Lymph Node Human Immunodeficiency Virus RNA Levels and Resistance Mutations in Patients Receiving High-Dose Saquinavir

Jonathan M. Schapiro, Mark A. Winters, Mark Vierra, Shannon Crawford, and Thomas C. Merigan

The development of resistance mutations and human immunodeficiency virus (HIV) RNA levels were compared in lymph nodes and plasma of patients receiving antiretroviral therapy. Ten HIV-positive patients receiving high-dose saquinavir monotherapy (3600 or 7200 mg/day) underwent 14 lymph node biopsies before and during therapy. HIV RNA levels and appearance of resistance mutations to saquinavir were determined in simultaneous lymph node and plasma samples. HIV RNA levels were found to be consistently higher (mean, 3.16 log RNA copies; SD, 1.04; range, 2.23–5.59) in lymph nodes than in simultaneous plasma samples. Saquinavir therapy resulted in a reduction in HIV RNA levels in both plasma and lymph nodes. The presence or absence of a resistance mutation to saquinavir at codons 48 or 90 of the HIV-1 protease gene was identical in 13 of 14 biopsies, suggesting that resistance mutations to saquinavir appear within close temporal proximity in lymph nodes and plasma.

Recent studies with combination antiretroviral therapy have shown suppression of plasma human immunodeficiency virus (HIV) RNA levels to <500 copies in many patients [1, 2]. This suppression of replication is believed to reduce the virus’s potential for generating drug-resistant mutant strains and thus is hoped to lead to prolonged and possibly chronic suppression of viral replication and disease manifestation [3].

The concern that viral replication may be continuing at levels below 500 copies has led to the development of ultrasensitive plasma assays capable of detecting as low as 20 copies [4]. A number of studies have shown lymph node HIV RNA levels to be greater than those found in plasma [5–7]. This would suggest that even in patients with plasma HIV RNA levels of <20 copies, continued HIV replication may be present in the lymphoid tissue, allowing for the eventual development of drug resistance mutations and ultimately to drug failure and loss of virus suppression. The degree to which higher virus loads may effect the development of resistance mutations in the lymph nodes requires further investigation.

To study the relationship between HIV RNA levels and the development of resistance mutations in plasma and lymph nodes, we examined lymph node biopsies and simultaneous plasma samples from patients receiving high doses of the HIV-1 protease inhibitor saquinavir.

Methods

Study population. Patients were enrolled in the Stanford saquinavir high-dose study, in which HIV-positive volunteers with CD4 cell counts of 200–500 were treated with saquinavir monotherapy, 3600 or 7200 mg/day, in six divided daily doses for a minimum of 24 weeks [8]. Patients showing continuing response were allowed to continue to receive therapy.

Lymph node biopsies. Fourteen biopsies were done on 10 patients. Biopsies were done at three time points: 2 at baseline before starting therapy, 7 early in the course of therapy (weeks 4–12), when plasma virus load levels which had initially dropped first began rising, and 5 late in the course of therapy (weeks 24–56), when plasma virus load levels were returning to baseline values. Patients had biopsies performed at one or two of these time points. Both early (weeks 4–12) and late weeks (weeks 24–56) were chosen for sampling, since plasma virus from patients at the time of first failure often lacks detectable drug resistance mutations, whereas sampling later on after weeks or months of failure often reveals drug-resistant virus [8].

Biopsy procedure. Excisional biopsy of a single whole lymph node was done under local anesthetic using standard technique by a staff surgeon as an outpatient procedure. Biopsies were done on either inguinal or cervical lymph nodes at the preference of the patient. Simultaneous blood samples were drawn at the time of biopsy for virologic studies.

Lymph node processing. Lymph node tissue was weighed and then disrupted by gently pressing the tissue through a 100-mesh tissue sieve (Cellctor; Belco Glass, Vineland, NJ). The sieve was rinsed with 1 mL of PBS (pH 7.2), and the resulting cell suspension was collected and centrifuged for 5 min at 400 g. The acellular supernatant was collected, aliquoted, and stored at −70°C. The cellular fraction was counted, and aliquots were stored as dry cell pellets at −70°C or as cell suspensions (in RPMI 1640 + 15% fetal bovine serum + 10% dimethylsulfoxide) in liquid nitrogen.

Resistance mutations. The presence of the G48V and L90M resistance mutations in the protease gene were determined from the RNA of plasma and acellular lymph node fraction by complete
Table 1. Correlation of resistance mutations at codons 48/90 of the HIV-1 protease gene in different compartments (plasma, lymph node DNA and RNA).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Baseline (before therapy)</th>
<th>Early (weeks 4–12 of therapy)</th>
<th>Late (weeks 24–56 of therapy)</th>
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<tr>
<td></td>
<td>Lymph node</td>
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<td>Plasma DNA RNA</td>
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<td>MU/WT MU/WT MU/WT</td>
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<td>10</td>
<td>WT/WT WT/WT WT/WT</td>
<td>MU/WT MU/WT MU/WT</td>
<td>MU/WT MU/WT MU/WT</td>
</tr>
</tbody>
</table>

NOTE. Data are mutation at codon 48/codon 90; WT, wild type genotype; MU, mutant genotype.

population base-pair sequencing of the HIV protease gene with the M13 forward primer and dye-labeled dideoxy-terminators in a sequencer (model 370A; Applied Biosystems, Foster City, CA). All sequences were proofread manually. Sequencing of the HIV proviral DNA was done in a similar manner from the cellular lymph node fraction.

