Identification and characterization of primate P-glycoprotein

Sir,

Achieving the therapeutic goal of human immunodeficiency virus (HIV) infection is complex in nature due to the potential drug–drug interactions, modulations in the cytochrome P450 enzyme and/or drug transporters such as P-glycoprotein. Pharmacological activity of P-glycoprotein has been extensively studied and is under significant investigation in humans and laboratory animals. The sequence of the P-glycoprotein encoding gene, MDR1, is well characterized in humans; however, no complete sequence has been reported for the MDR1 gene in non-human primates particularly of the most versatile noble model such as *Macaca nemestrinas*. As most of the preclinical studies for therapeutic assessment of new chemical compounds are carried out in human closest species, non-human primates, it is therefore necessary to identify and characterize primate P-glycoprotein.

Rationale for the current study was that, P-glycoprotein and cytochrome P450 enzymes (especially of CYP3A) are prominent factors identified as being important regulators of oral drug absorption.[1,2] We hypothesized the limited oral absorption and variable tissue distribution of protease inhibitors (PIs) in primates is in part due to the presence of efflux membrane transporters particularly of P-glycoprotein.

To demonstrate the assumption, specimens from freshly frozen liver, brain, kidney, and intestine of *M. nemestrinas* ($n = 3$) were screened for the expression of P-glycoprotein. Tissues were provided by the Washington Regional Primate Research Center at the University of Washington. tRNA was extracted from 50 mg of each tissues by stratagen RNA extraction according to the manufacturers protocol and amplified by superscript II one step RT-PCR protocol from *Invitrogen*. Existing human MDR1 full-length sense (F6) and antisense (R11) primers were selected and subsequently, each tissue was tested as shown in the gel electrophoresis [Figure 1] where two different sizes, approximately 2kb and 3.8kb, fragments of P-glycoprotein were observed for brain and kidney. Reproducibility for the presence of the fragments was confirmed in additional run as in Figure 2. All the forward and reverse primers were acquired from *Invitrogen* (Life technologies, Grand Island, NY). These findings suggest that in addition to the full length P-glycoprotein, there could be a possibility of existence of a smaller size transporter at least in the brain and kidney of the nemestrinas. Substantiating these findings, the existence of another form of P-glycoprotein with shorter length (mini p-glycoprotein) in murine leukemia cells and human natural killer cells has been reported in some articles with similar function to that of the classic 3.8kb P-glycoprotein.[3,4]

Primate *MDR1*cDNA Sequence for *M. nemestrinas* was constructed for sequence homology analysis with the human MDR1. Both forward and reverse primers were designed as pF11- AGT GTC CAG GTC GGA GCA AAG CGC CAG TGA A and pR11- TTC ACT GGC GCT TTG CTC CAG CCT GGA CAC T, based on *M. fascicularies* MDR1 cDNA sequence and acquired from *Invitrogen*. 3.2 pmol primers and 150 ng purified PCR gel (Qiagen kit) [Figure 3] product were used for sequencing (ABI Prism, Model 3100, Version 3.7). After analyzing the electropherogram for its nucleotide signals and purity, sequence text files were blasted with the *M. fascicularies* MDR1 coding region sequence (accession #AF537134) and *M.nemestrinas* MDR1 sequence was constructed by aligning both the forward and reverse primers. A total of 3843 bases were found for all the macaques ($n = 3$). Sequence analyses of human MDR1 and *M. nemestrinas* pMDR1 coding region from liver cDNA were then conducted and there were found more than 99% sequence homology [Table 1] with four nucleotide alterations at position 540, 544/5, and 3829. Further amino acid blast analysis indicated the presence of changes in amino acids at 185 and 1277 positions. While the changes in nucleotide at positions 540, and 544/5 are the most frequently observed polymorphisms in the human MDR1gene,[5] the single nucleotide polymorphism (SNP) at position 3829 A → G could be the significant variant between the two species (Homo sapiens and *M. nemestrinas*) leading to an amino acid change at position1277 Thr → Ala which could in turn affect the fate of an experimental drug PK/PD.

In this regard, there have been increasing evidence that polymorphism of the ABCB1 (MDR1) gene contributes to inter-individual variability in bioavailability and tissue distribution of P-glycoprotein substrates. Significant data have been reported on the most widely studied SNPs in MDR1 such as C3435CT and its association with Lopinavir/Ritonavir monotherapy failure in HIV-1 patients,[6] G2677T polymorphism in susceptibility of myeloid leukemia,[7]

