Effect of treating Cynomolgus monkeys with prototypical CYP inducers on liver CYP1A mRNA levels:

- BNF caused an increase in liver CYP1A1 and 1A2 mRNA levels, but as a CYP1A mRNA inducer OMP was less effective than BNF.
- Though to a smaller extent compared to BNF, OMP also caused increases in microsomal CYP1A1/2 activity (3.7- and 4.3-fold in male and female monkeys, respectively).

Effect of treating Cynomolgus monkeys with prototypical CYP inducers on liver CYP2B mRNA levels:

- Treatment with BNF caused an increase in CYP2A4 mRNA levels (66- and 240-fold in males and females), and CYP2A2 mRNA levels (51- and 160-fold in males and females). Treatment with OMP caused an increase in CYP2A1 mRNA levels (2.2- and 5.8-fold in males and females) and CYP2A2 mRNA levels (14.9- and 17.3-fold in males and females). Similarly, BNF and OMP caused increases in liver microsomal CYP1A1/2 activity by BNF was similar between male and female monkeys (11- and 9.4-fold, respectively) whereas induction of intestinal CYP1A1 activity by BNF was much lower in male than female monkeys (2.7- and 19-fold, respectively). Though to a smaller extent compared to BNF, OMP also caused increases (3.5-fold in CYP1A1/2 activity) for male and female monkeys, respectively. Treatment with PB caused a large increase in liver CYP2B6 mRNA levels (21.6- and 52.5-fold in males and females, respectively), and caused less pronounced increases in CYP2A4 (5.6- and 7.4-fold), CYP2B17 (3.6- and 8.2-fold), CYP2C18 (6.9- and 8.1-fold), CYP3A4 (6.7- and 2.5-fold), and CYP3A5 (6.7- and 2.5-fold) mRNA levels in males and females, respectively. PB also caused an increase in liver microsomal CYP2B17 and CYP2C43 activity (3.2- and 2.5-fold, respectively). Rifampin treatment caused an increase in liver CYP2B6 (3.6- and 8.2-fold), CYP2C18 (6.9- and 8.1-fold), CYP3A4 (6.7- and 2.5-fold), and CYP3A5 (6.7- and 2.5-fold) mRNA levels in males and females, respectively. Except for a small increase (3.5-fold in CYP2B6 and 2.7-fold in CYP3A4 mRNA levels), pyrazole did not cause an increase in the liver mRNA levels of any of the CYP enzymes examined. Results from this study indicate that BNF and OMP are in vivo inducers of CYP1A1 and CYP1A2 in monkeys, while PB and rifampin are inducers of CYP2B, CYP2C, and CYP3A enzymes.
**INTRODUCTION**

Evaluation of the potential of drug candidates to cause drug interactions is a requirement of the regulatory drug development process (Huang et al. 2008). This ability of a drug to inhibit, induce or be a substrate of drug-metabolizing enzymes may be predictive of its ability to affect disposition, efficacy or toxicity when co-administered with other drugs. Cynomolgus monkeys are commonly used to study metabolism of drugs during drug development. The objective of the present study was to determine the effects of known prototypical CYP inducers on the activity and gene expression of various CYP enzymes in monkey liver and intestine.

**MATERIALS & METHODS**

**Animal Treatment and Tissue Collection**

Male and female Cynomolgus monkeys (Macaca fascicularis) (2-4X Years old/sex) were treated orally (gastric intubation) once daily for four consecutive days with vehicle control (saline) or prototypical CYP inducers. The treatment groups included saline (vehicle control), β-Naphthoflavone (10 mg/kg/day for CYP1A), phenobarbital (25 mg/kg/day) for CYP2B, pyrazole (200 mg/kg/day) for CYP2C6, rifampin (25 mg/kg/day) for CYP3A and omeprazole (400 mg/kg/day) for CYP4A. Following the final day of treatment, animals were anesthetized by intramuscular ketamine and euthanized by intravenous barbiturate overdose. The livers were removed and snap frozen in liquid nitrogen. The upper half of the small intestine (duodenum and jejunum) was collected, placed in chilled saline solution and processed within two hours of collection.

**Microsomal CYP Enzyme Assays**

Microsomes were isolated from monkey livers by differential centrifugation based on methods described in Lu and Levin (1972). Microsomal protein concentrations were determined with a BCA Protein Assay Kit (Pierce Chemical Company, Rockford, IL) based on method described by Smith et al. (1965). Microsomal incubations were conducted in duplicate at 37 ± 1°C in 200 μl incubation mixtures containing potassium phosphate buffer (50 mM), MgCl2 (3 mM all but CYP2C5, 1 mM), NADPH-generating system (1 mM NADP, 5 mM glucose-6-phosphate, 1 Unit/mL glucose-6-phosphate dehydrogenase, and marker substrate at the final concentrations indicated). The testosterone incubations also contained 1 mM NADPH, 5 mM NADP, 0.5 mM diethyl-4-methyl-3-isoxazo-4-azon-1-benzoxazole (4-Me) (Sondén and Parkinson, 1988). Reactions were started by addition of the NADPH-generating system, and were stopped after 10 or 30 minutes by the addition of acetonitrile containing 0.1% trifluoroacetic acid. Samples were analyzed by HPLC/FLD (or HPLC/MS/MS or photometrically).

**CYP mRNA Analysis**

Messenger RNA (mRNA) levels in liver were determined by RT-PCR with an Applied Biosystems 7300 Real Time PCR sequence detection system. The relative quantity of the target mRNA compared with that of the control mRNA (β-actin) was determined by the ΔCt method (Applied Biosystems User Bulletin 4). Relative quantification measures the change in mRNA expression in a test sample relative to that in a control sample (i.e., DMSO). This method assumes that the efficiency of the target amplification and the efficiency of the endogenous control amplification are approximately equal. Calculations were as follows:

1. ΔCt = Ct (target) − Ct (endogenous control)
2. ΔΔCt = ΔCt (treated sample) − ΔCt (untreated control)
3. Fold change in expression = 2^(-ΔΔCt)

**Data Processing**

Data were processed and graphed with a validated, custom software program for Microsoft Excel (Office 2003). Individual rates of reaction from like treatment groups were averaged, and for those groups with n ≥ 3, standard deviations were calculated.

**RESULTS**

**Enzyme Activity and mRNA Expression**

The effects of treating monkeys with prototypical CYP inducers on liver and intestinal CYP activity are shown in Table 1 and Figures 1-5. The effects of treating monkeys with prototypical CYP inducers on intestinal CYP mRNA expression are shown in Table 2 and Figures 6-10.

**CONCLUSION**

The results of this study indicate that β-Naphthoflavone (BNF) and omeprazole (OMP) are in vivo inducers of CYP1A1 and CYP1A2 in monkeys, whereas phenobarbital (PB) and rifampin (RIF) are inducers of CYP3A, CYP2C and CYP2A enzymes. In both liver and intestine at the dosages tested, BNF was a more effective inducer of CYP1A than OMP, PB was the most effective inducer of CYP2C, whereas RIF was the most effective inducer of CYP3A.