Intracellular and Plasma Pharmacokinetics of Saquinavir-Ritonavir, Administered at 1,600/100 Milligrams Once Daily in Human Immunodeficiency Virus-Infected Patients

Jennifer Ford,¹* Marta Boffito,² Adrian Wildfire,² Andrew Hill,³ David Back,¹ Saye Khoo,¹ Mark Nelson,² Graeme Moyle,² Brian Gazzard,² and Anton Pozniak²

Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool L69 3GF,¹ St. Stephen’s Centre, Chelsea and Westminster Hospital, London SW10 9NH,² and Roche Products Ltd., Welwyn Garden City, Hertfordshire AL7 3AY,³ United Kingdom

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Ritonavir-boosted saquinavir (SQV/r) is currently licensed as a twice-daily regimen. Reducing the pill burden with once-daily dosing may improve adherence. Intracellular concentrations of drugs must be related to the clinical efficacy of protease inhibitors. The aims of the study were to determine the cellular and plasma pharmacokinetics of saquinavir and ritonavir concentrations, to determine the half-lives ($t_{1/2}$) of the drugs in each compartment, and to examine relationships between drug accumulation and lymphocyte subset P glycoprotein (P-gp) expression. Venous blood samples from 12 human immunodeficiency virus-infected patients receiving a hard-gel formulation of SQV/r (1,600/100 mg once daily) were collected at 2, 6, 12, and 24 h after dosing. Peripheral blood mononuclear cells were separated by density gradient centrifugation, and P-gp expression was measured by dual-color flow cytometry. Plasma and intracellular (cell-associated) drug concentrations were measured by high-performance liquid chromatography–tandem mass spectrometry. The ratio of the intracellular drug area under the concentration-time curve from 0 to 24 h (AUC0–24 h) to plasma drug AUC0–24 h was calculated to determine cellular drug accumulation. The median (range) AUC0–24 h of saquinavir in plasma was 16.2 (5.7 to 39.3) mg·h·liter⁻¹, and that in cells was 46.3 (24.7 to 114.6) mg·h·liter⁻¹. Corresponding ritonavir values were 7.5 (1.5 to 4.6) mg·h·liter⁻¹ and 10.4 (3.2 to 13.7) mg·h·liter⁻¹, respectively. The median accumulation ratios of cellular AUC to plasma AUC for saquinavir and ritonavir were 3.31 (range, 1.49 to 6.69) and 1.46 (range, 0.83 to 4.15), respectively. Significant differences between the plasma and intracellular saquinavir $t_{1/2}$ (4.5 h [range, 2.5 to 9.3 h] and 5.9 h [range, 4.0 to 17.7 h]; $P = 0.034$) and between the plasma and intracellular ritonavir $t_{1/2}$ (4.1 h [range, 2.6 to 8.3 h] and 6.2 h [range, 3.9 to 18.6 h]; $P = 0.032$) were observed. No relationship was observed between the accumulation of saquinavir or ritonavir and lymphocyte subset P-gp expression. The intracellular $t_{1/2}$ of saquinavir and ritonavir were longer than the plasma $t_{1/2}$, indicating that intracellular drug may be available at a time when concentrations in plasma are below the minimum effective concentration.

Saquinavir boosted with ritonavir (SQV/r) is currently licensed in Europe as a twice-daily regimen at a dosage of 1,000/100 mg in combination with other antiretroviral agents. Clinical efficacy has been observed with this dosage in trials as part of highly active antiretroviral therapy (HAART) (9). Pharmacoenhancement of protease inhibitors (PIs) with low-dose ritonavir due to the potent inhibition of CYP3A4 (18) has been extensively reported (1, 8). However, approximately 50% of human immunodeficiency virus (HIV)-infected patients receiving HAART experience therapeutic failure within 2 years (2, 26). Complex regimens associated with pill burden and a high dosage frequency make long-term adherence to therapy a challenge (10). Insufficient adherence to HAART may result in a suboptimal concentration, allowing for drug-resistant viral strains to evolve and contribute to therapeutic failure (24, 31). Since SQV/r has favorable pharmacokinetics for once-daily dosing, optimizing therapy with convenient and easy-to-follow regimens may increase adherence and improve long-term treatment success.

