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

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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 saquinavir and ritonavir concentrations, to determine the half-lives ($t_{1/2s}$) 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 ($\text{AUC}_{0–24}^\text{h}$) to plasma drug $\text{AUC}_{0–24}^\text{h}$ was calculated to determine cellular drug accumulation. The median (range) $\text{AUC}_{0–24}^\text{h}$ 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 14.6) 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 $t_{1/2s}$ (4.1 h [range, 2.6 to 8.3 h] and 5.9 h [range, 4.0 to 17.7 h]; $P = 0.034$) and between the plasma and intracellular ritonavir $t_{1/2s}$ (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 $t_{1/2s}$ of saquinavir and ritonavir were longer than the plasma $t_{1/2s}$, 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 SOV/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 SOV/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 monoclonal antibody UIC2-rPE was obtained from Immunotech (Marseilles, 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 Hydropurity 5C11 column was purchased from Hypersil (Manchester, United Kingdom). The internal standard (Ro 31-9564) was a gift from Roche (Basel, Switzerland). Hanks’ balanced salt solution (HBSS) and all other compounds were purchased from Sigma Chemical Company, Ltd. (Poole, United Kingdom).

Subjects. Twelve subjects (11 male and 1 female), with a mean age of 42 years (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 10⁷ to 10⁸ 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 SOV/r hard-gel formulation regimen (Invirease) with a starting dose of 1 h, ensuring that sampling conditions were ice cold to prevent drug loss (21).

For high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) analysis, an internal standard (Ro 31-9564; 20 μl, 100 ng·ml⁻¹) was added to dried PBMC extracts (resuspended in 100 μl of distilled water). To heat-inactivated plasma samples (100 μl, 58°C, 40 min), samples containing standard doses (range for saquinavir, 50 to 10,000 ng·ml⁻¹; for ritonavir, 100 to 20,000 ng·ml⁻¹), and quality control samples (saquinavir at 200, 1,000, and 5,000 ng·ml⁻¹; ritonavir at 400, 2,000, and 10,000 ng·ml⁻¹), an internal standard (Ro 31-9564; 20 μl, 1 μg·ml⁻¹) was added prior to further extraction with diethyl ether (3 ml). The aqueous layer was frozen, and the organic layer was transferred to a clean tube and evaporated to dryness. Standards, quality control samples, and plasma samples were reconstituted in the mobile phase (1.5 ml) (20 mM ammonium formate buffer–sodium acetate, 30% methanol, 70% water), and PBMCs were resuspended in a 150-μl volume. Each sample (100 μl) was transferred into auto sampler vials prior to injection onto the column. Saquinavir and ritonavir were eluted on a Hypersil Elite SC18 column (5-μm inside diameter, 250 by 4.6 mm) protected by a precolumn guard (silica Si 60; 5-μm inside diameter) with the mobile phase maintained at 1.2 ml·min⁻¹. The internal standard (retention time, 8.57 min) was measured with an ion trap MS/MS detection system that monitors simultaneous daughter ions (m/z 674/4573; 388.2).

Saquinavir (retention time, 4.58 min) was analyzed by fragmentation of the parent compound and quantification of resulting fragments, monitoring ions m/z 671.4/4571, 453.2. Ritonavir (retention time, 4.18 min) was also analyzed by fragmentation of the parent compound and quantification of resulting fragments, monitoring ions m/z 522.4/304.5. Drug assays were internally validated within the Liverpool laboratory, which holds Clinical Pathology Accreditation status, and externally validated by the International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV Infection (KKGT, Nijmegen, The Netherlands). The lower limits of quantification were 50 and 100 ng·ml⁻¹ for 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) that was programmed to recognize specific peaks and to quantify the intensity of the ion signal on an LCO Duo ThermoQuest 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.

Detection of cell-specific P-gp expression on lymphocytes. PBMCs isolated by density gradient centrifugation (4 × 10⁶ to 7 × 10⁶ cells·ml⁻¹) were fixed (1:10 dilution of CellFIX, 1.0 ml, 25 min, 4°C) and then washed (1 ml of HBSS; centrifugation at 4°C and 700 × g for 5 min) and resuspended in HBSS to a concentration of 2 × 10⁶ cells·ml⁻¹ as described previously (13). Aliquots (200 μl) of cell suspension were transferred into 14- by 5-ml plastic sample tubes to be incubated with different combinations of antibodies. Four cell samples were stained with either CD4-, CD8-, or CD56-FITC (2.5 μg·ml⁻¹) primary conjugated antibodies, so that two samples from each subpopulation could be counterstained with UIC2-rPE (375 ng·ml⁻¹) or with the isotypically matched control IgG2a-rPE (2.5 μg·ml⁻¹). Unstained cell samples were used as a negative control. Following (10 μl, 90 min), 100 μl of CellFIX (1:10 dilution) was transferred twice (1 ml of HBSS, 4°C) and centrifuged (700 × g, 6 min, 4°C). Samples were then fixed (1:10 dilution of CellFIX, 0.5 ml) and analyzed by dual-color flow cytometry.

