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Cerebrospinal fluid HIV-1 RNA during treatment with ritonavir/saquinavir or ritonavir/saquinavir/stavudine

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\textit{AIDS (in press)}
Abstract

Objective: To assess the HIV-1 RNA response and drug concentrations in the cerebrospinal fluid (CSF) and serum during treatment with saquinavir (SQV)/ritonavir (RTV) or SQV/RTV plus stavudine (d4T) in HIV-1 infected patients.

Design: Multicenter, open label, randomized controlled trial.

Methods: Two hundred and eight protease inhibitor (PI)- and d4T-naive, HIV-1 infected patients were treated with RTV 400 mg bid and SQV 400 mg bid +/- d4T 40 mg bid. Intensification with reverse transcriptase inhibitors was allowed if serum HIV-1 RNA remained >400 copies/ml after 12 weeks. In twenty-seven volunteers both CSF and serum HIV-1 RNA were measured at baseline, week 12 and 48, using the Roche Amplicor assay and the Ultrasensitive HIV-RNA assay. In 22 patients, serum and CSF drug concentrations were determined at week 12.

Results: The median baseline serum and CSF HIV-1 RNA concentrations were 4.81 and 3.21 log_{10} copies/ml respectively. A significant difference in proportion of patients with a CSF HIV-1 RNA level below the limit of quantification (<LLQ) after 12 weeks was found: 4/14 (RTV/SQV) versus 12/13 (RTV/SQV/d4T) (p=0.001). Similar results were found using the ultrasensitive assay. Patients with a baseline CSF HIV-1 RNA level <LLQ remained <LLQ, regardless of the treatment regimen. Treatment with RTV/SQV alone was the only independent predictor of a CSF HIV-1 RNA level >LLQ at week 12 (p=0.005) in logistic regression analysis. CSF RTV and SQV concentrations were <LLQ in most patients.

Conclusion: RTV/SQV alone cannot suppress detectable CSF HIV-1 RNA levels to <LLQ after 12 weeks of treatment in the majority of patients. CSF drug concentrations of RTV and SQV <LLQ may explain the suboptimal antiretroviral effect in the CSF.
INTRODUCTION

The rate of progression of HIV disease and the time to death is predicted by the magnitude of active HIV replication. Therefore, there is consensus that the goal of antiretroviral treatment is maximal suppression of viral replication as reflected by the lowest possible HIV-1 RNA levels in plasma. Preliminary reports have suggested that a combination of saquinavir (SQV) and ritonavir (RTV) alone can produce a sustained decrease of plasma HIV-1 RNA to below 200 copies/ml and a substantial increase in CD4 cell counts. However, the central nervous system (CNS) may represent an important sanctuary site for HIV, as the blood-brain-barrier restricts the passage of a number of antiretroviral drugs. Nucleoside analogue reverse transcriptase inhibitors (NRTIs) have been reported to penetrate the cerebrospinal fluid (CSF) well. In contrast, low RTV and SQV concentrations in the CSF have been reported. We investigated the effect of RTV/SQV combination therapy with or without stavudine on HIV-1 RNA levels in blood and CSF.

METHODS

Study Design

The Prometheus study is an open label, randomized controlled, multicenter trial among HIV-1 infected patients in the Netherlands and Belgium. Patients received orally either RTV 400 mg twice daily (bid) plus SQV 400 mg bid or RTV 400 mg bid plus SQV 400 mg bid plus stavudine (d4T) 40 mg bid (30 mg bid if body weight was below 60 kg). Participants had to be protease inhibitor (PI)- and d4T-naive before the start of the study. Intensification of study medication with NRTIs was allowed if serum HIV-1 RNA remained > 400 copies/ml after 12 weeks of treatment, confirmed at week 18.

Lumbar punctures were performed in 27 study participants at baseline and after 12 and 48 (12 pts) weeks of treatment to assess HIV-1 RNA and drug concentrations in both serum and CSF.