Although therapeutic drug monitoring has been suggested to have the potential to both reduce toxicity and optimize individual therapy (3), it should be noted that the major target of PIs is within cells infected with HIV, and therefore clinical outcome ultimately must be related to intracellular drug concentrations. Intracellular pharmacokinetics provides information regarding the access of drugs to a compartment where HIV replication occurs and, combined with plasma pharmacokinetics data, is useful in understanding therapeutic failure in relation to cellular resistance.

P glycoprotein (P-gp) encoded by the MDR-1 (ABCB1) gene functions as a protective barrier to potential toxic agents, lowering the intracellular concentration of a broad range of chemically unrelated substrates (11), a phenomenon known as multidrug resistance (5). PIs are substrates for P-gp (22, 27), and therefore P-gp-expressing cells, such as CD4⁺ lymphocytes, may accumulate less intracellular drug than cells that do not express P-gp (7). The affinity of transporters for PIs and the expression of P-gp and other transport proteins on lymphocytes, the main sites of viral replication, may hinder antiretroviral efficacy.

Previous investigations have determined the plasma pharmacokinetics of both soft- and hard-gel formulations of SQV/r...
administered once and twice daily (25; E. P. Acosta, M. S. Saag, and J. S. G. Montaner, 2nd Int. Workshop Clin. Pharmacol. HIV Ther., abstr. 3.14, 2001), and some have described intracellular SQV/r pharmacokinetics following twice-daily regimens (28, 21) or a saquinavir soft-gel formulation (G. Peytavin, R. Landman, C. Lamotte, F. Mentre, J. Gerbe, E. Dohlin, F. Boue, G. Spiridon, M. A. Valantin, C. Michelet, E. Bouvet, and P. Yeni, 2nd Int. Workshop Clin. Pharmacol. HIV Ther., abstr. 3.16, 2001). However, the intracellular pharmacokinetics of hard-gel SQV/r administered once daily is currently unknown. In this study, we determined cellular and plasma saquinavir and ritonavir concentrations over the dosage interval and calculated key pharmacokinetic parameters. In addition, we examined the relationship between P-gp expression on lymphocyte subsets and intracellular drug accumulation of saquinavir and ritonavir.

MATERIALS AND METHODS

Materials. Lymphoprep was purchased from Nycomed Pharma AS (Oslo, Norway). CellFIX was purchased from Becton Dickinson (Oxford, United Kingdom). The negative control mouse immunoglobulin G2a (IgG2a)-recombinant phycoerythrin (rPE), mouse anti-human CD4-fluorescein isothiocyanate (FITC), CD8-FITC, and mouse anti-human CD56-FITC were purchased from Serotec Ltd. (Oxford, United Kingdom). The anti-human P-gp monomonal antibody UIC2-rPE was obtained from Immunotech (Marseille, France). Phosphate-buffered saline tablets were purchased from Gibco Life Technologies, Ltd. (Paisley, United Kingdom). Ammonium formate, acetonitrile, and methanol were purchased from Fisher Scientific (Loughborough, United Kingdom). A Hypurity c到期 (range, 22 to 57 years), a median CD4 cell count at screening of 336 cells/mm³ (range, 118 to 947/mm³), and viral loads of 10^5 copies/ml (with the exception of 1 patient with a detectable viral load of 61 copies/ml) were enrolled in the study. Volunteers provided written informed consent prior to participation in the study, and ethics committee approval was obtained. Study participants had received a twice-daily SQV/r hard-gel formulation regimen (Invirease) with saquinavir and ritonavir, respectively. Inter- and intra-assay variabilities were 9% and 6% for saquinavir and 9% and 8% for ritonavir, respectively.

The HPLC-MS/MS data were recorded and quantified by Xcalibur software (version 1.0.1) that was programmed to recognize specific peaks and to quantify the intensity of the ion signal on an LCO Duo Thermoscan Finnigan MS. The saquinavir and ritonavir contents in total plasma (i.e., bound and unbound) and intracellular samples were determined by interpretation of data from the standard curve using peak area-to-internal standard ratios.

