Protease Inhibitor Resistance Analysis in the MONARK Trial Comparing First-Line Lopinavir-Ritonavir Monotherapy to Lopinavir-Ritonavir plus Zidovudine and Lamivudine Triple Therapy

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The MONARK study was a pilot randomized trial comparing the safety and efficacy of lopinavir-ritonavir (LPV/r) monotherapy to those of LPV/r-zidovudine-lamivudine triple therapy for antiretroviral-naïve human immunodeficiency virus type 1 (HIV-1)-infected patients. Resistance testing was performed at the time of initial screening and at the time of virological failure (defined to include low-level viremia with >50 and <400 HIV-1 virus RNA copies/ml of plasma). Changes from the baseline sequences, including mutations noted on the 2008 International AIDS Society—USA list of resistance-associated protease mutations, were considered. Drug resistance testing was performed for 38 patients (5 of 53 on triple therapy and 33 of 83 on monotherapy). By week 96 (W96), virus samples from 18 of 33 patients in the monotherapy arm showed changes from baseline sequences, and 5 of these patients had viruses with major protease inhibitor (PI) resistance-associated mutations (M46I at W40, L76V at W48, M46I and L76V at W48, L10F and V82A at W72, and L76V at W84). Data on virus phenotypes detected at the time of initial screening and at the time of virological failure were available for four patients in whom major PI resistance mutations developed, and these data revealed a mean increase of 2.2-fold (range, 0.75- to 4.6-fold) in the LPV 50% inhibitory concentration. All three patients in whom the L76V PI resistance mutation developed were infected with HIV-1 subtype CRF02_AG. In the triple-therapy group, no major PI resistance mutation was selected among the three patients with protease changes by W48. No association between the baseline CD4 cell count and the viral load, the W4 and final viral loads, or the final LPV trough concentration and the emergence of a major PI resistance mutation was found. Major PI resistance-associated mutations were detected in 5 (6%) of 83 patients treated with LPV/r monotherapy, suggesting that LPV/r monotherapy is an inappropriate first option. The mutation L76V may be considered in further studies of lopinavir resistance.

Highly active antiretroviral therapy (HAART) has dramatically improved the prognoses of patients infected with human immunodeficiency virus type 1 (HIV-1). However, because of the absence of HIV-1 eradication, patients require lifelong therapy, making long-term cumulative toxicity and cost critical issues in the treatment of HIV infection. Maintaining effective therapy is mandatory to avoid viral replication and subsequent drug resistance emergence.

In the context of simplification, different strategies of boosted-protease-inhibitor (boosted-PI) monotherapy have been evaluated. Lopinavir-ritonavir (LPV/r) was evaluated for use in these simplification strategies on the basis of its virological potency and high barrier to the development of resistance. Previous analyses of induction/maintenance strategies showed that, after full viral suppression is obtained with HAART, the efficacy of maintenance with LPV/r monotherapy is comparable to that with triple therapy (1, 2, 18). Another approach is to use boosted-PI monotherapy initially as a first-line regimen, thereby avoiding nucleoside reverse transcriptase inhibitor (NRTI) exposure entirely. The MONARK study was a prospective, open-label, randomized trial comparing the safety and efficacy of LPV/r monotherapy to those of a standard regimen of LPV/r plus zidovudine (ZDV) and lamivudine (3TC) as an initial treatment for 48 weeks (3). The main results demonstrated a lower rate of virological suppression by LPV/r monotherapy than by the LPV/r-based triple-drug regimen at week 48 (W48). Patients randomized into the LPV/r monotherapy arm were offered the option of remaining on the study treatment and were monitored up through W96.

In the context of antiretroviral monotherapy, it is of major concern to study the risk of drug resistance emergence. Indeed, combination therapy with LPV/r rarely selects for PI resistance in antiretroviral-naïve patients (13). The prevalences of PI
resistance during single-drug maintenance therapy with LPV/r in the previously mentioned randomized studies were comparable to those observed during LPV/r-based triple therapy, with the selection of major PI resistance mutations such as M46I, V82A, and L90M (1, 2, 18). In the first 48 weeks of the MONARK study, resistance mutations in the HIV protease gene were detected in 3 (3.6%) of 83 patients receiving LPV/r monotherapy and resistance mutations in the HIV reverse transcriptase gene were detected in 1 of 53 patients receiving LPV/r triple therapy (3). The objectives of the present study were to analyze the PI resistance outcomes for patients in the MONARK study up through the 96-week follow-up period and to identify risk factors associated with PI resistance selection in patients receiving LPV/r monotherapy.

