JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2002, p. 3865–3866 Vol. 40, No. 10
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.10.3865–3866.2002

Rate of Virological Treatment Failure and Frequencies of Drug Resistance Genotypes among Human Immunodeficiency Virus-Positive Subjects on Antiretroviral Therapy in Spain

Oscar Gallego,[1] Lidia Ruíz,[2] Alex Vallejo,[3] Bonaventura Clotet,[2] Manuel Leal,[3] and Vincent Soriano[1]*

Service of Infectious Diseases, Hospital Carlos III, Instituto de Salud Carlos III, Madrid,[1] Fundació IrsiCaixa, Barcelona,[2] and Hospital Virgen del Rocío, Seville,[3] Spain

Received 19 February 2002/Returned for modification 17 June 2002/Accepted 19 July 2002

The knowledge of which drug-resistant human immunodeficiency virus (HIV) genotypes are the most prevalent in a community may be helpful for designing the best salvage regimens. A total of 540 individuals on antiretroviral therapy attending 18 different outclinics in Spain were examined in a cross-sectional study conducted during June 2000. The overall rate of virologic failure (>50 HIV RNA copies/ml) was 54%. Among the subjects showing treatment failure, 79% harbored resistant HIV genotypes, 77% showed resistance to nucleoside analogues, 53% showed resistance to protease inhibitors, and 42% showed resistance to nonnucleoside reverse transcriptase inhibitors. Overall, 78.5% of individuals harbored HIV strains which showed resistance to two or more drug classes. Moreover, nucleotide substitutions causing broad cross-resistance among compounds within each drug family were quite common. These findings suggest that drug resistance mutations are very prevalent among subjects who have experienced several treatment failures. Therefore, facilitating the arrival of compounds belonging to new drug classes should be considered a priority.

The availability of new and more potent antiretroviral drugs has dramatically improved the life expectancy of human immunodeficiency virus (HIV)-infected patients (1). However, the long-term benefit of therapy is frequently limited by selection for resistant mutant HIV quasispecies (7). For this reason, surveillance of drug resistance in a community may provide useful information for the design and selection of preferred drug combinations for rescue interventions.

Cross-sectional drug resistance surveys have been performed in Spain over the last four years (4, 5, 11, 12). The results of these studies have shown that the rate of genotypic resistance is above 70% for nucleoside analogues (NA) and 27% for protease inhibitors (PI) for subjects previously exposed to antiretroviral drugs. Information on the rate of resistance to nonnucleoside reverse transcriptase (RT) inhibitors (NNRTI) is not available yet.

A cross-sectional study was carried out during June 2000 in 18 clinical centers widely distributed in Spain. At each location, the first 30 consecutive patients on antiretroviral therapy were recruited. Using an automatic sequencer (ABI Prism 3100; PE Biosystems, Foster City, Calif.), genetic sequence analyses were performed for all specimens harboring plasma viremia above 1,000 HIV RNA copies/ml. All nucleotide changes of the types considered by the International AIDS Society USA Resistance Testing Panel to be associated with drug resistance (7, 8) were recorded.

Overall, 46% (248 of 540) of patients under antiretroviral therapy in Spain showed complete virological suppression (<50 HIV RNA copies/ml). A total of 240 (44.4%) samples harbored a viral load above 1,000 HIV RNA copies/ml. Genotypic data were obtained for 221 (92%) of them. Resistant genotypes were recognized in 175 (79%) samples (Table 1). The rate of occurrence of resistant genotypes differed among the different drug families, totaling 77% for NA, 53% for PI, and 42% for NNRTI.

Table 2 summarizes the most prevalent resistance mutations affecting compounds of each of the three antiretroviral drug families. For the RT gene, mutations associated with resistance to zidovudine and lamivudine were the most frequently found, including the newly described lamivudine resistance genotypes 44D and 118I (6), which were present in 20% of the samples. It should be pointed out that 20% of patients harbored more than three classical zidovudine mutations, including 41L, 67N, 70R, 210W, 215Y/F, and 219Q/E. This feature has been associated with a reduced susceptibility to other NA such as abacavir, stavudine, and didanosine (2, 9, 10; B. Larder and S. Bloor, abstr. from the 5th Int. Workshop on HIV Drug Resistance and Treatment Strategies, Scottsdale, Ariz., 2001). Moreover, these so-called nucleoside-associated mutations (NAMs) may differ in the extent of their susceptibility to tenofovir, the first nucleotide analogue that appeared in the market.

