Randomized Trial Comparing Saquinavir Soft Gelatin Capsules Versus Indinavir As Part Of Triple Therapy (CHEESE study)

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Abstract

Objective: To compare efficacy and tolerability of saquinavir soft gelatin capsule formulation (SQV-SGC) and indinavir, both given as part of a triple drug regimen containing zidovudine and lamivudine, in HIV-1-infected individuals.

Design: Randomized, open label, multicenter study.

Patients: In total 70 patients were included who were antiretroviral-naive and who had a CD4 cells count <500/µL and/or >10.000 HIV RNA copies/ml plasma and/or HIV-related symptoms. Subjects were assigned randomly to zidovudine 200 mg t.i.d. plus lamivudine 150 mg b.i.d. plus either SQV-SGC 1200 mg t.i.d. (SQV-SGC group) or indinavir 800 mg t.i.d. (indinavir group). Data are presented for all patients up to week 24.

Results: Mean baseline CD4 counts (± SE) were 301±29 cells/µL and 310±43 cells/µL in the SQV-SGC and indinavir groups, respectively. The log10 median baseline HIV RNA load was 5.00 copies/ml in the SQV-SGC group and 4.98 copies/ml in the indinavir group. No difference in antiretroviral effect between the treatment arms could be demonstrated. Intention-to-treat analysis (LOCF) at week 24 revealed that RNA levels decreased to < 50 copies/ml in 74.3% of patients in the SQV-SGC group and in 71.4% of the patients in the indinavir group (p=0.78). In the on-treatment analysis the proportion of patients < 50 copies/ml at week 24 was 88.0% in the SQV-SGC group and 84.6% in the indinavir group (p=0.725). Intriguingly, the mean increase of CD4 cells (ITT) in the first 24 weeks was 162±20 cells/µL in the SQV-SGC group and 89±21 cells/µL in the indinavir group (p=0.01), but preliminary data indicate that this difference in CD4 gain may disappear after 24 weeks of treatment. Both regimens were generally well tolerated.

Conclusion: During the first 24 weeks of the study, we found no difference in antiviral potency between the indinavir group and the SQV-SGC group. A significantly higher CD4 response in the SQV-SGC group was observed.

Keywords: clinical trials, combination therapy, indinavir, saquinavir-SGC
Introduction

Saquinavir is a potent and specific inhibitor of the protease of human immunodeficiency virus (HIV) types 1 and 2 in vitro. Saquinavir was initially formulated as a hard-gelatin-capsule (SQV-HGC) which, as part of a combined antiretroviral treatment has been shown to be clinically effective (1). However, SQV-HGC has limited oral bioavailability, resulting in relatively low plasma levels. The recently developed soft-gelatin-capsule formulation of saquinavir (SQV-SGC) has improved oral bioavailability over SQV-HGC. At a dosage of 1200mg t.i.d., SQV-SGC achieves an approximately eight-fold higher plasma exposure to saquinavir compared with SQV-HGC (600mg t.i.d.) (2). It has been shown that SQV-SGC provides significantly more reduction of plasma HIV RNA levels in antiretroviral naive patients than SQV-HGC (3), consistent with the observation that the antiviral activity of saquinavir is positively correlated to plasma drug levels (4). Preliminary data from several clinical trials have demonstrated the antiviral potency of SQV-SGC in combination with two reverse transcriptase (RT) inhibitors (5,6).

No previous randomized clinical trial has compared two protease inhibitors in triple therapy background with the same RT inhibitors. The objective of this study was to compare the antiviral efficacy and tolerability of SQV-SGC with indinavir, as part of a triple therapy containing zidovudine and lamivudine in antiretroviral-naive HIV-1-infected individuals.
Methods

Study design
This was a randomized, parallel arm, open label, multi center study comparing antiviral efficacy and safety of zidovudine plus lamivudine plus SQV-SGC or zidovudine plus lamivudine plus indinavir, in HIV-1 infected patients. A stratified randomization procedure was used. Patients were stratified according to plasma HIV RNA level (either > 100,000 or < 100,000 copies/ml). Data up to week 24 of all patients are presented. The study was approved by the internal review boards at each site and all participating patients gave written informed consent.

