An Integrated Pharmacokinetic Model for the Influence of CYP3A4 Expression on the *In Vivo* Disposition of Lopinavir and Its Modulation by Ritonavir

ROB TER HEINE,[1] ROBERT A. B. VAN WATERSCHOOT,[2] RON J. KEIZER,[1] JOS H. BEIJNEN,[1] ALFRED H. SCHINKEL,[2] ALWIN D. R. HUITEMA[1]

[1]Department of Pharmacy and Pharmacology, Slotervaart Hospital, Amsterdam, the Netherlands

[2]Division of Experimental Therapy, The Netherlands Cancer Institute, Amsterdam, the Netherlands

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ABSTRACT: Lopinavir, a human immunodeficiency virus protease inhibitor, has a very low oral bioavailability, which can be enhanced with a low dose of the CYPA4 inhibitor ritonavir. Our aim was to separately quantify the role of intestinal and hepatic cytochrome P450 3A (CYP3A4) expression on lopinavir disposition in a novel mouse model. Lopinavir and ritonavir were administered to mice selectively expressing human CYP3A4 in the intestine and/or liver. Using nonlinear mixed-effects modeling, we could separately quantify the effects of intestinal CYP3A4 expression, hepatic CYP3A4 expression, and the presence of ritonavir on both the absorption and elimination of lopinavir, which was previously not possible using noncompartmental methods. Intestinal, but not hepatic, CYP3A4-related first-pass metabolism was the major barrier for systemic entry of lopinavir. Relative oral bioavailability of lopinavir in mice expressing both hepatic and intestinal CYP3A4 was only 1.3% when compared with mice that were CYP3A deficient. In presence of ritonavir, relative bioavailability increased to 9.5% due to inhibiton of intestinal, but not due to inhibition of hepatic first-pass metabolism. Hepatic CYP3A4 related systemic clearance was inversely related to ritonavir exposure and not only hepatic but also intestinal CYP3A4 expression contributed to systemic clearance of lopinavir. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:2508–2515, 2011

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INTRODUCTION

Lopinavir is currently one of the most widely used antiretroviral drugs from the class of human immunodeficiency virus (HIV) protease inhibitors (PIs). Lopinavir itself has a very low and variable oral bioavailability due to extensive metabolism by cytochrome P450 3A (CYP3A4) enzymes located in both the intestine and the liver. To overcome this low and variable bioavailability as well as to obtain adequate viral suppressive concentrations, lopinavir is coadministered with a low dose of ritonavir. The PI ritonavir is a potent inhibitor of CYP3A4-mediated metabolism and thereby increases the exposure to lopinavir.[1–3] The inhibition of CYP3A4 by ritonavir has been investigated in both *in vivo* and *in vitro* but the exact mechanism of CYP3A4 inhibition by ritonavir remains unclear. It is thought that CYP3A4 inhibition by ritonavir is both time and concentration dependent.[1,4]

First-pass metabolism of lopinavir can occur in both the intestine and the liver, but the individual contribution of each organ on first-pass metabolism remains unknown. Once in the systemic circulation, lopinavir undergoes hepatic metabolism, and the drug and its metabolites are predominantly eliminated from the body via the hepatobiliary route and excreted in the feces.[2] Simultaneous inhibition of both intestinal and hepatic CYP3A4 by ritonavir is thought to play an important role in increasing the oral bioavailability of lopinavir, not only by decreasing the presystemic metabolism but also by inhibiting systemic clearance of ritonavir from the body. However, the relative contribution of intestinal and hepatic metabolism on

Correspondence to: Rob ter Heine (Telephone: +31-20-512-4481; Fax: +31-20-5124753; E-mail: rob@terheine.nl)

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lopinavir disposition and the influence of ritonavir have not been quantified hitherto. Insight into these mechanisms may contribute to development of better oral drug treatment regimens because higher and more reproducible bioavailability may lead to a reduced pill burden, better tolerability, and increased adherence.

