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Cocktail approach for assessing CYP450 drug-drug interaction liabilities including assessment of differential inhibition of liver and gut CYP3A4.

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INTRODUCTION

The “Cocktail” approach is an industry accepted tool which uses the co-administration of probe drugs to assess the liability of a drug to inhibit a number of different Cytochrome P450 (CYP450) enzymes. One of its main benefits is that it is an efficient approach for assessing multiple CYP450 isoforms in a single clinical study, should preclinical data indicate that a drug may inhibit a number of CYP450 isoforms. Probe drugs need to be safe and tolerated following single administration, ideally they are specific for single CYP450 isoforms, commercially available and must not interact with the other drugs in the cocktail. Many different cocktails have been assessed[1,2,3] and this poster describes the use of the cocktail methodology to assess the potential of a perpetrator drug to inhibit CYP1A2, CYP2C9, CYP2C19, CYP3A4 and CYP2D6, to understand the mechanism of the interaction, and to investigate the differential inhibition of gut and liver CYP3A4.

STUDY DESIGN

The study was an open-label, single period study. Subjects were genotyped to exclude CYP2D6 and CYP2C9 poor metabolisers. Table 1 lists the probes used within the cocktail, their associated CYP450 enzyme activity, the dose and route of administration.

Table 1

Probe	CYP450 Isoform	Main Metabolite	Dose	Route Administration
Midazolam	Hepatic CYP3A4	1-Hydroxymidazolam	1 mg	Intravenous (IV)
Midazolam	Hepatic and gastrointestinal CYP3A4	1-Hydroxymidazolam	2 mg	Oral (solution)
Caffeine	Hepatic CYP1A2	Paraxanthine	150 mg	Oral
Tolbutamide	Hepatic CYP2C9	4-Hydroxytolbutamide	500 mg	Oral
Omeprazole	Hepatic CYP2C19	5-Hydroxyomeprazole	20 mg	Oral
Dextromethorphan	Hepatic CYP2D6	Dextrorphan	30 mg	Oral

Subjects were administered to the clinic on Day -1, and received the following treatments;

Day 1; midazolam IV bolus simultaneously with oral administration of caffeine, tolbutamide, omeprazole, and dextromethorphan.
Day 2; single oral dose of midazolam.
Days 3 – 7; daily dosing of perpetrator.
Day 8; midazolam IV bolus simultaneously with oral administration of caffeine, tolbutamide, omeprazole, dextromethorphan and perpetrator.
Day 9; single oral dose of midazolam and perpetrator.

Samples were collected to 24 hours post dose on Days 1, 2, 8 and 9 for analysis of the plasma concentration of each probe drug and its main metabolite. Predose samples taken on Days 3 – 8 confirm that perpetrator drug had achieved steady state. The maximum plasma concentration (Cmax), area under the plasma concentration-time curve (AUC), terminal half life (T1/2) and parent:metabolite ratios (molecular weight-adjusted AUC) of each probe on Days 8/9 were compared to those calculated on Days 1/2, to assess perpetrator affects on the exposure of the probe drug and to elucidate the mechanism of the effect. Absolute bioavailability (F) and IV clearance (Cl_{IV}) obtained from the oral and IV administration of midazolam, in the presence and absence of the perpetrator drug, were used to assess the differential inhibition of intestinal and hepatic CYP3A4.

METHODS

The bioavailability of midazolam is obtained from Equation 1

$$F = (AUC_{oral}/AUC_{IV}) \times (Dose_{IV}/Dose_{oral}) \times 100\ \% \text{ - Equation 1;}$$

where AUC_{oral} and AUC_{IV} are the area under the plasma concentration-times following oral dosing and IV dosing, respectively

METHODS (continued)

The hepatic extraction ratio (E_H) of IV midazolam, and (1 – E_H), an estimate of the fraction of drug surviving hepatic metabolism (F_H) can be calculated from Equation 2. The fraction of drug surviving gut metabolism (Fg) can be calculated from Equation 3. Calculation of F, F_H and F_g in the presence and absence of perpetrator will enable assessment of the impact of the perpetrator on hepatic and gastrointestinal CYP3A4, assuming Fraction of drug absorbed (Fabs) stays constant.

$$E_H = Cl_{IV}/Q_H \text{ – Equation 2;}$$

Where, and Q_H is Hepatic blood flow (1450 ml/min[4]),assuming clearance is primarily hepatic, metabolic .

$$F_{abs} \times F_g = F/(1 - E_H) \text{ – Equation 3;}$$

where F_{abs} is fraction of drug crossing into gastrointestinal epithelial cells and F_g the fraction of drug surviving gut metabolism.

RESULTS

Exposure data obtained on Days 8/9 compared to Days 1/2 showed that the perpetrator drug inhibited CYP1A2, CYP2D6, CYP2C9, CYP2C19 and CYP3A4.

When the data were examined, the pattern of effect on the exposure of the orally administered probe drugs and their measured metabolites, fell into 2 categories.

Dextromethorphan, omeprazole and midazolam (oral) had significantly elevated exposure (Cmax and AUC), (1.5 – 10 fold), following dosing with the perpetrator drug, although T1/2 was unaffected.

Tolbutamide and caffeine had significantly elevated overall exposure (AUC) and prolonged T1/2, following dosing with the perpetrator drug although the Cmax was unaffected.

For all of the orally administered drugs parent:metabolite ratio (molecular weight-adjusted AUC) of the main measured metabolite was increased following administration of the probe with the perpetrator drug.

Midazolam (IV) had a significantly reduced clearance. Exposure of the 1-hydroxymidazolam metabolite was also reduced, following dosing with the perpetrator drug.

Examination of F, F_H and F_g for midazolam in the absence and presence of perpetrator drug, showed that all three of these parameters were increased by co-administration with the perpetrator drug. However, the magnitude of effect on gut metabolism (F_g) was greater than the effect on hepatic metabolism (F_H).

DISCUSSION

The perpetrator drug was found to inhibit all the CYP450 enzymes assessed, to varying extents. The fold change in exposure of the probe drug enables assessment of the classification of the inhibitor as a strong, moderate or weak inhibitor for each of the CYP450s tested.

Furthermore, it is possible to elucidate the mechanism of interaction from the pattern of change of the exposure parameters. Dextromethorphan, omeprazole and midazolam (oral) all had elevated exposures but no change in T1/2, whilst Tolbutamide and caffeine had significantly elevated overall exposure (AUC) and prolonged T1/2 although the Cmax was unaffected. In the former situation the data is indicative of the inhibitor primarily effecting first pass metabolism, whilst in the latter scenario the effect appears to be mediated after first pass.

Dosing of midazolam IV and orally, enabled an assessment the impact of the perpetrator drug on gastrointestinal and hepatic CYP3A4. The greater increase in F_g compared to F_H in the presence of the perpetrator, indicates that the resultant increase in systemic exposure of oral midazolam can mainly be attributed to inhibition of gastrointestinal CYP3A4 with a much smaller effect on hepatic CY3A4.

CONCLUSIONS

- This poster has illustrated the value of a probe cocktail approach for assessing the in vivo inhibitory potential of a perpetrator drug on multiple CYP450 isoforms, in a single clinical study.
- Furthermore, the data generated enables assessment of the mechanism by which the perpetrator effects the change in exposure of the probes.
- Assessment of the extent of gut and hepatic CYP3A4 inhibition is possible by including both IV and oral midazolam in the study.

REFERENCES

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