ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2008, p. 1545–1548 Vol. 52, No. 4
0066-4804/08/$08.00+0 doi:10.1128/AAC.01284-07

In Vitro Phenotypic Susceptibility of Human Immunodeficiency Virus Type 2 Clinical Isolates to Protease Inhibitors▽

Delphine Desbois,[1] Bénédicte Roquebert,[1] Gilles Peytavin,[2] Florence Damond,[1] Gilles Collin,[1] Antoine Bénard,[5] Pauline Campa,[4] Sophie Matheron,[3] Geneviève Chêne,[5] Françoise Brun-Vézinet,[1] and Diane Descamps[1]* for the French ANRS HIV-2 Cohort (ANRS CO 05 VIH-2)

Laboratoire de Virologie, Service de Microbiologie, Hôpital Bichat-Claude Bernard, Paris, France[1]; Laboratoire de Toxicologie, Service de Pharmacie, Hôpital Bichat-Claude Bernard, Paris, France[2]; Service de Maladies Infectieuses et Tropicales, Hôpital Bichat-Claude Bernard, Paris, France[3]; Service de Maladies Infectieuses et Tropicales, Hôpital Saint-Antoine, Paris, France[4]; and ISPED, Université Victor Segalen Bordeaux 2, INSERM U593, Bordeaux, France[5]

Received 4 October 2007/Returned for modification 19 November 2007/Accepted 14 January 2008

We determine phenotypic susceptibility of human immunodeficiency virus type 2 (HIV-2) isolates to amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, saquinavir, and tipranavir. Saquinavir, lopinavir, and darunavir are potent against wild-type HIV-2 isolates and should be preferred as first-line options for HIV-2-infected patients. Other protease inhibitors are less active against HIV-2 than against HIV-1.

Few data are available on human immunodeficiency virus type 2 (HIV-2) susceptibility to antiretroviral agents. In France, as recommended by the national expert group on the treatment of HIV infection, HIV-2-infected patients receive highly active antiretroviral therapy (HAART) regimens as HIV-1-infected individuals do, except without nonnucleoside reverse transcriptase inhibitors or fusion inhibitor classes (17, 12). However, a recent study showed that CD4 cell recovery was poor in antiretroviral-naive HIV-2-infected patients starting treatment with HAART (8). Thus, it appears crucial to determine HIV-2 susceptibility to the current protease inhibitors (PIs) available in order to define the optimal regimen to be recommended.

(This work was presented at the 14th Conference on Retroviruses and Opportunistic Infection, Los Angeles, CA, 25 to 28 February 2007.)

We selected nine PI-naive HIV-2-infected patients from the French HIV-2 ANRS cohort. Six of these patients subsequently received HAART regimens including a PI (indinavir, nelfinavir, saquinavir, and/or ritonavir) for a median of 13 months (range, 2 to 36) and had plasma viruses harboring mutations in the protease gene. Peripheral blood mononuclear cell (PBMC) coculture isolates were collected from these patients before (T0, $n = 6$) and during (T1 [time of collection of first plasma specimen during PI exposure], $n = 6$; T2 [time of collection of second plasma specimen during PI exposure], $n = 2$) PI exposure. Protease gene sequences of plasma and PBMC isolates were determined as previously described (3). Amino acid changes were compared with those associated with drug resistance in HIV-1 (International AIDS Society-USA [IAS-USA]). We determined the in vitro phenotypic susceptibility of clinical HIV-2 isolates and HIV-2 reference strain ROD and HIV-1 reference strain BRU to amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, saquinavir, and tipranavir using the ANRS PBMC assay (4). Phenotypic inhibitory quotients (PIQs) were calculated for each clinical HIV-2 isolate as the ratio between the trough plasma PI concentration and the 50% inhibitory concentration (IC_{50}). The PIQs were not adjusted for protein binding. Table 1 shows the phenotypic results and protease gene sequences of the HIV-2 clinical isolates and the phenotypic results of the HIV-2 and HIV-1 reference isolates.