The concentration of saquinavir and ritonavir was determined by interpretation of data from the standard curve using peak area-to-internal standard ratios.
RESULTS

Plasma and intracellular saquinavir and ritonavir concentrations administered once daily. The intracellular pharmacokinetic profiles of saquinavir and ritonavir (Fig. 1) showed maximum concentrations ($C_{\text{max}}$) with median values of 3.86 and 0.68 mg·liter$^{-1}$, respectively. The corresponding $C_{\text{max}}$ of saquinavir and ritonavir in plasma were 1.54 and 0.76 mg·liter$^{-1}$ (Table 1), respectively, within the range of previously published data (M. Bofilto, L. Dickinson, A. Hill, C. Higgs, C. Fletcher, C. Johnson, S. Mandalia, D. Back, B. Gazzard, and A. Pozniak, 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-1612, 2003). The total plasma and intracellular exposures (AUC$_{0-24\text{h}}$) to saquinavir demonstrated a significant relationship ($r^2 = 0.63; P = 0.002$; 95% confidence interval [CI] of the $r$ value, 0.398 to 0.939) (Fig. 2a). The intracellular saquinavir AUC$_{0-24\text{h}}$ was higher than the plasma saquinavir AUC$_{0-24\text{h}}$, resulting in a median intracellular drug accumulation ratio of 3.31 (range, 1.49 to 6.69). Total plasma and intracellular exposures to ritonavir showed a trend towards a relationship with borderline significance ($r^2 = 0.33; P = 0.053$; 95% CI of the $r$ value, −0.005 to 0.862) (Fig. 2b). The intracellular ritonavir AUC$_{0-24\text{h}}$ was greater than the plasma ritonavir AUC$_{0-24\text{h}}$ with a median intracellular drug accumulation ratio of 1.46 (range, 0.83 to 4.15). The accumulation of saquinavir and ritonavir expressed as a ratio (cellular AUC$_{0-24\text{h}}$ to plasma AUC$_{0-24\text{h}}$) showed a direct relationship ($r^2 = 0.65; P = 0.0016$; 95% CI of the $r$ value, 0.428 to 0.943) (Fig. 2c). Although one of the data points gave rise to a skewed data set (Fig. 2a and c), when the outlier was removed, a significant relationship was observed, with an $r$ value of 0.45 and a $P$ value of 0.025 and an $r^2$ value of 0.33 and a $P$ value of 0.047 for the data shown in Fig. 2a and c, respectively. Saquinavir accumulation was significantly higher than ritonavir accumulation ($P = 0.034$), in accordance with the results of previous studies (S. H. Khoo, M. Hennessy, F. Mulcahy, S. Clarke, D. J. Back, P. G. Hoggard, J. F. Tjia, E. G. Wilkins, P. Carey, I. Williams, B. Peters, and M. G. Barry, 8th Conf. Retroviruses Opportunistic Infect., abstr. 258, 2001). The coefficients of variation of AUC$_{0-24\text{h}}$ for saquinavir in plasma and intracellular compartments were 57.6 and 58.6%, respectively, and the coefficients of variation of AUC$_{0-24\text{h}}$ for ritonavir in plasma and intracellular compartments were 55.9 and 36.0%, respectively. The pharmacokinetic parameters of saquinavir and ritonavir in plasma and cells are displayed in Table 1.

The median (range) terminal $t_{1/2}$ of saquinavir in plasma and cells were 4.5 h (2.5 to 9.3 h) and 5.9 h (4.0 to 17.7 h), respectively, while those of ritonavir were 4.1 h (2.6 to 8.3 h) and 6.2 h (3.9 to 18.6 h) (Table 1). Saquinavir and ritonavir $t_{1/2}$ in plasma were similar to those found in previous reports investigating the same once-daily dosage (R. S. Autar, J. Ananworanich, W. Apaterarpong, J. Sankote, A. Hill, B. Hirschel, D. Cooper, J. Lange, P. Phanuphak, K. Ruxrungtham, and D.

