The Impact of Pharmacologic and Genetic Knockout of P-Glycoprotein on Nelfinavir Levels in the Brain and Other Tissues in Mice

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ABSTRACT: Insufficient concentrations of protease inhibitors such as nelfinavir may reduce the effectiveness of HIV dementia treatment. The efflux transporter *mdr1* product P-glycoprotein (P-gp) has been demonstrated to play a role in limiting nelfinavir brain levels. The goal of this study was to compare the effect of GF120918 (10 mg/kg, IV), a P-gp inhibitor, on intravenous nelfinavir (10 mg/kg) *in vivo* disposition and tissue penetration in P-gp-competent *mdr1a/1b* (+/+) mice versus P-gp double knockout *mdr1a/1b* (−/−) mice. Intravenous administration with the P-gp inhibitor GF120918 to *mdr1a/1b* (+/+) mice increased nelfinavir concentrations over a range of 2.3- to 27-fold, whereas nelfinavir distribution in *mdr1a/1b* (−/−) mice was 2- to 16-fold higher than that in their wild counterparts. Nelfinavir levels after GF120918 coadministration were higher in the heart, liver, and kidneys than those detected with *mdr1a/1b* knockout mice. In contrast, *mdr1a/1b* knockout mice exhibited higher nelfinavir levels in the brain (16.1-fold vs. 8.9-fold increase) and spleen (4.1-fold vs. 2.3-fold increase) compared to pharmacological inhibition with GF120918 in wild mice. Most notably, GF120918 provided tissue-specific effects in *mdr1a/1b* knockout mice with enhanced ($p < 0.05$) drug accumulation in the brain (~21-fold) and heart (3.3-fold). Our results suggest *mdr1a/1b*-independant mechanisms may also contribute to nelfinavir tissue distribution in mice. J Pharm Sci 94:1216–1225, 2005

Keywords: nelfinavir; P-glycoprotein; tissue distribution; P-gp inhibitor; GF120918

INTRODUCTION

P-glycoprotein (P-gp) is a 170 kDa efflux transporter that plays a significant role in minimizing the absorption and tissue distribution of structurally diverse therapeutic agents. Efflux modulation with select transporter inhibitors may improve the oral absorption and distribution of drugs that are P-gp substrates into the central nervous system (CNS). In mice, two *mdr1* genes—*mdr1a* and *mdr1b*—are responsible for P-gp-mediated efflux. Together, they exhibit a function similar to human P-gp,[1] which is a single gene product of *MDR1*. The mouse *mdr1a* RNA is expressed predominantly in the intestine, liver, brain, and testis. Whereas *mdr1b* RNA is detected predominantly in adrenal, placenta, ovarium, and uterus, both *mdr1a* and *mdr1b* RNA are found in kidney. The mouse heart, lung, thymus, and spleen also contain similar and significant levels of both *mdr1a* and *mdr1b* RNA. In all major tissues, at least some *mdr1a* and *mdr1b* transcripts were detectable by RNase protection.[2–4]

Successful anti-HIV therapy is sometimes impeded by low or variable absorption and tissue distribution, which could lead to the development of drug resistant strains of the virus.[5] Over the past 10 years, therapies that attack HIV at many key points of viral replication have been developed to maximally reduce viral levels found in the

Abbreviations: CNS, central nervous system; GI tract, gastrointestinal tract.

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plasma. The key anti-HIV drugs that are routinely used as highly active antiretroviral therapy (HAART) include nucleoside and non-nucleoside analogs that are reverse transcriptase inhibitors and protease inhibitors.[5] Protease inhibitors (PIs) are high molecular weight, anti-HIV compounds.[5] Although protease inhibitors often exhibit a high degree of hydrophobicity (logP range = 2.9–5.2),[5] which favors membrane penetration, their overall absorption range is about 10-fold less than the expected values because transporter-mediated drug efflux and first pass metabolism attenuate the net absorption.[5] In addition, *in vitro* intestinal permeability of a series of similar peptide derivatives has been shown to be inversely proportional to the number of hydrogen bonding groups, a characteristic pertaining to PIs (one or more amide bonds and other hydrogen bonding groups).[5] These factors have a combined effect on the absorptive permeability and, hence, bioavailability of these drugs (e.g., bioavailability of saquinavir is only 3%–4%[6]). As HIV^+ subjects on HAART are expected to live longer than HIV^+ subjects not on HAART, controlling viral replication in the brain to prevent HIV-dementia, which depends on PI availability in the brain tissue, is a significant clinical problem.

