjustments when moderate CYP3A inhibitors are added to or withdrawn from the regimen. Routine everolimus therapeutic drug monitoring should be sufficient to determine whether dose adjustments are needed when weak CYP3A inhibitors are coadministered. This rational and systematic approach to drug interactions on everolimus yielded clinically useful, structured guidelines for dose adjustment. Copyright © 2006 John Wiley & Sons, Ltd.

Key words: drug interactions; enzyme inhibition; everolimus

Introduction

A large proportion of drugs used in clinical medicine—up to 60% by some estimates—utilize the cytochrome P450 (CYP) 3A pathway for biotransformation [1]. Consequently, the potential for pharmacokinetic drug interactions when coadministering agents that share this pathway is clearly of clinical relevance. It is understandable that drug development scientists and regulatory authorities share the desire to harmonize approaches to assess drug interactions and to develop a CYP3A classification system to communicate risk to health care providers and patients. While several regulatory

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agencies worldwide have issued general drug interaction guidances for industry [2–4], the specifics of study design and methodology have not been addressed in a comprehensive manner.

The drug metabolism and clinical pharmacology technical working groups of the Pharmaceutical Research and Manufacturers of America have issued a consensus statement to begin to address some of these issues and to define a drug interaction data package that can be expected by regulatory authorities in the submission dossier of a new drug [5]. In addition to defining currently preferred and alternate probe CYP substrates to use in clinical drug interaction studies, they also cite a CYP3A inhibitory classification system to allow ranking the magnitude of interactions via this pathway. Using midazolam as a prototype CYP3A substrate, this system classifies the inhibitory potential of a new drug as strong if the plasma area-under-the-curve (*AUC*) of midazolam increases $\geqslant$5-fold, moderate if the increase is >2.0- to 4.9-fold, and weak if the increase is $\leqslant$2.0-fold [5]. This system could also be used to characterize a new drug as a substrate of CYP3A, placing it in reference to preferred probe inhibitors in the three classes.

With the exception of midazolam, which was used to define the system, there are to our knowledge no examples in the scientific literature applying this classification system in a consistent manner during the development of a new drug. We present our attempt to do so with everolimus, a macrolide immunosuppressant, that acts as a proliferation signal inhibitor on T-lymphocytes. Everolimus is used to prevent acute rejection episodes after kidney and heart transplantation. It is primarily biotransformed via CYP3A with metabolites eliminated in the bile and is also a substrate of P-glycoprotein [6]. A series of clinical drug interaction studies were performed during the development of everolimus demonstrating that it fits into this classification system as a CYP3A substrate. The classification system provided a useful structure to rank the potential for interactions from coadministered drugs on everolimus and to recommend everolimus dose adjustments.

Methods

Five open-label crossover drug interaction studies were performed in healthy subjects using the strong CYP3A inhibitor ketoconazole [7]; the moderate inhibitors erythromycin [8] verapamil [9] and cyclosporine microemulsion [10]; and the weak inhibitor atorvastatin [11]. All study protocols were approved by medical ethics committees and all subjects gave written informed consent to participate.

In these studies the reference treatment was a 2 mg single dose of everolimus (Certican, Novartis Pharmaceuticals) administered after an overnight fast of at least 10 h. The test treatment consisted of single-dose everolimus administered simultaneously with the inhibitor either under single-dose conditions or after steady state was reached during multiple-dose administration of the inhibitor as itemized in Table 1. Blood

Table 1. Clinical drug interaction studies of CYP3A inhibitors on everolimus

Inhibitor class	Number of subjects	Test regimen	Everolimus *AUC* alone (μg.h/l)	Everolimus *AUC* with inhibitor (μg.h/l)	Fold-increase in everolimus *AUC*[a]	Ref
Strong	12	Ketoconazole 200 mg b.i.d. × 8 days, Everolimus on day 4	90 ± 23	1324 ± 232	15.0 (13.6–16.6)	[7]
Moderate	16	Erythromycin 500 mg t.i.d. × 9 days, Everolimus on day 5	116 ± 37	524 ± 225	4.4 (3.5–5.4)	[8]
Moderate	16	Verapamil 80 mg t.i.d. × 6 days, Everolimus on day 2	115 ± 45	392 ± 142	3.5 (3.1–3.9)	[9]
Moderate	12	Cyclosporine 175 mg single dose, Everolimus on day 1	74 ± 26	193 ± 47	2.7 (2.2–3.2)	[10]
Weak	12	Atorvastatin 20 mg single dose, Everolimus on day 1	120 ± 37	118 ± 46	1.0 (0.8–1.2)	[11]

[a]Fold-increase is point estimate and 90% confidence interval.
AUC, area under the concentration-time curve; b.i.d., twice daily; t.i.d., thrice daily.