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MATERIALS AND METHODS

MONARK study design. The MONARK study design has been described elsewhere (3). Briefly, patients were randomly assigned to receive first-line LPV/r monotherapy or LPV/r plus ZDV-3TC if they were naive with respect to antiretroviral therapy, had a CD4 cell count above 100 cells/mm³, a plasma HIV-1 RNA load below 100,000 RNA copies/ml, and no evidence of drug resistance at the screening visit. The primary end points were the reductions of plasma HIV-1 RNA loads to <400 copies/ml at W24 and <50 copies/ml at W48. Follow-up until W96 was planned for the evaluation of the long-term safety and efficacy of the LPV/r monotherapy. A suboptimal response was defined as (i) failure to achieve a decline in the viral load of at least 1.0 log₁₀ copies/ml by W4, (ii) failure to achieve a viral load below 400 copies/ml by W24, and (iii) any viral rebound of ≥1 log after the achievement of a plasma HIV-1 RNA load of <400 copies/ml, confirmed by a second measurement at least 14 days later.

Resistance testing and pharmacological measurements. Genotypic resistance testing for all participants was centralized at the Necker Virology Laboratory and was performed by population-based sequencing according to the ANRS method (4). Resistance tests were performed at the time of screening and at the time of virological failure, as previously recommended in the protocol. The resistance analysis was extended to include patients with low-level viremia (a virus load between 50 and 400 copies/ml), both those that did not reach an HIV-1 RNA load of <50 copies/ml at W24 and those that had a confirmed rebound of the HIV-1 RNA load to >50 copies/ml after reaching an HIV-1 RNA load of <50 copies/ml at W24. For patients with these episodes of low-level viremia, the samples obtained at the time of failure were analyzed. If mutations were detected, the results were compared to those for previously tested samples. Any change in the protease gene between screening and failure, including minor and major protease mutations identified previously by the International AIDS Society—USA (IAS), was noted (12).

Phenotypic resistance tests of all available samples from participants in whom at least one major PI resistance mutation was selected were performed at the baseline and at the time of virological failure by using the PhenoSense GT system (Monogram Biosciences, San Francisco, CA).

The LPV concentration in plasma before drug intake was measured by a validated high-performance liquid chromatography assay as described previously (3).

Viral subtype determination. The HIV-1 subtype was determined by phylogenetic analyses of reverse transcriptase sequencing (600 bp). The sequences were aligned with those from 37 HIV-1 reference strains (www.hiv-web.lanl.gov) according to the different HIV-1 group M subtypes by using the Clustal X program.

Statistical analyses. Logistic regression was used to analyze risk factors associated with any change in the protease gene from the time of screening, both minor and major IAS-identified PI resistance mutations, selected during LPV/r monotherapy or triple therapy. The investigated variables included the baseline HIV-1 RNA load, the baseline CD4 cell count, the viral subtype, the viral load reduction at W4, the last-measured viral load, and the LPV trough concentrations within 4 weeks of genotype testing.

RESULTS

Eighty-three patients were randomized to receive LPV/r monotherapy, and 53 were randomized into the LPV/r triple-therapy arm. In the LPV/r monotherapy arm, 33 patients were qualified for genotypic resistance testing. Of these 33 patients, 18 patients had samples showing a change in the protease gene from the time of screening. Five of 18 had a virus with a major PI resistance mutation. In the LPV/r triple-combination arm, among the five patients qualified for genotypic testing, three had a virus with a change in the protease gene and no patient had a virus with a major PI resistance mutation during the follow-up period of 48 weeks.

With respect to HIV-1 subtype distribution, the two treatment groups were well balanced at the baseline, with the majority of patients (68% of patients receiving LPV/r monotherapy and 56% of those receiving LPV/r triple therapy) having subtype B viruses. Non-B subtypes were distributed among patients in the LPV/r monotherapy and LPV/r triple-therapy arms as follows: subtype CRF02_AG, 16 and 12%; subtype A, 2 and 14%; subtype C, 4 and 2%; and other subtypes, 10 and 16%, respectively. The difference between the two arms in the proportion of patients with subtype A was due probably to the low numbers of subtype A-positive patients (one in the monotherapy group and two in the triple-therapy group). In the monotherapy group, of the five viruses with major PI resistance mutations, two belonged to the B subtype and three belonged to the CRF02_AG subtype.