Study Assessments

In twelve CSF samples obtained at week 12, protein and white cell count were determined. Serum and CSF HIV-1 RNA levels were measured using a commercially available PCR-based assay with a variable lower limit of quantification (LLQ) (median 230 copies/ml, range: 94-841 copies/ml) (Amplicor HIV Monitor Test, Roche Diagnostic Systems Inc., Branchburg, New Jersey, USA). If HIV-1 RNA values were < LLQ, the cut-off values were used as the individual’s HIV-1 RNA value in all analyses. If HIV-1 RNA values were < LLQ in the standard assay and enough sample was available, the serum or CSF was retested using the Roche Ultrasensitive HIV-1 RNA assay with a variable LLQ, which was 50 copies/ml or lower.

RTV, SQV and d4T concentrations in serum and CSF were analyzed by validated high performance liquid chromatographic assays with ultraviolet detec-
tion as described before. The LLQ for RTV, SQV and d4T in these assays were 25, 2.5, and 10 ng/ml respectively.

Statistical Analysis

A log\(_{10}\) transformation was performed on all HIV-1 RNA concentration values. Results are reported as medians and interquartile ranges. Differences in baseline variables and differences in proportions of patients with HIV-1 RNA in the CSF < LLQ between the treatment arms were tested using the two-tailed Fisher's exact test and the Wilcoxon's signed rank sum test, where appropriate. Correlation between baseline HIV-1 RNA in serum and CSF was tested using an unweighted linear regression model.

We examined the correlation between baseline characteristics (baseline serum and CSF HIV-1 RNA, treatment arm and pre-treatment status) and the study outcome 'CSF HIV-1 RNA > LLQ at week 12', using univariate logistic regression models. Then, the best fitted multivariate logistic regression model was constructed using both forward and backward selection. Reported P-values are all two-tailed and were considered statistically significant if ≤ 0.05. Analyses were performed using SAS, version 6.12 (SAS Institute, Cary, North Carolina, USA).

The study was approved by the Protocol Review Board of the National AIDS Therapy Evaluation Center and the local Medical Ethical Committees of the participating sites. All participants gave separate written informed consent for this neurological sub study.

RESULTS

Baseline Characteristics

Two hundred and eight patients were randomized between January 1997 and January 1998. Paired serum/CSF samples, obtained at week 0 and week 12, were available from 27 participants (RTV/SQV:14, RTV/SQV/d4T:13). Eight patients (30%) had been treated with one or more RTIs before start of the study. One patient in the RTV/SQV group was diagnosed with progressive multifocal leukoencephalopathy before the start of the study, which was progressive despite the initiation of antiretroviral therapy. None of the study participants was diagnosed with a neurological disease during the study.

At baseline, the only significant difference between the treatment groups was the number of patients with a baseline serum HIV-1 RNA > 5 log\(_{10}\) copies/ml (RTV/SQV:57%, RTV/SQV/d4T:0%, p=0.002) (Table 1). Baseline CSF HIV-1 RNA levels did not correlate with CSF protein or white cell count (data not shown).

HIV-1 RNA Levels in Serum and CSF

All patients had a detectable serum HIV-1 RNA level at baseline (median 4.81 log\(_{10}\) copies/ml, range 3.20-5.95). The median CSF HIV-1 RNA level at baseline was 3.22 log\(_{10}\) copies/ml (range <2.32-4.86) (Table 1). Seven patients, including 5 patients who had never used any antiretroviral drug before start of the study,
Table 1
Baseline characteristics of individuals randomized to receive treatment with RTV/SQV or RTV/SQV/d4T

