Assessing Safety and Efficacy of Directed P-Glycoprotein Inhibition to Improve the Pharmacokinetic Properties of Saquinavir Coadministered with Ritonavir

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ABSTRACT

Using a mouse model, we tested the effects of in vivo P-glycoprotein inhibition to enhance the oral uptake and penetration into pharmacological sanctuary sites of the human immunodeficiency virus protease inhibitor (HPI) saquinavir. The HPI ritonavir is frequently coadministered with saquinavir to improve saquinavir plasma levels since it strongly reduces the cytochrome P450 3A4-mediated metabolism of saquinavir. Previously, we demonstrated that ritonavir is not an efficient P-glycoprotein inhibitor in vivo, evidenced by the limited oral uptake of saquinavir and its penetration into brain and fetus. Increasing drug concentrations in these sites using more effective P-gp inhibitors might improve therapy but could also lead to toxicity. We orally coadministered ritonavir and saquinavir to mice, with or without the potent P-glycoprotein inhibitor N-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isouquinolinyl)ethyl]-phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918). Upon GF120918 coadministration, two of seven P-glycoprotein-deficient animals died. Using a decreased ritonavir dose, GF120918 coadministration led to a 4.4-fold increase in the saquinavir plasma area under the curve in wild-type mice, whereas no such effect was observed in P-glycoprotein-deficient mice. Despite the decreased ritonavir dose, all mice did suffer from impaired gastric emptying. Including GF120918 in a multiple (bidaily) dosing regimen, we found continued accumulation of saquinavir in brain over several days, resulting in 10-fold higher levels compared with vehicle-treated mice. Transient ritonavir-related neurotoxicity, however, was observed after the fourth and final drug dosing. Clinical attempts to efficiently inhibit P-glycoprotein function for improved HPI disposition may therefore be feasible, but they should be performed without ritonavir and monitored carefully for unexpected toxicities.

The suboptimal pharmacokinetic properties of saquinavir and other HIV protease inhibitors (HPIs) form an impediment to the optimal treatment of HIV/AIDS. The oral bioavailability of HPIs, and especially of saquinavir, is quite low, and consequently, patients must take these drugs frequently and in high doses (Perry and Noble, 1998). The relation between a frequent and high drug-dose intake and poor therapy compliance has been well described (Eisen et al., 1990). The interrupted intake of drugs can have dramatic consequences for HIV/AIDS patients since the accompanying decreased trough drug concentrations may facilitate the selection of drug-resistant viruses. Currently, two mechanisms have been described that limit the oral bioavailability of saquinavir (Williams and Sinko, 1999).

The most important cause for the low oral bioavailability of saquinavir is an extensive first-pass effect, which is mainly due to cytochrome P450 3A4 (CYP3A4)-mediated metabolism (Cameron et al., 1999; Huisman et al., 2001; Kaufmann et al., 1998; Koudriakova et al., 1998). Cyp3A4, strategically located in the enterocytes and hepatocytes (Guengerich, 1999), metabolizes saquinavir very efficiently (Fitzsimmons and Collins, 1997). It has been demonstrated that the HPI ritonavir is a potent inhibitor of CYP3A4 and other cytochrome P450 isoforms and that coadministration of ritonavir with saquinavir leads to highly elevated saquinavir plasma levels both in animals and humans (Eagling et al., 1997; Fitzsimmons and Collins, 1997; Cameron et al., 1999; Dresser et al., 2000).

Another factor limiting the oral uptake of saquinavir is P-glycoprotein (P-gp) function (Alsens et al., 1998; Kim et al., 2000).
Materials and Methods

Chemicals. [14C]Saquinavir (41.3 μCi mg⁻¹) and saquinavir were provided by Roche Discovery Welwyn (Welwyn Garden City, UK). Ritonavir was purchased from Abbott Laboratories, Inc. (Abbott Park, IL) as Norvir (80 mg ml⁻¹). GF120918 was generously provided by GlaxoSmithKline (Uxbridge, Middlesex, UK). Metofane (methoxyflurane) was from Medical Developments Australia, Pty. Ltd. (Melbourne, Australia). Deionized water was obtained using the Milli-Q Plus system (Millipore Corp., Bedford, Massachusetts). Bovine serum albumin was from Boehringer Mannheim GmbH (Mannheim, Germany).