Study patients
Patients of 18 years and older who were seropositive for HIV type 1 and who were antiretroviral naive (with exception of zidovudine use for less than 12 months), were screened for enrollment at eight locations in The Netherlands. Patients were eligible for study treatment if, at the moment of screening plasma HIV RNA levels were at least 10,000 copies/ml (AmpliCor HIV monitor test, Roche Diagnostics) and/or if CD4 counts were less than 500 cells/µL and/or if they had a history of HIV related symptoms (CDC stage B or C).

Exclusion Criteria
Criteria for exclusion were a hemoglobin level less than 7.0 mmol/l (in males) or 6.5 mmol/l (in females), a platelet count of less than 25 x 10^9 / l, a neutrophil count of less than 0.75 x 10^9 / l, serum creatinine level exceeding 1.5 fold the upper normal limit or levels of hepatic aminotransferases (ASAT, ALAT) exceeding 5 fold the upper normal limit. In addition, patients were excluded if they required acute therapy for an active opportunistic infection or if they required systemic antineoplastic chemotherapy and/or radiotherapy. Also excluded were patients with malabsorption or inadequate oral intake or patients with unexplained, chronic diarrhea persisting for 14 days or more. Pregnant or breastfeeding women were also excluded as were patients who participated in other investigational studies within 30 days prior to screening.
Treatment Regimens
Eligible patients were assigned randomly to either: (i) indinavir (Crixivan, Merck, West Point, Pa) 800mg t.i.d. plus zidovudine (Retrovir, Glaxo-Wellcome, Research Triangle Park, N.C.) 200mg t.i.d. plus lamivudine (Epivir, Glaxo-Wellcome, Research Triangle Park, N.C.) 150 mg b.i.d. or : (ii) Saquinavir Soft-Gelatin-Capsules (Fortovase, Hoffmann-La Roche, Inc., Nutley, New Jersey) 1200mg t.i.d. plus zidovudine 200mg t.i.d. plus lamivudine 150 mg b.i.d..

Assessments
The patients were assessed every 4 weeks through week 24. At screening, baseline and every visit, medical history was reviewed and standard biochemical and hematological tests were conducted. Plasma concentrations of the protease inhibitors were determined each study visit.

Virologic and Immunologic Studies
At each site, plasma (EDTA) was processed, stored at -70°C and assayed later for HIV RNA by a quantitative reverse transcriptase polymerase chain reaction assay (Roche Amplicor Monitor Standard Assay). The lower limit of detection was 400 HIV RNA copies/ml in this assay. An investigational version of an ultrasensitive RT-PCR assay with a lower limit of detection of 50 copies/ml (Roche Amplicor Monitor) was performed at a central laboratory.
Absolute numbers of CD4+ and CD8+ T-lymphocytes were determined performed at a central laboratory by flowcytometry.

Statistical Analysis
The primary parameter of antiretroviral efficacy was HIV RNA (log transformed to the base 10). Secondary parameters were CD4 counts, improvement in or progression to AIDS defining illnesses, the incidence of adverse events and biochemical and haematological safety parameters. Baseline values of HIV RNA levels and CD4 counts were calculated as the geometric mean of two consecutive pretreatment determinations, the first between day -14 and day -7 and the second on day 0.
Analyses of the variables pertaining to efficacy (plasma HIV RNA and
CD4 response) were performed on an intention-to-treat basis that included data of all randomized patients who have received at least one dose of study medication. The last observation was carried forward as the imputation method. On treatment analyses, representing all those patients for whom data were available at that time point, are also presented.

A repeated measures analysis-of-variance-model was used to detect differences between the two treatment arms in area under the curve of plasma HIV RNA levels and CD4 counts with repeated equally spaced observations within the patients. In the analyses of the proportions of patients below limit of detection and incidence of adverse events, the treatments were compared by Fisher’s exact tests. All reported P values are two-sided.

Results

Study Patients
Seventy patients were enrolled in the study from January 1997 to February 1998, 35 in each treatment group. The baseline characteristics of the patients were not different between treatment groups, as is shown in Table 1. Ten patients discontinued the study after 4-24 weeks for the following reasons: an adverse event (5) (table 2), lost to follow up (1), consent withdrawn (1), receiving of erroneous study medication (1), protocol violation (2). The number of discontinuations was the same between the two treatment groups.