<table>
<thead>
<tr>
<th></th>
<th>RTV/SQV</th>
<th>RTV/SQV/d4T</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>male (%)</td>
<td>12 (86)</td>
<td>12 (92)</td>
<td>1.00</td>
</tr>
<tr>
<td>median age in years (range)</td>
<td>44 (25-54)</td>
<td>36 (31-70)</td>
<td>.42</td>
</tr>
<tr>
<td>pre-treated before start study (%)</td>
<td>4 (29)</td>
<td>4 (31)</td>
<td>1.00</td>
</tr>
<tr>
<td>CD4 cell count /μL, median (range)</td>
<td>245 (10-500)</td>
<td>100 (10-870)</td>
<td>.63</td>
</tr>
<tr>
<td>serum HIV-1 RNA log_{10} copies/ml, median (range)</td>
<td>4.98 (4.23-5.95)</td>
<td>4.64 (3.88-4.95)</td>
<td>.30</td>
</tr>
<tr>
<td>CSF HIV-1 RNA log_{10} copies/ml, median (range)</td>
<td>3.42 (&lt;2.32-4.38)</td>
<td>2.94 (&lt;2.35-4.86)</td>
<td>.75</td>
</tr>
</tbody>
</table>

* p-value for difference between the two study arms, using the two-tailed Fisher’s exact test for categorical variables and the Wilcoxon’s signed rank sum test for continuous variables.

Figure 1 - Box and whisker plots of HIV-1 RNA
Box and Whisker plot for serum (a) and CSF (b) HIV-1 RNA before start of the study and after 12 weeks of treatment. The solid line across the box indicates the median, values in the box are values within the second and third quartiles; minimal and maximal values are connected by the whiskers.
had a CSF HIV-1 RNA level < LLQ at baseline (RTV/SQV: 3 pts, RTV/SQV/d4T: 4 pts). Baseline serum and CSF HIV-1 RNA levels did not correlate ($r^2=0.05$, $p=0.24$). The median serum HIV-1 RNA change after 12 weeks of treatment was -2.11 and -2.24 log$_{10}$ copies/ml for the RTV/SQV and RTV/SQV/d4T group, respectively ($p=0.42$) (Figure 1a). After 12 weeks, 7/14 patients in the RTV/SQV group and 9/13 patients in the RTV/SQV/d4T group had a serum HIV-1 RNA level < LLQ ($p=0.44$).

The median CSF HIV-1 RNA change over time was +0.15 log$_{10}$ copies/ml in the RTV/SQV group, compared to -0.46 log$_{10}$ copies/ml in the RTV/SQV/d4T group ($p=0.03$) (Figure 1b). A significantly different proportion of patients with CSF HIV-1 RNA level < LLQ was found after 12 weeks of treatment: 4/14 patients in the RTV/SQV group and 12/13 patients in the RTV/SQV/d4T group ($p=0.001$). Baseline serum and CSF HIV-1 RNA concentrations were not significantly different in patients on RTV/SQV alone who had a CSF HIV-1 RNA < LLQ at week 12, when compared to patients on the same treatment regimen with CSF HIV-1 RNA > LLQ at week 12 ($p=0.29$). All patients with CSF HIV-1 RNA < LLQ at baseline remained < LLQ in CSF throughout 12 weeks, regardless of their treatment regimen.

In 23 patients, week 0 and 12 CSF and serum samples were available for retests using the ultrasensitive HIV-1 RNA assay. One patient had a CSF HIV-1 RNA < LLQ in the ultrasensitive assay at baseline. The median HIV-1 RNA change in serum was -2.4 log$_{10}$ copies/ml in the RTV/SQV group and -2.7 log$_{10}$ copies/ml in the RTV/SQV/d4T group ($p=0.58$). The number of patients that reached a serum HIV-1 RNA <50 copies/ml after 12 weeks of treatment was 3/12 in the RTV/SQV arm versus 5/11 in the RTV/SQV/d4T arm ($p=0.40$). The median HIV-1 RNA change in the CSF was -0.2 log$_{10}$ copies/ml in the RTV/SQV arm versus -1.5 log$_{10}$ copies/ml in the RTV/SQV/d4T arm ($p=0.006$). In the CSF only 1/12 patients in the RTV/SQV arm reached HIV-1 RNA <50 copies/ml at week 12, versus 7/11 in the RTV/SQV/d4T arm ($p=0.009$).

