A Pilot Study of Nevirapine, Indinavir, and Lamivudine among Patients with Advanced Human Immunodeficiency Virus Disease Who Have Had Failure of Combination Nucleoside Therapy

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The effects of nevirapine, indinavir, and lamivudine in combination were studied among 22 human immunodeficiency virus (HIV)-infected patients with CD4 cell counts ≤50/mm³, whose options for antiretroviral therapy were limited by clinical or laboratory failure or toxicity with previous regimens. Median plasma HIV RNA was 5.16 log₁₀ copies/mL at baseline, decreasing by a median of 3.12 log₁₀ copies/mL at 24 weeks. Median baseline CD4 cell count was 30/mm³, increasing by a median of 95/mm³ at week 24. Adverse reactions led to drug discontinuation in 4 cases. Steady-state pharmacokinetic analysis in 17 patients was consistent with an interaction between nevirapine and indinavir. Nevirapine plasma levels were within the expected range, while indinavir levels were lower than expected. Despite this interaction, the combination of nevirapine, indinavir, and lamivudine was safe and well-tolerated and had substantial antiviral and immunologic effects lasting for the 24-week study.

Combination antiretroviral therapy has been clearly shown to be superior to monotherapy [1–3]. More recently, triple-drug combinations including two nucleoside analogues plus a nonnucleoside reverse transcriptase inhibitor or a protease inhibitor have demonstrated superiority to double-nucleoside therapy in terms of surrogate marker effect and clinical outcome [4–7]. On the basis of these results, triple-drug combination therapy is widely recommended for the treatment of human immunodeficiency virus (HIV) infection [8–11].

Concomitant use of drugs, however, increases the chance of pharmacokinetic interactions. With the increasing use of protease inhibitors and nonnucleoside reverse transcriptase inhibitors, concern has arisen regarding possible pharmacokinetic interactions between them [12–14]. Nevirapine is a hepatic enzymatic inducer and as such would be expected to decrease the effective serum concentrations of protease inhibitors [15]. Only recently has preliminary information become available concerning the effect of nevirapine on the pharmacokinetics of currently available protease inhibitors. As expected, nevirapine was found to reduce the area under the curve (AUC) for indinavir, ritonavir, and saquinavir by ~10%–30% when administered in combination [13, 14]. However, the clinical and virologic implications of this interaction have not been fully characterized.

Therefore, we conducted a 24-week pilot study to assess the safety, antiviral and immunologic effects, and pharmacokinetics of nevirapine, indinavir, and lamivudine administered in combination among patients with advanced HIV disease who have had failure of combination nucleoside therapy.

Methods

Patients. HIV-infected patients with CD4 cell counts ≤50/mm³ were eligible if they had demonstrated intolerance, toxicity, or disease progression with nucleoside analogue–based antiretroviral therapy. Eligible patients had no prior exposure to nevirapine or indinavir. Prior use of lamivudine was allowed.

Study design. This was a prospective, open-label study conducted within the framework of the expanded access programs for nevirapine and indinavir. Patients received a combination of nevirapine, indinavir, and lamivudine in standard doses commenced simultaneously. During 24 weeks of study treatment, patients were monitored for safety by assessment of clinical events and standard laboratory parameters, for antiviral response by measurement of plasma HIV RNA, and for immunologic response by measurement of CD4 cell counts. Pharmacokinetics were assessed by measuring peak and trough plasma levels of nevirapine and indinavir on a single day after 6 weeks of study therapy. The impact of the pharmacokinetic interaction was assessed by comparison with previously published data, as historical controls.

Drug therapy. Patients received treatment with a combination of nevirapine, indinavir, and lamivudine for 24 weeks. Nevirapine was given at a dose of 200 mg daily for 2 weeks, escalating to...
Clinical follow-up. A medical history and physical examination were completed at the screening visit (4–8 weeks before starting study therapy) and again at the baseline visit and at weeks 1, 2, 4, 6, 8, 12, 16, 20, and 24. Information regarding adverse events and HIV-related illnesses was collected at each clinic visit.