HIV RNA
Over the initial 24 weeks, plasma HIV RNA levels declined (median) 2.40 log10 in the SQV-SGC group and 2.38 log10 in the indinavir group (Figure 1). No significant differences were observed between the treatment groups in the decrease of plasma HIV RNA levels.

Figure 2A shows the proportion of patients who achieved plasma HIV RNA levels below 400 copies/ml, according to treatment group. At week 24, using an intention-to-treat analysis (ITT), 82.9% (29/35) in the SQV-SGC-arm and 85.7% (30/35) in the indinavir arm had plasma HIV RNA
Table 1. Base-line Characteristics of the study patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SQV-SGC Group (n=35)</th>
<th>indinavir Group (n=35)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender - no. of patients (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>32 (91.4)</td>
<td>31 (88.6)</td>
<td>p=0.99 §</td>
</tr>
<tr>
<td>female</td>
<td>3 (8.6)</td>
<td>4 (11.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Mean age (±SD) - yr</strong></td>
<td>38 (± 8.46)</td>
<td>37 (± 9.02)</td>
<td>p=0.45 ¶</td>
</tr>
<tr>
<td><strong>Race (ethnic group) - no of patients (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>30 (85.7)</td>
<td>33 (94.3)</td>
<td>p=0.42 §</td>
</tr>
<tr>
<td>Afro-european</td>
<td>4 (11.4)</td>
<td>1 (2.9)</td>
<td>p=0.35 §</td>
</tr>
<tr>
<td>Oriental</td>
<td>1 (2.9)</td>
<td>1 (2.9)</td>
<td>n.a</td>
</tr>
<tr>
<td><strong>Prior zidovudine therapy - no patients (%)</strong></td>
<td>2 (5.7)</td>
<td>1 (2.9)</td>
<td>p=0.99 §</td>
</tr>
<tr>
<td><strong>Prior AIDS defining illness - no of patients (%)</strong></td>
<td>7 (20)</td>
<td>10 (28.6)</td>
<td>p=0.58 §</td>
</tr>
<tr>
<td><strong>CD4 count - cells / µL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>301</td>
<td>310</td>
<td>p=0.775 ¶</td>
</tr>
<tr>
<td>Range</td>
<td>10-750</td>
<td>30-1075</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma HIV RNA (log_{10} copies/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>5.00</td>
<td>4.98</td>
<td>p=0.668 ¶</td>
</tr>
<tr>
<td>Range</td>
<td>3.41 - 6.87</td>
<td>3.10 - 6.23</td>
<td></td>
</tr>
</tbody>
</table>

¶: Independent-samples t-test
§: Fisher’s exact test
n.a.: not applicable
levels below 400 copies/ml (p=0.74). In the on-treatment (OT) analysis, the proportion of patients < 400 copies/ml at week 24 was 90.3% (28/30) in the SQV-SGC arm and 96.5% (28/29) in the indinavir arm (p=0.57). Figure 2B shows the proportion of patients with plasma HIV RNA levels below 50 copies/ml. At week 24, ITT analysis showed that 74.3% (26/35) in the SQV-SGC arm and 71.4% (25/35) in the indinavir arm had plasma HIV RNA levels below 50 copies/ml (p=0.78). In the OT analysis, the proportion of patients < 50 copies/ml at week 24 was 88.0% (22/25) in the SQV-SGC arm and 84.6% (22/26) in the indinavir arm (p=0.73).

