Susceptibilities of Simian Immunodeficiency Virus to Protease Inhibitors

Angelica C. Giuffre, Joanne Higgins, Robert W. Buckheit Jr., and Thomas W. North

Center for Comparative Medicine and Department of Veterinary Molecular Biosciences, University of California, Davis, Davis, California 95616, and Southern Research Institute, Fredrick, Maryland 21701

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We used a focal infectivity assay with HeLa H1-JC.37 cells to directly compare susceptibilities of simian immunodeficiency virus (SIV) and human immunodeficiency virus type 1 (HIV-1) to protease inhibitors. SIVmac239 was inhibited by indinavir, saquinavir, and ritonavir, with 50% effective concentrations (means ± standard deviations) of 39 ± 8, 55 ± 3, and 13 ± 5 nM, respectively. The corresponding values for inhibition of HIV-1 were 66 ± 4, 47 ± 10, and 25 ± 14 nM, respectively.

The development of protease inhibitors as potent antiretroviral drugs enabled the first successful drug combinations used for highly active antiretroviral therapy (HAART) (9, 12, 13), and protease inhibitors remain a major component of AIDS therapy. HAART provides long-term suppression of plasma human immunodeficiency virus type 1 (HIV-1) loads to undetectable levels (12, 13, 30) and increased CD4+ T-cell counts (9, 19, 23) in many patients. However, major problems remain, including the emergence of multidrug-resistant virus, latency or persistence, and residual replication of HIV-1, even in patients on suppressive HAART (5, 6, 17, 34, 44). Memory T cells have been identified as one site that harbors latent HIV-1 (10, 46), but it is likely that there are other sites. The sites of residual replication have not been determined and represent a major impediment to eradication of HIV-1 in patients (5, 18). New therapeutic strategies will be necessary to better control or eradicate HIV-1 infection. A highly relevant and predictive animal model of HAART would greatly facilitate development of innovative therapeutic strategies.

Although protease inhibitors have been successful in HAART, they have not been extensively studied in the available animal models of AIDS: feline immunodeficiency virus (FIV) infection of cats or simian immunodeficiency virus (SIV) infection of rhesus macaques. Both of these models have been used extensively for studies of nucleoside analogs (14, 15, 26, 31, 39, 41). However, FIV is not susceptible to the protease inhibitors used in AIDS therapy (36). SIV is susceptible to protease inhibitors that inhibit HIV-1 (1, 3, 25), but direct comparisons of these two viruses by using the same cell line with a quantitative infectivity assay have not been made. The proteases of HIV-1 and SIV have similar biochemical properties (11, 27), but there are substantial differences in several amino acids in the active sites (47). The SIV protease was inhibited by one preclinical inhibitor, SB203386, but the Ki for inhibition was 10 times higher than the Ki for inhibition of the HIV-1 protease (20). In the work reported here, we directly compared the in vitro susceptibilities of SIVmac239 and HIV-1 to three Food and Drug Administration-approved protease inhibitors: indinavir, saquinavir, and ritonavir.

For these comparisons, we used a focal infectivity assay (FIA) (4, 32) with a cell line, HeLa H1-JC.37, that is permissive to infection by both SIV and HIV-1 (21, 33). These cells naturally express CXCR4 and have been engineered to express human genes for CD4 and CCR5 (33). Conditions for the FIA were recently described (29). The viruses used in these studies were SIVmac239 and HIV-1 NL4-3, provided by Paul Luciw (University of California, Davis); SIVmac251, provided by Koen Van Rompay (University of California, Davis); and RT-SHIV (made with a 5’-half clone obtained from Joseph Sodroski [Dana-Farber Cancer Institute, Harvard Medical School] [40] and the 3’-half clone of SIVmac239 [24, 35]). Virus stocks were prepared and stored as previously described (29, 41). Indinavir, saquinavir, and ritonavir used in these studies were provided by Raymond F. Schinazi (Emory University, Decatur, Ga.) and by Mohamed Nasr (Division of AIDS, National Institute of Allergy and Infectious Diseases). Schinazi also provided 3’-azido3’-deoxythymidine (AZT) and 2’,3’-dideoxy-3’-thiacytidine (3TC).

For initial validation of the FIA with SIVmac239, we compared it with two p27 antigen-based assays that utilize either CEMx174 cells or peripheral blood mononuclear cells (PBMC), both of which have previously been used for studies of drug susceptibility of SIV (41–43). The dose-response curves for inhibition of SIVmac239 by indinavir, saquinavir, and ritonavir that were obtained with these three assays are shown in Fig. 1. The concentrations required to inhibit focus formation or p27 production by 50% (EC50) were determined directly from the linear portions of those plots. The results are summarized in Table 1. For each drug, the dose-response curves and EC50 values obtained with the three assays were similar. With any of the drugs, there was no more than a twofold difference in the EC50 values among the three assays.