Laboratory monitoring. Plasma HIV RNA was quantitated with the Amplicor HIV-1 Monitor assay (Roche Molecular Systems, Branchburg, NJ) from suitable specimens collected twice at baseline and at weeks 1, 2, 4, 6, 8, 12, 16, 20, and 24. Specimens having readings <500 copies/mL by the standard Amplicor assay were retested with the Ultra Direct assay, which has a lower limit of quantification of 20 copies/mL [16]. CD4 cell counts were done twice at baseline and at 2- to 6-week intervals thereafter. Routine hematology and chemistry testing were done at baseline and every 4 weeks thereafter.

Pharmacokinetic sampling. Patients who received study drugs for a minimum of 6 consecutive weeks during the first 16 weeks of the study were eligible for pharmacokinetic sampling. This was done four times on a single day. Patients were requested to take their usual oral doses of nevirapine at 8:00 P.M. and indinavir at 11:00 P.M. the night before testing, while they continued to take lamivudine on a regular schedule. The following day, a sample was drawn for trough nevirapine and indinavir levels at 7:00 A.M. 200 mg twice daily thereafter. Indinavir was given at 800 mg every 8 h on an empty stomach. Lamivudine was given at 150 mg twice daily. Concurrent use of other antiretroviral therapies was not allowed. No washout period was required before starting study therapy.

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Table 1. Indinavir, nevirapine, and lamivudine for advanced HIV disease: baseline characteristics of study patients.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Pharmacokinetic subgroup</th>
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<tr>
<td>Number</td>
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<td>17</td>
</tr>
<tr>
<td>Male/female</td>
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<td>17/0</td>
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<tr>
<td>HIV RNA, median (range)</td>
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<tr>
<td>CD4 cell count, median</td>
<td>cells/mm³</td>
<td>30 (10–50) 35 (10–50)</td>
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<tr>
<td>CD8 cell count, median</td>
<td>cells/mm³</td>
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<tr>
<td>Previous protease inhibitor experience</td>
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<td></td>
</tr>
<tr>
<td>Previous nonnucleoside reverse transcriptase inhibitor experience</td>
<td>2 (loviride) 1 (loviride)</td>
<td></td>
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<td>Previous lamivudine</td>
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<td>AIDS diagnosis</td>
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Figure 1. Median changes in CD4 cell counts and plasma HIV RNA (pVL) versus time in patients during study drug treatment. Bars represent 25th and 75th percentiles. CD4 cell counts are not included at 1, 2, and 8 weeks because n < 10.
indinavir level was obtained at 4:00 P.M. Patients continued to take their usual concomitant medications on the sampling day. Plasma was separated and frozen within 1 h of drawing and stored at −70°C for batch testing.

Plasma samples were analyzed for nevirapine concentrations at Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT) by use of a validated high-performance liquid chromatographic assay with UV detection (wavelength = 280 nm). Standard curves for the analytical method covered the range of 25–10,000 ng/mL. The limit of assay quantitation was 25 ng/mL. Quality control samples analyzed with each analytical run had coefficients of variation for precision and accuracy of <15%. Plasma samples were analyzed for indinavir concentrations at BAS Analytics (West Lafayette, IN) by a proprietary validated high-performance liquid chromatographic assay with a limit of assay quantitation of 12 ng/mL.

Statistics. Median CD4 cell counts and plasma HIV RNA measurements were calculated at baseline for the entire group and for the subset of patients included in the pharmacokinetic analyses. At every follow-up visit, the change shown by each person from his baseline CD4 cell count and plasma HIV RNA level was calculated. The median of these changes at each time point is expressed as “median change.” The proportions of patients with plasma HIV RNA measurements <500 copies/mL and <20 copies/mL were also tabulated at each follow-up visit. In cases in which Amplicor HIV-1 test results were <500 copies/mL, Ultra Direct results were used in their place. Ultra Direct results below the quantitation limit of this assay (20 copies/mL) were set to 20.