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samples were obtained before each everolimus dose and then at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96 and 120 h postdose. Everolimus whole blood concentrations were determined by a validated liquid chromatography method with mass spectrometric detection as previously described [12]. The assay limit of quantification was 0.3 ng/ml. The area under the concentration-time curve extrapolated to infinity (*AUC*) was derived by noncompartmental methods. The *AUC*s were log-transformed and compared between treatments by conventional bioequivalence testing to yield the test/reference ratio of the geometric means and 90% confidence interval. The ratio served as the estimate of the magnitude of the drug interaction on everolimus expressed as the fold-increase in everolimus exposure. The intersubject variability in the magnitude of the interaction was derived as the standard deviation divided by the mean of these *AUC*-ratios (*AUC* with inhibitor/*AUC* alone). Relationships between the reference everolimus *AUC* and the fold-increase in the test *AUC* were explored by graphical evaluation and fitting a descriptive log function through the data.

Results

Interaction magnitude

As summarized in Table 1 the population average fold-increase in everolimus *AUC* in the presence of strong, moderate and weak CYP3A inhibitors fell within the bounds of the classification system [5] in all cases. A related rapamycin macrolide, sirolimus, reported similar fold-increases in sirolimus *AUC* of 10.9 in the presence of ketoconazole, 4.2 in the presence of erythromycin, 3.3 in the presence of cyclosporine microemulsion, 2.2 in the presence of verapamil, and no relevant change in the presence of atorvastatin [13]. The fold-increases in everolimus and sirolimus *AUC* were similar to that of the prototype CYP3A substrate, midazolam, in the presence of these inhibitors. Published studies reported mean fold-increases in midazolam *AUC* in the presence of multiple-dose ketoconazole of 8.7, 11 and 16 [14–16], in the presence of multiple-dose erythromycin of 4.4 and 3.8 [17–18] and in the presence of multiple-dose verapamil of 2.9 [19]. The lack of change in the *AUC* of atorvastatin—a recommended and sensitive probe substrate of CYP3A—in the presence of everolimus [11] indicates that everolimus does not inhibit CYP3A to a clinically relevant extent.

Interaction variability

Figure 1 depicts the individual fold-increases in everolimus *AUC*. The associated intersubject variability was generally moderate to high at 20% in the presence of ketoconazole, 57% for erythromycin, 28% for verapamil, 34% for cyclosporine microemulsion and 36% for atorvastatin. While all subjects exhibited a strong interaction magnitude (⩾5-fold increase) with ketoconazole and a weak interaction magnitude (<2.0-fold) with atorvastatin, some subjects receiving moderate inhibitors had fold-increases which transgressed the categorical boundaries. Specifically, for cyclosporine microemulsion 3 of 12 subjects (25%) had weak interactions between 1.5- to 1.9-fold. For erythromycin 6 of 16 subjects (38%) had a strong interaction ranging from 5.1- to 12.6-fold and for verapamil 1 of 16 subjects (6%) had a strong interaction of 6.3-fold.

Exposure-response associations

The pharmacokinetic data were explored to look for predictive or explanatory factors for the