Other common RT mutations were 103N (27.2%), 181C/I (16.7%), and 190A/S (13.6%), which compromise susceptibility to NNRTI. In contrast, mutations classically associated with resistance to didanosine (L74V), zalcitabine (T69D/N), or stavudine (V75T) were recognized in less than 5% of instances (data not shown). Three subjects carried multiple nucleoside-

* Corresponding author. Mailing address: Service of Infectious Diseases, Hospital Carlos III, Instituto de Salud Carlos III, Calle Nueva Zelanda 54, 4 B, Madrid 28035, Spain. Phone: 34 91 4532500. Fax: 34 91 7336614. E-mail: vsoriano@dragonet.es.

TABLE 1. Genotypic resistance to antiretroviral drugs in subjects on therapy

Category	No. (%) of pretreated subjects showing resistance (n = 221)
Overall	175 (79)
NA	171 (77)
NNRTI	93 (42)
PI	117 (53)
Two drug families	121 (55)
All three drug families	52 (23.5)

resistant genotypes (one subject with a codon 151 complex and two subjects with codon 67 and 69 insertions).

For the protease gene, the most frequent resistance genotypes were 90M (31%), 82A/F/I/T (23.5%), and 46I/L (18.5%). In more than 80% of the cases, primary PI mutations were associated with more than three secondary resistance mutations. This observation should be evaluated in the light of recent observations from clinical trials in which the response to ritonavir booster PI regimens (C. De Mendoza, L. Martín-Carbonero, P. Barreiro, B. Diaz, E. Valencia, M. Núnez, V. Soriano, and J. Gonzalez-Lahoz, Abstr. 8th Eur. Conf. Clin. Aspects. Treatm HIV Infect., abstr. 235, 2001, and L. Valer, D. Gonzalez, C. de Mendoza, P. Labarga, A. García-Henarejos, F. Guerrero, A. Vergara, V. Soriano, and the Fortogene Spanish Team, Abstr. 8th Eur. Conf. Clin. Aspects. Treatm HIV Infect., abstr. 246, 2001) seems to have been significantly compromised in the presence of five or more PI resistance mutations.

This study provides an overview of the rate of virological success among subjects on antiretroviral therapy in Spain as well as of the frequency of resistant viruses in patients failing therapy. Overall, nearly half (46%) of the patients showed complete virological suppression (<50 HIV RNA copies/ml). This good news was counterbalanced by the fact that nearly 80% of nonresponders carried resistant genotypes. Nucleotide substitutions causing broad cross-resistance among compounds within each drug family were the most common (NAMs for NA, K103N for NNRTI, and L90M and/or ≥5 resistance mutations for PI). These findings suggest that drug resistance mutations are very prevalent among subjects who have experienced multiple treatment failures. For those patients, the arrival of compounds belonging to new drug classes is particularly important. For the remaining 21% of subjects who show virological failure despite lacking resistance mutations, the most appropriate interventions are likely to be those oriented toward improvement of treatment adherence; such interventions should be particularly emphasized for subjects on therapy for long periods of time (3).

TABLE 2. Main genotypes conferring drug resistance in 221 subjects failing antiretroviral therapy

Drug family	Mutation	No. (%) of subjects
NA	T215Y/F	112 (50.7)
	M184V/I	109 (49.3)
	M41L	91 (41.2)
	E44D/A ± V118I	45 (20.4)
	≥4 NAMs	42 (20)
NNRTI	K103N	60 (27.2)
	Y181C	37 (16.7)
	G190A	30 (13.6)
PI	L90M	68 (31)
	V82A/F/T	52 (23.5)
	M46L/I	41 (18.5)
MNR[a]	Insert 67/69[b]	2 (0.9)
	Q151M complex	1 (0.45)

[a] MNR, multinucleoside resistance.
[b] Insert 67/69, codon 67 and 69 insertions.