The protease sequences of the wild-type HIV-2 strains, compared to the HIV-1 clade B consensus sequence, contained several amino acids associated with HIV-1 PI resistance, such as 10I/V, 16E, 20V, 32I, 33V, 35G, 36I, 43T, 46I, 47V, 58E, 62V, 69K, 71V, 73A, 82I, and 93L. Other differences were observed at positions involved in but not associated with HIV-1 resistance, such as 13A, 34A, 60K, 63E, 76M, 77T, 85F, and 89I. Relative to the HIV-1 reference strain, the median IC_{50} values of the HIV-2 wild-type isolates were 31-fold higher for amprenavir, eightfold higher for atazanavir, sevenfold higher for tipranavir, and threefold higher for indinavir and nelfinavir. Darunavir, lopinavir, and saquinavir median IC_{50} and IC_{90} values were similar for HIV-1 and the wild-type HIV-2 isolates (Table 1). Viruses isolated from the six PI-experienced patients at T1 and T2 harbored the I82F, I84V, and L90M substitutions, alone or in combination with minor HIV-1 PI mutations, such as V10I, V33I, I54M, I64V, V71I, and I89V. Compared to the corresponding wild-type isolates, the six mutants showed increases of fourfold to >10-fold in the IC_{50} and/or IC_{90} values of all tested PIs, at both T1 and T2. The PIQ values of amprenavir, atazanavir, indinavir, nelfinavir, and tipranavir were, respectively, 33-fold, eightfold, threefold, threefold, and sevenfold lower for HIV-2 wild-type strains than for HIV-1. Darunavir, lopinavir, and saquinavir PIQ values were similar.

* Corresponding author. Mailing address: Laboratoire de Virologie, Hôpital Bichat Claude Bernard, 46 rue Henri Huchard, 75018 Paris, France. Phone: 33 1 40 25 61 54. Fax: 33 1 40 25 67 69. E-mail: diane.descamps@bch.aphp.fr.

▽ Published ahead of print on 28 January 2008.

TABLE 1. Phenotypic susceptibilities to PIs and/or protease mutations of the HIV-2 subtype A and B consensus sequences of the nine wild-type isolates and HIV-1 and HIV-2 reference isolates before (T0) and during (T1 and T2) PI treatment[a]