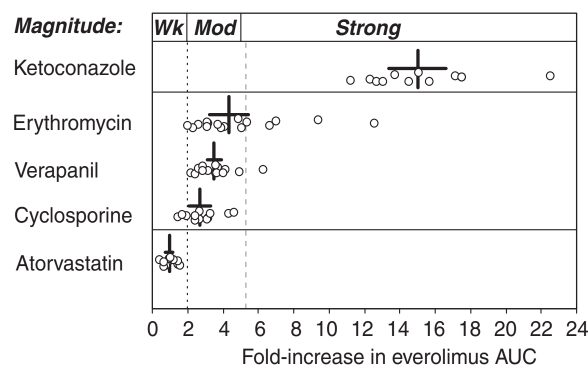

Figure 1. Fold-increase in everolimus *AUC* in the presence of various CYP3A inhibitors against a categorical background for weak inhibition (*Wk*, ⩽2.0-fold increase), moderate inhibition (*Mod*, 2.0- to 4.9-fold increase) and strong inhibition (*Strong*, ⩾5-fold increase). Shown are the individual fold-increases (open circles), the population average (vertical bar), and the 90% confidence interval (horizontal bar)

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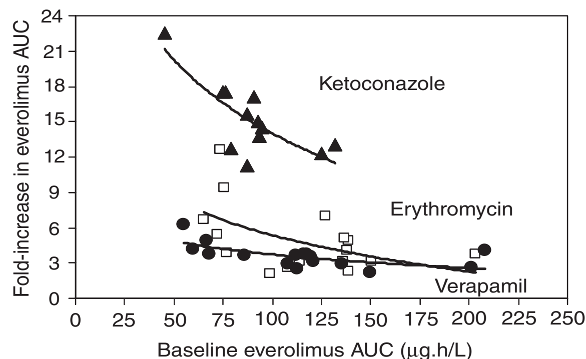

Figure 2. Relationship between everolimus *AUC* in the absence of coadministered drugs (baseline *AUC*) and the fold-increase in everolimus *AUC* in the presence of CYP3A inhibitors ketoconazole (filled triangles), erythromycin (open squares) and verapamil (filled circles). Shown is the best fit of a logarithmic trendline to the data: for ketoconazole, $y = -9\ln(x) + 55$ ($r^2 = 0.617$); for erythromycin, $y = -1.6\ln(x) + 11.3$ ($r^2 = 0.423$); for verapamil, $y = -4.5\ln(x) + 26$ ($r^2 = 0.275$)

magnitude of the interaction on everolimus or its intersubject variability. The blood or plasma level of the inhibitor during the test treatment was in no case predictive of the magnitude of the interaction on everolimus [7–11]. The baseline everolimus *AUC*, however, was inversely related to the fold-increase in everolimus *AUC* in the presence of the inhibitor as shown in Figure 2 for ketoconazole, erythromycin, and verapamil. In each case a linear regression did not adequately account for the curvature in these relationships; hence, they were described with logarithmic trendlines as specified in the figure legend. In general, the lower the baseline everolimus *AUC*, the greater the interaction magnitude in the presence of the inhibitor. There was no apparent relationship for everolimus with cyclosporine for which the scatterplot appeared flat. Since atorvastatin essentially did not change the everolimus *AUC*, no relationship was observed for this weak inhibitor.

Clinical implications

Everolimus is marketed at dose strengths of 0.25, 0.5, 0.75 and 1 mg tablets and 0.1 and 0.25 mg dispersible tablets [20]. In organ transplant medicine, everolimus dosing is individualized by monitoring predose blood levels along with the clinical condition of the patient [6]. Given the small influence of weak CYP3A inhibitors on everolimus pharmacokinetics, it is anticipated that routine therapeutic drug monitoring would indicate whether an individual patient needs an everolimus dose adjustment when a weak CYP3A inhibitor is added to or removed from the regimen. At the other extreme, the large influence of strong CYP3A inhibitors on everolimus exposure would make compensatory dose adjustments difficult to achieve given the tablet strengths available. Hence, coadministration of strong CYP3A inhibitors with everolimus is not recommended.

Addition or removal of moderate CYP3A inhibitors to an everolimus regimen should be accompanied by therapeutic drug monitoring to guide compensatory everolimus dose adjustments. The study data shown in Figure 1 indicate that most everolimus *AUC* increases were in the range of 2- to 4-fold and this can serve as a general indicator of what to expect when coadministering a moderate CYP3A inhibitor. However, some increases in everolimus exposure may extend into the strong interaction range. Figure 2 suggests that patients who may be more susceptible to a drug interaction from a CYP3A inhibitor appear to be those with low baseline everolimus exposure. Such patients would likely require a higher everolimus dose than the population average dose in order to maintain everolimus blood levels in the therapeutic range in the absence of CYP3A inhibitors. Clinicians treating such patients should be more alert for drug interactions when adding a moderate CYP3A inhibitor to the regimen until everolimus therapeutic drug monitoring results are available from the analytical laboratory to confirm the actual magnitude of the interaction.

