Susceptibility to protease inhibitors in HIV-2 primary isolates from patients failing antiretroviral therapy

Berta Rodés*, Julie Sheldon, Carlos Toro, Victoria Jiménez, Miguel Ángel Álvarez and Vincent Soriano

Molecular Biology Laboratory, Department of Infectious Diseases, Hospital Carlos III, Madrid, Spain

Received 22 August 2005; returned 1 November 2005; revised 2 December 2005; accepted 24 January 2006

Background: Current protease inhibitors (PIs) are designed against HIV-1, and information on their performance against HIV-2 clinical isolates is scarce.

Methods: Genetic and phenotypic analyses using all available PIs were performed in five HIV-2 primary isolates from two patients on regular follow-up who failed PI-HAART.

Results: HIV-2 proteases before therapy showed amino acids associated with resistance in HIV-1 (pro10V, pro32I, pro36I, pro46I, pro47V, pro71V and pro73A). Phenotypic results showed that indinavir, saquinavir, lopinavir and tipranavir had full activity against wild-type HIV-2. However, a susceptibility reduction was noticed for nelfinavir (6.6-fold) and amprenavir (31-fold). During therapy with lopinavir, one patient developed proV47A, which translated into high-level resistance (13.4- to 41-fold) to indinavir, lopinavir and amprenavir, and hypersusceptibility to saquinavir. All isolates from the other patient had multiple mutations after several PIs failed (proV10I, proV33L, proI54M, proV71I and proI82F). The acquisition of mutations 54M and 82F along with naturally occurring changes resulted in multi-PI-resistant viruses (33- to >1000-fold), and only saquinavir retained full activity.

Conclusions: Naturally occurring secondary mutations or polymorphisms in the HIV-2 protease may decrease the activity of nelfinavir and amprenavir. Moreover, upon selection of primary resistance mutations, pre-existing secondary changes might play an important role in the acquisition of a multi-PI resistance phenotype in HIV-2.

Keywords: retroviruses, resistance, HAART

Introduction

Although eventually their effects are clinically and immunologically indistinguishable, human immunodeficiency virus type 2 (HIV-2) infection progresses to AIDS at a slower rate than HIV-1 infection.1,2 HIV-2-infected patients are equally eligible for antiretroviral therapy; however, the number of subjects who receive treatment is limited owing to the lower prevalence of HIV-2 infection worldwide and the fact that most infected individuals live in regions where antiretroviral therapy has not been available until recently.3

Current approved antiretroviral drugs have been designed against HIV-1, and little information on their activity against HIV-2 is available. It is known that HIV-2 shows natural resistance to non-nucleoside reverse transcriptase inhibitors.4-6 Genetic differences between HIV-1 and HIV-2 proteases suggest that current HIV-1 protease inhibitors (PIs) may not be equally effective against HIV-2, and several clinical reports have shown a lower efficacy of PIs against HIV-2 than against HIV-1.7,8 However, information about PI susceptibility of either HIV-2 wild-type or mutant clinical isolates is rather scarce. Taking into account that antiretroviral therapy is expanding to regions where HIV-2 is circulating, it is important to define the activity of these drugs against HIV-2. For this purpose, we selected HIV-2 patients failing PI-based regimens in our clinic and performed in vitro PI susceptibility analyses.

Patients and methods

Two HIV-2-infected patients on regular follow-up at our institution failing PI-based regimens were identified, and written informed consent was obtained. These patients were enrolled in a program designed to perform in vitro susceptibility analyses for PIs against HIV-2 isolates. Antiretroviral therapy was suspended for at least 14 days before the experiments were conducted.

*Corresponding author. Tel: +34-91-453-2586; Fax: +34-91-733-6614; E-mail: brodes.hciii@salud.madrid.org

© The Author 2006. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org
consent was obtained from them. The present study was approved by the Ethics Committee of the Hospital Carlos III. The patients' main characteristics are summarized in Table 1. Clinical data, CD4+ T cell counts and HIV-2 viral load were recorded. Plasma HIV-2 RNA was measured retrospectively in stored samples using EasyQ version 1.1 (BioMérieux, Boxtel, The Netherlands).9,10

Sequence analysis

The HIV-2 protease region was amplified from plasma RNA using primers and conditions described previously.11 Amplified products were sequenced using the ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA), following the manufacturer’s instructions. DNA sequences were analysed, edited and translated using the Sequence Navigator software, version 1.0.1 (Applied Biosystems). Encoded HIV-2 protein sequences were then aligned and compared with HIV-2 wild-type consensus sequences obtained from GenBank. Observed mutations were compared with those associated with resistance in HIV-1 and listed in the latest International AIDS Society (IAS) resistance guidelines.12 The HIV-2 subtype was further characterized by phylogenetic analyses using protease sequences.