Table 2. Adverse events According To treatment Group

<table>
<thead>
<tr>
<th></th>
<th>SQV-SGC Group</th>
<th>indinavir Group</th>
<th>Fishers’s Exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate or severe Adverse Events:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical nephrolithiasis</td>
<td>0</td>
<td>2</td>
<td>N.S.</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>5</td>
<td>1†</td>
<td>N.S.</td>
</tr>
<tr>
<td>Nausea</td>
<td>5</td>
<td>5</td>
<td>N.S.</td>
</tr>
<tr>
<td>Serious Adverse Events:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastritis</td>
<td>0</td>
<td>1</td>
<td>N.S.</td>
</tr>
<tr>
<td>Hematemesis</td>
<td>1</td>
<td>0</td>
<td>N.S.</td>
</tr>
<tr>
<td>Urine bladder polyp</td>
<td>0</td>
<td>1</td>
<td>N.S.</td>
</tr>
<tr>
<td>Grade 4 Anemia</td>
<td>0</td>
<td>1†</td>
<td>N.S.</td>
</tr>
<tr>
<td>CDC Events:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non Hodgkin lymphoma</td>
<td>1†§</td>
<td>1∗§</td>
<td>N.S.</td>
</tr>
<tr>
<td>PCP</td>
<td>0</td>
<td>1†</td>
<td>N.S.</td>
</tr>
<tr>
<td>Herpes Zoster</td>
<td>2</td>
<td>3</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total CDC Events</td>
<td>3</td>
<td>5</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

† Resulted in study discontinuation
§ Resulted in death
CD4 Cell Counts
During the initial 24 weeks of the study, mean numbers of CD4⁺ T cells in the peripheral blood (±SEM) increased 162±20 cells / µL and 89±21 cells / µL (Figure 3) in the SQV-SGC group and the indinavir group, respectively (ITT). The increase of CD4⁺ T lymphocytes was significantly greater in the group assigned to the SQV-SGC containing regimen (repeated measures analysis, p=0.01). In each group, CD4 count response pattern was biphasic with a higher rate of increase in the first four weeks of treatment as compared to the later four-week intervals.

Adverse Events and AIDS defining events
Both study treatments were generally well tolerated (Table 2). Five patients withdrew from the study because of an adverse event: In two patients a non-Hodgkin lymphoma was diagnosed, requiring systemic antineoplastic chemotherapy. One patient developed severe anemia, one patient was intolerant to AZT (nausea) and one patient had *Pneumocystis carinii* pneumonia. There were three deaths among the study patients (Table 2).

The incidence of adverse events with moderate or severe intensity shown in table 2. The most common adverse event experienced by the patients was

![Figure 1](image.png)

*Figure 1. Median plasma HIV RNA levels during the initial 24 weeks of the study. Median values are shown. Bars are 25th and 75th percentiles.*
Figure 2A. Proportion of patients with plasma HIV RNA levels of less than 400 copies / milliliter.

Figure 2B. Proportion of patients with plasma HIV RNA levels of less than 50 copies / milliliter.
mild-moderate gastro-intestinal discomfort (dyspepsia, nausea, flatulence, abdominal pain or diarrhoea) with no difference in the reporting of symptoms between the two treatment groups.

There were 8 new AIDS defining events during the first 24 weeks of the study, with no difference in the incidence between treatment groups (p=0.45)(table 2).

Virologic Failures
In four patients treatment failure was observed (one patient receiving indinavir, three receiving SQV-SGC). Treatment failure was defined as absence of virologic response or having rebound of HIV RNA levels above 400 copies/ml on two or more consecutive study visits following a virological response. Virological response was defined as achieving HIV RNA levels < 400 copies/ml.

One patient from the indinavir-arm with rebound of plasma HIV RNA levels reported intermittent adherence to the study regimen. Later on, this patient adhered to the study regimen as recommended and again achieved plasma HIV RNA levels < 400 copies/ml.
Two patients from the SQV-SGC arm with rebond of plasma HIV RNA levels had low saquinavir plasma levels on several study visits although patient report and pill count did not show low adherence to study medications. On several occasions, estimated saquinavir trough levels in these two patients were less than 50 ng / ml, the calculated EC90 of saquinavir in vivo (28). In one of these patients, malabsorption of SQV-SGC was confirmed by recording a full eight-hour pharmacokinetic curve after observed intake of SQV-SGC with meal.

One patient in the SQV-SGC arm did not achieve plasma HIV RNA levels below 400 copies/ml at any time during the initial 24 weeks of treatment. Adequate saquinavir plasma levels could be detected in this patient. To determine to what extent drug resistance contributed to the treatment failure, genotypic resistance measurements were performed on plasma samples from baseline and week 18. At baseline, no drug resistance mutations were observed in the reverse transcriptase (RT) gene or the protease gene of the HIV RNA population derived from this patient's plasma. At week 18, the HIV RT gene harbored the M184V mutation, conferring high level resistance to 3TC. The absence of a virological response in this patient may be best explained by the high HIV RNA plasma levels before initiation of therapy (day -14: 3,200,000 copies/ml and day 0: 11,500,000 copies/ml).

