Drug resistance mutations in patients infected with HIV-2 living in Spain

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Background: In contrast with HIV-1, information about drug resistance in HIV-2 is scarce and mainly derived from small series of patients failing antiretroviral therapy.

Methods: The spectrum of changes in the reverse transcriptase (RT), protease (PR) and integrase (INT) genes was examined in HIV-2 individuals enrolled in the HIV-2 Spanish register.

Results: From a total of 236 HIV-2-infected individuals registered in Spain from 1989 to June 2010, 53 PR, 44 RT and 8 INT sequences were obtained. Low plasma viraemia precluded collection of this information from most of the remaining cases. No major mutations associated with drug resistance in HIV-1 were recognized in 29 PR, 20 RT and 5 INT sequences from antiretroviral-naïve HIV-2 individuals, although natural polymorphisms with potential effects on susceptibility to PR inhibitors were recognized at 10 positions (L10V/I, V32I, M36I, M46I, I47V, Q58E, A71V/I, G73A, V82I and L89I/V) and for nucleoside reverse transcriptase inhibitors at three positions (T69N, V75I and K219E). In 24 antiretroviral-experienced patients with virological failure the most frequent major RT resistance mutations were M184V (58%), Q151M (33%) and K65R (21%), which are rarely seen thymidine analogue mutations. In PR the most frequent major changes were V47A (17%), I54M (17%), I82F (13%), L90M (29%) and L99F (29%). Two of the three patients who failed on raltegravir had N155H in the INT region.

Conclusions: Drug resistance mutations in HIV-2 are selected at the same positions as in HIV-1, although with different frequency. Polymorphisms in the RT and PR associated with drug resistance in HIV-1 as compensatory changes are common in untreated HIV-2 subjects. These findings highlight the need for specific guidelines for interpreting genotypic resistance patterns in HIV-2 infection.

Keywords: reverse transcriptase, protease, integrase

Introduction

HIV-2 infection is endemic in certain areas of West Africa where rates of 10% have been described in some settings. Infection with HIV-2 can ultimately lead to AIDS, although disease progression is much slower than with HIV-1. Most individuals with HIV-2 infection in North America and Europe are immigrants from endemic countries or natives who have lived in or have had sexual partners from those regions. HIV-2 and HIV-1 share 60% of the amino acids in the proteins encoded by the pol gene, and the structures of the reverse transcriptase (RT), protease (PR) and integrase (INT) of the two viruses are relatively similar, especially when considering the catalytic sites. Overall, it is important to distinguish HIV-1 from HIV-2 infection because the natural history, transmissibility and response to therapy are quite different.

The development of drug resistance in HIV-2 has been examined in several studies. However, information is scarce compared...
with HIV-1 and is mainly derived from limited series of patients failing antiretroviral therapy.\textsuperscript{6} Natural resistance of HIV-2 to non-nucleoside reverse transcriptase inhibitors is mainly due to the presence of changes 181I/V, 188L and 190A in the HIV-2 RT.\textsuperscript{7,8} Likewise, significant differences in the transmembrane protein structure explain why HIV-2 is naturally resistant to enfuvirtide.\textsuperscript{9} On the other hand, the susceptibility of HIV-2 to PR inhibitors (PIs) seems to differ from that of HIV-1, the activity of fosamprenavir being especially compromised against HIV-2.\textsuperscript{10–13} In contrast, the antiviral activity of INT inhibitors seems to be quite similar against HIV-1 and HIV-2.\textsuperscript{5,14}

Finally, while transmission of drug-resistant HIV-1 strains is a well-documented phenomenon, occurring in approximately 10\% of subjects in Western countries,\textsuperscript{15,16} only anecdotal cases of transmission of resistant HIV-2 have been reported so far.\textsuperscript{17,18} Herein, we examine the prevalence of drug resistance mutations in both antiretroviral-naive and antiretroviral-experienced individuals infected with HIV-2 recorded in the Spanish HIV-2 national register.

**Methods**

The Spanish HIV-2 national register is a publicly funded database that collects information on all individuals diagnosed with HIV-2 infection in Spain since 1989. A centralized repository of stored clinical samples, peripheral blood mononuclear cells and plasma functions in parallel was used for the current study. This study was approved by the Ethics Committee of all hospitals participating in the study.

Amplification of sequences of the PR, RT and INT genes was attempted in plasma specimens. HIV-2 RNA was extracted by the QIAamp Viral RNA Mini Extraction Kit (Qiagen, Hamburg, Germany) following the manufacturer’s instructions. Specimens underwent RT–PCR to amplify the three regions of the pol gene corresponding to PR, RT and INT. Primers and conditions have been described previously.\textsuperscript{5,19} PCR amplicons were purified using the High Pure PCR Product Purification Kit (Roche, Mannheim, Germany) and directly sequenced using the ABI PRISM 3100 Genetic Analyzer using the ABI PRISM Rhodamine Terminator Reaction Kit (Applied Biosystems, Foster City, CA) and inner primers for each genetic region. Finally, sequences were analysed using SeqScape version 2.5 using ROD HIV-2 as a reference strain. Major and compensatory drug resistance mutations were defined using the information for HIV-1 derived from the 2008 International AIDS Society–USA panel mutation list.\textsuperscript{20} Further virological characterization of the HIV-2 group was done using a genotyping tool that uses a sliding window to generate multiple overlapping segments of a query sequence and its reference dataset.\textsuperscript{21}

