Relationship between P-Glycoprotein Activity Measured in Peripheral Blood Mononuclear Cells and Indinavir Bioavailability in Healthy Volunteers

GUILLERMO F. BRAMUGLIA,[1] CATALINA M. CORTADA,[2] VERÓNICA CURRAS,[1] CHRISTIAN HÖCHT,[1] FABIÁN BUONTEMPO,[3] GABRIEL MATO,[3] VIVIANA NISELMAN,[4] MODESTO RUBIO,[1,5] MARTA CARBALLO[2]

[1]Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 5to piso (C1113AAD), Buenos Aires, Argentina

[2]Citogenética Humana y Genética Toxicológica (CIGETOX), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

[3]Área de Farmacia Hospital de Pediatría Juan P Garrahan, Buenos Aires, Argentina

[4]Cátedra de Matemática, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

[5]Instituto de Investigaciones Farmacológicas (ININFA-CONICET), Buenos Aires, Argentina

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ABSTRACT: Indinavir, a HIV-1 protease inhibitor, showed large inter-individual pharmacokinetic variability. It has been proposed as a substrate of P-glycoprotein (P-gp), an efflux transporter, that may contribute to limit indinavir bioavailability. A liquid formulation of indinavir was developed from indinavir capsules in order to study indinavir pharmacokinetics in healthy volunteers. Compartmental and noncompartmental analysis of indinavir plasma concentrations showed high inter-individual variability in terms of area under the curve (AUC) and maximal plasma concentration (C_{max}). A significant negative association between AUC normalized to body weight (AUC × weight) and lymphocyte P-gp activity, using Rh123 efflux assay, was observed ($p = 0.008$; $r = -0.75$). AUC normalized to elimination rate constant (AUC × beta) also showed a significant negative relationship with lymphocyte P-gp activity ($p = 0.03$, $r = -0.64$). Apparent clearance (CL/[F × weight]) and volume of distribution (VD/[F × weight]) showed a positive correlation with P-gp activity. Conversely, elimination rate constant did not correlate with P-gp activity. Although there is not enough evidence of a correlation between lymphocitary and intestinal function of P-gp, our results suggest a relationship between a P-gp phenotype marker, Rh123 efflux assay in lymphocytes, and indinavir bioavailability. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:327–336, 2009

Correspondence to: Guillermo F. Bramuglia (Telephone: +54-11-4964-8265; Fax: +54-11-4508-3645; E-mail: gbram@ffyb.uba.ar)

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INTRODUCTION

The small intestine represents the principal site of absorption for any ingested compound, whether dietary, therapeutic, or toxic. Oral administration is the most popular route for drug administration since dosing is convenient and noninvasive and most drugs are well absorbed by the gastrointestinal tract. As well as degrading and absorbing nutrients and solutes from the intestinal lumen, intestinal enterocytes form a selective barrier to drugs and xenobiotics. This barrier function largely depends upon specific membrane transport systems and intracellular metabolizing enzymes. The extent to which a compound is absorbed by the intestinal epithelium is therefore a critical factor in determining its overall bioavailability.

Among the different membrane transport systems, the ATP-binding cassette (ABC) family of transport proteins represents one of the largest families of proteins in living organisms.[1–3] P-glycoprotein (P-gp), that obtains the energy required for the vectorial transport of drug substrates across the membrane via the hydrolysis of ATP, is responsible for the efflux of several drug compounds across the cell membrane.[4] P-gp is widely expressed in different tissues, including small intestine, kidney tubules, adrenal glands, blood–brain barrier, muscle, lung, pancreas, placenta, testis, stomach, and liver.[5–7] P-gp expressed in the enterocyte cells effluxes its substrates across the apical membrane back into the intestine, decreasing intestinal drug absorption and increasing drug metabolism in the enterocyte cells.[8,9]

Several reports have shown the existence of large inter-individual differences in P-gp expression in tissues such as the small intestine[10] and liver.[11]

Considering the role of intestinal P-gp activity in drug bioavailability, large inter- individual variability in P-gp activity could affect the therapeutic outcome of pharmacological treatments with drugs with narrow therapeutic index.

Indinavir (IDV), a HIV-1 protease inhibitor and substrate of P-gp, showed a large inter-individual variability in plasmatic levels attained after oral administration in healthy volunteers and patients.[12] Previous works have shown clear evidence for interactions between P-gp activity and protease inhibitors. Thus, given the tissue distribution of P-gp, it might lower protease inhibitors bioavailability and could be responsible for the existence of sanctuary sites, such as the brain and testes, by limiting the levels of accumulation of protease inhibitors in these tissues.[13,14] Accordingly, it is reasonable to hypothesize that variability of this transporter could influence drug disposition, as well as antiretroviral treatment efficacy.

Different studies have attempted to measure the effect of P-gp-mediated efflux on the oral bioavailability of drugs.[15–17] Anyway, there are no standardized methodologies to asses P-gp expression or function[18] and its relationship with P-gp substrates bioavailability.

Various single nucleotide polymorphisms (SNPs), in the MDR1 gene, which codifies for P-gp, have been identified, including a silent mutation in exon 26 (C3435T) that have been correlated with duodenal expression of P-gp.[19]

Among the different methods used to evaluate P-gp activity, 99mTc-MIBI has been reported to be a substrate for P-gp and it is used for *in vivo* functional detection of P-gp in different tumors.[20] Furthermore, it has been suggested that the distribution of this tracer correlates with the active efflux mediated by P-gp in excretory organs.[21]

In addition, flow citometry detection of P-gp measuring dye or drug efflux in the presence or absence of a P-gp modulator is one of the recommended techniques for MDR1 phenotype measurement in peripheral blood mononuclear cells and has been largely used in hematological malignancies as a prognosis marker.[22]

Since there is not enough evidence of a correlation between lymphocitary and intestinal function of P-gp, the aim of this work was to study the relationship between the pharmacokinetic variability of IDV after a single oral dose in healthy volunteers and P-gp activity determined by Rhodamine 123 efflux assay in peripheral blood mononuclear cells.

MATERIALS AND METHODS

Sample Collection

Eleven healthy volunteers, enrolled previously in a collaborative P-gp activity clinical study that