HIV RNA polymerase chain reaction (PCR) quantification. Plasma and acellular lymph node fraction HIV RNA levels were determined by a previously described externally controlled reverse transcription–PCR technique [9]. Dilutions of plasma or acellular lymph node fractions were tested, when necessary, to obtain values within the linear range of the assay.

Results

Lymph node mutations. Mutations in the protease gene at codons 48 and 90, which have been associated with resistance to saquinavir, were determined in the plasma virion RNA and virion RNA and DNA from simultaneous lymph node biopsies (table 1). All baseline and early samples from both plasma and lymph nodes showed the wild type genotype (WT) at codons 48 and 90. From the 5 late samples, 2 plasma samples showed the mutant genotype (MU) at codon 48 and three showed WT. The simultaneous lymph node samples showed identical results, with the same 2 samples having MU and the remaining 3 having WT. Two plasma samples showed MU at codon 90 and 3 showed WT. Of lymph node samples, 1 was MU and 4 were WT. In one set of simultaneous samples in which the plasma showed a MU, the corresponding lymph node sample was WT. Altogether, of the 28 codons assayed in the 14 samples, 27 had an identical determination of WT or MU in the simultaneous plasma and lymph node samples. Four late plasma samples and 3 late lymph node samples showed MU. One MU determination was found in plasma without a corresponding lymph node MU. In no patient did lymph node mutations appear without a corresponding mutation in the plasma. Lymph node RNA and DNA showed identical results in all samples.

Lymph node virus load. The mean HIV RNA load determined in the lymph node samples was 7.7 log copies/g (SD, 0.97; range, 5.91–9.02). Simultaneous plasma HIV RNA levels showed a mean of 4.44 log copies/mL (SD, 0.75; range, 2.8–5.53). Individual lymph node levels were consistently higher than those of the simultaneous plasma samples, although the magnitude of this difference varied (figure 1). The mean difference between plasma and lymph node samples was 3.16 (SD, 1.04; range, 2.23–5.59).

Two patients had lymph nodes biopsied before and after 4 weeks of therapy (figure 2). Both showed a reduction in HIV RNA load in their plasma accompanied by a corresponding reduction in their lymph nodes. In the patient shown at the left in figure 2, the reduction in plasma virus load was 1.41 log copies/mL and in lymph node virus load was 1.47 log copies/mL. In the patient shown at the right, the reduction in plasma virus load was 0.71 log copies/mL and in lymph node virus load was 0.49 log copies/mL.

Discussion

We found lymph node HIV RNA levels to be consistently higher than those found in the plasma. Although there was a large variation in the magnitude of this difference in individual patients, the average lymph node level was ~1000-fold greater than the simultaneous plasma sample. This is in general agreement with the findings of other studies, in which lymph node virus load was found to be 100- to 1000-fold higher than plasma virus load [6, 7, 10, 11]. This finding is strengthened by the
The fact that although a variety of technically distinct assays were used in these studies, the results were quite similar, with substantially greater HIV RNA found in the lymphoid tissue [6, 7, 10]. Our assay detects total RNA and therefore measures both viable and possibly nonviable virus. As much nonviable viral RNA may be trapped by the lymphoid tissue, these measurements may overestimate the actually amount of replicating virus present. This would have major implications regarding the correlation between these relatively high HIV RNA levels and their actual potential to generate drug resistance mutations. In addition, the rate of mutations seen in plasma may actually reflect resistant virus generated in the lymphoid tissue. Our data suggest that drug resistance mutations develop within close temporal proximity in lymph nodes and plasma. This is in agreement with some, but not all, previous studies [11–13]. From the sparse data available to date, it appears that more potent antiretroviral regimens (including those used in our study, in which high doses of saquinavir were used to obtain greater plasma drug levels) have a substantial effect on lymph node HIV RNA levels and therefore result in similar mutations in lymph node and plasma. Less potent regimens, such as those containing only nucleoside analogue monotherapy, have little impact on lymph node HIV RNA and mutations [11]. This is supported by our findings that high-dose saquinavir reduced lymph node HIV RNA levels in the 2 patients for whom biopsies were done both before and after initiating therapy and by the substantial reductions in lymph node virus load seen with protease inhibitor–containing and combination therapy [14–16].

Whether these greater HIV RNA levels seen in lymph nodes will ultimately result in the development of drug resistance in patients with undetectable plasma virus load will be better determined in current studies, in which lymphoid tissues from patients with prolonged undetectable plasma HIV RNA are examined on an ongoing basis [17].
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References


