Prevalence of darunavir resistance mutations in HIV-1-infected patients failing other protease inhibitors

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Background: To estimate to what extent darunavir might be effective in patients failing distinct protease inhibitors (PIs), the genotypic resistance scores recently reported for the drug were examined in a large clinical HIV-1 drug resistance database.

Methods: All clinical specimens from HIV-infected patients failing PI-based regimens referred for drug resistance testing between 1999 and 2007 to a reference centre in Madrid were analysed. Darunavir-specific resistance mutations listed by the September 2006 IAS-USA panel update were considered.

Results: A total of 1021 genotypes from patients failing lopinavir (39.2%), nelfinavir (28.1%), saquinavir (14.5%), indinavir (13.7%), atazanavir (6.6%), fosamprenavir (5.3%) and tipranavir (1.1%) were identified. The prevalence of major darunavir resistance mutations was I50V 2.1%, I54M 1.3%, L76V 2.7% and I84V 14.5%. For minor darunavir resistance mutations, the rates were V11I 3.3%, V32I 3.9%, L33F 11%, I47V 2.1%, I54L 2.3%, G73S 12.8% and L89V 2.4%. Overall, 6.7% (n = 68) of the genotypes had three or more darunavir resistance mutations, which corresponded to a mean total number of PI resistance mutations of 12.3 ± 1.9. In the multivariate analysis, prior fosamprenavir failure, prior saquinavir failure, the total number of PI resistance mutations and the number of prior PIs used were all independently associated with having more darunavir resistance mutations.

Conclusions: The prevalence of darunavir resistance mutations is low in patients failing other PI-based regimens, although prior failure to amprenavir and saquinavir might produce more cross-resistance to darunavir. Thus, darunavir may be a good option for patients who have failed other PI-based regimens.

Keywords: new antiretrovirals, genetic barrier, salvage therapies

Introduction

Protease inhibitors (PIs) are potent antiretroviral agents which in combination with other drugs have reduced dramatically the morbidity and mortality of persons with HIV infection. However, virological failure continues to occur in a substantial proportion of HIV-infected individuals on highly active antiviral therapy (HAART). Cross-resistance is extensive within the PIs class and the efficacy of ritonavir-boosted PIs is greatly influenced by the extent of baseline protease resistance mutations. In general, the presence of ≥5 resistance mutations within the protease gene has been associated with a diminished response to all currently available PIs. Therefore, the development of a new PI with a greater genetic barrier to resistance is crucial.

Darunavir, formerly TMC-114, is the latest approved PI for the treatment of HIV infection. It was originally designed to be active against HIV strains resistant to other currently available PIs. The POWER trials have evaluated the safety and efficacy of darunavir in highly treatment-experienced patients using as comparators other ritonavir-boosted PIs chosen as the most appropriate by the investigators. In all these studies, darunavir has demonstrated significantly greater reductions in plasma HIV-RNA and increases in CD4 counts over the active controls in patients with extensive PI resistance. Overall, 45% of patients on darunavir had plasma HIV-RNA below 50 copies/mL at week 48, more than twice seen in controls, with a similar profile of clinical and laboratory adverse events.

Information on darunavir resistance is still scarce and mainly derived from clinical trials used for the registration of the drug. In the latest International AIDS Society-USA panel list (www.iasusa.org, last update in September 2006), a total of 11 mutations were defined as specifically associated with darunavir...
resistance. They were segregated as major (I50V, I54M, L76V and L89V) or minor (V11I, V32I, L33F, I47V, I54L, G73S and L89V) resistance mutations. In the POWER trials, the virological response to darunavir was strongly predicted by the baseline number of darunavir-associated resistance mutations, with a significantly impaired response seen in patients harbouring viruses with three or more darunavir-associated resistance mutations.

The aim of this study was to assess the prevalence of darunavir-associated resistance mutations in patients who had failed different PI-based regimens outside the context of clinical trials and estimate to what extent darunavir could be an effective therapeutic option for these patients.

Materials and methods

Study population

All HIV drug resistance tests performed from January 1999 until March 2007 on antiretroviral-experienced individuals with plasma HIV-RNA >1000 copies/mL in a reference HIV laboratory located in Madrid, Spain, were retrospectively examined. Only patients failing PI were selected for this analysis. Prior treatment exposure and drug regimens at the time of failure were recorded in a case report form for each sample. All specimens collected from patients failing darunavir were excluded from this analysis.