**Results**

From 1989 to June 2010 a total of 236 HIV-2-infected individuals were reported in the national HIV-2 Spanish register. Their main characteristics are shown in Table 1. Two-thirds of individuals were male, with a median age at diagnosis of 40 years. Although more than three-quarters of them originated in sub-Saharan Africa, 17\% were native Spaniards. The main route of infection was heterosexual contact (at least 61\% of cases), followed by homosexual relationships (5\%) and intravenous drug use in only 1\% of subjects. Virological characterization of HIV-2 strains was obtained for 73 individuals, and HIV-2 group A was by far the predominant variant (89\%). Finally, co-infection with HIV-1 was demonstrated in up to 10\% of cases.

Overall, 44 HIV-2 RT sequences could be obtained from 44 subjects. Low plasma viraemia precluded obtaining results in most of the remaining subjects due to unsuccessful amplification of genetic material. Besides the well-described changes that naturally occur in the RT (181I/V, 188L and 190A), another three mutations that have been associated with drug resistance in HIV-1 (T69N, V75I and K219E) were found in all 20 antiretroviral-naive HIV-2-infected individuals examined. In the remaining 24 antiretroviral-experienced patients, 22 infected by group A and 2 by group B, the presence of drug resistance changes was universal, with mutations K65R (5; 21\%), Q151M (8; 33\%) and M184V (14; 58\%) being the most frequently found, occasionally in combination (Figure 1). In contrast, thymidine analogue mutations (TAMs) were rarely found, except for K219E, which occurs naturally in HIV-2. The only TAMs that were selected under antiretroviral therapy were D67N (1; 4\%) and K70R (1; 4\%). Other changes at positions 69, 75, 219 and 321 that have been associated with drug resistance in HIV-1 (T69N, V75I and K219E) were found in all 20 antiretroviral-naive HIV-2-infected individuals examined. In the remaining 24 antiretroviral-experienced patients, 22 infected by group A and 2 by group B, the presence of drug resistance changes was universal, with mutations K65R (5; 21\%), Q151M (8; 33\%) and M184V (14; 58\%) being the most frequently found, occasionally in combination (Figure 1). In contrast, thymidine analogue mutations (TAMs) were rarely found, except for K219E, which occurs naturally in HIV-2. The only TAMs that were selected under antiretroviral therapy were D67N (1; 4\%) and K70R (1; 4\%). Other changes at the RT potentially acting as compensatory drug resistance mutations are recorded in Figure 1.

Overall, HIV-2 PR sequences were obtained from 53 HIV-2 patients. All sequences from 29 antiretroviral-naive individuals displayed changes already known to influence PI susceptibility in HIV-1 at 10 positions (L10V/I, V32I, M36I, M46I, I47V, Q58E, A71V, G73A, V82I and L89I/V). Using the Spanish genotypic interpretation algorithm for HIV-1,\textsuperscript{22} the estimated susceptibility

<table>
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<tr>
<th>Variables</th>
<th>Study population</th>
<th>Total HIV-2 cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>73</td>
<td>236</td>
</tr>
<tr>
<td>Gender</td>
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</tr>
<tr>
<td>male</td>
<td>49 (67%)</td>
<td>155 (66%)</td>
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<td>female</td>
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<td>4 (2%)</td>
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<tr>
<td>Median age, years (range)</td>
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<td>40 (2–84)</td>
</tr>
<tr>
<td>Risk group</td>
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<tr>
<td>heterosexual contact</td>
<td>56 (77%)</td>
<td>145 (61%)</td>
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<tr>
<td>men who have sex with men</td>
<td>5 (7%)</td>
<td>12 (5%)</td>
</tr>
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<td>intravenous drug users</td>
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<td>3 (1%)</td>
</tr>
<tr>
<td>vertical</td>
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</tr>
<tr>
<td>transfusion</td>
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<td>unknown</td>
<td>9 (12%)</td>
<td>74 (31%)</td>
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<td>Country of origin</td>
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<tr>
<td>native Spaniards</td>
<td>15 (21%)</td>
<td>40 (17%)</td>
</tr>
<tr>
<td>sub-Saharan Africa</td>
<td>49 (67%)</td>
<td>179 (76%)</td>
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<td>Portugal</td>
<td>5 (7%)</td>
<td>6 (3%)</td>
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<td>France</td>
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<td>Latin America</td>
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<td>unknown</td>
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<td>7 (3%)</td>
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<td>65</td>
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<tr>
<td>HIV-2 group B</td>
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<td>8</td>
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<tr>
<td>unknown</td>
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<td>163</td>
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</table>
score for distinct PIs in this PI-naive HIV-2 population was saquinavir (0) – atazanavir (2) – darunavir (3) – indinavir (3) – lopinavir (4) – fosamprenavir (6) – tipranavir (6.2). It should be noted that the interpretation is as follows: scores 0–2 are considered as drug susceptible; scores 3–4 as intermediate resistance; and scores ≥5 as drug resistance.