Dual-color flow cytometry. Flow cytometry was conducted with a Coulter epics XL-MCL flow cytometer that groups individual cells according to size, granularity, fluorescence, and intensity of fluorescence. Forward scatter and side scatter signals were detected on a linear scale dot plot, and fluorescence was detected on a logarithmic-scale histogram. Lymphocytes from the total PBMC population were electronically gated by using light-scattering properties, and 8,000 events were collected for each sample. P-gp expression on lymphocytes was determined from a histogram measuring FL2 fluorescence that detects lymphocytes conjugated to the phycoerythrin fluorochrome. Expression of P-gp on lymphocytes was calculated by subtracting the median fluorescence intensity value obtained for the nonspecific isotype control (IgG2a-rPE) from the median fluorescence intensity value of the P-gp-specific UIC2-rPE antibody, referred to as the median increase in fluorescence.

Data analysis. Intracellular concentrations of saquinavir and ritonavir were calculated based on the single PBMC volume of 0.4 μl and the total cell density (14). To date, the volume of a single cell is the best surrogate marker to calculate intracellular concentrations. The calculated intracellular concentration of drug is the total drug concentration associated with the cells. The areas under the curve (AUC) and t½,dr for drug in plasma and cells over the 24-h dosage interval were evaluated by noncompartamental modeling using the linear trapezoid method (OPFIT) computer software (version 1.0). The same number of PBMCs were used to calculate both plasma and intracellular drug t½,ds from the linear slope of the profile. In order to balance variable concentrations of PBs in the plasma, intra-
cellular accumulation data were quantified and expressed as the ratio of the intracellular AUC to the total plasma AUC over a whole dosage interval to determine the extent of intracellular penetration of the drug. Correlations and comparisons between data sets were analyzed by simple linear regression and the Wilcoxon signed rank test, respectively. Statistics were analyzed with Arcus.

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. Boffito, 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 \(r^2\) 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. Apateerapong, J. Sankote, A. Hill, B. Hirschel, D. Cooper, J. Lange, P. Phanuphak, K. Ruxrungtham, and D.

![Image](http://aac.asm.org/) 

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.

<table>
<thead>
<tr>
<th>TABLE 1. Pharmacokinetic data for saquinavir and ritonavir in plasma and cellular compartments for 12 patients (^a)</th>
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<tbody>
<tr>
<td>Drug and compartment</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td><strong>Saquinavir</strong></td>
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<tr>
<td>Plasma</td>
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<td>Intracellular</td>
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<tr>
<td><strong>Ritonavir</strong></td>
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<td>Plasma</td>
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<td>Intracellular</td>
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\(^a\) \(C_{\text{max}}\), 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 pl).
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Significant differences between the t1/2 of saquinavir in plasma and cells (P = 0.034) and between the t1/2 of ritonavir in plasma and cells (P = 0.032) were observed, so that the ratios of both saquinavir and ritonavir concentrations in cells to those in plasma increased over the dosing interval (Table 2).

**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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<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>
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<tr>
<td>24</td>
<td>7.64 (1.55–19.2)</td>
</tr>
</tbody>
</table>

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

**FIG. 2.** Relationship between cellular (IC) and plasma exposure to saquinavir (a), cellular and plasma exposure to ritonavir (b), and intracellular accumulation of saquinavir and ritonavir (c) in 12 HIV-infected subjects. Exposure is expressed as the AUC0–24h, and accumulation is expressed as the ratio of cellular AUC0–24h to plasma AUC0–24h.

**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 \( C_{24h} \) of saquinavir in plasma \( (0.08 \text{ mg} \cdot \text{litr}^{-1}) \) was below the minimum effective concentration (MEC) recommended by therapeutic drug monitoring \( (0.1 \text{ mg} \cdot \text{litr}^{-1}) \), and the median \( \text{AUC}_{0-24h} \) was below the target for optimal suppression \( (20 \text{ mg} \cdot \text{h} \cdot \text{litr}^{-1}) \) (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).

In summary, this paper describes the intracellular pharmacokinetics of saquinavir and ritonavir in patients receiving a hard-gel formulation of SQV/r \( (1,600/100 \text{ mg}) \) 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 \( t_{1/2} \) and an increasing accumulation ratio over the dosage interval.

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REFERENCES