We used the FIA with HeLa H1-JC.37 cells to directly com-
pare the susceptibilities of SIVmac239 and HIV-1 to these three protease inhibitors. Assay conditions were identical except that foci of HIV-1-infected cells were detected with the HIV-1-specific antibody 22-6 (16), whereas foci of infection by SIV or RT-SHIV were detected with SIV-specific antibodies in serum from SIV-infected rhesus macaques (29). SIV and HIV-1 were very similar in their susceptibilities to each of the three inhibitors (Table 2). All statistical analyses were performed according to the ANOVA analysis of variance. The difference in EC50 values between SIVmac239 and HIV-1 were not significantly different (P > 0.05), except for indinavir (P = 0.005). With all three drugs, the EC50 values obtained with these two viruses were different by no more than twofold. As controls, two nucleoside analogues, AZT and 3TC (Table 2), which are known to inhibit HIV-1 and SIV (2, 7, 28, 38), were evaluated. SIVmac239 and HIV-1 were more similar in susceptibilities to protease inhibitors than to AZT. We also evaluated the susceptibilities of uncloned SIVmac251 and RT-SHIV to these three protease inhibitors (Table 2). Both of these viruses were inhibited by all three protease inhibitors, with EC50 values being similar to those obtained with SIVmac239. There was no more than a twofold difference in EC50 values between SIVmac239 and either of these two viruses.

Our data demonstrate that SIVmac239 and HIV-1 are very similar in their susceptibilities to three protease inhibitors that are approved for use in AIDS therapy. These comparisons were made from infections of a single cell line under identical conditions. This precludes differences in cellular uptake or metabolism of drugs, enabling direct comparisons of drug susceptibilities of the two viruses. The SIV-rhesus macaque model has been widely used to study nucleoside inhibitors (26, 41, 45), and our in vitro data suggest that this model may be more broadly useful for studies of HAART combinations that in-

TABLE 1. Inhibition of SIVmac239 by protease inhibitors determined with three drug susceptibility assays

<table>
<thead>
<tr>
<th>Protease inhibitor</th>
<th>Mean EC50 ± SD (nM)</th>
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<tbody>
<tr>
<td></td>
<td>HeLa H1-JC.37</td>
</tr>
<tr>
<td>Indinavir</td>
<td>39 ± 8</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>13 ± 5</td>
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</tbody>
</table>

* The assays used were the FIA with HeLa H1-JC.37 cells and p27 assays with CEMx174 cells or PBMC. For the three assays, the mean EC50 values were determined from at least three separate experiments.

Drug Concentration (nM)

FIG. 1. Dose-response curves comparing the susceptibilities of SIVmac239 to indinavir , saquinavir , and ritonavir in three different assays. (A) Susceptibility of SIV in HeLa cells determined by the FIA. (B) Susceptibility of SIV in CEMx174 cells determined by p27 enzyme-linked immunosorbent assay. (C) Susceptibility of SIV in PBMC determined by p27 enzyme-linked immunosorbent assay. The values are means of at least three experiments (± standard deviations). EC50 values were determined from the best-fit line of the linear portion of the graph. Data were plotted as percentages of control (no drug) versus inhibitor concentration.

TABLE 2. Comparison of drug susceptibilities of SIVmac239, HIV-1, SIVmac251, and RT-SHIV

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>SIVmac239</th>
<th>HIV-1</th>
<th>SIVmac251</th>
<th>RT-SHIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indinavir</td>
<td>39 ± 8</td>
<td>66 ± 4</td>
<td>45 ± 3</td>
<td>52 ± 5</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>55 ± 3</td>
<td>47 ± 10</td>
<td>55 ± 9</td>
<td>63 ± 10</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>13 ± 5</td>
<td>25 ± 14</td>
<td>25 ± 4</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>AZT</td>
<td>470 ± 40</td>
<td>120 ± 40</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3TC</td>
<td>550 ± 50</td>
<td>360 ± 60</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* These comparisons were made using the FIA. Mean EC50 values were determined from at least three separate experiments.

ND, not determined.
clude protease inhibitors. There have been attempts to study HAART combinations that include protease inhibitors in SIV-macaque models (22, 37); however, the efficacy of monotherapy with a protease inhibitor was not demonstrated at the dose used in those studies. Our data suggest that drug combinations that include Food and Drug Administration-approved protease inhibitors can be studied in the SIV-rhesus macaque model. This may enable the study of complications in HAART that are difficult or impossible to investigate in humans, such as more detailed analysis of reservoirs and sites of residual replication in tissues.

As expected, RT-SHIV was similar to SIV in susceptibility to these three protease inhibitors. This is important because RT-SHIV is susceptible to nonnucleoside reverse transcriptase inhibitors whereas SIV is not. The RT-SHIV–rhesus macaque model offers an opportunity to study HAART with combinations of drugs that include all of the classes currently approved for use in therapy of HIV-1.

We plan to characterize mutations in the SIV protease that confer resistance to each of these protease inhibitors. This will provide important structure-function comparisons of the SIV and HIV-1 proteases. If drug-resistant SIV mutants are similar to clinically important HIV-1 mutants, then the model will be useful for evaluation of the virulence and pathogenicity of protease inhibitor-resistant mutants.

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