Drug Distribution Studies. Mice used in all experiments were males, except for the mice that were used in the fetal distribution studies, and between 10 and 14 weeks of age. Animals were housed and handled according to institutional guidelines complying with Dutch legislation under a 12-h light/dark cycle at a temperature of 22°C. Wild-type, Mdr1a⁻/⁻/1b⁻/⁻, and Mdr1a⁻/⁻/1b⁻/⁺ mice were of a >99% FVB genetic background (Schinkel et al., 1994, 1997). The mice received a standard chow (AM-III; Hope Farms, Woerden, The Netherlands) and acidified water ad libitum. At t = −30 min, the mice received ritonavir and/or GF120918 (10 mg kg⁻¹), and at t = 0 min, the mice received 5 mg kg⁻¹[14C]saquinavir. All drugs were dosed orally in a volume of 2.5 μl/gm b.w.

Norvir was diluted with a control vehicle for Norvir containing 43% v/v ethanol. This vehicle resembles the matrix of liquid Norvir and contains Cremophor EL (105 mg ml⁻¹) (Sigma-Aldrich, St. Louis, Missouri), propylene glycol (0.25 ml ml⁻¹), peppermint oil (3.5 mg ml⁻¹), and water-free citric acid (2.8 mg ml⁻¹). Vehicle pH was 4.3. Animals received 1 to 2 μCi (37–74 kBq) of the radiolabeled drugs, and 40-μl blood samples were taken from the tail vein at the appropriate time points, or mice were sacrificed by orbital bleeding or cardiac puncture under Metofane anesthesia, followed by cervical dislocation.

Tissues were collected and processed as described previously by Smit et al. (1999). In short, scintillation counting was applied to Ultima Gold scintillation cocktail (Packard Bioscience B.V., Groningen, The Netherlands) mixed with a fraction of in 4% bovine serum albumin (w/v) homogenized tissue. Tissue concentrations of [14C]saquinavir were thus assessed by total radioactivity measurements. Unchanged saquinavir and ritonavir were determined in plasma by HPLC according to Van Heeswijk et al. (1998). The within-day precision ranged from 1.8 to 6.7%, and between-day precision ranged from 0.7 to 7.6%. The lower limits of quantification for saquinavir and ritonavir were 25 and 50 ng/ml, respectively. Unchanged fetal saquinavir concentrations were too low to be determined by HPLC and were for that reason determined as the radioactive drug equivalent per weight. Genotype analysis was done by polymerase chain reaction, according to Smit et al. (1999).

Statistical Analysis. Statistical analysis was performed using the Student’s t test (unpaired and two-tailed). The modified Bonferroni procedure was applied to the data presented in Fig. 2. The regression coefficient R² was calculated using Pearson’s standard linear regression. Differences between two sets of data were considered statistically significant if P < 0.05. Unless indicated otherwise, errors are represented as the standard deviation.

Results

Increased Ritonavir Toxicity Caused by Coadministration of GF120918. We first set out to determine whether 100 mg kg⁻¹ of the potent P-gp inhibitor GF120918 was able to efficiently inhibit P-gp function at various tissue barriers. GF120918 was coadministered with ritonavir (50 mg kg⁻¹) at t = −30 min and [14C]saquinavir (5 mg kg⁻¹) at t = 0 min to wild-type and P-gp-deficient mice. This high dose of ritonavir, close to the maximum tolerated dose, was used to inhibit saquinavir metabolism to the highest possible extent. The HPI doses were equal to those used in our previous study (Huisman et al., 2001) in which we demonstrated that at this dose of ritonavir P-glycoprotein function was not abrogated. With the P-gp inhibitor GF120918 included, however, two of seven P-gp-deficient mice died within 1 h after drug administration showing signs of ataxia and tremor. The wild-type mice showed no signs of toxicity. Since we observed similar toxicity signs previously when determining the maximum tolerated dose of ritonavir in mice (Huisman et al., 2001), the toxicity was most likely due to ritonavir overexposure. When the ritonavir dose was lowered to 25 mg kg⁻¹, toxicity effects were not observed in any of the animals.

The Effect of GF120918 on the [14C]Saquinavir Plasma Concentration. We first determined whether 100 mg kg⁻¹ of the potent P-gp inhibitor GF120918 could in-
crease the [14C]saquinavir plasma concentration after oral saquinavir administration. Wild-type and P-gp knockout mice received oral ritonavir (12.5 mg kg\(^{-1}\)) and [14C]saquinavir (5 mg kg\(^{-1}\)) with either GF120918 (100 mg kg\(^{-1}\)) or vehicle (Fig. 1). Subsequently, we determined the [14C]saquinavir plasma concentration every hour up to 11 h. Note that we demonstrated previously that even a ritonavir dose of 50 mg kg\(^{-1}\) does not abrogate P-glycoprotein function (Huisman et al., 2001).