Isolate[b]	Amprenavir		Atazanavir		Darunavir		Indinavir	
	IC_{50} (μM)	IC_{90} (μM)	IC_{50} (μM)	IC_{90} (μM)	IC_{50} (μM)	IC_{90} (μM)	IC_{50} (μM)	IC_{90} (μM)
HIV-1 reference isolate BRU	0.02	0.20	0.007	0.03	0.004	0.009	0.005	0.04
HIV-2 reference isolate ROD	0.60	4.60	0.03	0.10	0.005	0.02	0.03	0.30
HIV-2 clinical isolates from:								
Patient 1 (subtype H)								
T0: 10I-40P-41Y-60H-63N-70T-73G-89L-92E	0.90	2.70	0.10	0.50	0.004	0.04	0.02	0.50
T1: 10I-34E*-40P-41Y-60H-63N-70T-73G-82F*-89L-92E	10.0 (12)	82.0 (30)	1.90 (16)	15.0 (32)	0.90 (225)	4.70 (126)	12.0 (621)	99.0 (193)
Patient 2 (subtype A)								
T0: 14H-17D-43T-68N/D	0.60	3.80	0.07	0.20	0.009	0.03	<0.01	<0.01
T1: 5L/F*-14Y/H*-17G/D*-43T-54I/M*-62V/A*-70R/K*-71I*	4.40 (8)	13.0 (3)	0.30 (4)	2.10 (11)	0.60 (71)	7.30 (222)	18.0 (/)	2,794 (/)
Patient 3 (subtype A)								
T0: 14H-60K/N-65E	0.90	6.80	0.04	0.30	0.01	0.03	0.03	0.40
T1: 54M*-65E-71I*-74N*-90M*	4.00 (5)	60.0 (9)	0.40 (11)	1.80 (6)	0.20 (18)	1.40 (45)	10.0 (304)	82.0 (206)
Patient 4 (subtype A)								
T0: 10I-17D-40D-43I-46V-66V/A-70R/K	0.40	3.50	0.10	0.30	0.004	0.03	0.004	0.10
T1: 10I-17D-40D-43I-45K/R*-46V-54M*-64I/V*-69K/R*-71V/I*-90M*	9.50 (22)	31.0 (9)	0.20 (2)	1.80 (6)	0.80 (216)	3.00 (88)	0.20 (15)	3.10 (28)
Patient 5 (subtype A)								
T0: 14H-40D-70K-72R/K-91T/S	0.30	1.60	0.04	0.20	0.004	0.02	0.02	0.40
T1: 10I*-40D-43I*-70K-82F*-84V*-85L*-89V*-90M*-91T/L*-98N/K*	12.0 (36)	96.0 (60)	0.04 (0.8)	1.30 (6)	0.50 (124)	2.40 (105)	6.40 (400)	51.0 (143)
T2: 10V/I*-40D-43I*-56V*-70K-82F*-84V*-89V*-90M*	12.0 (35)	95.0 (60)	0.40 (9)	3.00 (14)	1.00 (254)	8.10 (351)	11.0 (705)	90.0 (252)
Patient 6 (subtype B)								
T0: 14Y-61N-99L	0.60	4.60	0.06	0.30	0.002	0.01	0.10	0.60
T1: 14Y-19P*-33I*-61N-71I*-75M*-84V*-90M*	14.0 (24)	110 (24)	2.20 (38)	17.0 (51)	0.50 (246)	3.90 (327)	3.80 (31)	30.0 (50)
T2: 14Y-19P*-61N-64V*-71I*-90M*-95I*	17.0 (30)	140 (30)	0.20 (3)	1.40 (4)	0.70 (324)	5.10 (429)	12.0 (100)	95.0 (161)
Patient 7 (subtype B)								
T0: 12T-14Y-19P-40N-41D-61N-62I-96S-99L	0.40	2.40	0.20	0.40	0.004	0.02	0.004	0.02
Patient 8 (subtype B)								
T0: 12Q-14R-17G/D-19P-61N-62I-92A	0.40	2.80	0.03	0.30	0.01	0.05	0.40	2.60
Patient 9 (subtype A)								
T0: 41D	0.80	3.30	0.03	0.30	0.10	0.50	0.006	0.20
Median IC_{50}/IC_{90} value of clinical isolates at T0 (±SD)	0.60 (±0.20)	3.30 (±1.50)	0.06 (±0.05)	0.30 (±0.08)	0.004 (±0.04)	0.03 (±0.16)	0.02 (±0.10)	0.40 (±0.80)

[a] Values in parentheses are the fold increases over the values for T0.
[b] Protease gene sequences are given for the indicated time points. *, substitutions selected between T0 and T1/T2.

PIs are designed to fit the active site of the HIV-1 protease and are sensitive to structural changes in the viral protein. It has been reported that the therapeutic outcome of HIV-2-infected patients might be influenced by the choice of PIs (1, 15). For amprenavir, our data are in keeping with those reported elsewhere, showing significantly less activity against HIV-2 wild-type strains than against HIV-1 (9, 14, 16). These phenotypic results could be explained by the natural presence in HIV-2 protease of amino acids associated with resistance to HIV-1, which might influence the binding affinity of the PIs for HIV-2 protease (2, 3, 9, 10, 11). In our study, all the HIV-2 wild-type strain protease sequences naturally presented the amino acids 32I and 47V, associated with resistance in HIV-1 infection to amprenavir, according to the IAS-USA list and to different genotypic resistance interpretation algorithms (www.hivfrenchresistance.org, http://hivdb.stanford.edu and www.kuleuven.be/rega/cev/links/rega_algorithm). We found that clinical HIV-2 isolates and HIV-1 reference strains had similar phenotypic susceptibility to saquinavir, lopinavir, and darunavir. As amprenavir and darunavir are structurally close, we expected darunavir to be relatively ineffective in HIV-2. Crystallographic studies with HIV-1 showed that darunavir interacts directly with the main chains of aspartic acid residues (Asp-29 and Asp-30), whereas other PIs interact with side chains in the S2 subsite of the HIV-1 enzyme (6, 7). Moreover, it has been reported for HIV-1 that the binding affinity of darunavir for the wild-type protease was >100-fold higher than those of other PIs due to the slower dissociation rate of this molecule