![FIG. 1. Concentration of saquinavir (a) and ritonavir (b) within plasma and cellular (IC) compartments over the 24-h dosage interval, expressed as the mean and standard error of the mean on a logarithmic scale for 12 HIV-infected subjects.](image-url)

<table>
<thead>
<tr>
<th>Drug and compartment</th>
<th>$C_{\text{max}}$ (mg·liter$^{-1}$)</th>
<th>$C_{24\text{h}}$ (mg·liter$^{-1}$)</th>
<th>$t_{1/2}$ (h)</th>
<th>AUC$_{0-24\text{h}}$ (mg·h·liter$^{-1}$)</th>
<th>Coefficient of variance of AUC$_{0-24\text{h}}$ (%)</th>
<th>Accumulation ratio$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saquinavir</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Plasma</td>
<td>1.54 (0.26-4.95)</td>
<td>0.08 (0.03-0.50)</td>
<td>4.5 (2.5-9.3)</td>
<td>16.2 (5.7-39.3)</td>
<td>57.6</td>
<td>3.31 (1.49-6.69)</td>
</tr>
<tr>
<td>Intracellular</td>
<td>3.86 (1.09-15.3)</td>
<td>0.71 (0.20-1.28)</td>
<td>5.9 (4.0-17.7)</td>
<td>46.3 (24.7-114.6)</td>
<td>58.6</td>
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<tr>
<td><strong>Ritonavir</strong></td>
<td></td>
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<tr>
<td>Plasma</td>
<td>0.76 (0.16-1.93)</td>
<td>0.04 (0.01-0.08)</td>
<td>4.1 (2.6-8.3)</td>
<td>7.5 (1.5-14.6)</td>
<td>55.9</td>
<td>1.46 (0.83-4.15)</td>
</tr>
<tr>
<td>Intracellular</td>
<td>0.68 (0.23-1.73)</td>
<td>0.13 (0.04-0.44)</td>
<td>6.2 (3.9-18.6)</td>
<td>10.4 (3.2-13.7)</td>
<td>36.0</td>
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</tbody>
</table>

$^a$ $C_{\text{max}}$, concentrations at 24 h ($C_{24\text{h}}$), $t_{1/2}$, AUC$_{0-24\text{h}}$, and the accumulation ratios are expressed as medians (ranges).

$^b$ Accumulation is expressed as a ratio of the cellular AUC$_{0-24\text{h}}$ to plasma AUC$_{0-24\text{h}}$. Intracellular concentrations of saquinavir and ritonavir were calculated using the volume of a single PBMC (0.4 μl).
TABLE 2. Ratio of saquinavir and ritonavir concentration in cells to that in plasma over the dosing interval

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Concn ratio (in cells/in plasma) of:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Saquinavir</td>
</tr>
<tr>
<td>2</td>
<td>2.92 (1.43–19.9)</td>
</tr>
<tr>
<td>6</td>
<td>2.74 (1.05–5.92)</td>
</tr>
<tr>
<td>12</td>
<td>4.07 (1.50–22.8)</td>
</tr>
<tr>
<td>24</td>
<td>7.64 (1.55–19.2)</td>
</tr>
</tbody>
</table>

* Median values (ranges) for 12 subjects are shown.

so that the ratios of both saquinavir and ritonavir concentrations in cells to those in plasma increased over the dosing interval (Table 2).

**Relationship between cellular accumulation of saquinavir and ritonavir and P-gp expression on lymphocyte subsets.**

Saquinavir accumulation within cells from HIV-infected subjects demonstrated no relationship to cell surface expression of total lymphocyte P-gp (total cells, \( r^2 = 0.02; P = 0.643; 95\% \) CI of the \( r \) value, \(-0.666 \) to \( 0.464 \)) or cell subset P-gp (\( CD4^+ \) cells, \( r^2 = 0.009; P = 0.774, 95\% \) CI of the \( r \) value, \(-0.508 \) to \( 0.633 \); \( CD8^+ \) cells, \( r^2 = 0.044; P = 0.515; 95\% \) CI of the \( r \) value, \(-0.699 \) to \( 0.415 \)) (\( CD56^+ \) cells, \( r^2 = 0.111; P = 0.740; 95\% \) CI of the \( r \) value, \(-0.642 \) to \( 0.497 \)). Similarly, no relationship was observed between the intracellular accumulation of ritonavir and P-gp expression on total lymphocytes (total cells, \( r^2 = 0.0085; P = 0.943; 95\% \) CI of the \( r \) value, \(-0.589 \) to \( 0.558 \)) or cell subset P-gp (\( CD4^+ \) cells, \( r^2 = 0.0007; P = 0.935; 95\% \) CI of the \( r \) value, \(-0.591 \) to \( 0.556 \); \( CD8^+ \) cells, \( r^2 = 0.124; P = 0.262; 95\% \) CI of the \( r \) value, \(-0.770 \) to \( 0.278 \); \( CD56^+ \) cells, \( r^2 = 0.210; P = 0.134; 95\% \) CI of the \( r \) value, \(-0.817 \) to \( 0.157 \)).