Figure 1 depicts virological and pharmacological outcomes for the five patients with the emergence of major PI resistance mutations during LPV/r monotherapy. Major PI resistance mutations were detected between W40 and W90. For three patients, the detection of major PI resistance mutations occurred before or at W48, and for the last two patients, it occurred during the long-term follow-up period through W96. In these five patients, low levels of viremia ranging between 2.5 and 3.1 log₁₀ copies/ml were observed. Protease mutations M46I and V82A have been found previously in patients experiencing the failure of triple-combination therapy including LPV/r. Interestingly, in three of the five patients, the protease mutation L76V had been selected, and all three were infected with the HIV-1 CRF02_AG subtype. The testing of samples from four patients for phenotypic susceptibility to LPV was performed at the initial screening and at the time of failure. A mean increase of 2.2-fold (range, 0.75- to 4.6-fold) of the 50% inhibitory concentration of LPV was observed. In one patient (no. 3002), the trough plasma LPV concentration was measured at 1,433 ng/ml before a small increase in the viral load occurred (Fig. 1). Among the five patients in whom major PI resistance mutations developed, three had viral loads intensifi-
iation by 4 patients, viral loads below 50 copies/ml in 9 patients, a missing viral load measurement in 1 patient, and viral loads above 50 copies/ml in 2 patients (11).

Baseline viral loads and CD4 cell counts, early virological responses (at W4), and final viral loads and LPV trough concentrations were investigated to identify risk factors for the selection of minor or major PI resistance mutations (Table 1). No significant difference in these variables was found among the 15 patients with no change in the protease gene, the 13 patients with new but no major PI resistance mutations, and the 5 patients in whom the selection of a major PI resistance mutation occurred. The patterns of distribution of HIV-1 subtypes between patients with and without major PI resistance mutations were not significant. Similarly, no significant association was found between the same variables (the viral load, CD4 cell count, viral subtype, and trough plasma LPV concentration) and the occurrence of protease changes in patients treated with the LPV/r triple therapy during the 48-week period of follow-up with this arm.

FIG. 1. Virological and pharmacological outcomes for the five patients in the LPV/r monotherapy arm with major PI resistance mutation selection. Sequences of protease (PRO) and reverse transcriptase (RT) were available at screening and at the time of virological failure. Major mutations that emerged are indicated in bold. NA, not available; IC50, 50% inhibitory concentration; Ctrough, trough concentration in plasma; c/ml, copies per milliliter.
**TABLE 1.** Factors not associated with protease mutation selection

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. of patients</th>
<th>Baseline*</th>
<th>Change in viral load*</th>
<th>Last measurement of:</th>
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<td></td>
<td></td>
<td>Viral load (log_{10} copies/ml)</td>
<td>CD4 cell count (cells/mm³)</td>
<td>Viral load (log_{10} copies/ml) at W4</td>
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<td>LPV/r monotherapy group</td>
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<td>Patients with virus sequences showing no change from time of screening</td>
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<td>-1.95</td>
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<td>4.5</td>
<td>222</td>
<td>-2.08</td>
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<td>4.8</td>
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<td>-2.11</td>
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<tr>
<td>LPV/r-ZDV-3TC group</td>
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<tr>
<td>Patients with virus sequences showing no change from time of screening</td>
<td>2</td>
<td>4.6</td>
<td>242</td>
<td>-1.6</td>
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<tr>
<td>Patients with virus sequences showing a new mutation but no major IAS mutation</td>
<td>3</td>
<td>4.3</td>
<td>180</td>
<td>-2.04</td>
</tr>
</tbody>
</table>

* Values shown are medians.

** Values in parentheses are the number of patients meeting the criterion among the number of patients from whom samples were available for testing.

