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. 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.

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 MDR1 gene,[9] 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 position 1277 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]

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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.

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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]
C1236T SNP in HIV-1 positive children causing significant reduction in Lopinavir plasma concentration affecting the virological response to highly active antiretroviral therapy\(^{[5]}\) are some of the direct impacts of polymorphisms influencing the pharmacodynamics and pharmacokinetics outcome of a therapy.

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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Yimam M. Identification and characterization of tacrolimus MR in renal transplant recipients have been faced by the patients. Advagraf® (Astellas Pharma US, Inc.) is available as a once daily modified release (MR) tacrolimus. Comparable clinical outcome, trough concentration and financial constraints are reported.

Patients (case no. 1, 2, 3 and 4) were initiated on Advagraf® (Panacea Biotec Ltd., India). Two days prior to transplantation (dose between 0.192 and 0.202 mg/kg/day), along with prednisolone and mycophenolate mofetil. Patients (case no. 1, 3 and 4) were from the North east of India and case no. 2 was from the South of India. Whole blood tacrolimus concentration was measured by the LC-MS/MS.

Patients (case no. 1, 2, 3 and 4) were initiated on Advagraf® (Panacea Biotec Ltd., India). Two days prior to renal transplantation and subsequently converted to the twice daily, PanGraf® (Panacea Biotec Ltd., India). Therapeutic drug monitoring (TDM) of tacrolimus in four transplant recipients who were initiated on Advagraf®, once daily prior to renal transplantation and subsequently converted to the twice daily, PanGraf®.

In comparison to the conventional formulation (Advagraf) is subject to wet granulation and capsule filling to delay drug release of tacrolimus. Modified release formulation (Pangraf) of tacrolimus, the modified release tacrolimus MR (Advagraf®) with a release period of about 12 hours has been studied.

The cost of 1 mg tacrolimus is INR 100 for the innovator, Prograf® (Astellas Pharma US, Inc.) in comparison to INR 43 for PanGraf® (Panacea Biotec Ltd., India). The cost of 1 mg tacrolimus was INR 4.8 for generic tacrolimus (tacrolimus twice daily, generic) is widely prescribed.

Studies used Prograf® (Astellas Pharma US, Inc.) for the efficacy of Lopinavir/Ritonavir (LPV/r) monotherapy in HIV-1 patients. Potential implications of CYP3A4, CYP3A5 and MDR-1 genetic variants on the efficacy of Lopinavir/Ritonavir (LPV/r) monotherapy in HIV-1 patients. J Int AIDS Soc 2014;17(Suppl 3):19589.

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