As can be seen in Fig. 1a, the [14C]saquinavir plasma concentration and AUC were considerably increased (4.4-fold) in GF120918-treated wild-type animals compared with the vehicle-treated wild-types (\(P < 0.0001\)). Next, we determined the effect of GF120918 on the [14C]saquinavir plasma concentration in P-gp knockout animals under otherwise identical conditions, although one would of course not expect to find an effect (Fig. 1b). The [14C]saquinavir plasma AUCs for these groups of animals were identical (\(P = 0.67\)), indicating that the effect of GF120918 in wild-type mice was primarily mediated by P-gp inhibition.

The overall [14C]saquinavir plasma concentration seemed to be slightly higher in GF120918-treated wild-type animals than in GF120918-treated knockouts, but the AUCs were not significantly different (\(P > 0.1\)), indicating that GF120918 inhibited P-gp efficiently. Noteworthy, GF120918-treated knockout mice showed considerable fluctuation in the [14C]saquinavir plasma concentration in time (Fig. 1b). Based on the data for individual mice, we were under the impression that, in this specific group of five animals, emptying of the stomach was somewhat delayed in a few mice (data not shown).

In this experiment, impaired gastric emptying might be due to several causes, but both saquinavir (Washington et al., 2000) and GF120918 (Polli et al., 1999) have been given at very high dosages to rodents without obvious adverse side effects. We hypothesized therefore that it was most likely that ritonavir was responsible for this toxic effect and that GF120918 possibly exacerbated this.

To test whether ritonavir can indeed cause delayed gastric emptying and to determine a drug-dose combination that does not cause this possible toxic effect, wild-type and P-gp-deficient mice received various dosages of ritonavir, ranging...
from 0 to 50 mg kg\(^{-1}\), with or without 100 mg kg\(^{-1}\) GF120918, followed 30 min later by oral [\(^{14}\)C]saquinavir (5 mg kg\(^{-1}\)). The mice were sacrificed 8 h later, after which the percentage of the initially applied [\(^{14}\)C]saquinavir dose remaining in the stomach was determined (Fig. 2).

Focusing on the wild-type data in Fig. 2, it is obvious that 25 mg kg\(^{-1}\) of ritonavir or 100 mg kg\(^{-1}\) GF120918 alone does not lead to markedly impaired gastric emptying. In wild-type mice treated with GF120918, however, increasing dosages of ritonavir up to 25 mg kg\(^{-1}\) caused increasingly delayed gastric emptying, demonstrating the role of ritonavir and the involvement of GF120918 in the toxicity (\(R^2 = 0.893\)). Similar ritonavir dose-dependent toxicity was observed in knockout mice without (\(P < 0.05\)) or with (\(R^2 = 0.889\)) GF120918 treatment (Fig. 2), although knockout mice without GF120918 treatment appeared to be somewhat less sensitive than wild-type or knockout mice treated with GF120918.

**Accumulation of [\(^{14}\)C]Saquinavir in Pharmacological Sanctuary Sites upon Multiple Drug Treatment.** Since HIV/AIDS patients on highly active antiretroviral therapy take drugs life-long and at least once daily, we wanted to determine whether GF120918 could be included in a long term ritonavir-saquinavir coadministration regimen. The aim was to achieve improved HPI penetration in the pharmacological sanctuary sites brain, testis, and glandula vesicularis but without causing unacceptable toxicity due to possible long-term accumulation of drugs in the CNS or other organs.

To test this, wild-type mice were treated every 12 h with 5 mg kg\(^{-1}\) ritonavir, with or without 100 mg kg\(^{-1}\) GF120918, followed 30 min later by 5 mg kg\(^{-1}\) [\(^{14}\)C]saquinavir. The ritonavir dose was decreased to 5 mg kg\(^{-1}\) to minimize effects on the gastric release of drugs, although this dose may be too low to inhibit metabolism of [\(^{14}\)C]saquinavir fully. One group of animals was sacrificed 12 h after the first [\(^{14}\)C]saquinavir administration, and the remaining group received the drug combination four times (at 0, 12, 24, and 36 h) and was sacrificed 12 h after the final drug administration. Animals in the latter group were thus exposed to the drugs for 48 h. All animals were visually checked for toxicity signs for at least 1.5 h after each drug dosing.

