In Vitro Phenotypic Susceptibility of HIV-2 Clinical Isolates to CCR5 Inhibitors

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HIV-2 is naturally resistant to nonnucleoside reverse transcriptase inhibitors, to a fusion inhibitor, and to some of the protease inhibitors. Maraviroc is the first drug of the new anti-CCR5 drug class and is effective only on CCR5-tropic (R5) HIV-1. No previous studies concerning HIV-2 susceptibility to maraviroc have been reported yet. We developed a phenotypic maraviroc susceptibility test using a peripheral blood mononuclear cell (PBMC) model. We analyzed the maraviroc susceptibility of 13 R5 HIV-2, 2 X4R5 (dual) HIV-2, and 2 CXCR4-tropic (X4) HIV-2 clinical isolates. We also tested, with the same protocol, 1 X4 HIV-1 and 4 R5 HIV-1 clinical isolates. For the R5 HIV-2 clinical isolates, the 50% effective concentration (EC₅₀) for maraviroc was 0.80 nM (interquartile range [IQR], 0.48 to 1.39 nM), similar to that observed for the R5 HIV-1 isolates. The median maximum percentage of inhibition in the R5 HIV-2 isolates was 93% (IQR, 84 to 98%), similar to that observed in the R5 HIV-1 isolates. As expected, both X4 HIV-1 and HIV-2 were highly resistant to maraviroc. Our study showed for the first time that maraviroc is active *in vitro* against R5 HIV-2. The new tools we developed will allow identification of HIV-2-infected patients eligible for CCR5 inhibitor use and management of virological failure when receiving a maraviroc-based regimen.

HIV-2 is a retrovirus discovered a few years after HIV-1. HIV-2 infections are restricted mainly to West Africa, including Guinea-Bissau, Gambia, Senegal, and Guinea. Some European countries are also concerned with HIV-2 infection, which represents 5% of HIV infection in a series of patients in Portugal (17) and 2% of the new HIV infections in France (3).

The HIV-1 and HIV-2 genomes differ by about 50 to 60% at the nucleotide level. Such differences may be correlated with differential responses to some antiretrovirals, as observed with the natural resistance of HIV-2 to nonnucleoside reverse transcriptase inhibitors and to the fusion inhibitor enfuvirtide and the decreased susceptibility to some protease inhibitors (13, 14, 21). The new class of CCR5 inhibitors, including maraviroc (7), is the first one to have a cellular target, and so, it is expected not to be impacted by HIV genetic diversity and may provide a new therapeutic opportunity for HIV-2 infection. However, previous studies showed that HIV-2 isolates can use a broad range of coreceptors *in vitro*, including CCR5 and CXCR4 as well as alternative coreceptors, as follows: CCR1-5, GPR15 (BOB), CCR8, and CXCR6 (BONZO) (5, 8, 10, 12). The *in vivo* role of these alternative coreceptors is still under question (2, 9, 11, 22); however, it suggests that HIV-2 clinical isolates may be able to access more coreceptors than those of HIV-1, a mechanism that might impact the antiviral activity of maraviroc against HIV-2.

The only study reporting on the *in vitro* phenotypic susceptibility of HIV-2 to CCR5 inhibitors was carried out using nonlicensed CCR5 inhibitors, precursors of maraviroc, with only two CCR5-using HIV-2 isolates used (20). *In vivo*, two case reports described an early virological response with a multiple-drug salvage regimen containing maraviroc in two HIV-2-infected patients with a multiresistant virus (1, 16).

The aims of this study were to determine the viral tropism and phenotypic susceptibility to maraviroc of HIV-2 clinical isolates.

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MATERIALS AND METHODS

Study patients and virus stocks. All strains assessed in the study were issued from distinct HIV-2-infected maraviroc-naïve patients of the French Agence Nationale de Recherches sur le SIDA et les Hépatites Virales (ANRS) HIV-2 cohort (CO 05) and were isolated by cocultivation of the phytohemagglutinin (PHA)-activated peripheral blood mononuclear cell (PBMC) pool obtained from healthy blood donors, as previously described (15). Written consent was provided by all patients at the time of inclusion in the French ANRS CO 05 HIV-2 cohort.