**DISCUSSION**

Although previous investigations have determined the intracellular pharmacokinetics of saquinavir and ritonavir administered twice daily, this study presents the intracellular pharmacokinetics for both compounds in patients receiving a hard-gel formulation of SQV/r administered once daily. We have used the term intracellular drug concentration; however, we are aware that what we have determined represents the total level of drug that is cell associated. This concentration is unlikely to reflect the free cytosolic concentration but provides important information on the drug’s access to the cellular compartment. Earlier intracellular drug studies showed that boosting saquinavir with ritonavir results in a lower intracellular saquinavir accumulation ratio, even though intracellular saquinavir concentrations and AUC values are higher. Moreover, ritonavir accumulation was increased when ritonavir was administered with saquinavir in comparison to the sole administration of ritonavir (21; S. H. Khoo, S. H., P. G. Hoggard, P. Newton, E. R. Meaden, A. Smith, I. Williams, J. Lloyd, J. F. Tjia, H. Reynolds, E. G. Wilkins, N. J. Beeching, B. Peters, and D. J. Back, 8th Eur. Conf. Clin. Aspects Treatment HIV Infect., abstr. 159, 2001). In this study, the median intracellular accumulation ratios of saquinavir and ritonavir were 3.31 (range, 1.49 to 6.69) and 1.46 (range, 0.83 to 4.15), respectively, indicating that saquinavir and ritonavir enter the intracellular compartment and accumulate at rates approximately 3.3 and 1.5 times higher than those in plasma. All PIs, excluding indinavir,
are lipophilic and can penetrate the phospholipid bilayers of cellular membranes (20). The accumulation ratios reported in this study concur with those found in studies measuring accumulation ratios of coadministered saquinavir and ritonavir (21, 28). Saquinavir accumulated intracellularly to a greater extent than ritonavir, in accordance with former observations in vivo (Khoo et al., 8th Eur. Conf. Clin. Aspects Treatment HIV Infect.). Differential accumulation of PIs has been demonstrated within lymphoblastoid cell lines in vitro (19), in PBMCs in vivo (Khoo et al., 8th Eur. Conf. Clin. Aspects Treatment HIV Infect.), and in subcellular fractions of cells ex vivo (C. Lamotte, G. Peytavin, F. Clavel, and R. Farinotti, 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-1801, 2003). The accumulation of PIs depends on a dynamic balance between several factors, including physiochemical properties of PIs, their affinity for plasma proteins, and transport by influx and efflux proteins.

A correlation was observed between plasma and intracellular saquinavir exposure, and borderline significance was found for the relationship between plasma and intracellular ritonavir exposure (Fig. 2a and b). This association suggests that the extrapolation of intracellular concentrations from simultaneous levels in plasma may be a possibility, in agreement with a previous study (Peytavin et al., 2nd Int. Workshop Clin. Pharmacol. HIV Ther.), with more confidence in the extrapolation of saquinavir concentrations than ritonavir concentrations. An association between saquinavir and ritonavir accumulation was observed, so that greater intracellular saquinavir exposure was linked to greater intracellular ritonavir exposure (Fig. 2c), in accordance with the results of other studies (21). This suggests the possibility of a common means or mechanism of intracellular accumulation, such as passive diffusion, sequestration inside the cell via protein binding or ion trapping, or active influx-efflux transport.