**Discussion**

This trial has a unique design: it is the first randomized prospective trial in HIV infected patients comparing two different protease inhibitors given as part of a triple therapy with the same RT-inhibitor background. Both regimens displayed a powerful antiretroviral effect. We observed no significant difference in antiretroviral activity between the SQV-SGC group and the indinavir group during the initial 24 weeks of the study. At week 24, 74.3% of the intention-to-treat population in the SQV-SGC group and 71.4% in the indinavir group had plasma HIV RNA levels below 50 copies/ml. Eight AIDS defining illnesses were diagnosed with no difference in incidence between the two treatment groups.
Surprisingly, a significantly higher CD4 cell response during the first 24 weeks was observed in the SQV-SGC group as compared with the indinavir group, but preliminary data from the post 24 week period show that this difference may disappear at week 32 of treatment. At week 32, mean CD4 change from baseline (±SEM) was 168±24 cells / μL in the SQV-SGC group (n=23) and 169±25 cells / μL in the indinavir group (n=26).

The explanation for this difference in immunologic response during the first 24 weeks remains unclear. Low baseline CD4 count and large magnitude of viral load reduction during treatment are two determinants of good initial CD4 response during HAART (7,8,9). However, neither baseline CD4 count nor virus load reduction differed between the two treatment groups and therefore these parameters do not explain the difference in CD4 cell response. The rise of CD4+ T cells in blood during HAART has been explained by two mechanisms: proliferation of CD4+ T lymphocytes (7) and/or the recirculation of CD4 T lymphocytes which resided in lymphoid tissue prior to therapy (9). Alternatively, the CD4 count increase during HAART may be explained by a direct influence of antiretroviral agents on recirculation or proliferation of lymphocytes. In needlestick-injured personnel given AZT prophylaxis for one month increases of CD4 counts have been reported (10,11). However, administration of indinavir in two healthy volunteers did not result in a rise of CD4 counts (12). We hypothesize that each antiretroviral drug may have a direct pharmacologic effect on either lymphocyte proliferation and/or trafficking and that saquinavir-SGC may be different from indinavir in this aspect, which may explain the initial difference of the CD4+ T lymphocyte response.

Although the majority of patients achieved plasma HIV RNA levels below 400 copies/ml in both treatment groups, three patients (one receiving indinavir, two receiving SQV-SGC) had rebound of HIV RNA levels above 400 copies/ml on two or more consecutive study visits. Non compliance explains the viral rebound in the patient from the indinavir-arm. In two patients from the SQV-SGC arm, the viral rebound may be explained by low saquinavir plasma levels. In one of these two patients, malabsorption of SQV-SGC was observed.
One patient (receiving SQV-SGC) did not achieve plasma HIV RNA levels below 400 copies/ml, probably due to the extremely high baseline HIV RNA plasma levels. The potency of AZT/3TC/SQV-SGC was not sufficient to prevent residual HIV replication and emergence of resistant virus variants in this patient with a high HIV replication level. In several studies it was demonstrated that high baseline viral loads are associated with less efficient viral suppression (13,14) and a slower rate of HIV clearance from plasma (15). As it has been shown that a five drug regimen provides improved suppression of HIV replication over triple therapy (16), five (or four) drug regimens may be required to attain a durable antiviral response in patients with high pretreatment plasma HIV RNA levels.

In summary, we showed by head-to-head comparison that during the initial 24 weeks of treatment saquinavir soft gelatin capsule formulation shows equivalent antiretroviral efficacy to indinavir, when given as part of a triple therapy containing zidovudine and lamivudine, in antiretroviral-naive HIV-1-infected patients. SQV-SGC therefore is an appropriate choice of protease inhibitor for first line therapy. SQV-SGC is generally well tolerated, which is crucial for long term adherence to antiretroviral regimens. Prolongation of follow up is required to determine whether over the long term SQV-SGC and indinavir may differ with respect to toxicity, antiviral potency or immunologic effect. Studies on a large population may be performed to confirm our findings.

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