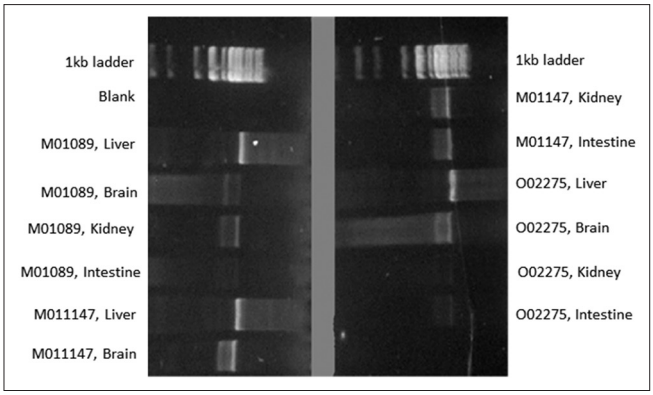

Figure 1: Tissue expression of P-glycoprotein in primates. Total RNA was extracted from 50mg of liver, brain, kidney and intestine using stratagen RNA extraction protocol and amplified by superscript II one step RT-PCR protocol from *Invitrogen*

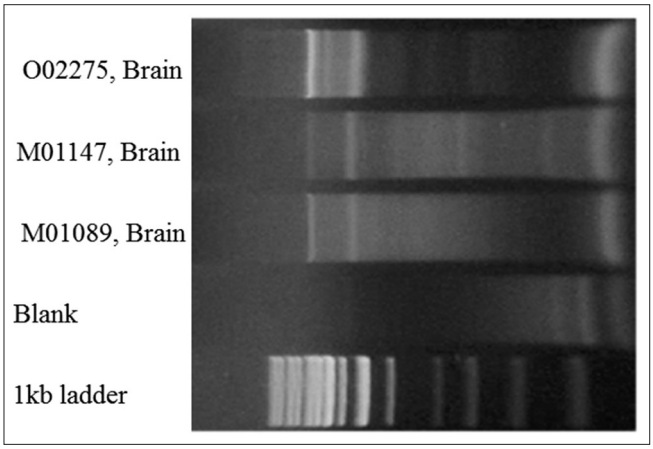

Figure 2: Expression of possible mini and full length P-glycoprotein in the brain of *M. nemestines*. Total RNA was extracted from 50mg brain tissue using stratagen RNA extraction protocol and amplified by superscript II one step RT-PCR procedure from *Invitrogen*

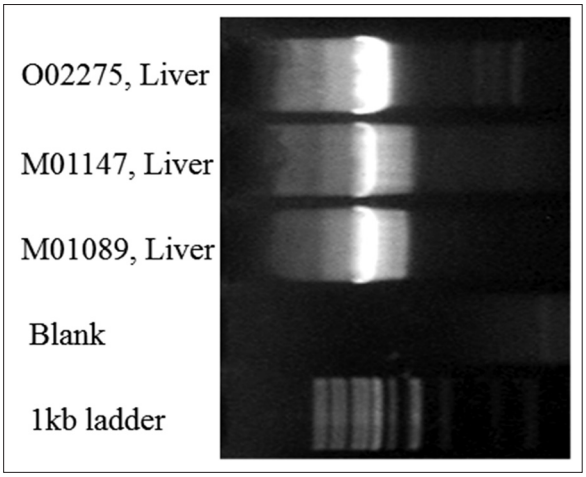

Figure 3: Primate MDR1 cDNA gel electrophoresis from Liver. cDNA extracted and purified by Qiagene kit was amplified by fail safe PCR

C1236T SNP in HIV-1 positive children causing significant reduction in Lopinavir plasma concentration affecting the virological response to highly active antiretroviral therapy[8] are some of the direct impacts of polymorphisms influencing the pharmacodynamics and pharmacokinetics outcome of a therapy.

Table 1: Multiple nucleotide alterations and amino acid changes of *M. nemestrinas* P-gp encoding gene isolated from liver comparing to human MDR1

Subject	MDR1 coding sequence	
	Nucleotide and position	Amino acid and position
H.sapiens	T at 540	Ser at 180
	TT at 554/5	Val at 185
	A at 3829	Thr at 1277
M. nemestrinas	C at 540	Ser at 180
	GA at 554/5	Gly at 185
	G at 3829	Ala at 1277

Therefore, the data depicted here suggest that the limited oral absorption and variable tissue distribution of PIs in primates could be in part by the presence of efflux membrane transporters particularly of P-glycoprotein. P-glycoprotein may limit penetration of PIs into several therapeutically relevant compartments and thus diminishing the chance of achieving a curative treatment regimen. As a result, identifying and characterizing the presence of P-glycoprotein, an ATP-dependent multidrug efflux membrane pump with extensive substrate specificity, could guide in designing a target specific therapeutic regimen in patients favoring a good pharmacological outcome specifically for those organs that provide potential HIV sanctuary sites in the body. However, further elucidation for sequence confirmation of the mini-P-glycoprotein in the brain and/or kidney and functional analysis of A3829G are a necessity.

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Conflicts of interest

There are no conflicts of interest.

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