The cumulative antiviral treatment effect over 24 weeks, or the proportion of potential improvement in plasma HIV RNA achieved by each patient, was defined as 1 − NAUC (normalized AUC) [17, 18]. A person with no change from the baseline HIV RNA value over the 24-week observation period would have a value of 0, while a person whose plasma HIV RNA was reduced to the quantitation limit of the Ultra Direct assay very quickly would have a value close to 1. A negative value would result from increases in virus load relative to baseline, if the duration and size of the increases were large enough to counteract any decreases.

Results for drug levels are reported as means ± SDs. The relationships between peak and trough levels of nevirapine and indinavir and patients’ virologic response to therapy, as measured by 1 − NAUC, were summarized with scatter plots and Spearman’s correlation coefficients. For indinavir, for which two values for peak and trough were available for each patient, the correlation was examined for 1 − NAUC and absolute peak or trough levels (i.e., the higher of the two peaks and the lower of the two troughs). Lowess (locally weighted scatter plot smoother) curves were drawn on the plots to summarize the relationships of pairs of variables. The loess curve, which is akin to a moving average, was chosen instead of fitting a straight line to the data, since the relationships were nonlinear [19].

Results

Patients. A total of 22 patients were enrolled, and 17 were available for the pharmacokinetic study (table 1). Only 1 patient had previously used a protease inhibitor (ritonavir), and the drug had been discontinued after 14 weeks because of dizzy-
ness, hot flashes, fatigue, and diarrhea. Two patients had previously received loviride (an experimental nonnucleoside reverse transcriptase inhibitor) as part of their participation in the CAESAR trial [3]. All patients except 3 had prior experience with lamivudine.

There were no withdrawals attributable to clinical progression or laboratory treatment failure (i.e., increasing plasma virus load) during the study period. Three patients withdrew for personal reasons, unrelated to toxicity or lack of efficacy.

Safety. No unexpected clinical or laboratory adverse events occurred during the study.

No serious (grade ≥3) clinical adverse events to the study medications were encountered during the study. Adverse events of moderate severity (grade 2) led to the discontinuation of nevirapine or indinavir in 4 patients. In 2 of these cases, the events (nausea/vomiting and rash) were attributed to nevirapine, and in the other 2 cases the events (urinary frequency/nocturia and nausea/vomiting) were attributed to indinavir. No cases of nephrolithiasis were observed. A single new AIDS-defining opportunistic infection occurred during the 24-week follow-up period: a case of Pneumocystis carinii pneumonia diagnosed during week 3 of the study in a patient with a previous AIDS diagnosis.

Two grade ≥3 laboratory toxicities occurred during the study. One patient developed hyperbilirubinemia (maximum level, 65 μmol/L = 3.8 mg/dL), which resolved after a 4-day interruption of indinavir; therapy was resumed without recurrence. Another patient developed worsening of preexisting neutropenia (decreasing from 900 at baseline to 300 cells/mm³ at week 16 during therapy). Thereafter, lamivudine was replaced by stavudine, while indinavir and nevirapine were continued. Pharmacokinetic sampling was done for this patient before the change in therapy. No cases of chemical hepatitis were observed.

Virologic response. Plasma virus load decreased rapidly from baseline, and this decrease was maintained for the 24-week study period (figure 1). The median plasma virus load was 5.16 log₁₀ at baseline. Median changes in plasma virus load were −2.42 log₁₀ copies/mL at 4 weeks, −3.04 log₁₀ copies/mL at 12 weeks, and −3.12 log₁₀ copies/mL at 24 weeks. The plasma virus load changes in the pharmacokinetic subgroup followed a similar pattern (data not shown).

Virus load was suppressed to below the limits of quantitation of the HIV RNA assays in a substantial proportion of patients (figure 2). At week 24, 73% of patients (11/15) had plasma virus load <500 copies/mL, and 40% (6/15) of patients had plasma virus load <20 copies/mL.