**DISCUSSION**

To our knowledge, this is the first randomized study including patients receiving LPV/r monotherapy with a follow-up period of at least 96 weeks. Five (6%) of the 83 patients included in the LPV/r monotherapy arm and monitored for 96 weeks and none of the 53 patients included in the LPV/r triple-therapy arm and monitored for 48 weeks exhibited the selection of major PI resistance mutations. The emergence of protease mutations occurred late, between W40 and W90, at a low level of viremia but after several weeks of almost continuous viral replication. It is important that intermittent viremia (an HIV-1 RNA load of more than 50 but less than 500 copies/ml) was evident in a higher proportion of patients in the LPV/r monotherapy arm than in the triple-combination arm (3), not only in this study of antiretroviral-naïve patients but also in maintenance studies of patients with previously suppressed viral loads (1, 2, 18). However, patients with advanced infections, high viral loads (above 100,000 copies/ml), and low CD4 cell counts (below 100 cells/mm³) were excluded from this study. Prolonged periods of low-level viremia may favor the development of resistance mutations, even with boosted LPV. The mutations selected yielded neither significant phenotypic nor full genotypic resistance to LPV/r at the time of failure. Indeed, the high genetic barrier of LPV/r against resistance generally requires the accumulation of a high number of mutations to confer PI resistance. Nevertheless, our results showed that the process of PI resistance development is ongoing even with low-level viremia. Moreover, in our study, resistance was detected even by a population-based sequencing method that is not an ultrasensitive approach. Recently, in the LPV/r maintenance therapy OK04 study, viral variants carrying major LPV resistance mutations at the time of virological rebound were detected more frequently by single-genome sequencing than by standard population genotyping (15).

The barrier for the selection of PI resistance with LPV/r monotherapy appeared to be lower than that with the LPV/r-based three-drug regimen. The first hypothesis to explain this observation is that the absence of an NRTI may decrease the potency of the antiretroviral regimen in naïve patients, even those who have baseline HIV-1 RNA levels below 100,000 copies/ml. In other trials evaluating NRTI-sparing regimens, the rate of resistance selection by the NRTI-containing regimen was lower (7, 19). The second hypothesis is that the polymorphism of HIV-1 non-B subtype protease genes decreases the genetic barrier because some baseline polymorphism mutations may impact LPV susceptibility, thus increasing the risk for the development of resistance. Recently, an analysis of predictive factors in the MONARK study revealed that viral non-B subtypes were associated independently with a poor virological response in the LPV/r monotherapy arm (8). But the analysis of adherence to therapy showed that the HIV-1 subtype could be a confounding factor associated with the virological response. While few data on the lack of efficacy of the combined antiretroviral regimen in non-B-subtype-infected patients were available, recent data showed similar patterns of virological success for HAART regimens including boosted atazanavir, boosted darunavir, and boosted lopinavir in naïve patients infected with subtypes B, CRF01_AE, CRF12_BF, C, and F (5, 6, 14). However, limited data on PI efficacy for the CRF02_AG subtype, the most prevalent HIV-1 non-B subtype in our study, are available.

With a limited number of patients with major PI resistance mutation selection, our study did not show that the viral subtype is a risk factor in selecting for protease mutations during LPV/r monotherapy. But interestingly, the three patients in whom an L76V PI resistance mutation developed were infected with HIV-1 strains belonging to the CRF02_AG subtype. Recently, Nijhuis et al. described a novel pathway involving the mutation A431V within the Gag cleavage site, followed by mutations M46I and L76V in protease, leading to LPV resistance in three naïve patients infected with HIV-1 subtypes B and K (17). Two patients were treated with LPV/r-based...
combination therapy, and one patient was treated with LPV/r monotherapy. Clonal analysis and site directed mutagenesis showed that the Gag NC/P1 cleavage site mutation A431V is a primary LPV resistance mutation conferring a 2.6-fold increase in LPV resistance. The single mutation L76V significantly reduces infectivity. The introduction of the M46I mutation could restore infectivity, and this mutation emerged before L76V. The A431V-M46I-L76V mutant viruses had a 10-fold increase in LPV resistance. Nijhuis et al. reported elsewhere that Gag cleavage site mutations on their own can explain high levels of resistance to a new PI, without mutations in the protease gene (16). Research into Gag cleavage site-based resistance in the MONARK study as well as other clinical trials may provide additional insight into resistance pathways associated with viral subtypes (9, 10).

Our study did not find predictive factors associated with the selection of major PI resistance mutations, but in the context of monotherapy, the genetic polymorphism of the protease gene and treatment adherence difficulties may lead to low-level viremia and resistance emergence.

It is noteworthy that full viral resuppression was achieved in the five patients in whom a major PI resistance mutation was selected during LPV/r monotherapy, after either the intensification of treatment with ZDV-3TC, a change to ZDV-3TC-nevirapine, or continuation on LPV/r. To conclude, our data confirm that LPV/r monotherapy should not be considered a preferred treatment option for widespread use in antiretroviral-naive patients because of lower rates of virological suppression and higher risks for resistance emergence with this therapy than with other regimens.

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