A total of 24 HIV-2-infected individuals had failed on PI-based regimens. The drugs used were lopinavir/ritonavir (10), darunavir/ritonavir (6), atazanavir/ritonavir (3), fosamprenavir/ritonavir (2), indinavir/ritonavir (1), tipranavir/ritonavir (1) and unreported (1). Changes considered as major PI resistance mutations in HIV-1 were found in 71% of cases, some of them alone and others in combinations. They were V47A (4; 17%), I54M (4; 17%), I82F (3; 13%), L90M (7; 29%) and L99F (7; 29%). Other secondary or compensatory mutations were frequent and are shown in Figure 1.

From a total of eight INT sequences examined, five belonged to antiretroviral-naive HIV-2 patients and none harboured primary resistance mutations. The other three patients, all infected with HIV-2 group A, failed on raltegravir-containing regimens, and two of them harboured mutation N155H in the INT region (Figure 1). Other INT resistance changes in these patients were E92Q (n = 1), T97A (1), A153G (1) and S163G/D (2).

**Discussion**

This study shows that selection of drug resistance mutations in HIV-2 patients failing antiretroviral therapy is generally seen at the same positions as in HIV-1. The frequency of changes seems to differ from that seen in HIV-1, and accordingly RT mutations Q151M and K65R were much more frequent than TAMs in our treated HIV-2 study population. Instead of TAMs, zidovudine resistance in HIV-2 often involves selection of the Q151M complex, which might emerge faster and more frequently than in HIV-1. This observation has already been reported previously, but stresses the problem of the frequent emergence of multinucleoside resistance profiles in HIV-2.

Selection of PR resistance mutations was also demonstrated in all individuals who failed a PI-containing regimen. Besides changes at positions known to be associated with resistance in HIV-1, such as V47A, I54M, I82F and L90M, an L99F mutation was selected in up 29% of our patients. This change must be considered to be a specific major HIV-2 PI resistance mutation, although it has also been detected in antiretroviral-naive individuals infected with HIV-2 group B. The other PR changes, considered as minor HIV-2 resistance mutations, that were selected in our study population were K7R, V10I, V33I, T56V, V62A and V71I.

Recently, Bayesian networks were used to identify mutations selected during therapy and investigate direct dependencies between them in HIV-2. Changes I54L/M, I82F, L90M and L99F at the PR were found to represent the four main resistance pathways for PIs in HIV-2. With respect to resistance to nucleoside analogues, as previously highlighted by others, the most common pathways were K65R, Q151M, M184V and probably S215F/Y. The clinical interpretation of these findings is that in the presence of these primary mutations, if therapy is not modified during virological failure, further accumulation of accessory mutations will occur in an attempt to either increase resistance or ameliorate the fitness cost of primary resistance mutations.

The recognition of major PR mutations in 71% of patients failing PIs in our series merits some further discussion. Virological failure of ritonavir-boosted PIs in HIV-1 infection is often seen in the absence of PR resistance changes. Kinetic inhibition assays have shown that lopinavir, saquinavir, tipranavir and darunavir are the PIs exhibiting the highest potency in HIV-2; however,
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there are 84, 2, 24, and 17 times weaker than the corresponding values against the HIV-1 PR, respectively.12 Moreover, besides changes in antiviral activity, changes in the genetic barrier for resistance to PIs may differ between HIV-2 and HIV-1. In this regard, natural polymorphisms at the HIV-2 PR might facilitate selection of resistance changes.27 This is the case for lopinavir, which in HIV-2 is more prone to selection mutation I47A, as it only requires one nucleotide change at this position compared with two in HIV-1.30 In addition, HIV-2 susceptibility following interpretation of the Spanish HIV-1 algorithm resulted in a surprisingly high level of resistance to tipranavir, which is in agreement with the information recently reported by others testing phenotypically wild-type HIV-2 strains and concluding that HIV-2 was naturally resistant to tipranavir and amprenavir.51

With respect to INT inhibitors, selection of N155H occurred in two out of three patients who failed on raltegravir. Phenotypic studies have already shown the large impact of this change in reducing susceptibility to this drug in HIV-2 isolates.5,32 Moreover, other INT changes at positions known to be associated with resistance to INT inhibitors in HIV-1, such as E92Q, T97A, A153G and S163G/D, were recognized in these individuals.

Finally, we did not find major drug resistance mutations in antiretroviral-naive individuals infected with HIV-2, although a few natural polymorphisms were frequently found. According to these data, baseline HIV-2 drug resistance testing does not seem to be warranted before prescription or antiretroviral therapy, although continuous surveillance is advisable given the presence of resistance changes.17,18 In contrast, drug resistance testing in HIV-2 patients failing antiretroviral therapy is warranted because the success of subsequent rescue interventions may largely be influenced by adequate selection of drugs. Since no commercial HIV-2 drug resistance tests exist, efforts to develop standardized methods and interpretation systems are needed.

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Transparency declarations

None to declare.

References