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ANTIVIRAL AGENTS

In Vitro Antiviral Activity of Cabotegravir against HIV-2

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ABSTRACT We examined the antiviral activity of the integrase inhibitor (INI) cabotegravir against HIV-2 isolates from INI-naive individuals. HIV-2 was sensitive to cabotegravir in single-cycle and spreading-infection assays, with 50% effective concentrations (EC_{50}s) in the low to subnanomolar range; comparable results were obtained for HIV-1 in both assay formats. Our findings suggest that cabotegravir should be evaluated in clinical trials as a potential option for antiretroviral therapy and preexposure prophylaxis in HIV-2-prevalent settings.

KEYWORDS HIV-2, PrEP, West Africa, antiretroviral therapy, cabotegravir, human immunodeficiency virus, treatment

Human immunodeficiency virus type 2 (HIV-2) is endemic in West Africa and has spread to other locales with socioeconomic ties to the region (1, 2). Relative to HIV-1, HIV-2 infection involves a slower rate of CD4 cell decline, lower plasma viral loads, and slower disease progression (3–7). Nevertheless, significant numbers of HIV-2 and HIV-1/2 dually infected individuals eventually progress to AIDS and can benefit from antiretroviral therapy (ART) (7–11).

There are important differences between HIV-1 and HIV-2 with regard to antiretroviral (ARV) drug sensitivity (12, 13). HIV-2 is intrinsically resistant to nonnucleoside reverse transcriptase inhibitors (NNRTIs) (14, 15) and shows relatively poor sensitivity to several HIV-1-active protease inhibitors (PIs); saquinavir, darunavir, and lopinavir appear to be the only PIs with clinically effective potency against HIV-2 (16–20). These distinctions complicate HIV treatment in West Africa and other regions where HIV-1 and HIV-2 cocirculate. Difficulties in differentiating HIV-2 or HIV-1/2 dual infection from HIV-1 infection can lead to the inappropriate use of NNRTI-based regimens in HIV-2-infected patients and to premature use of PI-based regimens as first-line ART in patients infected solely with HIV-1 (21–23). Efforts are needed to simplify ART in areas where HIV-1/HIV-2 discriminatory testing is unreliable and stockouts of HIV-2-active antivirals are commonplace (24).

ARV regimens containing two nucleoside reverse transcriptase inhibitors (NRTIs) plus an integrase inhibitor (INI) or an NNRTI are currently recommended by the World Health Organization for first-line treatment of HIV-1 infection (25). A growing body of evidence suggests that INI-based regimens might fulfill the need for universally active first-line ART in settings where HIV-2 is endemic. HIV-2 is susceptible to the INIs raltegravir, elvitegravir, and dolutegravir, with 50% effective concentrations (EC_{50}s) in the low-nanomolar to picomolar range (26–31). Data from case studies and small case series indicate that raltegravir- and elvitegravir-based regimens can suppress HIV-2 viral loads in ART-naive individuals (32, 33) and in ART-experienced patients whose treatment history does not include an INI (32, 34–39). More recently, two groups conducting clinical trials in ART-naive HIV-2-infected patients reported favorable immunovirologic

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outcomes in response to INI-based regimens (40, 41). In addition, some evidence suggests that dolutegravir might be effective in a subset of HIV-2-infected patients who have developed resistance to raltegravir (42–44).

Cabotegravir (S/GSK1265744; Shionogi/GlaxoSmithKline) is an investigational INI currently in development for the prevention and treatment of HIV-1 infection (45, 46). The antiviral potency and pharmacokinetic properties of cabotegravir render the drug amenable to once-daily oral dosing, and long-acting injectable formulations of the drug have been evaluated in nonhuman primate models of HIV-1 infection and in clinical trials (47–58). In contrast, there are no published data regarding the activity of cabotegravir against HIV-2, although one group reported a mean EC_{50} of 0.12 nM for four HIV-2 isolates at an international meeting (59).

In the current study, we tested the susceptibility of 15 different HIV-2 isolates (8 from group A, 6 from group B, and 1 A/B intergroup recombinant) to cabotegravir in single-cycle infections of MAGIC-5A indicator cells. A detailed description of the single-cycle assay has been published elsewhere (60). We further tested a subset of our HIV-2 library in 6-day spreading infections of an immortalized T-cell line (CEMss) as described below. In both assay formats, HIV-1 isolates from ART-naive individuals were included for comparison. The 50% cytotoxic concentrations (CC_{50}) of cabotegravir in MAGIC-5A and CEMss cells were >1 and >10 μM, respectively, as assessed by CellTiter-Glo luminescent cell viability assay (Promega) (see Fig. S1 in the supplemental material).

