ISSX Meeting - Noordwijk aan Zee, 2012

Validation of an in Vivo Phenotyping Cocktail for the Early Detection of Inductive or Inhibitory Potential of New Drugs

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Background

Introduction

Most of the cytochrome P450 (CYP) enzymes are subject to induction or inhibition. Therefore, focusing on this system during early drug development provides opportunities for early risk assessment.

Results of pre-clinical in vitro studies used to assess the drug-drug interaction (DDI) potential of new molecules, may need to be confirmed in vivo using probe substrates.

These probe substrates are selectively metabolized to a single metabolite by a given CYP isoform.

The probe substrates can be administered simultaneously in a so-called **"cocktail-approach"** to assess several enzymes simultaneously in one experiment and in the same individual.

Objectives

- To test a cocktail containing caffeine (1A2), mephenytoin (2C19), dextromethorphan(2D6), and endogenous cortisol (3A4) as a predictor for inhibitory and inductive potential of new drugs.
- To use a single time point or collection interval to assess enzyme activities instead of a whole PK curve as a practical approach.

Methods

Study Design

- 24 volunteers received the probe substrates (see table) once individually (Period 1-6) and once simultaneously (Period 7) to study possible interactions.
- After this baseline activity, volunteers were challenged with a known (selective) inducer for 10 consecutive days (Period 8).
- The (selective) inhibitors were dosed orally only on Day 10, followed by administration of the cocktail (Period 8).
- The changes in baseline activity after administration of the inducer or inhibitor were monitored.
- Return to baseline of the CYP enzyme activity was evaluated by a cocktail assessment 21 days later (Period 9).
- Plasma samples were taken after 2h, 4h, 6h and 8h post dose
- Urine samples were collected during the 0-4h, 4-6h, and 6-8h intervals post dose.

Overview of the probe and doses

CYP	Substrate / Metabolite	Dose (mg)	Inhibitor	Inducer
1A2	Caffeine (CAF)/Paraxanthine (PX)	100	Fluvoxamine	Omeprazole
2C9	Tolbutamide (TB)/Hydroxy-Tolbutamide (OH-TB) + carboxytolbutamide (COOH-TB)	500	Fluvoxamine	Rifampicin
2C19	Mephenytoin (ME)/Hydroxy-Mephenytoin (OH-ME)	100	Fluvoxamine	Rifampicin
2D6	Dextromethorphan (DM)/Dextrorphan (DX)	30	Paroxetine	Rifampicin
2E1	Chlorzoxazone (CZ)/Hydroxy-Chlorzoxazone (OH-CZ)	250		Phenytoin
3A4	Cortisol (CORT)/Hydroxy-Cortisol (OH-CORT)	Endogenous	Ketoconazole	Phenytoin, Rifampicin

Study Design

	Block I							Block II			
	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7*	Period 7*	Period 8		Period 9
	Day 1	Day 8	Day 15	Day 22	Day 29	Day 35	Day 43	Day 0	Day 1-9	Day 10	Day 31
dosing parts of cocktail	x	x	x	x	x	x					
cocktail assessment							x	x		x	x
dosing inhibitors										x	
dosing inducers									x	x	

*(Day 43 - Block I) = (Day 0 - Block II)

Analysis of Results

- For each CYP (except for CYP2C9), enzyme activity was studied by the change in amount of parent (P), amount of metabolite (M) and the ratio metabolite/parent (M/P)
- Ratio's were calculated for each sampling time point:
  (1) $P_{Period\ 8}/P_{Period\ 7}$, (2) $M_{Period\ 8}/M_{Period\ 7}$,
  (3) $(M/P)_{Period\ 8}/(M/P)_{Period\ 7}$
- For CYP2C9 enzyme activity was studied by the change in amount of metabolite M1 and M2 and for the sum of the metabolites (M1+M2).
- For CYP2C9, the ratio's were
  (1) $M1_{Period\ 8}/M1_{Period\ 7}$, (2) $M2_{Period\ 8}/M2_{Period\ 7}$,
  (3) $(M1+M2)_{Period\ 8}/(M1+M2)_{Period\ 7}$
- Return to baseline of CYP activity was assessed by plotting the M/P ratio in Period 9 versus Period 7, or $M1+M2_{Period9}$ to $M1+M2_{Period7}$ for TB around a line of identity.
- A graphical evaluation of ratio (3) at the 6h time point or 4-6h collection interval was performed for each inducer or inhibitor and CYP isoform.

Results

Individual versus cocktail administration

- For all the probe substrates, the concentration/amount excreted of the parent compound was not significantly altered (except for ME).
- For all the probe substrates, the concentration/amount excreted of the metabolite was significantly altered for at least one time point or collection interval (except for PX).
- As a result, the metabolite/parent ratio's (or sum of metabolite ratio's) were also significantly altered.