Recently, Cyp3a knockout and transgenic CYP3A4 mouse models have been generated that provide *in vivo* models to investigate the separate contribution of intestinal and hepatic metabolism on the disposition of oral drugs.[5] We have investigated the influence of drug transporters and CYP3A4 expression on the *in vivo* lopinavir disposition in mice, and we proved that both intestinal and hepatic CYP3A4 expressions were important for lopinavir disposition and that ritonavir modulated both hepatic and intestinal lopinavir metabolism.[6] However, this analysis was performed using noncompartmental pharmacokinetic methods describing total exposure by calculating the area under the concentration–time curve (AUC). The separate influence of hepatic CYP3A4 expression, intestinal CYP3A4 expression, and the influence of ritonavir on the absorption and the clearance of lopinavir could not be estimated using noncompartmental methods. The aim of the present study was, therefore, to develop an integrated pharmacokinetic model to quantify these processes separately.

MATERIALS AND METHODS

Animals

Mice were housed and handled according to institutional guidelines complying with Dutch legislation. Animals used in this study were CYP3A4-knockout ($Cyp3a^{-/-}$), homozygous $Cyp3a^{-/-}$A (human CYP3A4 in the liver), and $Cyp3a^{-/-}$V (human CYP3A4 in the intestine). $Cyp3a^{-/-}$A and $Cyp3a^{-/-}$V mice were crossed to obtain homozygous $Cyp3a^{-/-}$AV mice (human CYP3A4 in both the liver and intestine) and genotypes were verified as described.[5] All mice used in this study were male, had a greater than 99% FVB (an inbred mouse strain preferable for transgenic analyses) genetic background, and were between 8 and 14 weeks of age. Animals were kept in a temperature-controlled environment with a 12-h light/12-h dark cycle and received a standard diet (AM-II; Hope Farms, Woerden, the Netherlands) and acidified water *ad libitum*. To minimize variation in absorption, mice were fasted for 2 h before lopinavir was administered, and approximately 1.5 h after administration, the mice were fed again.

Pharmacokinetic Sampling

Lopinavir with or without ritonavir (Sequoia Research Products Ltd., Pangbourne, UK) was formulated in ethanol/propylene glycol [19:81 (v/v)] in a concentration of 15 mg/mL (lopinavir) and 3.75 mg/mL (ritonavir), and drugs were administered by oral gavage into the stomach of the mice. Four animals of each strain were dosed lopinavir (100 mg/kg) and another four animals of each strain were dosed lopinavir with ritonavir (100 and 25 mg/kg). Multiple blood samples ($\sim$40 μL) were collected from the tail vein just before and at 0.5, 1, 2, 4, 8, and 24 h after administration of lopinavir or lopinavir/ritonavir using heparinized capillary tubes (Oxford Labware, St. Louis, Missouri). Blood samples were centrifuged at 2100 $\times$ *g* for 10 min at 4°C, and the plasma fraction was collected and stored at −20°C until analysis.

Bioanalysis

Lopinavir and ritonavir concentrations in plasma samples were determined using a previously described sensitive assay using liquid chromatography coupled to tandem mass spectrometry.[7] The accuracies and precisions of this assay for determination of lopinavir and ritonavir were within ±9% for both compounds.

Pharmacokinetic Analysis

Lopinavir pharmacokinetics was analyzed using nonlinear mixed-effect modeling with NONMEM VI 2.0 (ICON Development Solutions, Ellicott City, Maryland). Concentrations below the limit of quantification (signal-to-noise ratio < 7) were left out the analysis. The laplacian estimation method with interaction was used, and the covariance option was used to calculate the relative standard error of estimates. The minimal value of the objective function (OFV, equal to minus twice the log likelihood) was used as goodness of fit characteristic to discriminate between hierarchical models using the log likelihood-ratio test. A p value of 0.01 representing a decrease in OFV of 6.64 points was considered statistically significant. XPose and Perl-speaks-NONMEM were used for graphical and statistical model diagnostics.[8,9]

The pharmacokinetic model is schematically depicted in Figure 1. The interindividual error and residual error were described using an exponential error model. Data were log transformed, and a one-compartment model was used with first-order oral absorption and first-order elimination from the central compartment. To correct for differences in weight *a priori*, the oral clearance (Cl/*F*, where *F* represents the oral bioavailability) and volume of distribution (*V*/*F*) were allometrically scaled to a typical mouse weighing 25 g (see below). Ritonavir area under plasma concentration–time curves were calculated by noncompartmental methods without extrapolating to infinity.