Single-cycle assays: HIV-1_{NL4-3} and HIV-2_{ROD9}. We initially compared the susceptibility of two prototypic HIV strains to cabotegravir, i.e., HIV-1_{NL4-3} (group M, subtype B) and HIV-2_{ROD9} (group A). These viruses were derived from 293T/17 cultures that were transfected with corresponding full-length plasmid molecular clones as previously described (61). Both strains were tested against cabotegravir from two sources, GlaxoSmithKline (GSK) and Selleck Chemicals, Inc. All dilutions of the drug were prepared in 10% vol/vol dimethyl sulfoxide (DMSO); the final concentration of DMSO in the assay wells was 1%.

Cabotegravir from both suppliers was highly active against HIV-1_{NL4-3} and HIV-2_{ROD9}, with EC_{50}s ranging from 1.2 to 1.7 nM (see Table S1 in the supplemental material). These values are consistent with the EC_{50}s reported for HIV-1_{NL4-3}-based vectors in a single round of replication (EC_{50}s of 0.5 nM [47] and 1.6 nM [62]). Altogether, HIV-1_{NL4-3} and HIV-2_{ROD9} were similar in their susceptibility to cabotegravir; after 15 and 27 independent determinations, respectively, the mean EC_{50}s for these two strains differed by $<$1.1-fold (Fig. 1A and Table 1). For HIV-2_{ROD9}, the antiviral potency of cabotegravir was comparable to that of dolutegravir but greater than that of raltegravir and elvitegravir, as determined in the single-cycle assay (Fig. 1B).

Single-cycle assays: other HIV-1 and HIV-2 isolates. Next, we tested other HIV isolates from ARV-naive individuals in single-cycle infections. Cabotegravir inhibited group M HIV-1 strains from subtypes A, B, C, and D, as well as the group O isolate HIV-$1_{MVP5180-91}$, with EC_{50}s ranging from 1.3 to 2.2 nM (Table 1). A similar range of EC_{50}s was observed for eight group A HIV-2 strains (0.92 to 2.7 nM) (Table 1). Slight reductions in cabotegravir sensitivity relative to HIV-2_{ROD9} were apparent for group B isolates HIV-$2_{CDC310319}$ and HIV-2_{EHO} (EC_{50}s, 4.0 $\pm$ 0.84 and 4.1 $\pm$ 1.4 nM, respectively; $P <$ 0.0001, analysis of variance with Sidak's posttest). However, four other HIV-2 group B isolates yielded EC_{50}s that were similar to those seen for HIV-1 and HIV-2 group A (range, 1.0 to 2.7 nM) (Table 1). In addition, the A/B intergroup recombinant HIV-2_{7312A} (CRF01_AB), which contains a group B integrase sequence, was fully susceptible to the drug (EC_{50}, 1.6 nM) (Table 1). Altogether, the average EC_{50}s ($\pm$1 standard deviation) for HIV-1, group A HIV-2, and group B HIV-2 were 1.7 $\pm$ 0.38, 1.8 $\pm$ 1.0, and 2.6 $\pm$ 1.3 nM, respectively.

As an additional control, we determined the susceptibility of each of the HIV-1 and HIV-2 isolates discussed above to the NNRTI efavirenz. All HIV-2 strains were highly resistant to efavirenz in single-cycle infections, whereas all HIV-1 group M strains were susceptible to the drug (Table 1; see also Fig. S1 in the supplemental material). HIV-$1_{MVP5180-91}$ (group O)