Drug phenotypic analyses

Five primary HIV-2 isolates obtained from patients SP-2-p1 and SP-2-p2 at different time points were used to infect phytohaemagglutinin–interleukin-2 (PHA/IL-2) stimulated peripheral blood mononuclear cells (PBMCs) from HIV-seronegative blood donors under different concentrations of the drug.13 Susceptibility was tested for amprenavir (provided by GlaxoSmithKline), indinavir (provided by Merck), nelfinavir (obtained from Roche), tipranavir (obtained from Boehringer-Ingelheim), saquinavir (obtained from Roche), atazanavir (obtained from Bristol-Myers Squibb) and lopinavir (obtained from Abbott). A 1000 TCID50 (50% of the tissue culture infective dose) of each virus stock was used to infect 10^6 PBMCs. The effect of drug inhibition on viral replication was determined by measuring p24 antigen levels in culture supernatants 7 days after the infection. Susceptibility data were reported in terms of the concentration of the drug required to inhibit 50% of virus-induced cell killing (IC50). The IC50 value was calculated from at least three independent experiments performed in triplicate and was expressed as mean ± SD. Intermediate- and high-level resistance were defined as >5- and >10-fold increase in the IC50 value with respect to HIV-2 wild-type, respectively.

Cell toxicity assessment

The toxicity was measured 7 days after infection. Drug concentrations ranging from 0 to 100 μM were exposed to healthy PBMCs in parallel to the susceptibility analyses. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma) was added to each well at a final concentration of 500 μg/mL 5 h before dissolving the crystals in 200 μL of DMSO, and measurements were recorded at 550 nm UV wavelength.

Results

Protease sequences from both HIV-2 patients before therapy showed the presence of some amino acids that have been

<table>
<thead>
<tr>
<th>Patient</th>
<th>Subtype</th>
<th>Months of follow-up</th>
<th>Antiretroviral regimen</th>
<th>Months from initiation of respective HAART</th>
<th>Plasma HIV-2 RNA (copies/mL)</th>
<th>CD4 (cells/mm³)</th>
<th>Mutations selected in the protease during treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP-2-p1</td>
<td>B</td>
<td>0</td>
<td>none</td>
<td>0</td>
<td>&lt;50</td>
<td>323</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td>68</td>
<td>none</td>
<td>0</td>
<td>12 303</td>
<td>224</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>ZDV + 3TC + ABC</td>
<td>7</td>
<td>336</td>
<td>180</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td>81</td>
<td>ZDV + 3TC + ABC + LPV/r</td>
<td>6</td>
<td>867</td>
<td>255</td>
<td>V47A, S19P, K45R, I64V</td>
</tr>
<tr>
<td></td>
<td></td>
<td>91</td>
<td>ZDV + 3TC + ABC + LPV/r</td>
<td>16</td>
<td>&lt;50</td>
<td>135</td>
<td>V47A, S19P, K45R, I64V</td>
</tr>
<tr>
<td>SP-2-p2</td>
<td>A</td>
<td>0</td>
<td>none</td>
<td>0</td>
<td>20 000</td>
<td>50</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>ZDV + ddC</td>
<td>34</td>
<td>170</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>102</td>
<td>d4T + 3TC + IDV</td>
<td>40</td>
<td>8250</td>
<td>341</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>113</td>
<td>ddl + ABC + LPV/r</td>
<td>8</td>
<td>4500</td>
<td>180</td>
<td>V10I, I54M, I82F, D17G, V33L, T56V, E79D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>147</td>
<td>ZDV + 3TC + ABC + TPV</td>
<td>18</td>
<td>110</td>
<td>52</td>
<td>V10I, I54M, I82L, D17G, V33L, T56V, I64V, V71I, E79D</td>
</tr>
</tbody>
</table>

ND, not determined; ZDV, zidovudine; 3TC, lamivudine; ABC, abacavir; LPV/r, lopinavir boosted with ritonavir; ddC, zalcitabine; d4T, stavudine; IDV, indinavir; ddl, didanosine; TPV, tipranavir.
associated with resistance in HIV-1, such as pro46I (primary mutation), pro10V, pro32I, pro36I, pro46V, pro71V and pro73A (secondary mutations or polymorphisms). Other changes at positions involved in but not associated with resistance in HIV-1, such as pro20V, pro33V, pro63E, pro77T and pro82I, were also observed in both the wild-type HIV-2 proteases. Following exposure to PI-containing antiretroviral regimens, these patients selected several mutations associated with resistance in HIV-1 and other mutations of unknown impact on PI resistance. Table 1 shows amino acid changes that appeared during the therapy.

