Prevalence and factors associated with darunavir resistance mutations in multi-experienced HIV-1-infected patients failing other protease inhibitors in a referral teaching center in Brazil

ABSTRACT

Information about resistance profile of darunavir (DRV) is scarce in Brazil. Our objectives were to estimate the prevalence of DRV resistance mutations in patients failing protease inhibitors (PI) and to identify factors associated with having more DRV resistance mutations. All HIV-infected patients failing PI-based regimens with genotyping performed between 2007 and 2008 in a referral teaching center in São Paulo, Brazil, were included. DRV-specific resistance mutations listed by December 2008 IAS-USA panel update were considered. Two Poisson regression models were constructed to assess factors related to the presence of more DRV resistance mutations. A total of 171 HIV-infected patients with available genotyping were included. The number of patients with lopinavir, saquinavir, and amprenavir used in previous regimen were 130 (76%), 83 (49%), and 35 (20%), respectively. The prevalence of major DRV resistance mutations was 50V: 5%; 54M: 1%; 76V: 4%; 84V: 15%. For minor mutations, the rates were 11I: 3%; 32I: 7%; 33F: 23%; 47V: 6%; 54L: 6%; 74P: 3%; 89V: 6%. Only 11 (6%) of the genotypes had ≥ 3 DRV resistance mutations. In the clinical model, time of HIV infection of > 10 years and use of amprenavir were independently associated with having more DRV resistance mutations. In the genotyping-based model, only total number of PI resistance mutations was associated with our outcome. In conclusion, the prevalence of DRV mutations was low. Time of HIV infection, use of amprenavir and total number of PI resistance mutations were associated with having more DRV mutations.

Keywords: antiretroviral therapy; highly active; HIV protease inhibitors; acquired immunodeficiency syndrome; Brazil.

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INTRODUCTION

Over 90% of people with HIV/AIDS live in developing countries, where only a minority of patients who need treatment has access to first and second-line antiretroviral (ARV) drugs.

Darunavir (DRV) was approved by the Food and Drug Administration in June 2006 for use in treatment-experienced patients.[1,2] Since 2008, DRV was included among the ARV drugs available in Brazil,[3] a middle-income country with a well-structured National Program of AIDS, including free access to drugs and genotyping. However, information about this protease inhibitor (PI) is scarce in our setting. The objectives of this study were to estimate the prevalence of DRV resistance mutations in patients failing other PIs and to identify associated factors with having more DRV resistance mutations.

PATIENTS AND METHODS

All adult HIV-1-infected patients from the AIDS Clinic of School of Medicine, *Universidade de São Paulo,* a referral teaching center in Brazil, with genotyping performed between January 2007 and December 2008, were retrospectively examined. Only patients failing PI-based regimes were selected for this analysis. Demographic, prior treatment exposure and drug regimens at the time of failure were recorded. Darunavir-specific resistance mutations listed by the December 2008 IAS-USA panel update were considered.[4] They were classified as major (I50V, I54M, L76V, and I84V) or minor (V11I, V32I, L33F, I47V, L74P). These mutations have been mainly derived from information recorded from the POWER and DUET trials.[5]

All data are reported as absolute numbers and percentages, as well as mean ± SD.

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Comparisons were made using the Student's t test or Wilcoxon rank sum tests for continuous variables, and the Pearson qui-square or the Fisher's exact tests for categorical variables. We performed two multivariable Poisson models assessing factors related to the presence of more DRV mutations: I) a clinical model, including clinical variables only; and II) a genotyping-based model, including clinical variables and the variable "total number of PI resistance mutations". The values of $p < 0.05$ were considered statistically significant. All statistical analysis was performed using the S-Plus 7.0 (Insightful, WA, USA).

RESULTS

A total of 171 HIV-infected patients failing PI-based regimens and with available genotyping were included. The mean age was 43 ± 8.6 years old and 112 (65%) were male. The mean time of HIV diagnosis was 11.5 ± 4.0 years. Most patients (n = 114, 68%) had ≥ 10 years of HIV diagnosis. Most patients had a history of AIDS-defining diseases (n = 100, 58%).