We thank Angélica Corral for excellent technical assistance and Juan González-Lahoz for his continuous support.

REFERENCES

1. **Carpenter, C. C. J., D. A. Cooper, M. A. Fischl, J. M. Gatell, B. G. Gazzard, S. M. Hammer, M. S. Hirsch, D. M. Jacobsen, D. A. Katzenstein, J. S. G. Montaner, D. D. Richman, M. S. Saag, M. Schechter, R. T. Schooley, M. A. Thompson, S. Vella, P. G. Yeni, and P. Volberding.** 2000. Antiretroviral therapy in adults: update recommendations of the International AIDS Society-USA panel. JAMA **283:**381–390.
2. **Coakley, E., J. Gillis, and S. Hammer.** 2000. Phenotypic and genotypic resistance patterns of HIV-1 isolates derived from individuals treated with didanosine and stavudine. AIDS **14:**F9–F16.
3. **Friedland, G., and L. Andrews.** 2001. Adherence to antiretroviral therapy. AIDS Rev. **3:**111–120.
4. **Gallego, O., L. Ruiz, A. Vallejo, E. Ferrer, A. Rubio, B. Clotet, M. Leal, V. Soriano, and the ERASE-3 Group.** 2001. Changes in the rate of genotypic resistance to antiretroviral drugs in Spain. AIDS **15:**1894–1896.
5. **Gómez-Cano, M., A. Rubio, T. Puig, M. Pérez-Olmeda, L. Ruiz, V. Soriano, J. A. Pineda, L. Zamora, N. Xaus, B. Clotet, and M. Leal.** 1998. Prevalence of genotypic resistance to nucleoside analogues in antiretroviral-naive and antiretroviral-experienced HIV-infected patients in Spain. AIDS **12:**1015–1020.
6. **Hertogs, K., S. Bloor, V. De Vroey, and B. Larder.** 2000. A novel HIV-1 RT mutational pattern confers phenotypic lamivudine resistance in the absence of mutation M184V. Antimicrob. Agents Chemother. **44:**568–573.
7. **Hirsch, M., F. Brun-Vèzinet, R. D'Aquila, S. Hammer, V. A. Johnson, D. R. Kuritzkes, C. Loveday, J. W. Mellors, B. Clotet, B. Conway, L. M. Demeter, S. Vella, D. M. Jacobsen, and D. D. Richman.** 2000. Antiretroviral drug resistance testing in adult HIV infection. JAMA **283:**2417–2426.
8. **International AIDS Society-USA Resistance Testing Panel.** 2001. Antiretroviral resistance mutations. HIV Clin. Trials **2:**346–355.
9. **Izopet, J., A. Bicart-See, and C. Pasquier.** 1999. Mutations conferring resistance to zidovudine diminish the antiviral effect of stavudine plus didanosine. J. Med. Virol. **59:**507–511.
10. **Miller, V., M. Ait-Khaled, and C. Stone.** 2000. HIV-1 RT genotype and susceptibility to RT inhibitors during abacavir monotherapy and combination therapy. AIDS **14:**163–171.
11. **Pérez-Olmeda, M., J. Del Romero, A. Rubio, L. Ruiz, C. Rodríguez, M. Leal, B. Clotet, and V. Soriano.** 2001. Prevalence of drug resistance in Spain before and after the introduction of protease inhibitors. J. Med. Virol. **63:**85–90.
12. **Puig, T., M. Pérez-Olmeda, A. Rubio, L. Ruiz, C. Briones, J. M. Franco, M. Gómez-Cano, L. Stuyver, L. Zamora, C. Alvarez, M. Leal, B. Clotet, V. Soriano, and the ERASE-2 Study Group.** 2000. Prevalence of genotypic resistance to nucleoside analogues and protease inhibitors in Spain. AIDS **14:**727–732.