Several studies have shown that improved brain, testis, or fetal distribution/accumulation of various PIs (saquinavir, amprenavir, and nelfinavir) could be achieved with coadministration of P-gp inhibitors.[7–9] Kim et al.[10] reported that brain levels were significantly increased for radiolabeled indinavir (10.6-fold), saquinavir (7.4-fold), and nelfinavir (36.3-fold) after intravenous administration in *mdr1a* (–/–) knockout mice compared to wild-type mice. In addition, clinical studies in HIV-infected patients showed that P-gp expressed on the surface of lymphocytes (a major site of HIV replication and antiretroviral drug action) may further contribute to disease progression by limiting the accumulation of PIs in lymphocytes.[11] As such, *in vivo* studies suggest that the enhanced P-gp activity indeed has a negative effect on the antiretroviral activity of PIs.

The potential role of P-gp in reducing the oral bioavailability and CNS penetration of many drugs[12] has triggered research in P-gp-deficient mice to evaluate the role of P-gp in the *in vivo* disposition and tissue penetration of PIs. Comparison studies on the effect of P-gp on drug distribution generally have adopted one of two approaches (1) comparing drug distribution in P-gp deficient (genetic knockout) animals versus normal wild-type animals,[10,13,14] or (2) comparing drug distribution in the presence and absence of a P-gp inhibitor (often referred to as pharmacological knockout). Although studies using these two approaches have produced voluminous information, the two approaches need to be compared and their relationship elucidated and verified so that the voluminous information can be integrated.

Studies with *mdr1a* (–/–) mice with digoxin, a P-gp probe substrate, had suggested that these mice behave pharmacologically similar to *mdr1a/1b* (–/–) mice.[1] However, digoxin brain levels were reported to increase 200-fold in *mdr1a* (–/–) mice ($t = 3$ days)[15] compared to a 1.4-fold increase in *mdr1b* (–/–) mice and a 27.2-fold increase in *mdr1a/1b* (–/–) mice.[1] Further evaluation of the *mdr1a* (–/–) mice uncovered the upregulation of *mdr1b* gene expression in several tissues, such as the kidney and liver,[16] but not in others such as the intestine.[16] On the basis of these studies, it is likely that *mdr1a/1b* (–/–) mice are a better model system to test the pharmacological roles of P-gp and to analyze the specificity and effectivity of P-gp inhibitors.[1]

Currently, no study has addressed the effect of pharmacological inhibition by a P-gp inhibitor versus genetic P-gp double knockout in the same species for the HIV protease inhibitors, particularly nelfinavir, the most commonly prescribed anti-HIV PI.[1] Therefore, the goal of this study was to investigate the effects of P-gp inhibition both by pharmacological inhibition (via GF120918) and complete genetic knockout [*mdr1a/1b* (–/–)] in mice and to elucidate the differences between the two approaches for nelfinavir.

MATERIALS AND METHODS

Materials

Ketamine HCl injection and Xylazine were supplied as Keta-Ject® and Xyla-Ject®, respectively, from Phoenix Pharmaceuticals (St. Joseph, MO). All surgical supplies were purchased from World Precision Instruments (Sarasota, FL). Experimental supplies were purchased from Fisher

[1]Upon completion of this study, Doran et al.[40] reported a study with ritonavir in *mdr1a/1b* (–/–) mice after subcutaneous administration as part of studying the impact of P-gp in *mdr1a/1b* (–/–) mice on the disposition of drugs targeted to the CNS. Their study revealed a differential effect of P-gp-mediated efflux in the CNS with most brain/plasma (B/P) AUC ratios ranging from 1.1 to 2.6-fold greater B/P ratios in the knockout mice versus the wild mice.