The patient with prior ritonavir experience and 1 of the patients with prior loviride experience showed good virologic responses to the study combination, achieving plasma virus loads of 33 and <20 copies/mL, respectively, at week 24. The other loviride-experienced patient withdrew early because of a nevirapine-induced rash. With regard to lamivudine, 10 of 11 patients with virus load <500 copies/mL and all 6 with virus load <20 copies/mL at 24 weeks had received this agent before the study.

Immunologic response. CD4 cell counts increased substantially over the 24-week study period (figure 1). The median CD4 cell count was 30/mm³ at baseline. The median change in CD4 cell count was +30 cells/mm³ at week 4 and +95 cells/mm³ at week 24. Changes in CD4 cell counts for the 17 patients eligible for the pharmacokinetic study followed a similar pattern (data not shown).

Pharmacokinetics. Observed nevirapine plasma levels varied little between patients and within individuals during the course of the day and were not affected by coadministration of indinavir (figure 3). Nevirapine peak was 21.6 ± 8.3 μM and nevirapine trough was 18.3 ± 5.7 μM. These values did not differ significantly from published data for nevirapine monotherapy [20] (P = .074 and .25, respectively).

In contrast, observed indinavir plasma levels varied widely between patients and within individuals during the course of the day and appeared to be reduced substantially in the presence of nevirapine (figure 4). Indinavir peak levels were 6401 ± 4416 nM for the morning dose and 3285 ± 3373 nM for the afternoon dose. These represent 51% and 26%, respectively, of the published levels for indinavir when given alone [21] (P < .001 and < .001, respectively). The observed indinavir trough levels were 109 ± 66.6 nM in the morning and 109 ± 56.3 nM in the afternoon, representing 43% of published levels for indinavir monotherapy [21] (P = .0075 and .0068, respectively).

Correlation of pharmacokinetics and virologic responses. The cumulative antiviral effect, represented by 1 − NAUC for HIV RNA over 24 weeks, did not show a statistically significant correlation with nevirapine peak (Spearman’s correlation coeffi-

![Figure 3](https://example.com/figure3.png)
Figure 4. Indinavir peak (A) and trough (B) plasma levels for morning (AM) and afternoon (PM) draws for study patients (n = 17). Solid circles represent individual patient samples; mean value is indicated by horizontal line. Peak and trough levels (mean ± SD) are also shown from published data for indinavir, 800 mg every 8 h as monotherapy (peak, 12,617 ± 4037 nM; trough, 251 ± 178 nM; n = 16) [21].

cient = .27, P = .29), with nevirapine trough (correlation coefficient = .38, P = .13), nor with absolute indinavir peak levels (correlation coefficient = .42, P = .10). However, a statistically significant correlation was found between the 24-week cumulative antiviral effect and the absolute indinavir trough levels (correlation coefficient = .53, P = .03) (figure 5).

Discussion

The results of this pilot study demonstrate that standard doses of nevirapine, indinavir, and lamivudine are generally well-tolerated and can lead to substantial reductions in plasma virus load and increases in CD4 cell count. This effect was demonstrated among patients with advanced HIV disease who previously had either disease progression or virologic failure while receiving nucleoside analogue–based combination therapy, often including lamivudine. In addition, our data support the existence of a pharmacokinetic interaction between nevirapine and indinavir, characterized by indinavir peak and trough concentrations being significantly lower than expected compared with historical controls; however, this interaction does not preclude a substantial immunologic and virologic response.