Following the final drug administration at \(t = 36\) h, all GF120918-treated mice showed transient signs of passivity and unresponsiveness, reminiscent of a mild form of previously observed ritonavir toxicity (data not shown). The animals fully recovered within 90 min. There were no pronounced abnormalities observed in the percentages of the dose in any part of the gastrointestinal tract, indicating that the ritonavir dose was below the level at which it causes considerable delayed gastric emptying (data not shown). The applied ritonavir dose was not sufficient to inhibit [\(^{14}\)C]saquinavir metabolism fully since the plasma levels of [\(^{14}\)C]saquinavir, saquinavir, and ritonavir were below the detection limit at the 12 and 48 h time points.

Since in our experience the relative drug accumulation in the spleen is not significantly affected by P-gp function, we used the [\(^{14}\)C]saquinavir spleen concentration as a reference reflecting possible differences in plasma exposure between the treatment groups (Fig. 3).

The [\(^{14}\)C]saquinavir brain concentration was almost 4-fold higher in the GF120918-treated group than in the vehicle-treated group after 12 h. At \(t = 48\) h, there was a 10-fold difference in brain penetration, whereas brain concentration in the vehicle-treated group was not significantly different.

**Fig. 2.** Effect of ritonavir and GF120918 on the percentage of the [\(^{14}\)C]saquinavir dose remaining in the stomach. Wild-type and P-gp-deficient (knockout) mice were analyzed 8 h after oral administration of 5 mg kg\(^{-1}\) [\(^{14}\)C]saquinavir and pretreatment with or without oral GF120918 (100 mg kg\(^{-1}\)) and different doses of oral ritonavir (0 to 50 mg kg\(^{-1}\)) as indicated. Data are expressed as average ± S.D. (n = 3–6). *, \(P < 0.05\); **, \(P < 0.01\); ***, \(P < 0.001\).
from that at 12 h. Testis concentrations were 1.5- and 3.9-fold increased due to GF120918 at t = 12 and 48 h, respectively. In contrast, reference spleen concentrations were only 1.7- and 2.6-fold increased due to GF120918 treatment at 12 and 48 h. Effects of GF120918 treatment on glandula vesicularis [14C]saquinavir levels were similar to those for spleen. Of note, the absolute brain and testis levels of [14C]saquinavir had clearly increased between 12 and 48 h in the GF120918-treated mice but not in vehicle-treated mice. In contrast, continued [14C]saquinavir accumulation was not observed for spleen or glandula vesicularis either with or without GF120918.

**Increased [14C]Saquinavir Fetal Penetration upon GF120918 Pretreatment.** We previously demonstrated that despite high ritonavir plasma concentrations in mice P-gp function in the materno-fetal barrier still limits penetration of orally administered [14C]saquinavir into fetuses, resulting in an 18-fold higher [14C]saquinavir penetration in P-gp-deficient fetuses compared with wild-type fetuses (Huisman et al., 2001). We now investigated whether in a similar setup P-gp function in the materno-fetal barrier could be blocked efficiently by oral coadministration of GF120918.

Since P-gp is expressed in trophoblasts, which are of fetal origin, the genotype of the fetus determines the expression of placental P-gp. Heterozygous dams (Mdr1a+/−/Ib+/−) were mated to heterozygous males (Mdr1a+/−/Ib+/−), resulting in a fetal offspring of all three genotypes (Mdr1a+/−/Ib+/−, Mdr1a+/−/Ib−/−, and Mdr1a−/−/Ib−/−). Intrinsic to this setup, all fetuses in one dam are exposed to the same maternal plasma concentrations of drugs. Pregnant dams at gestation day 15 received 100 mg kg⁻¹ GF120918 and 25 mg kg⁻¹ ritonavir orally at t = −30 min, followed by 5 mg kg⁻¹ oral [14C]saquinavir at t = 0 min. The maternal plasma and fetal [14C]saquinavir concentrations were determined 4 h after the [14C]saquinavir administration. The maternal [14C]saquinavir plasma concentrations at t = 4 h varied widely [i.e., 0.11−3.37 μg ml⁻¹ (Table 1)]. The same was true for unchanged saquinavir and ritonavir concentrations (data not shown). In retrospect, this effect was presumably caused by various degrees of impaired gastric emptying due to ritonavir (see above). Fortuitously, however, this wide variation in maternal plasma levels allowed us to assess the efficacy of placental P-gp inhibition at a wide range of plasma exposure levels, as fetuses of all P-gp genotypes are usually represented within each mother and can thus be directly compared with each other. Table 1 shows the [14C]saquinavir data collected for all individual dams and for the corresponding fetuses of each genotype. It is clear that relative to wild-type fetuses on average only 1.08- and 1.25-fold more [14C]saquinavir accumulated in heterozygous and knockout fetuses.