TABLE 1—*Continued*

Lopinavir		Nelfinavir		Saquinavir		Tipranavir	
IC_{50} (μM)	IC_{90} (μM)	IC_{50} (μM)	IC_{90} (μM)	IC_{50} (μM)	IC_{90} (μM)	IC_{50} (μM)	IC_{90} (μM)
0.03	0.10	0.002	0.10	0.01	0.04	0.05	0.40
0.02	0.07	0.05	0.90	0.01	0.09	0.40	3.50
0.06	0.50	0.04	0.30	0.03	0.30	0.40	0.80
3.20 (57)	8.70 (17)	9.00 (236)	24.0 (79)	7.30 (252)	31.0 (114)	7.30 (19)	16.0 (20)
0.03	0.08	0.03	0.30	<0.006	<0.006	0.30	2.20
0.04 (1)	0.40 (5)	14.0 (463)	110 (450)	>12.5 (/)	>12.5 (/)	6.70 (24)	26.0 (12)
0.04	0.20	0.06	0.80	0.005	0.05	0.30	2.50
0.40 (12)	3.30 (21)	3.80 (68)	53.0 (65)	>12.5 (/)	>12.5 (/)	4.70 (15)	14.0 (5)
0.02	0.20	0.05	0.40	0.002	0.02	0.30	2.70
1.70 (75)	8.50 (42)	1.20 (24)	15.0 (37)	0.09 (45)	0.70 (33)	4.10 (12)	23.0 (9)
0.02	0.08	0.20	0.40	0.005	0.02	0.40	4.20
0.10 (5)	0.80 (10)	>17.6 (/)	>17.6 (/)	6.40 (1,287)	51.0 (2,221)	2.80 (8)	16.0 (4)
0.10 (7)	1.10 (14)	10.0 (66)	81.0 (193)	22.0 (4,421)	180 (7,635)	4.80 (13)	29.0 (7)
0.04	0.07	0.20	1.00	14.0	82.0	0.40	1.70
0.20 (4)	1.50 (20)	14.0 (67)	107 (107)	8.20 (0.6)	65.0 (0.8)	4.80 (11)	17.0 (10)
0.10 (3)	0.9 (12)	>17.6 (/)	>17.6 (/)	6.70 (0.5)	52.0 (0.6)	4.00 (10)	12.0 (7)
0.03	0.10	0.06	0.20	0.007	0.02	0.40	3.00
0.02	0.20	0.04	0.80	0.06	0.30	0.30	2.60
0.04	0.10	0.20	2.10	0.02	0.20	0.30	0.7
0 0.03 (±0.01)	0.14 (±0.10)	0.06 (±0.08)	0.40 (±0.60)	0.008 (±4.60)	0.05 (±27.0)	0.30 (±0.05)	2.50 (±1.10)

from the protease active site (5). In the same way, crystallography structure studies of the HIV-2 protease and binding affinity experiments might help us to understand the difference observed in the natural susceptibilities of HIV-2 strains to these two drugs as well as phenotypic resistance in HIV-2 mutated strains. The IC_{50} and IC_{90} values of atazanavir, indinavir, nelfinavir, and tipranavir for the HIV-2 isolates were higher than those observed for HIV-1, suggesting the hypothesis of the lower activities of these PIs against HIV-2. However, these values were lower than their respective trough plasma concentrations. This might be explained by the fact that HIV-2 wild-type isolates harbored several amino acids associated with PI resistance in HIV-1.