**Predictors of a CSF HIV-1 RNA concentration > LLQ at Week 12**

Patients were not stratified before randomization for their participation in this sub study. Therefore, differences in baseline characteristics could influence the study outcome ‘CSF HIV-1 RNA > LLQ at week 12’. Treatment arm, baseline CSF and serum HIV-1 RNA, and pre-treatment with NRTIs were considered possible factors. In a multivariate analysis only treatment with RTV/SQV was an independent predictor of CSF HIV-1 RNA > LLQ at week 12 (odds ratio 30, 95% CI: 2.8-313; $p=0.005$) (Table 2).

**Drug Concentrations in Serum and CSF**

In 22 patients both serum and CSF concentrations of RTV and SQV were measured at week 12. The median RTV concentration in serum was 5807 ng/ml (range: 1100-17210 ng/ml). In 19/22 samples concentrations were above the minimal recommended threshold (MRT) of 2100 ng/ml for RTV. The median serum SQV concentration was 886 ng/ml (range: 95-3368 ng/ml), all above the MRT of 50 ng/ml.
Table 2
Predictors of a CSF HIV-1 RNA concentration > LLQ at week 12, using logistic regression analysis

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Univariate analysis, p-value</th>
<th>Multivariate analysis, p-value</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment arm RTV/SQV</td>
<td>0.005</td>
<td>0.005</td>
<td>30 (2.8-313)</td>
</tr>
<tr>
<td>Baseline serum log_{10} HIV-1 RNA</td>
<td>0.141</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline CSF log_{10} HIV-1 RNA</td>
<td>0.210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment with NRTIs before start study</td>
<td>0.824</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Possible predictors of a CSF HIV-1 RNA level > LLQ at week 12 were tested in a univariate logistic regression model. The best fitted multivariate model only contained treatment arm RTV/SQV.

LLQ = lower limit of quantification

Table 3
Long term (48 week) follow up

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n of patients with CSF HIV-1 RNA &lt; LLQ / total n of patients observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTV/SQV for 48 weeks</td>
<td>1/4 week 0, 1/4 week 12, 2/4 week 48</td>
</tr>
<tr>
<td>RTV/SQV + d4T/3TC after week 18</td>
<td>0/3 week 0, 0/3 week 12, 3/3 week 48</td>
</tr>
<tr>
<td>RTV/SQV/d4T</td>
<td>2/5 week 0, 4/5 week 12, 4/5 week 48</td>
</tr>
</tbody>
</table>

Table shows the result of the twelve patients who underwent lumbar punctures at week 0, 12 and 48.

n = number
LLQ = lower limit of quantification

The RTV concentrations in the CSF were <25 ng/ml in 19/22 patients (range <25-57 ng/ml). The SQV concentrations in the CSF were <2.5 ng/ml in 20/22 patients (range <2.5-14.7). The median d4T concentration of 8 patients on RTV/SQV/d4T was 488 ng/ml (range: 72-969) and 213 ng/ml (range: 81-367) in serum and CSF respectively.

Week 48 Results

In 12 patients, a third lumbar puncture was performed 48 weeks after initiation of treatment. Patients, who started with a CSF HIV-1 RNA < LLQ at baseline, remained < LLQ throughout 48 weeks (n=3). In 3 patients, who had intensified treatment with RTV/SQV with d4T and lamivudine (3TC) between week 18 and 24, as prescribed in the protocol, CSF HIV-1 RNA levels became < LLQ by week 48. Two out of three patients, who continued RTV/SQV treatment, had a CSF HIV-1 RNA > LLQ through 48 weeks (Table 3).

DISCUSSION

This is the first study comparing treatment with PIs only versus treatment with PIs plus a NRTI in their effect on CSF HIV-1 RNA. Patients with a CSF HIV-1 RNA < LLQ at baseline remained < LLQ after 12 weeks of treatment with RTV
and SQV alone. However, RTV/SQV alone was unable to suppress detectable baseline CSF HIV-1 RNA to undetectable levels in all but one patient in the same time period.