### Table 1

<table>
<thead>
<tr>
<th>[14C]Saquinavir Concentration</th>
<th>Maternal Plasma</th>
<th>P-gp (+/+)/ WT Fetus</th>
<th>P-gp (+/−)/HET Fetus</th>
<th>P-gp (−/−)/KO Fetus</th>
<th>Ratio HET/WT Fetus</th>
<th>Ratio KO/WT Fetus</th>
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<tr>
<td>μg ml⁻¹</td>
<td>ng g⁻¹</td>
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<tr>
<td>0.11</td>
<td>154 ± 16 (2)</td>
<td>178 ± 16 (6)</td>
<td>171 ± 15 (3)</td>
<td>1.16</td>
<td>1.11</td>
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<tr>
<td>0.16</td>
<td>14 ± 3 (2)</td>
<td>14 ± 2 (5)</td>
<td>18 ± 1 (3)</td>
<td>1.03</td>
<td>1.32</td>
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<tr>
<td>0.72</td>
<td>43 ± 8 (4)</td>
<td>54 ± 4 (4)</td>
<td>84 (1)</td>
<td>1.25</td>
<td>1.96</td>
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<tr>
<td>1.25</td>
<td>440 ± 65 (2)</td>
<td>460 ± 25 (3)</td>
<td>470 ± 67 (2)</td>
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<tr>
<td>1.48</td>
<td>267 ± 7 (2)</td>
<td>278 ± 13 (3)</td>
<td>323 ± 16 (4)</td>
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<tr>
<td>1.75</td>
<td>464 ± 4 (4)</td>
<td>504 ± 15 (5)</td>
<td>552 ± 49 (3)</td>
<td>1.08</td>
<td>1.19</td>
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<td>2.05</td>
<td>727 ± 53 (2)</td>
<td>692 ± 38 (3)</td>
<td>695 ± 81 (5)</td>
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<tr>
<td>3.13</td>
<td>1382 (1)</td>
<td>1494 ± 59 (4)</td>
<td>1904 ± 35 (4)</td>
<td>1.10</td>
<td>1.18</td>
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<tr>
<td>3.37</td>
<td>316 ± 29 (5)</td>
<td>331 ± 17 (6)</td>
<td>(0)</td>
<td>1.05</td>
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</table>

Average ratios 1.08 1.25

Fig. 3. Effect of P-gp inhibition on the distribution of saquinavir in a repeated administration regimen. Concentration of [14C]saquinavir in wild-type mice is expressed as nanograms per gram. Mice received 5 mg kg⁻¹ ritonavir, with or without GF120918 (100 mg kg⁻¹), followed 30 min later by [14C]saquinavir. One half of the animals were sacrificed after 12 h; the other half received the appropriate drug combination every 12 h for four times and was sacrificed 12 h after the final drug dosing. This latter group is referred to as “48 h”. Data are expressed as average ± S.D. (n = 5).
In our previous study, without GF120918 (Huisman et al., 2001), in which 50 mg kg⁻¹ ritonavir was used but under otherwise identical conditions, these ratios were 1.33 and 18.1 for heterozygous and knockout fetuses, respectively. Taken together, our data show that P-gp function at the maternal-fetal barrier in mice can be inhibited almost fully over a wide range of saquinavir plasma concentrations, following coadministration of oral GF120918, ritonavir, and saquinavir.

**Discussion**

Several pharmacological factors may limit the therapeutic efficacy of HPIs, including low oral bioavailability and poor penetration into pharmacological sanctuary sites. In this study, we have tested in mice the safety and efficacy of a regimen to optimize the pharmacokinetic behavior of saquinavir, using ritonavir as an effective inhibitor of metabolism and GF120918 as an effective P-gp inhibitor. All drugs were administered orally to emulate the clinical setup as much as possible.