Phenotypic results with the wild-type HIV-2 isolate (SP-2-p1-75) showed that most PIs (indinavir, tipranavir, lopinavir and saquinavir) had full activity against it (Table 2) compared with HIV-1 (reported IC\textsubscript{50} values in HIV-1 are 0.023, 0.18, 0.027 and 0.011 \textmu M, respectively).\textsuperscript{5,14–16} However, a substantial reduction in susceptibility was seen for nelfinavir (IC\textsubscript{50} = 0.37 ± 0.21 \textmu M) and ampranavir (IC\textsubscript{50} = 2.93 ± 1.22 \textmu M) compared with the IC\textsubscript{50}\textsubscript{8} reported for the HIV-1 IIIB strain (IC\textsubscript{50} values of 0.056 and 0.094 \textmu M, respectively).\textsuperscript{5} Therefore, in this HIV-2 isolate, susceptibility to nelfinavir and to ampranavir was reduced by 6.6- and 31-fold, respectively (Table 2). The IC\textsubscript{50} value for atazanavir could not be compared since no IC\textsubscript{50} has been reported in HIV-1 for atazanavir using conventional phenotypic assays.

In the course of a first-line treatment containing lopinavir boosted with ritonavir, patient SP-2-p1 showed detectable levels of plasma viraemia and selected the proV47A mutation along with other changes in the protease region, although in positions not associated with resistance (Table 1). No further changes appeared while the patient was on lopinavir. He admitted poor adherence to medication and showed sub-therapeutic lopinavir plasma levels (0.06 \textmu g/mL). Subsequent analyses with the primary HIV-2 isolate carrying the proV47A mutation (SP-2-p1-91) revealed high-level phenotypic resistance (between 13- and 41-fold increase in IC\textsubscript{50}) to indinavir, lopinavir and amprenavir, whereas susceptibility was retained for tipranavir and atazanavir. Unexpectedly, this mutant isolate showed hypersusceptibility to saquinavir (0.5-fold). The susceptibility to nelfinavir was further reduced 4.4-fold (Table 2).

Table 2. Susceptibility of different HIV-2 isolates to protease inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Patient SP-2-p1</th>
<th></th>
<th>Patient SP-2-p2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC\textsubscript{50} (\textmu M)</td>
<td>fold increase\textsuperscript{a}</td>
<td>IC\textsubscript{50} (\textmu M)</td>
<td>fold increase\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amprenavir</td>
<td>2.93 ± 1.22</td>
<td>&gt;100 (34)</td>
<td>97.3 ± 5.57 (33.2)</td>
<td>&gt;100 (34)</td>
</tr>
<tr>
<td>Indinavir</td>
<td>0.041 ± 0.006</td>
<td>0.551 ± 0.096 (13.4)</td>
<td>82 ± 9.1 (2000)</td>
<td>37.3 ± 2.46 (909)</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>0.37 ± 0.21</td>
<td>1.62 ± 0.28 (4.4)</td>
<td>&gt;20.5 (&gt;55)</td>
<td>&gt;20.5 (&gt;55)</td>
</tr>
<tr>
<td>Tipranavir</td>
<td>0.329 ± 0.017</td>
<td>0.93 ± 0.03 (2.8)</td>
<td>1.806 ± 0.1 (5.5)</td>
<td>1.864 ± 0.15 (5.7)</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>0.011 ± 0.001</td>
<td>0.0055 ± 0.003 (0.5)\textsuperscript{b}</td>
<td>0.022 ± 0.016 (2)</td>
<td>0.052 ± 0.011 (4.7)</td>
</tr>
<tr>
<td>Atazanavir</td>
<td>0.047 ± 0.014</td>
<td>0.165 ± 0.14 (3.5)</td>
<td>2.008 ± 0.37 (43)</td>
<td>2.093 ± 0.30 (44)</td>
</tr>
<tr>
<td>Lopinavir</td>
<td>0.027 ± 0.005</td>
<td>1.365 ± 0.09 (41)</td>
<td>18.5 ± 7.6 (577)</td>
<td>18.7 ± 3.8 (584)</td>
</tr>
</tbody>
</table>

ND, not determined.
\textsuperscript{a}Fold increase in the IC\textsubscript{50} value compared with that obtained for the HIV-2 wild-type (SP-2-p1-75) specimen. Antiviral activity above 5-fold increase compared with HIV-2 wild-type is shown in bold.
\textsuperscript{b}A 0.5-fold increase denotes hypersusceptibility.