PI treatment-associated amino acid changes in the HIV-2 protease gene occurred at positions known to confer PI resistance in HIV-1 and were not associated with the use of a particular PI without any order of accumulation. They altered the phenotypic susceptibility of the isolates to all the PIs tested here. These results are in keeping with data published elsewhere (2, 3, 9, 11, 13, 15). Mutagenesis experiments coupled with phenotypic susceptibility testing might help to determine the impact of each substitution on PI resistance. Saquinavir, lopinavir, and darunavir appear to be the best choices for first-line therapy of HIV-2 infection, while amprenavir should not be used. Atazanavir and tipranavir might be used with care (17). Our results suggest that treatment guidelines for HIV-1-infected patients should not be directly extrapolated to HIV-2-infected patients. Virological efficacy data in vivo might help us to evaluate the place of PIs in HIV-2 antiretroviral strategy.

Drugs and sources. Amprenavir was provided by Glaxo-SmithKline (Marly-le-Roi, France), atazanavir by Bristol-Myers Squibb (Rueil-Malmaison, France), darunavir by Tibotec

(Mechelen, Belgium), indinavir by Merck Sharp & Dohme-Chibret (West Point, PA), lopinavir by Abbott (Rungis, France), nelfinavir and saquinavir by Roche (Neuilly sur Seine, France), and tipranavir by Boehringer-Ingelheim (Ridgefield, CT).

This work was supported by Agence Nationale de Recherche sur le SIDA et les Hépatites Virales (ANRS).

We thank Laetitia Stephant for her technical skills.