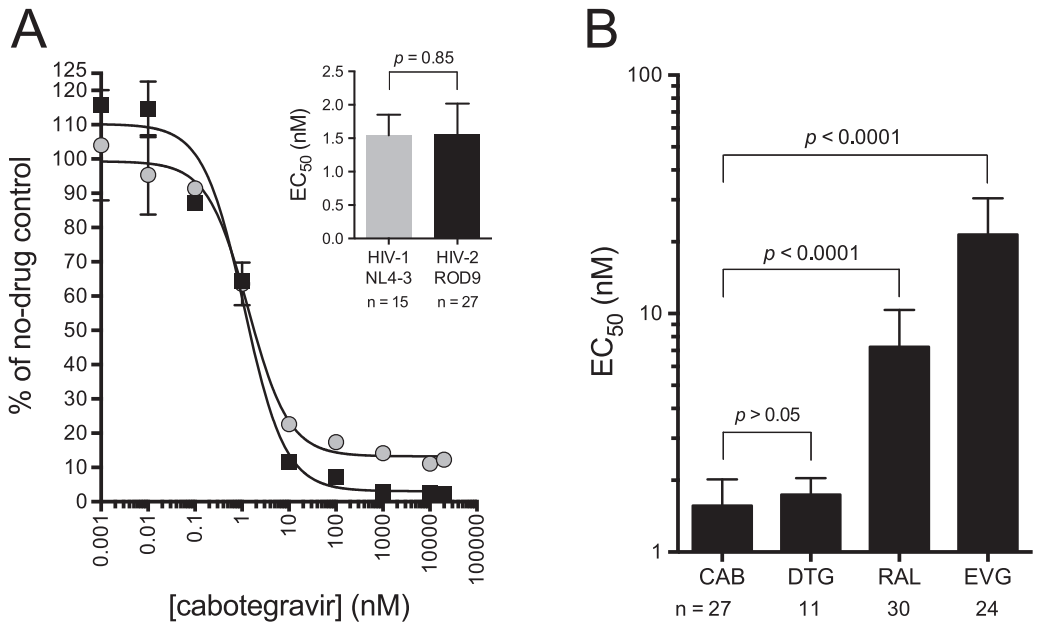

FIG 1 Antiviral activity of cabotegravir against HIV-1_{NL4-3} and HIV-2_{ROD9}. All data in the figure are from single-cycle infections of MAGIC-5A cells. Error bars indicate ±1 SD and, when not visible, are smaller than the symbols. (A) Results from a single assay in which HIV-1_{NL4-3} (gray circles) and HIV-2_{ROD9} (black boxes) were tested head-to-head. Data points represent the amount of β-galactosidase activity produced in HIV-infected cabotegravir-treated cultures relative to HIV-infected solvent-only (i.e., no-drug) controls. Each point is the mean of two cultures that were maintained in parallel. Curves were generated using a sigmoidal regression equation (GraphPad Prism 6.0 software). Mean EC_{50}s from multiple assay runs with HIV-1_{NL4-3} and HIV-2_{ROD9} are shown in the inset. Values below the *x* axis indicate the total number of assay runs that were performed for each strain. The *P* value was calculated using Welch's *t* test. (B) EC_{50}s obtained for HIV-2_{ROD9} tested against cabotegravir (CAB), dolutegravir (DTG), raltegravir (RAL), and elvitegravir (EVG). Data for raltegravir and dolutegravir are contemporaneous with the cabotegravir data set; data for elvitegravir were previously published (71). *P* values are from analysis of variance with Tukey's posttest.

also showed a reduction in efavirenz susceptibility relative to HIV-1 group M (EC_{50}, 54 ± 6.5 nM); this result is consistent with a previous report showing that HIV-$1_{MVP5180-91}$ is intrinsically resistant to the NNRTIs delavirdine and nevirapine in culture (63).

To ensure that our single-cycle assay could detect subtle differences in cabotegravir susceptibility, we constructed and tested HIV-1 and HIV-2 variants that contained site-directed mutations in the integrase-encoding region of *pol*; these mutations encode amino acid changes that are known to confer low- to intermediate-level resistance to cabotegravir and/or other INIs *in vitro* (27–31, 62, 64–67). The combination of replacements E92Q and N155H in HIV-1_{NL4-3} integrase conferred a 3.9-fold increase in the EC_{50} for cabotegravir relative to the parental wild-type clone. In contrast, the Y143C mutation alone or in combination with T97A had no impact on cabotegravir susceptibility (Table 2). These results are concordant with previous findings for Y143C and E92Q+N155H mutants of HIV-1 in single-cycle assays (47, 62). In addition, the E138K+G140S+Q148R mutant of HIV-1_{NL4-3} was 10-fold resistant to cabotegravir. For HIV-2_{ROD9}, mutants E92Q+Y143C, E92Q+N155H, and G140A+Q148R were 1.5-, 7.5-, and 6.9-fold resistant to cabotegravir, respectively, relative to wild-type HIV-2_{ROD9} (Table 2). Collectively, these data show that the single-cycle assay can reliably detect low-level cabotegravir resistance in both HIV-1 and HIV-2.