Concentrations of PIs in plasma and viral outcome or CD4+ cell numbers have been routinely measured as analytical markers for disease progression in the long-term management of HIV infection. The findings of the CHEESE study demonstrated that 86% of patients were virologically suppressed despite suboptimal plasma saquinavir soft-gel concentrations throughout the study period (33). It was hypothesized that discrepancies between plasma drug concentrations and virological response may be related to the intracellular pharmacokinetics of the drugs. Furthermore, another study (21) established a disconnect between very low plasma saquinavir concentrations and high intracellular drug accumulation in a subset of patients who demonstrated durable virological suppression despite receiving low doses (600 mg every 8 h) of unboosted hard-gel saquinavir. In this study, by measuring hard-gel SQV/r administered once daily, a similar trend was detected. The median trough concentration (Cmin) of saquinavir in plasma (0.08 mg · liter⁻¹) was below the minimum effective concentration (MEC) recommended by therapeutic drug monitoring (0.1 mg · liter⁻¹), and the median AUC0–24h was below the target for optimal suppression (20 mg · h · liter⁻¹) (15). These patients remained virologically suppressed, with plasma HIV RNA levels of less than 50 copies/ml, and only one patient had a detectable viral load of 61 copies/ml. Previous data suggest that this regimen is durable, with 93% of patients virologically suppressed after 24 weeks of therapy (6).

Intracellular saquinavir trough concentrations were much higher (0.71 mg · liter⁻¹), suggesting that cellular drug concentrations may be present when plasma drug levels are low.

Saquinavir and ritonavir are both extensively protein bound in plasma (98% bound) and predominantly attached to α1-acid glycoprotein (4). Previous reports of intensive once-daily SQV/r (1,600/100 mg) pharmacokinetic data sets demonstrate saquinavir t1/2s of 4.6 and 4.68 h and ritonavir t1/2s of 4.9 and 3.95 h in plasma (Boffito et al., 43rd ICAAC, abstr. A-1612, and Autar et al., 9th Eur. Conf. Clin. Aspects Treatment HIV Infect., abstr. 4.1/1, respectively). This study illustrated similar results, with saquinavir and ritonavir t1/2s in plasma of 4.5 and 4.1 h, respectively, significantly shorter than the intracellular t1/2s of 5.9 and 6.2 h, respectively. In vitro, saquinavir exhibits a long intracellular t1/2, suggesting the possibility of the drug being trapped inside the cell or the existence of a greater affinity for influx transporters (29). In addition, the accumulation ratio of both saquinavir and ritonavir increased over time (Table 2), suggesting the possibility that intracellular drug may be available at a time when plasma drug concentrations are below the MEC. It is possible that relatively higher intracellular accumulation of saquinavir at trough concentrations may allow greater forgiveness for missed or late doses. Thus, the intracellular penetration of PIs is clinically important, and an understanding of intracellular pharmacology may improve long-term therapy by reducing cellular resistance.

Multidrug resistance transporters may play a role in reducing intracellular drug concentrations in a number of tissue and cellular compartments via an efflux mechanism, thus contributing to HIV sanctuary (16, 17). In addition, P-gp is expressed on lymphocytes and is differentially expressed on the various subsets (23), which may have an impact upon the cellular concentration of substrates. In this report, no relationship between lymphocyte subset P-gp expression and the intracellular drug accumulation of saquinavir and ritonavir was observed despite both drugs being substrates for the transporter. This result, in part, concurs with those of a previous study of a twice-daily SQV/r regimen, which demonstrated no relationship between saquinavir accumulation and total P-gp expression but which did demonstrate a weak relationship between ritonavir accumulation and P-gp expression (28). The difference between these results may reflect a difference in dosing, since the once-daily regimen achieves higher drug concentrations that may saturate P-gp. It is known that PIs are inhibitors of P-gp (30, 32, 34), and therefore once-daily regimens with higher achieved concentrations may increase their own accumulation by reducing efflux.

In summary, this paper describes the intracellular pharmacokinetics of saquinavir and ritonavir in patients receiving a hard-gel formulation of SQV/r (1,600/100 mg) administered once daily. Accumulation was unrelated to the lymphocyte surface expression of P-gp in this cohort of patients. Plasma drug concentrations were below the MEC; however, the intracellular pharmacokinetics of saquinavir and ritonavir were favorable, with greater cellular t1/2s and an increasing accumulation ratio over the dosage interval.

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REFERENCES