Discussion

This overview of the everolimus drug interaction program during drug development demonstrates that the influence of five different CYP3A inhibitors on everolimus fits into the proposed CYP3A inhibitory classification system proposed

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by the Pharmaceutical Research and Manufacturer's Association [5]. Such a classification system begins to address the desire expressed by industry sponsors to develop a ranking system to allow better assessment and comparison of different drugs in their drug interaction potential and to communicate risk to health care providers and patients [5].

With regard to comparing drug interaction potential among drugs, the two macrolide immunosuppressants, everolimus and sirolimus, differ in chemical structure and biotransformation pathways [21–22] which in theory could yield differences in their susceptibility to metabolic drug interactions. Nonetheless, comparing the everolimus results presented here with published data from sirolimus suggests that they share a generally similar risk for CYP3A inhibition drug interactions as interpreted against the background of this classification system.

This classification system also serves as a framework to communicate drug interaction risk to clinicians. The risk of increased drug exposure when coadministering strong CYP3A inhibitors led to the recommendation to avoid these comedications in the product labels of both everolimus [20] and sirolimus [13]. Coadministration of moderate CYP3A inhibitors, however, appears to be associated with a generally lower magnitude in the fold-increase of everolimus *AUC*; however, the healthy subject data indicate that some individuals are more susceptible to CYP3A inhibition interactions resulting in larger increases in exposure. The data further suggest that these individuals tend to have lower everolimus exposure in the absence of comedications as shown in Figure 2. This pattern has been observed in other drug interaction studies with CYP3A substrates [23–25]. It is speculated that individuals with higher expression of CYP3A (manifested by lower exposure when the drug is given alone) have more enzyme to inhibit and thereby elicit a stronger drug interaction in the presence of a CYP3A inhibitor [24–26]. In other words, intersubject variability in enzyme levels is likely a strong contributor to variability in the magnitude of drug interactions. In light of this underlying variability, moderate CYP3A inhibitors may generally be used with everolimus provided that it is accompanied by therapeutic drug monitoring to quantify the increase in everolimus exposure after adding the moderate inhibitor and to individually adjust the everolimus dose to compensate for the interaction [20].

An additional application of the CYP3A inhibitor classification system in risk management is as a qualitative guide to drug interactions not specifically tested by the sponsor. This is explicitly recognized in the European Agency for the Evaluation of Medicinal Products drug interaction guidance which states: 'It seems reasonable that *in vivo* studies with strong inducers/inhibitors may be used to extrapolate qualitatively to other inducers/inhibitors of the same enzyme' [4]. In the case of everolimus, the summary of product characteristics extends the recommendation to avoid strong CYP3A inhibitors based on ketoconazole data to include itraconazole, voriconazole, clarithromycin and ritonivir [20]. Similar qualitative extensions are made for moderate CYP3A inhibitors [20].

While the CYP3A inhibitor classification system has many desirable traits, some investigators cite points for caution as well [27]. For example, when using the system to classify a new drug as an inhibitor with midazolam as the probe substrate, the data might not be extrapolatable to other substrates. The classification results may be study design-specific if the magnitude of the interaction depends on the drug doses studied, administration under single-dose or multiple-dose conditions, or relative timing of coadministration (simultaneously versus delayed). Furthermore, the potency of an individual drug interaction may be understated if multiple 'mild' or 'moderate' inhibitors are coadministered in a patient's drug regimen leading to a stronger combined interaction or if the victim drug is a substrate of multiple CYP enzymes or transporters inhibited by the perpetrator drug.

Conclusions

Mindful of the caveats mentioned above, drug development scientists, regulatory reviewers and clinicians can use this CYP3A inhibitory classification system as a tool to design drug interaction programs, to manage regulatory risk and to guide pharmacotherapy in the patient care setting. The example of everolimus demonstrates

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that applying a rational and systematic approach to drug interactions on everolimus yielded clinically useful, structured guidelines for dose adjustment.

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