Discussion

The efficacy of different PIs in HIV-2-infected individuals is largely unknown. The HIV-2 protease shows a 50% divergence in amino acid composition compared with the HIV-1 protease, along with many secondary mutations and polymorphic changes at positions associated with PI-resistance in HIV-1.\textsuperscript{11,17–19} Since PIs are sensitive to minor structural changes in the binding
and since the available PIs are designed to fit the HIV-1 protease, the observed differences might affect their activity against HIV-2. Accordingly, some reports have shown differences in the clinical outcome of HIV-2-infected patients using distinct PIs. Therefore, evaluation of the in vitro activity of currently available PIs against HIV-2 clinical isolates is important to define the expected benefit of these compounds as part of the HIV-2 armamentarium and to clarify the rationale behind choosing one particular PI over another.

Although our study had the limitation of the small number of isolates studied, the results provide basic information about PI activity against HIV-2 primary isolates and the impact of some protease mutations in reducing their activity. We showed that naturally occurring polymorphic changes in the HIV-2 protease, some of which are considered to be secondary resistance mutations in HIV-1, by themselves are not enough to cause drug resistance to most PIs (indinavir, tipranavir, lopinavir and saquinavir), which maintained full activity against one wild-type HIV-2 isolate. However, increases in the IC_{50} value (5- to 10-fold) that were in the range of intermediate resistance were observed for nelfinavir. Our data are in agreement with those reported by Witvrouw et al., who also showed a slight reduction in the activity of nelfinavir against HIV-2. Furthermore, a significant reduction in antiviral activity against our HIV-2 isolate was recognized for amprenavir. This PI seems to be the most affected by naturally occurring mutations within and outside the active site in HIV-2, which might influence the binding affinity of the drug to a greater extent than for other PIs. In HIV-1, the presence of four or more mutations at positions 10, 32, 46, 47, 54, 73, 82, 84 and 90 confers resistance to amprenavir. The HIV-2 wild-type isolates already have four of these changes (at positions 10, 32, 46 and 47) and this could explain the resistance to amprenavir observed in this study.

The selection of the proV47A mutation in patient SP-2-p1 upon treatment with lopinavir, which was used as first-line PI, is of particular interest. This change resulted in a significant reduction of susceptibility to lopinavir and cross-resistance to indinavir, nelfinavir and amprenavir. However, it did not affect the susceptibility to tipranavir and atazanavir. The selection of this primary mutation in HIV-1 has been observed in vitro and rarely in vivo in patients exposed to lopinavir. In HIV-2-infected patients, the selection of this mutation has recently been observed in patients receiving lopinavir and may be favoured since one nucleotide change is required (proV47A) compared with two nucleotide changes in HIV-1 (pro47A). The hypersusceptibility we found for saquinavir in the HIV-2 isolate with proV47A is consistent with results reported in HIV-1.

Phenotypic results obtained with the other three HIV-2 mutants showed that the presence of proI54M and proI82F/L caused a multiple-PI-resistant phenotype in HIV-2. Interestingly, saquinavir was the only PI that remained active against these mutant isolates. Mutation proI82F is known to cause a slight reduction in the susceptibility to tipranavir in HIV-1. We found a partial virological response upon introducing tipranavir in our patient. He maintained low detectable HIV-2 plasma RNA while on tipranavir for 18 months, and during this period proI82F was substituted by pro82L. This change rendered the virus highly resistant to tipranavir.

In summary, natural changes in the HIV-2 protease might decrease its susceptibility to nelfinavir and amprenavir. Furthermore, pre-existing polymorphisms may significantly amplify the effect of a single primary mutation. Thus, the principles guiding the use of PIs in HIV-1 may not apply to HIV-2-infected patients.

Acknowledgements

This work was partly supported by Fundación Investigación y Educación en Sida (FIES) and Red de Investigación en Sida (RIS) G03/173.

Transparency declarations

None to declare.

References