We recently developed an HIV-2 tropism phenotypic assay (TPA) using two GHOST(3) cell lines expressing CD4-plus-CCR5 or CD4-plus-CXCR4 coreceptors, with a HIV-2 long terminal repeat (LTR)-driven green fluorescent protein (GFP) gene which is activated upon infection (18). Infection efficiency was quantified by flow cytometric analysis for GFP detection. TPA was performed on 53 HIV-2 clinical isolates obtained from maraviroc-naïve patients. The HIV-2 gp105 V3 loop was also sequenced from viral supernatants, and statistical analyses resulted in the identification of 4 genotypic determinants of CXCR4 coreceptor usage in the V3 loop, as follows: (i) mutation at residue 18, (ii) mutation V19K/R, (iii) V3 loop global net charge, and (iv) insertions at position 24. Among the 53 HIV-2 clinical isolates tested, 34 were classified as CCR5-tropic

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TABLE 1 Maraviroc phenotypic susceptibility of 17 HIV-2 and 5 HIV-1 clinical isolates

Clinical isolate	Type	Viral group	Phenotypic tropism ^a	EC ₅₀ (nM)	MPI (%)
10-033	HIV-2	B	R5	1.45	81
10-044		A	R5	1.39	85
10-046		A	R5	1.13	93
10-047		B	R5	0.04	98
10-048		A	R5	0.99	93
10-050		A	R5	3.58	70
10-051		A	R5	0.48	82
10-056		A	R5	0.58	90
10-058		B	R5	0.80	93
10-060		A	R5	0.38	84
10-064		A	R5	1.42	98
10-067		B	R5	0.27	99
10-074		A	R5	0.68	100
10-049		A	R5X4	9.40	55
10-069		A	R5X4	>1,000	12
10-045	HIV-1	A	X4	>1,000	0
10-055		A	X4	>1,000	0
CNR-39		M (B subtype)	R5	3.69	74
CNR-40		M (B subtype)	R5	5.63	70
CNR-41		M (B subtype)	R5	0.12	78
CNR-43		M (B subtype)	R5	1.05	90
CNR-37		M (B subtype)	X4	>1,000	28

^a Determined by the tropism phenotypic assay using GHOST(3) cells, as described in reference 18.

(R5) viruses, 11 as CXCR4-tropic (X4) viruses, and 8 as X4R5 (dual) viruses by TPA. Phenotypic assay was performed using HIV-2 supernatants, classified by TPA as R5 (*n* = 13), dual (*n* = 2), and X4 (*n* = 2) viruses. Among the HIV-2 R5 viruses, 9 belong to HIV-2 group A and 4 to HIV-2 group B. Phenotypic susceptibility to maraviroc of HIV-1 isolates was also determined (R5, *n* = 4, and X4, *n* = 1).

Phenotypic susceptibility determination. Phenotypic susceptibility of HIV-2 clinical isolates to maraviroc was determined using a ANRS PBMC method, as previously described (4). All maraviroc susceptibility assays were performed in RPMI 1640 medium containing 10% (vol/vol) heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, and 1,000 U/ml penicillin-streptomycin. All PBMCs were pooled from three to four donors and incubated for 3 days in culture medium containing PHA. PHA-activated PBMCs were washed and resuspended in medium containing human recombinant interleukin-2 (IL-2) immediately prior to use in an antiviral assay.

Drug susceptibility assays were performed in 96-well tissue culture plates. Six-point dilution series of maraviroc (final concentrations of 0, 0.1, 0.5, 2.5, 10, and 100 nM) were prepared in culture medium. PHA-stimulated PBMCs were preincubated with maraviroc at the appropriate concentrations for 1 h before viral infection. Cells were washed and infected with cell-free virus supernatant at 100 median tissue culture infective doses (TCID₅₀) for 1 h at 37°C. Cells were subsequently washed once and 2 × 10⁶ infected PBMCs were added to each well of assay plates containing maraviroc. Plates were incubated for 4 days at 37°C in a humidified 5% (vol/vol) CO₂ atmosphere. Each dilution was tested in quadruplicate. To assess viral titers, serial dilutions (1, 10⁻¹, and 10⁻²) of cell-free viral supernatant were also tested in quadruplicate during each assay. At day 4, viral supernatant was collected, and virus replication was quantified by HIV-2 specific viral load measurement, as previously described (6). All viral supernatants were tested at 100 TCID₅₀; when the titer obtained for serial dilutions was lower at day 4, viral supernatants obtained at day 5 were tested. The percent inhibition of viral replication for each concentration of maraviroc was calculated to determine the 50%

effective concentration (EC₅₀) and maximum percentage of inhibition (MPI).