The mean ± SD of $CD4^+$ T cell count at baseline was 251 ± 184 cells/mL. Mean $CD4^+$ T cell count nadir was 109 ± 93.1 cells/mL. Mean HIV RNA load at the moment of the failure was 88,538 ± 15,351 copies/mL or 4.5 ± 0.6 $\log_{10}$ copies/mL.

Most patients (n = 99, 58%) used at least five antiretroviral regimens previously. A group of 141 (82%) patients had used non-nucleoside reverse transcriptase inhibitors. The number of patients with LPV, SQV, and APV use at any time before genotyping (in the last regimen or prior to last regimen) were 130 (76%), 83 (49%), and 35 (20%), respectively. Patients failing lopinavir (n = 93, 54%), atazanavir (n = 56, 33%), saquinavir (13, 8%), amprenavir (n = 8, 5%), indinavir (n = 8, 5%), nelfinavir (n = 8, 5%), and tipranavir (n = 1, 1%) were identified to this study. Among these, 16 (9%) patients received regimens with double-boosted protease inhibitors. The prevalence of major DRV resistance mutations was 50V: 8 (5%); 54M: 1 (1%); 76V: 7 (4%); 84V: 26 (15%). For minor DRV resistance mutations, the rates were 11I: 5 (3%); 32I: 12 (7%); 33F: 40 (23%); 47V: 10 (6%); 54L: 10 (6%); 74P:5 (3%); 89V: 11 (6%). The number of mutations for DRV was as follows: No mutation: 89 (52%), one mutation: 52 (30%); two mutations: 19 (11%); three mutations: 4 (2%); four mutations: 2 (1%); and five mutations: 5 (3%). Only 11 (6%) of the genotypes had ≥ 3 DRV resistance mutations. The mean total number of PI resistance mutations was 8.4 ± 4.1. The patients with ≥ 3 DRV-specific resistance mutations had a mean total number of protease resistance mutations from the IAS-USA list of 13.5 ± 1.9, whereas individuals with < 3 DRV-specific resistance mutations had a mean number of 8.1 ± 4.0 ($p < 0.001$). The variables associated with more DRV-specific resistance mutations in the univariable analysis were: time of HIV diagnosis, nadir CD4 cell count, CD4 prior to genotyping, number of PI previously used, prior use of amprenavir, and total number of mutations for PIs (Table 1).

Table 1. Univariable analysis to identify associated variables with DRV-specific resistance mutations

Variable	Relative Risk (95% CI)	p
Age (per 5 year increase)	1.0 (0.9-1.1)	0.6
Time of HIV diagnosis > 10 years (*vs.* ≤ 10 years)	3.4 (2.0-5.7)	< 0.001
AIDS-defining disease (*vs.* No)	0.7 (0.5-1.0)	0.06
Nadir CD4 cell count < 100 (*vs.* ≥ 100) cells/µL	1.4 (1.0-2.0)	0.04
CD4 prior to genotyping (*vs.* > 200) cells/µL		
< 100 cells/µL	1.8 (1.2-2.6)	0.02
100-200 cells/µL	0.9 (0.5-1.4)	
Viral load prior to genotyping (*vs.* ≤ 10,000 copies/mL)		
> 100,000 copies/mL	1.0 (0.6-1.7)	0.8
10,000-100,000 copies/mL	0.9 (0.6-1.4)	
Number of ARVs previously used (per increase in one drug)	1.2 (1.0-1.5)	0.08
Number of PI previously used (per increase in one PI)	1.1 (1.1-1.2)	< 0.001
Prior use of APV either last ARV scheme or before (vs. No)	2.6 (1.8-3.7)	< 0.0001
Number of all mutations for PIs (per increase in one PI mutation)	1.3 (1.2-1.4)	< 0.00001

In the clinical model, time of HIV diagnosis of > 10 years (RR 2.8, 95% CI 1.6-4.9, $p < 0.001$) and prior use of amprenavir (RR 2.2, 95% CI 1.5-3.3, $p < 0.001$) were independently associated with having more DRV resistance mutations. In the genotyping-based model, only the total number of PI resistance mutations (RR per increase in one mutation 1.3, 95% CI 1.2-1.4, $p < 0.0001$) was associated with our outcome.