Drug resistance testing

Genetic sequences from both HIV protease (PR) and reverse transcriptase were obtained from plasma using the Viroseq HIV-1 kit (Abbott Laboratories, North Chicago, IL, USA) and an automatic sequencer (ABI Prism 3100; Celera Diagnostics, Alameda, CA, USA). Drug resistance mutations within the pol gene were interpreted following the latest International AIDS Society-USA panel list (www.iasusa.org, last update in September 2006). Accordingly, the following 11 resistance mutations were considered for darunavir: V11I, V32I, L33F, I47V, I50V, I54L/M, G73S, L76V, I84V and L89V. These changes have been mainly derived from information recorded from the POWER trials.

Statistical analyses

All data are reported as absolute numbers and percentages, as well as mean ± SD. Comparisons were made using the Student’s t-test for continuous variables, and the Pearson χ² or the Fisher’s exact tests for categorical variables. Univariate and multivariate linear logistic regression analyses were performed to assess which factors were related to the presence of more darunavir resistance mutations. Statistical significance was assumed for P < 0.05. All statistical analyses were performed using the SPSS v11.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

A total of 1021 genotypes corresponding to distinct patients failing PI regimens were recorded during the study period. They belonged to failures on lopinavir/ritonavir (n = 400; 39.2%), nelfinavir (n = 287; 28.1%), saquinavir/ritonavir (n = 148; 14.5%), indinavir/ritonavir (n = 140; 13.7%), atazanavir/ritonavir (n = 67; 6.6%), fosamprenavir/ritonavir (n = 54; 5.3%) and tipranavir/ritonavir (n = 11; 1.1%). Overall, 8.3% (n = 85) of patients received double PI combinations, mainly lopinavir/ritonavir plus saquinavir.

The mean number of PI resistance mutations was 5.3 ± 3.4 using the latest IAS-USA panel list. Only 3.5% of the genotypes (n = 33) did not harbour any PI resistance mutation. The prevalence of major and minor resistance mutations to darunavir is presented in Figure 1. Overall, there was a low prevalence of darunavir-associated resistance mutations, the least prevalent being I47V, I50V, I54L/M and L89V, all with a frequency below 2.5%. The most prevalent were L33F, G73S and I84V and had rates of 11%, 12.8% and 14.5%, respectively. It should be noted that within the more prevalent mutations, only I84V was a major mutation. This observation could be relevant since the mutations with the highest phenotypic impact were less prevalent.

In the POWER trials, the presence of three or more darunavir resistance mutations was negatively associated with darunavir response. Among the 1021 genotypes examined in this study, only 6.7% (n = 68) had three or more darunavir resistance mutations. The majority of patients (68%) did not harbour any darunavir-associated resistance change, 15.9% had one, 9.5% had two, 3.8% had three and 2.9% had four to six mutations. In addition, we examined the prevalence of darunavir resistance mutations among 354 genotypes tested between 2004 and 2007 and we obtained similar results. Only 9.3% had three or more darunavir resistance mutations and most of the genotypes (66%) did not harbour any darunavir-associated resistance change. Moreover, it should be highlighted that the subset of patients with three or more darunavir resistance mutations had a mean total number of protease resistance mutations from the IAS-USA list of 12.3 ± 1.9, whereas subjects with less than three darunavir resistance mutations had a mean number of 5.3 ± 3.4 (P < 0.001) (Figure 2). A high correlation was found between the number of darunavir-associated resistance mutations and the total number of protease resistance mutations, as recorded in the latest IAS-USA list (r = 0.7; P < 0.001). Altogether, these data suggest that the genetic barrier for resistance to darunavir/ritonavir 600/100 mg twice daily seems to be exceptionally high, since the total number of protease resistance mutations required to have three or more darunavir-associated mutations was on average at least 12 in our population.

Some darunavir resistance mutations were recognized more often in patients failing specific PIs. This was the case for
Darunavir resistance

![Graph showing the correlation between the number of darunavir (DRV) resistance mutations and the number of protease resistance mutations recorded in the IAS-USA panel.]