Several explanations may be given for this finding. First, differences in baseline characteristics between the two study groups, like baseline serum and CSF HIV-1 RNA and pre-treatment status could influence the outcome. None of these parameters was a significant independent predictor of a detectable CSF HIV-1 RNA > LLQ after 12 weeks. Second, a lower adherence to the double PI regimen might explain the differences found. However, self-reported adherence to both treatment regimens was not significantly different. Third, the CNS may harbor HIV strains with resistance patterns that differ from those isolated from the blood which leads to discordant HIV-1 RNA responses in CSF and blood. Evidence for independent development of NRTI resistance patterns in the CSF has been found in 30% of tested strains. Although very little is known about development of PI resistance in other compartments than blood, the selective pressure of the extremely low RTV and SQV concentrations in the CSF are probably insufficient to induce PI resistance within 12 weeks of treatment in this compartment. The treatment arm was the only significant independent predictor of CSF HIV-1 RNA response: treatment with RTV/SQV alone increased the risk of a detectable CSF HIV-1 RNA at week 12 by 30-fold (95% CI 2.89-313). This was highly significant (p=0.005), despite the small sample size. Therefore, we hypothesize that insufficient viral suppression in the CSF in the RTV/SQV group is caused by the low exposure to RTV and SQV in the CSF. Inhibition of the entry of PIs into the CNS by the membrane transporter P-glycoprotein at the level of the blood brain barrier can attribute to the low CSF drug levels. D4T penetrates into the CSF much better than both RTV and SQV. D4T can, at least in combination with 3TC, reduce CSF HIV-1 RNA to undetectable levels, and may be responsible for the superior suppression of HIV-1 RNA in the CSF observed in the d4T-containing regimen.

Our data contradict the findings of Cameron et al, who reported that CSF HIV-1 RNA was < LLQ in the standard Roche Amplicor assay in 14/15 (93%) PI naive patients, who had used SQV/RTV for a median of 60 weeks. Longer duration of treatment in their study could be an explanation for the different findings. Even with a 4-drug regimen it can take more than 2 months before CSF HIV-1 RNA declines to undetectable levels. Moreover, in one of our patients on RTV/SQV alone, CSF HIV-1 RNA became undetectable by week 48. A higher proportion of patients with CSF HIV-1 RNA < LLQ at baseline could be another explanation for the different results in their study, as baseline CSF HIV-1 RNA concentrations were not obtained.

Ongoing, low level viral replication, which could not be detected with the standard Amplicor assay, is associated with early viral rebound. Therefore we re-tested samples, which were <LLQ in the standard Roche Amplicor assay, using the Roche Ultrasensitive HIV-1 RNA assay. Analyses including these results confirmed the results of the standard Amplicor assay.
CSF HIV-1 RNA is frequently used as a surrogate marker of CNS infection, because it is relatively easy to obtain, and can be followed long-term. However, the relationship between CNS infection and CSF HIV-1 RNA is not totally clarified. CSF may also function as a sanctuary site for HIV-1 RNA, where from HIV-1 RNA may return in the blood. Therefore, the goal of antiretroviral treatment should be the suppression of viral replication in all compartments.

Staprans et al. suggested that CSF-penetrating drugs may not be necessary for adequate suppression of CSF HIV-1 RNA in early infection. This hypothesis could explain the observation of a rapid decline of CSF HIV-1 RNA in one patient on RTV/SQV alone who had undetectable CSF drug levels. We observed that CSF HIV-1 RNA remained < LLQ throughout the study in all patients with a CSF HIV-1 RNA < LLQ at baseline, regardless the treatment regimen. However, we don’t know the exact length of HIV infection in our patients and we found no correlation between baseline CD4+ lymphocyte counts and baseline CSF HIV-1 RNA or CSF HIV-1 RNA response. Once baseline CSF HIV-1 RNA was detectable, at least one CSF penetrating drug was necessary to suppress detectable CSF HIV-1 RNA.

We advise against the use of RTV/SQV alone as a first line antiretroviral therapy. However, in some cases (e.g. patients with adherence problems to more complex regimes or intolerance to all relevant NRTIs) RTV/SQV can be an alternative, provided that the patient is willing to undergo lumbar punctures, to check for low CSF HIV-1 RNA levels.

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References