A number of relevant conclusions can be drawn. By extensively inhibiting P-gp function in clinically important barriers, we were able to increase the saquinavir plasma AUC by 4.4-fold and the relative penetration into fetus as well. This regimen, however, also revealed a number of ritonavir dose-related adverse events resulting from the absence or pharmacological inhibition of P-gp activity. In extreme cases a lethal, probably CNS-related toxicity of ritonavir, necessitating a reduction in dose. And, even at a reduced dose, ritonavir can cause a marked delay in gastric emptying.

From our data, it appears that when ritonavir is present GF120918 also contributes to some toxicity events, independent of its inhibition of P-gp. It is unlikely that the lethal toxicity we observed was due to GF120918 or saquinavir by itself. GF120918 has been orally administered in high doses to both mice (Polli et al., 1999; Bardelmeijer et al., 2000) and patients (Malingré et al., 2001) without obvious toxicity, and saquinavir has also been given at very high oral dosages to P-gp-deficient mice (500 mg kg⁻¹; Washington et al., 2000) without adverse toxicity events. Furthermore, it is unlikely that GF120918 inhibits the CYP3A-mediated metabolism of ritonavir or saquinavir, as it is not a significant CYP3A4 inhibitor (Cummins et al., 2002). The exact mechanism behind the GF120918-related toxicity therefore remains to be investigated, but we note that the effects are limited compared with those caused by ritonavir.

We have further demonstrated that, in a multiple dosing regimen with a greatly reduced dose of ritonavir, GF120918 treatment resulted in a progressive accumulation of saquinavir in brain and testis, but again toxicity was encountered that was most likely caused by ritonavir. Previously, several groups including our own have demonstrated improved brain, testis, or fetal penetration of various HPIs (saquinavir, amprenavir, and nelfinavir) by coadministration of effective P-gp inhibitors (Polli et al., 1999; Smit et al., 1999; Choo et al., 2000). None of these studies, however, employed a clinically realistic administration schedule, involving (repeated) oral administration of all relevant drugs, nor did they reveal the toxicity risks that we encountered.

We note that the importance of active transporters in HPI therapy could well extend beyond oral bioavailability and tissue penetration, as illustrated for instance by Meaden et al. (2002) who provided data suggesting that active transporters may play a role in limiting accumulation of ritonavir and saquinavir in lymphocytes of HIV-infected patients. Inhibition of transport proteins may therefore improve this aspect of HPI-based therapy as well.

To our knowledge, we are the first to describe a severely delayed gastric emptying caused by ritonavir. It is well known that in humans ritonavir frequently causes severe nausea and vomiting, which can be a dose-limiting toxicity (Gatti et al., 1999). Perhaps the same pharmacodynamic toxic effect of ritonavir plays a role in the stomach of mice and humans, but as mice are physically incapable of vomiting, the toxicity reveals itself as a greatly delayed gastric emptying. As this toxicity was only observed in P-gp-deficient or GF120918-treated wild-type animals, it is tempting to speculate that it resulted from increased penetration of ritonavir in the CNS or some other sanctuary site. In our experiments and also in HIV/AIDS patients treated with highly active antiretroviral therapy, ritonavir is primarily coadministered to inhibit rapid metabolism of other HPIs like saquinavir. Obviously, from our data, we would advise to avoid ritonavir from any clinical regimen including highly effective P-gp inhibitors. For rapidly metabolized HPIs such as saquinavir, this would mean that alternative CYP3A inhibitors should be included that do not cause severe toxicity in the absence of P-gp activity. Perhaps, ketoconazole would be a good candidate (Jordan, 1998). Alternatively, one could consider the use of compounds that are both effective P-gp and CYP3A inhibitors (Wandel et al., 1999), but their in vivo toxicity profiles should first be carefully investigated.

In conclusion, our data show that pharmacological P-gp inhibition with the aim of enhancing the oral uptake and penetration of the HPI saquinavir into pharmacological sanctuary sites is feasible. This could mean that viral replication in sanctuary sites is more efficiently suppressed. The observed toxicities, most likely caused by ritonavir, however, demonstrate that great caution must be exercised when trying to inhibit P-gp in a clinical setting. Even when omitting ritonavir from such HPI treatment regimens, one should always be aware that other (co-)administered drugs may reveal unexpected side effects due to the effective inhibition of P-gp.

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