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Chimpanzee Cytochromes P450 (CYPs) and "Phase Zero" Study

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[Objective] The chimpanzee is unique among nonhuman primates in its similarity to man. Chimpanzee genes show 98% similarity of the DNA found in humans. *In vivo* pharmacokinetic/metabolic studies "Phase Zero" using chimpanzees would help fill the gap between routine animal data and Phase I of clinical trials. Chimpanzees would be invaluable for drug interaction studies. Drug metabolizing enzyme systems, especially cytochromes P450 (CYPs), play a major role in metabolic drug interactions. Since no information on CYP isoforms in chimpanzees is available, CYP substrates of human liver CYP isoforms (3A4, 2C19, 2D6, 2E1) were studied in liver biopsy samples from chimpanzees and compared with the human liver data. *In vitro* studies will complement the *in vivo* tests described below, but not all the substrates have to be identical between the two studies. The selection of substrates depends partly on the availability of suitable methods as well as on the anticipated need for studies using chimpanzees. For drug-drug interaction studies, 3A4 is the most important P450 enzyme isoform. For pharmacogenetic studies in humans, 2D6 and 2C19 are known to show deficiency with frequencies varying between ethnic groups.

In vivo probe drugs are convenient and this non-invasive method was used for testing the drug metabolizing capacity of chimpanzees. Many drugs are known to be metabolized by specific enzymes and monitoring of their metabolites and comparing to these of humans would provide a quick indication of the suitability of chimpanzees as an animal model. Initially the three probe drugs selected are dextromethorphan (DEX), mephenytoin (MP) and caffeine (CAF), with which 4 different CYP enzyme activities can be monitored. This *in vivo* approach needs to be complemented with *in vitro* verification of enzyme specificity using liver biopsy.

[Methods] Six chimpanzees were anesthetized with ketamine and needle biopsy samples of liver were obtained. Biopsy samples in plastic cryo-tubes were transferred immediately to -70 °C freezer. Tissue (100 mg) after thawing was suspended in 0.5 ml 1.15% KCl solution and homogenized by a Kontes pellet pestle (1.5 ml). Normally the incubation volume for CYP activity assay was 0.5 ml (containing about 0.15 µg of microsomal protein). The incubation was carried out by a standard method described previously (Inaba et al 1997). Briefly, radio-labelled substrates were used and the metabolites separated by TLC. [¹⁴C]Testosterone (TS) was used for CYP3A4 activity, [¹⁴C]chlorzoxazone for CYP2E1 and [¹⁴C]S-MP for CYP2C19 (4'-OH) and 2D6 (N-demethylation). The quantification of the metabolites was done by Molecular Dynamics PhosphorImager. The linearity of radioactivity determination was 10,000 fold.

For the *in vivo* study, a capsule containing a cocktail of 3 probe drugs was given to 3 chimpanzees. Each capsule contained 200 mg CAF, 100 mg MP and 120 mg DEX. Urine was collected for 24 hours. The LC-MS/MS methods were developed to quantify, in addition to the parent drug, 17X, 1X and 1U for CAF, 4'-hydroxy MP and nirvanol for MP, and dextrorphan and 3-methoxymorphinan for DEX.

[Results & Discussion] *In vivo* probe drugs were selected on the following basis. Genetic polymorphism is important to consider in drug development and monitoring urinary metabolites is one of the most convenient way of screening for PM subjects. DEX and MP are the widely used drugs for phenotyping CYP2D6 and CYP2C19, respectively. CAF is a useful probe not only because it is readily available in cola-drinks or coffee but also because it is the only probe that can

be used in humans to monitor CYP1A2. Inducibility is a great concern in drug development. Table 1 shows the *in vivo* profiles of probe drugs and their metabolites in urine of 3 chimpanzees and 4 human male subjects.

In vitro studies included additional CYP isoforms. Since more than 50% of drugs in use are metabolized by CYP3A4, this enzyme is frequently the site for drug-drug interaction. Initial emphasis was placed on chimpanzee CYP3A especially the presence and similarity to human 3A4. As shown in Table 2 the *in vitro* profiles of chimpanzee liver homogenate resembled closely to the human liver data. In addition, CYP3A in chimpanzee liver homogenate was detected by Western immunoblot (for 3A1 and 3A4). We cannot use the term 3A4, but the CYP3A enzyme in chimpanzees must be nearly identical to the human enzyme as we observed an indistinguishable inhibitory pattern with ketoconazole, a specific inhibitor of CYP3A4 at 1 μ M concentration. The radio-TLC results with CYP2C19 probe S-MP between chimpanzees and humans are very similar. The minor pathway of MP in humans, N-demethylation, however, was the major pathway in chimpanzees. These *in vitro* data were consistent with the *in vivo* data as shown above. Chlorzoxazone 6-hydroxylation found in human was detected in chimpanzee liver *in vitro*.

[Conclusion] Our studies indicate that the chimpanzee is unparalleled in its similarity to humans.

[References] 1) Inaba T. et al., in A.C. Cuello and B. Collier (eds), Pharmacological Sciences: Birkhauser, Basel, pp.169-175 (1995). 2) Inaba T. et al., Toxicol. Lett. **93**:215-219 (1997)
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Table 1. *In vivo* profiles of probe-drugs and their metabolites in urine of chimpanzees and human

		(Metabolites/Intact)									
		MP	4'OH	Nirv	DEX	O-dem	N-dem	CAF	17X	1X	IU
Human	A	1.00	2.91	0.33	1.00	12.18	LOQ	1.00	3.08	1.27	1.68
	B	1.00	6.32	0.46	1.00	8.33	LOQ	1.00	10.34	6.43	5.26
	C	1.00	LOQ	0.17	1.00	5.72	LOQ	1.00	1.96	1.82	7.35
	D	1.00	4.21	0.50	1.00	9.03	LOQ	1.00	7.36	2.32	3.15
Chimp	A	1.00	2.77	11.32	1.00	11.21	LOQ	1.00	31.52	29.74	108.82
	B	1.00	0.55	5.98	1.00	7.59	LOQ	1.00	23.09	15.78	42.51
	C	1.00	0.31	1.60	1.00	2.26	LOQ	1.00	4.18	2.97	6.42

LOQ: Below the limit of quantitation (<25.0 ng/ml)

Table 2. *In vitro* profiles of [14 C]testosterone and its metabolites in liver homogenate of various species

		(Area %)				
		T	6 β -OH	M-1	M-2	M-3
Human	A	57	24	9	0	0
	B	69	14	6	0	0
Chimp	A	60	15	10	0	0
	B	73	13	5	0	0
Rabbit		36	27	11	0	0
Rat (M)		22	30	1	1	0
Rat (F)		0	1	0	0	72
G. Pig		43	17	12	15	0

Substrate ([14 C]TS) at 50 μ M in a liver homogenate (7-10 mg wet wt)

M-1, M-2, M-3: Radioactive metabolites of [14 C]TS.