The management of HIV-infected persons continues to evolve at a rapid pace. Now that the single-drug therapy strategy is abandoned, multiple-drug combinations are currently used with the aim of suppressing viral replication as much as possible for as long as possible [6–11]. Patients who started treatment before these guidelines were developed often face difficult challenges when making therapeutic decisions. It is within this context that in the late spring of 1996, having gained access to two new promising antiretroviral agents, nevirapine and indinavir, we offered this combination to persons who had exhausted conventional treatment approaches. Because of the potential for drug interactions, we did so under close clinical and laboratory monitoring within this pilot study. Further, we incorporated steady-state peak and trough drug level determinations in an attempt to correlate pharmacokinetic variables with clinical laboratory changes and particularly with virologic effect. We elected to retain lamivudine within the therapeutic regimen despite the fact that a majority of the study participants had previously demonstrated evidence of treatment failure with this agent and likely carried drug-resistant virus isolates. Given the very favorable safety profile of lamivudine, we felt that retaining it would not compromise the safety of the combination regimen [3, 22–25]. Furthermore, there has been some evidence suggesting that HIV strains resistant to lamivudine may have decreased fitness [26]. In addition, in vitro evidence has been generated suggesting that there may be a synergistic interaction between lamivudine and nevirapine [27].

We found that nevirapine, indinavir, and lamivudine given in combination had very substantial antiviral and immunologic effects. Plasma virus load decreased rapidly on initiation of therapy, leading to a median reduction in plasma HIV RNA of >3 log10 copies/mL, which remained for the 24 weeks of the study. This was associated with a median increase in CD4 cell count of 95 cells/mm³, which remained at 24 weeks. The magnitude of these immunologic and virologic responses is particularly encouraging when considering the very advanced stage and extensive prior antiretroviral therapy use of our study group. Previous exposure to a nonnucleoside reverse transcriptase inhibitor (2 patients), a protease inhibitor (1 patient), or lamivudine (19 patients) did not preclude a favorable response to the combination. Furthermore, unexpected adverse effects were not encountered within this group of patients.

Our pharmacokinetic data support the existence of an interaction between nevirapine and indinavir, as recently reported [13]. This interaction is characterized by plasma concentrations of indinavir (both peak and trough levels) being significantly lower than expected in comparison to historical controls. Limited pharmacokinetic sampling in our study precludes a precise
estimation of the magnitude of the interaction. However, our results are consistent with those encountered in the context of pharmacokinetic interaction studies in which coadministration of nevirapine and indinavir led to an ~30% reduction in indinavir AUC [13].

A substantial degree of variability in the peak and trough concentrations of indinavir is illustrated by our data. This finding is consistent with available data from intensive pharmacokinetic studies [21]. Comparing the pharmacokinetic parameters encountered for our patients with those reported for patients receiving indinavir monotherapy, it is evident that although indinavir levels are significantly lower in the presence of nevirapine, there is a substantial degree of overlap with indinavir levels achieved without nevirapine. Taken together, these data suggest that a strategy of adjusting indinavir dose based on measurements of trough serum concentrations should be further evaluated, whether or not nevirapine is included in the regimen.

Our data also illustrate a relatively small variation between the peak and trough concentrations for nevirapine. These results are consistent with previous reports [20] and lead us to speculate that nevirapine could be effectively used in a once-daily regimen, in combination with other antiretroviral agents. Clinical trials assessing this issue are currently underway.

We performed an exploratory analysis in an attempt to characterize the relationship between pharmacokinetic parameters and antiviral effect. When the cumulative antiviral effect (represented by $1 - \text{NAUC}$ for HIV RNA over 24 weeks) was plotted against pharmacokinetic parameters for nevirapine and indinavir, a statistically significant correlation was present only for the absolute indinavir trough (figure 5). The higher the absolute trough level of indinavir for an individual within the study group, the greater tended to be the cumulative antiviral effect achieved by that individual over the 24-week study period. The results of this exploratory analysis suggest that increasing indinavir dose in some patients may optimize antiviral effect when given in combination with nevirapine. This issue needs to be further evaluated in prospective clinical trials.

In summary, our results demonstrate that standard doses of nevirapine, indinavir, and lamivudine given in combination are generally safe and well-tolerated and have substantial antiviral and immunologic effects among advanced, heavily pretreated HIV-infected patients. Our data also support the existence of a pharmacokinetic interaction between nevirapine and indinavir. The clinical implication of this interaction and the role of dosage adjustments for indinavir merit further study.

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References