REFERENCES

1. **Adjé-Touré, C. A., R. Cheingsong, J. G. Garcia-Lerma, S. Eholie, M. Borget, J. Bouchez, R. A. Otten, C. Maurice, M. Sassan-Morokro, R. E. Ekpini, M. Nolan, T. Chorba, W. Heneine, and J. N. Nkengasong.** 2003. Antiretroviral therapy in HIV-2-infected patients: changes in plasma viral load, $CD4^+$ cell counts and drug resistance profiles of patients treated in Abidjan, Cote d'Ivoire. AIDS **17:**S49–S54.
2. **Colson, P., M. Henry, M. Tivoli, H. Gallais, J. A. Gastaut, J. Moreau, and C. Tamalet.** 2003. Polymorphism and drug-selected mutations in the reverse transcriptase of HIV-2 from patients living in southern France, abstr. 145. XII Int. HIV Drug Resist. Workshop: Basic Princ. Clin. Implications, Los Cabos, Mexico.
3. **Damond, F., F. Brun-Vezinet, S. Matheron, G. Peytavin, P. Campa, S. Pueyo, F. Mammano, S. Lastere, I. Farfara, F. Simon, G. Chene, and D. Descamps.** 2005. Polymorphism of the human immunodeficiency virus type 2 (HIV-2) protease gene and selection of drug resistance mutations in HIV-2-infected patients treated with protease inhibitors. J. Clin. Microbiol. **43:**484–487.
4. **Damond, F., G. Collin, S. Matheron, G. Peytavin, P. Campa, S. Delarue, A. Taieb, A. Benard, G. Chene, F. Brun-Vezinet, and D. Descamps.** 2005. In vitro phenotypic susceptibility to nucleoside reverse transcriptase inhibitors of HIV-2 isolates with the Q151M mutation in the reverse transcriptase gene. Antivir. Ther. **10:**861–865. (Letter.)
5. **De Wit, M., I. Keuleers, E. Gustin, I. Dierynck, S. Hallenberger, and K. Hertogs.** 2007. Binding kinetics of PI to wild type and multi drug resistant HIV-1 proteases: a mechanistic study of the genetic barrier to resistance to darunavir, abstr. 605. XIV Conf. Retroviruses Opportunistic Infect., Los Angeles, CA, 25 to 28 February 2007.
6. **Koh, Y., H. Nakata, K. Maeda, H. Ogata, G. Bilcer, T. Devasamudram, J. F. Kincaid, P. Boross, Y. F. Wang, Y. Tie, P. Volarath, L. Gaddis, R. W. Harrison, I. T. Weber, A. K. Ghosh, and H. Mitsuya.** 2003. Novel *bis*-tetrahydrofuranylurethane-containing nonpeptidic protease inhibitor (PI) UIC-94017 (TMC114) with potent activity against multi-PI-resistant human immunodeficiency virus in vitro. Antimicrob. Agents Chemother. **47:**3123–3129.
7. **Kovalevsky, A. Y., F. Liu, S. Leshchenko, A. K. Ghosh, J. M. Louis, R. W. Harrison, and I. T. Weber.** 2006. Ultra-high resolution crystal structure of HIV-1 protease mutant reveals two binding sites for clinical inhibitor TMC114. J. Mol. Biol. **363:**161–173.
8. **Matheron, S., F. Damond, A. Benard, A. Taieb, P. Campa, G. Peytavin, S. Pueyo, F. Brun-Vezinet, and G. Chene.** 2006. CD4 cell recovery in treated HIV-2-infected adults is lower than expected: results from the French ANRS CO5 HIV-2 cohort. AIDS **20:**459–462.
9. **Ntemgwa, M., B. G. Brenner, M. Oliveira, D. Moisi, and M. A. Wainberg.** 2007. Natural polymorphisms in the human immunodeficiency virus type 2 protease can accelerate time to development of resistance to protease inhibitors. Antimicrob. Agents Chemother. **51:**604–610.
10. **Parreira, R., F. Monteiro, E. Padua, J. Piedade, T. Venenno, M. T. Paixao, and A. Esteves.** 2006. Natural polymorphisms of HIV type 2 *pol* sequences from drug-naive individuals. AIDS Res. Hum. Retroviruses **22:**1178–1182.
11. **Pieniazek, D., M. Rayfield, D. J. Hu, J. N. Nkengasong, V. Soriano, W. Heneine, C. Zeh, S. M. Agwale, C. Wambebe, L. Odama, and S. Z. Wiktor.** 2004. HIV-2 protease sequences of subtypes A and B harbor multiple mutations associated with protease inhibitor resistance in HIV-1. AIDS **18:**495–502.
12. **Ren, J., L. E. Bird, P. P. Chamberlain, G. B. Stewart-Jones, D. I. Stuart, and D. K. Stammers.** 2002. Structure of HIV-2 reverse transcriptase at 2.35-A resolution and the mechanism of resistance to non-nucleoside inhibitors. Proc. Natl. Acad. Sci. USA **99:**14410–14415.
13. **Rodés, B., A. Holguín, V. Soriano, M. Dourana, K. Mansinho, F. Antunes, and J. González-Lahoz.** 2000. Emergence of drug resistance mutations in human immunodeficiency virus type 2-infected subjects undergoing antiretroviral therapy. J. Clin. Microbiol. **38:**1370–1374.
14. **Rodés, B., J. Sheldon, C. Toro, V. Jimenez, M. A. Alvarez, and V. Soriano.** 2006. Susceptibility to protease inhibitors in HIV-2 primary isolates from patients failing antiretroviral therapy. J. Antimicrob. Chemother. **57:**709–713.
15. **van der Ende, M. E., K. Brinkman, M. Keuters, J. M. Prins, S. A. Danner, A. D. M. E. Osterhaus, and M. Schutten.** 2000. Antiretroviral therapy in HIV2 infected patients in the Netherlands: results in 17 patients. AIDS **14:**S104.
16. **Witvrouw, M., C. Pannecouque, W. M. Switzer, T. M. Folks, E. De Clercq, and W. Heneine.** 2004. Susceptibility of HIV-2, SIV and SHIV to various anti-HIV-1 compounds: implications for treatment and postexposure prophylaxis. Antivir. Ther. **9:**57–65.
17. **Yeni, P.** 2006. Rapport 2006: prise en charge médicale des personnes infectées par le VIH. Flammarion, Paris, France.