Return to baseline

- The ratios, assessed in Period 9 and Period 7, were fairly similar for PX/CAF, OH-ME/ME, DX/DM (plasma) and OH-CORT/CORT.
- Most of the urinary DX/DM ratios and all OH-CORT/CORT ratios showed high variability, but the results pointed in the expected direction.
- The sum of the amounts of COOH-TB and OH-TB, and the ratios OH-CZ/CZ in Period 9 were generally higher than those assessed in Period 7.

Induction or inhibition

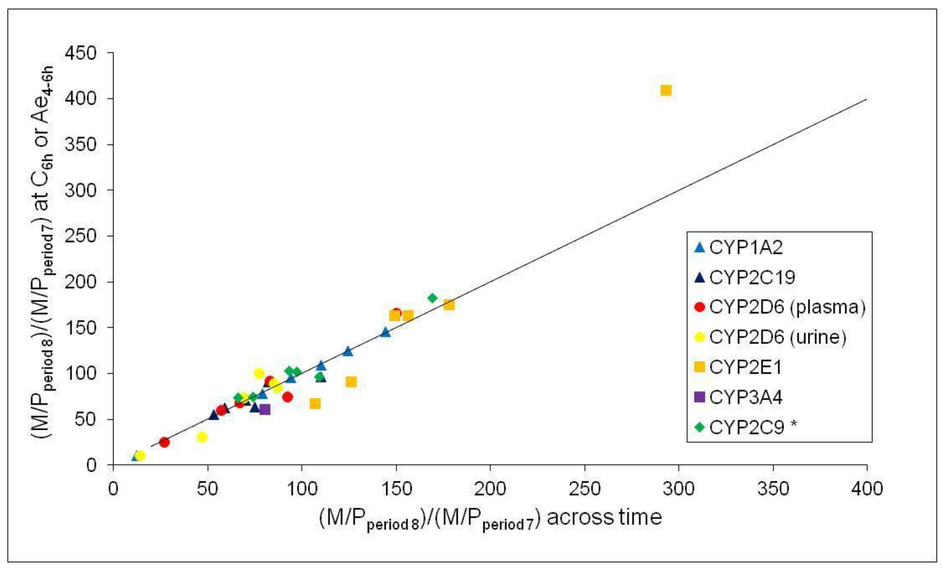
- After challenge with rifampicin induction was observed for 1A2, 2E1 and 2C9, but not on 2C19 and 2D6.
- After challenge with phenytoin, induction was observed for 2E1 and 3A4.
- After challenge with omeprazole, induction was observed for 1A2.
- An inhibitory effect was observed by fluvoxamine for 1A2, 2C9 and 2C19 and by paroxetine for 2D6.
- The presumed inhibitory effect of omeprazole on 2C19 was confirmed.
- Due to analytical difficulties , the known inhibitory effect of ketoconazole on 3A4 could not be deduced.

Across time versus one time point

- The Least Square means analysis between Period 7 and Period 8 at the 6h plasma time point or the 4-6 h urine collection interval showed a good correlation with the analysis across time points.

Differences between Period 7 and 8

			CYP1A2 Plasma PX/CAF	CYP2C19 Urine OH-ME/ME	CYP2D6 Plasma DX/DM	CYP2D6 Urine DX/DM	CYP2E1 Plasma OH-CZ/CZ	CYP3A4 Urine OH-CORT/CORT		CYP2C9 Urine COOH-TB + OH-TB
Inhibitor	Ketoconazole	P		↑				↓	M1	
	(3A4)	M	↓	↑	↑			↓	M2	↓
		M/P	↓				↑		M1+M2	
	Fluvoxamine	P	↑		↑				M1	
	(1A2, 2C9, 2C19)	M	↓						M2	
		M/P	↓	↓	↓		↑		M1+M2	↓
	Paroxetine	P			↑	↑			M1	
	(2D6)	M				↑			M2	
		M/P			↓	↓	↑		M1+M2	
Inducer	Rifampicin	P	↓		↓				M1	↑
	(3A4,2C9/19,	M		↓	↓	↓			M2	↑
	2D6)	M/P	↑				↑		M1+M2	↑
	Phenytoin	P	↓	↑					M1	↓
	(2E1, 3A4)	M	↓		↓	↓	↑	↑	M2	↓
	(2C19 inhibitor)	M/P	↑	↓	↓		↑		M1+M2	↓
	Omeprazole	P	↓	↑					M1	
	(1A2)	M							M2	
	(2C19 inhibitor)	M/P	↑	↓					M1+M2	



Scatter plot of the M/P ratios : correlation between the across time and single time point analysis.

* CYP2C9 { (M1+M2 period 8) / (M1+M2 period 7) }

Discussion

- Changes in probe metabolism were seen when cocktail administration was applied, suggesting an interaction between the some of the probes.
- These interactions could not conceal large effects on the enzyme activity. This nevertheless limits the use of the cocktail to the disclosure of large inhibiting or inducing effects, with a high potential of being relevant.
- The analysis at a single time point correlated well to the analysis across time points.

Conclusion

- In general, the expected effects were seen, but in some particular situations the results were different from initially expected.
- The possibility to evaluate the inducing or inhibiting property of a drug through the analysis of the enzyme activity at only one time point is a major advantage.

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