Figure 2. Correlation between the number of darunavir (DRV) resistance mutations and the number of protease resistance mutations recorded in the IAS-USA panel.4

fosamprenavir/ritonavir (V11I, L33F, I50V, I54L, G73S, L89V and I84V), tipranavir/ritonavir (V32I, I47V, I54M and L89V), lopinavir/ritonavir (V32I, L33F, I47V and L76V), saquinavir/ritonavir (L33F, I54M, G73S and I84V) and atazanavir/ritonavir (L33F, I54M and G73S). However, the mean number of darunavir resistance mutations was significantly greater only for the subset of patients who had failed fosamprenavir/ritonavir (1.8 ± 1.5 versus 0.5 ± 0.9; P < 0.001), tipranavir/ritonavir (1.2 ± 1.1 versus 0.5 ± 1.02; P < 0.001), saquinavir/ritonavir (0.8 ± 1.1 versus 0.5 ± 1, P = 0.003) and atazanavir/ritonavir (0.8 ± 1.1 versus 0.5 ± 1, P = 0.043) compared with the rest.

In order to identify which variables were independently associated with darunavir resistance mutations, a multivariate analysis was performed in which the PI at the time of failure, the total number of PI resistance mutations and the number of prior failed PIs were examined. The variables that were independently associated with the presence of darunavir resistance mutations were: failure on fosamprenavir/ritonavir (B = 0.51, 95% CI: 0.27–0.76), failure on saquinavir/ritonavir (B = 0.29, 95% CI: 0.043–0.55), the total number of protease resistance mutations (B = 0.19, 95% CI: 0.17–0.21) and the number of prior failed PIs (B = 0.11, 95% CI: 0.04–0.19).

Taking into account the mean number of darunavir resistance mutations, the lowest cross-resistance was recognized for patients who had failed on indinavir, nelfinavir and lopinavir. Conversely, the highest cross-resistance was seen for the subset of patients who had failed on fosamprenavir, tipranavir, saquinavir or atazanavir. An explanation for these results could be the higher number of PI resistance mutations accumulated in patients failing those drugs compared with the rest as well as an increased number of failures on prior PIs, which was the case for fosamprenavir/ritonavir, tipranavir/ritonavir and atazanavir/ritonavir. However, in the multivariate analysis, it became clear that prior failure on fosamprenavir and saquinavir was the most important predictor of harbouring viruses with darunavir-associated resistance mutations, and therefore other reasons are needed to explain this finding.

Both fosamprenavir and darunavir are structurally related molecules and therefore it is not surprising that they share some specific resistance mutations.5 150V, the primary mutation for fosamprenavir, has shown the highest impact on darunavir susceptibility.7,8 Moreover, molecular studies of the V82F and I84V double mutants show that they seem to distort the geometry of the binding site and preferentially affect the interaction of the protease with amprenavir and darunavir, whereas the natural substrate retains affinity.9 In our population, the mutation I84V was significantly more prevalent in patients failing fosamprenavir/ritonavir (38.9% versus 13.1%; P < 0.001) and saquinavir/ritonavir (29.7% versus 11.9; P < 0.001) compared with the other PI regimens. Therefore, the higher prevalence of resistance changes which provide the greatest loss of susceptibility to darunavir, such as I50V and I84V in patients failing fosamprenavir/ritonavir and saquinavir/ritonavir, most likely explain our observations.

Although data from the POWER studies showed only a minimal impact of prior fosamprenavir failure on the response to darunavir/ritonavir,10 the limited number of examined patients (73 in the three POWER trials) and differences in study design, patient population, enfuvirtide use and baseline protease resistance profiles most likely reduced the ability to accurately gauge this effect. The higher potency of darunavir/ritonavir demonstrated against multiple PI-resistant viruses compared with other drugs within this family in the POWER trials has been explained by the extremely high affinity of darunavir for the protease and elevated drug exposure using the approved doses of darunavir/ritonavir 600/100 mg twice daily, based on its wide therapeutic range.6 Only when multiple specific mutations are present may the activity of darunavir be substantially compromised, and this could be the case for viruses that have followed the fosamprenavir mutational resistance pathways.

In summary, the analysis of a relatively large number of clinical specimens derived from patients failing PI sent for drug resistance testing shows a low prevalence of darunavir-associated resistance mutations. Overall, only 6.7% of samples had three or more darunavir-associated resistance mutations, which has been established as the threshold above which the activity of the drug is abolished. Interestingly, it corresponded to a mean of at least 12 of the total number of protease resistance mutations. Altogether, these findings confirm the high genetic barrier for resistance of darunavir and that this drug will be a good therapeutic option for rescue interventions in patients who have failed different PI-based regimens.

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Transparency declarations

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888