Spreading-infection assays. To assess the robustness of our findings with the single-cycle assay, we evaluated the activity of cabotegravir against two HIV-1 and eight HIV-2 isolates (five from group A, three from group B) in spreading infections of CEMss cells (also referred to as the multicycle assay). Briefly, 96-well microcultures of CEMss cells were treated with various concentrations of cabotegravir, followed by infection with HIV-1 or HIV-2 at a multiplicity of 0.01 to 0.04 focus-forming units per cell. Half of the culture volume was removed at days 2 and 4 postinfection and replaced with an

TABLE 1 Susceptibility of HIV-1 and HIV-2 isolates to cabotegravir in single-cycle infections of MAGIC-5A cells

Isolate by HIV type	Group/subtype	EC_{50} (nM)[a] for:	
		Cabotegravir[b]	Efavirenz[c]
HIV-1			
92UG029	M/A	2.0 ± 0.43	2.9 ± 0.35
NL4-3	M/B	1.5 ± 0.31	2.2 ± 0.46
LAI	M/B	1.4 ± 0.45	1.7 ± 0.073
MJ4	M/C	1.3 ± 0.061	1.4 ± 0.16
92UG001	M/D	2.2 ± 1.3	2.0 ± 0.21
MVP5180-91	O	2.0 ± 0.11	54 ± 6.5
HIV-2			
ROD9	A	1.6 ± 0.45	>1,000
7924A	A	1.0 ± 0.15	>1,000
MVP15132	A	2.5 ± 1.2	>1,000
60415K	A	2.7 ± 0.70	>1,000
CBL-20	A	0.92 ± 0.048	>1,000
CBL-23	A	2.1 ± 0.79	>1,000
CDC77618	A	2.3 ± 0.81	>1,000
ST	A	1.6 ± 0.30	>1,000
CDC310072	B	1.0 ± 0.23	>1,000
CDC310319	B	4.0 ± 0.84	>1,000
EHO	B	4.1 ± 1.4	>1,000
DIL	B	2.5 ± 0.33	>1,000
COU	B	2.3 ± 0.46	>1,000
BER	B	2.7 ± 0.82	>1,000
7312A	CRF01_AB[d]	1.6 ± 0.46	>1,000

[a]EC_{50}, 50% effective concentration (mean ± SD).
[b]All EC_{50}s for cabotegravir were calculated from three or more independent assay runs. EC_{50}s for NL4-3 and ROD9 were obtained using cabotegravir from GlaxoSmithKline, Inc., and Selleck Chemicals, Inc. (see also Table S1 in the supplemental material). The remaining isolates listed above were tested against cabotegravir from Selleck Chemicals.
[c]The NNRTI efavirenz serves as a non-INI control. EC_{50}s for efavirenz are the results of 3 determinations for each HIV-1 isolate and ≥2 determinations for each HIV-2 isolate.
[d]Intergroup (A/B) recombinant. The integrase-encoding sequence of HIV-2_{7312A} is monophyletic with that of other isolates belonging to HIV-2 group B (72).

equivalent volume of fresh medium and drug. On day 6, the cultures were frozen at −80°C to ablate CEMss viability. Samples from the assay plates were then diluted in complete medium and transferred to MAGIC-5A cells to measure the level of infectious virus; this "scoring" phase utilized our previously described protocol for the MAGIC-5A single-cycle assay (60).

TABLE 2 Antiviral activity of cabotegravir against site-directed mutants of HIV-1 and HIV-2 integrase

Genotype[a] by HIV type	EC_{50} (nM)[b]	Fold change[c]
HIV-1		
Wild type	1.5 ± 0.31	
Y143C	1.2 ± 0.069	0.80
T97A+Y143C	1.2 ± 0.75	0.80
E92Q+N155H	**5.9 ± 0.81**	**3.9**
E138K+G140S+Q148R	**15 ± 3.1**	**10**
HIV-2		
Wild type	1.6 ± 0.45	
E92Q+Y143C	2.4 ± 0.48	1.5
E92Q+N155H	**12 ± 5.4**	**7.5**
G140A+Q148R	**11 ± 5.5**	**6.9**

[a]Amino acid changes in HIV-1 and HIV-2 integrase were engineered via site-directed mutagenesis of plasmid molecular clones pNL4-3 and pROD9, respectively. Wild type indicates virus stocks produced from the parental (nonmutated) copies of pNL4-3 and pROD9. The integrase-encoding region of each plasmid clone was confirmed by automated Sanger DNA sequencing.
[b]EC_{50} determined in the MAGIC-5A single-cycle assay (means ± SD from ≥3 independent assay runs). Values shown in boldface are significantly different from the corresponding wild-type EC_{50} ($P < 0.0001$, analysis of variance of $\log_{10}$-transformed EC_{50}s with Sidak's posttest).
[c]EC_{50} for the mutant divided by the EC_{50} for the corresponding wild-type virus.