The direct sequencing of the gp105 V3 loop was performed using viral supernatants at time of phenotypic assay. No changes were observed in gp105 V3 loop sequences for any of the HIV-2 clinical isolates between day 0 and day 4 of the ANRS PBMC phenotypic susceptibility assay.

RESULTS AND DISCUSSION

EC₅₀ and MPI values of the strains assessed in the study are depicted in Table 1. Phenotypic susceptibility testing showed the maraviroc EC₅₀ to be 0.80 nM (interquartile range [IQR], 0.48 to 1.39 nM) in the 13 R5 HIV-2 clinical isolates tested (Fig. 1). The two dual HIV-2 isolates showed EC₅₀s of 9.40 nM and greater than 1,000 nM, respectively. Differences observed in the results of the 2 dual HIV-2 viruses might be related to the proportion of X4 virus present in the mixture, resulting in widely different dose-response curves. Both of the X4 HIV-2 isolates tested showed EC₅₀s of greater than 1,000 nM, as observed with the X4 HIV-1 virus. R5 HIV-1 isolates showed an EC₅₀ value to maraviroc of 2.37 nM (IQR, 0.81 to 4.17 nM).

The median MPI of R5 HIV-2 isolates was 93% (IQR, 84 to 98%). The MPIs were 12 and 55% for the two dual HIV-2 isolates and 0% for the two X4 HIV-2 isolates. Under the same conditions, the median HIV-1 MPI of R5 HIV-1 was 76% (IQR, 73 to 81%).

No specific *env* position, in or near the gp105 CCR5 binding site, was found to be associated with the level of EC₅₀ or MPI.

In this study, based on a PBMC phenotypic susceptibility model, the EC₅₀ and MPI values obtained for R5 HIV-2 strains are similar to those obtained for R5 HIV-1 strains tested under the same conditions. Moreover, the EC₅₀s obtained with HIV-2 are also in a range similar to those previously described for HIV-1 (7).

Maraviroc is a competitive viral entry inhibitor that does not block the intracellular viral replication cycle. Thus, a small number of activated PBMCs may be infected, in the presence of maraviroc, and should be able to produce a nonnegligible number of viral particles. This mode of action might explain that in our study, the MPI varies between 70 and 100% in HIV-2 as well as in HIV-1. PBMC assay steps also have intrinsic variability, including (i) the numbers of infected cells during the infection step, (ii) the level of viral replicative capacity of the clinical isolate, and (iii) CCR5 density variations at the surfaces

FIG 1 Susceptibility of 13 R5, 2 X4, and 2 dual HIV-2 clinical isolates to maraviroc using a modified version of the Agence Nationale de Recherches sur le SIDA et les Hépatites Virales (ANRS) PBMC assay. The dashed lines correspond to the 2 X4 and 2 dual HIV-2 clinical isolates.

of PBMCs between different PBMC pools. However, these different points were taken into account in our assay by quadruplicate determination of each point and by the use of a PBMC pool of three to four patients in each experiment.

Until now, in HIV-1, MPI values have been reported using a PBMC model in maraviroc-resistant strains only (19). This study showed differences in MPI levels between the PBMC model and recombinant model that might be explained by (i) the detection method of infection (reporter gene in recombinant assays compared to HIV load or reverse transcriptase activity in PBMC assays) and (ii) the duration of the cultivation step (one single viral replication cycle in recombinant assays compared to 3 to 7 days in PBMC assays).

Discrepant results are reported in the literature about the use of alternative coreceptors by HIV-2, which could be related to the cell line used in the *in vitro* assay [U87 or GHOST(3) cell lines] (2). In addition, two studies using different approach with PBMCs, one with homozygous $\Delta 32$ -*CCR5* PBMCs and the other with specific antagonists, showed that alternative coreceptors did not significantly impact viral replication on PBMCs (11, 22). These findings, added to the similar antiretroviral activity of maraviroc observed against HIV-2 and HIV-1 and the similar MPI range observed in our study, strongly suggest that alternative coreceptors do not seem to play a major role in HIV-2 infection.

In the present study, we set up a phenotypic PBMC-based test and assessed for the first time that maraviroc is active *in vitro* against R5 HIV-2 clinical isolates. Finally, the new tools we developed will allow identification of HIV-2-infected patients eligible for CCR5 inhibitor use and management of virological failure when receiving a maraviroc-based regimen.

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