DISCUSSION

In this study, the prevalence of DRV mutations was low. Time of HIV infection, use of amprenavir and total number of PI resistance mutations were associated with having more DRV mutations.

Most genotypes were from patients with a prolonged history of HIV infection, low $CD4^+$ T cell nadir, triple-class ex-

perienced-patient, and predominant use of ritonavir-boosted PIs when the genotyping was performed. Therefore, the population profile included in this study presents the ideal scenario to use salvage regimens.

In the POWER studies, baseline phenotypic susceptibility to DRV was the strongest predictor of virologic response.[2] However, phenotyping is not available in the public health services in Brazil, where the National Genotyping Network (RENAGENO) was established in 2001.[3] In the POWER/DUET trials, the virologic response to DRV was strongly predicted by the baseline number of DRV-specific resistance mutations with the presence of ≥ 3 of these mutations associated with a decreased virologic response.[6] The frequency of DRV-specific resistance mutations in our study was low. Only 6% of the genotypes had ≥ 3 DRV-specific resistance mutations. These results confirm that DRV constitutes a viable option to structure a rescue regimen in multi-experienced HIV-infected patients failing other PI. The result was similar with a Spanish study that evaluated 1,021 genotypes from patients failing other PIs and 6.7% of them had ≥ 3 DRV-specific resistance mutations.[7] Other study reported that 96% of 1,175 PI-treated persons in a Northern California clinic population and 99% of 2,744 PI-treated persons listed in the Stanford HIV Drug Resistance Database have < 3 DRV-specific resistance mutations.[8]

In the present study, time of HIV infection, prior use of amprenavir and total number of PI resistance mutations were associated with having more DRV resistance mutations in two independent multivariate models. Few studies identified associated factors with the number of DRV-specific resistance mutations. A Spanish study identified that prior fosamprenavir failure, prior saquinavir failure, total number of PI resistance mutations and the number of prior PIs used were all independently associated with having more DRV-specific resistance mutations in the multivariate analysis.[7] A North American study identified in multivariate analysis that the number of DRV resistance-associated mutations depended on the number of previous PIs administered and on amprenavir/fosamprenavir treatment.[8] In contrast to these reports, a subanalysis of the POWER 1, 2, and 3 studies concluded that prior utilization or resistance to amprenavir at screening seemed to have only a minimal effect on the virological response to DRV/r at 48-weeks.[9] In addition, a study that evaluated the efficacy of DRV in twelve patients failing PI (5 of them with fosamprenavir) concluded that prior failure with this PI had only a minimal impact on the efficacy to DRV.[10]

Amprenavir, fosamprenavir, and DRV are structurally related molecules.[7] Moreover, the regression coefficients of the mutations associated with decreased susceptibility to DRV and fosamprenavir are strongly correlated.[11] DRV is a PI with a high genetic barrier. Thus, although amprenavir and fosamprenavir can select mutational resistance pathways to DRV, this drug need multiple specific mutations to be substantially compromised. A practical consideration to this observation could be the early discontinuation of a failing regimen containing amprenavir or fosamprenavir in order to avoid accumulating resistance that could compromise the future use of DRV. On the other hand, a recent study suggests that expected increased efficacy of DRV compared to amprenavir in PI-experienced patients is most likely a result of higher potency rather than unique cross-resistance profile.[12]

In conclusion, the prevalence of DRV-specific resistance mutations was low and similar to previous reports from developed countries. Time of HIV infection, prior use of amprenavir and total number of PI resistance mutations were associated with having more DRV resistance mutations. DRV may be an important component of a salvage treatment of patients who failed other PI-based regimens in our setting. Local clinical studies are necessary to confirm this finding.

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