TABLE 3 Susceptibility of HIV-1 and HIV-2 isolates to cabotegravir in spreading infections of CEMss cells

Isolate by HIV type	Group/subtype	EC_{50} (nM)[a]	No. of assays[b]
HIV-1			
92UG029	M/A	0.21 ± 0.072	3
NL4-3	M/B	0.15 ± 0.029	4
HIV-2			
ROD9	A	0.14 ± 0.056	5
ST	A	0.25 ± 0.014	3
CBL-20	A	1.0 ± 0.82	2
CBL-23	A	0.16 ± 0.059	3
CDC77618	A	0.85 ± 0.57	3
CDC310319	B	0.99 ± 0.90	6
EHO	B	0.20 ± 0.027	2
DIL	B	1.3 ± 1.1	3

[a]Values are means ± SD. These assays were performed using cabotegravir from GlaxoSmithKline, Inc.
[b]Independent dose-response assays performed for each strain.

Cabotegravir potently inhibited HIV-2 replication in the multicycle assay; EC_{50}s ranged from 0.14 to 1.0 nM for group A and 0.20 to 1.3 nM for group B HIV-2 isolates, respectively (Table 3). The control/comparator strains HIV-$1_{92UG029}$ and HIV-1_{NL4-3} were likewise sensitive to the drug (Table 3). Of note, for HIV-$1_{92UG029}$ and HIV-1_{NL4-3}, the EC_{50}s obtained in spreading infections were ~10-fold lower than those seen in single-cycle infections; a similar fold increase in cabotegravir sensitivity was observed for HIV-2_{ROD9}, HIV-2_{ST}, and HIV-2_{CBL-23} (compare Tables 1 and 3). EC_{50}s for HIV-$2_{CDC77618}$, HIV-$2_{CDC310319}$, and HIV-2_{DIL} were also 2- to 4-fold lower in the spreading assay compared with single-cycle infections, although run-to-run variation for these three strains was relatively high in the spreading-infection assay (Table 3). The tendency toward lower EC_{50}s in spreading infections relative to single-cycle assays is consistent with previous studies of INIs from our group and others (27, 31, 65) and has also been observed with inhibitors belonging to the NRTI drug class (60, 68). Overall, our findings from the single-cycle and spreading-infection assays indicate that HIV-2 is sensitive to cabotegravir *in vitro*, with EC_{50}s in the low to subnanomolar range.

Implications for HIV-2 prevention and treatment. The UNAIDS (Joint United Nations Programme on HIV and AIDS)/World Health Organization has set ambitious targets for HIV diagnosis, prevention, and treatment, with the ultimate aim of ending the global AIDS epidemic by 2030 (69). Efforts to attain these goals in West Africa and other areas will require a renewed commitment to clinical care for HIV-2-infected individuals (24). In particular, efforts are needed to improve HIV-2 patient access to fixed-dose, single-tablet formulations in which all antiretroviral components are active against HIV-2.

Cabotegravir is a novel strand transfer inhibitor that could potentially be coformulated with two NRTIs for once-daily oral administration (55, 58). Our findings suggest that such a regimen would be active in HIV-2-infected patients and therefore might simplify first-line treatment of HIV infection in settings in which HIV-2 is endemic.

Long-acting, injectable formulations of cabotegravir (CAB-LA) have been proposed for two modalities: (i) as maintenance therapy (in combination with the NNRTI rilpivirine [RPV-LA]) for HIV-1-infected patients who are virologically suppressed (55, 58) and (ii) as preexposure prophylaxis (PrEP) in individuals with a high risk of HIV acquisition (49, 53, 54, 57). With regard to maintenance therapy, CAB-LA/RPV-LA would likely be precluded in HIV-2-infected patients because of the intrinsic resistance of HIV-2 to rilpivirine and other NNRTIs (14, 15, 70). For PrEP, CAB-LA is currently being compared with daily oral tenofovir disoproxil fumarate-emtricitabine in phase 2b and phase 3 clinical trials (clinicaltrials.gov NCT02720094 and NCT03164564, respectively). Based on the locations of the study sites, participants will be at risk primarily for acquiring HIV-1; risk of HIV-2 acquisition will be minimal. If CAB-LA proves to be effective for PrEP, we believe that an evaluation of the drug should be performed in an HIV-2-prevalent setting, preferably in the context of a controlled clinical trial.

Altogether, our findings suggest that cabotegravir may be useful for HIV prevention and treatment in areas that harbor significant numbers of HIV-2-infected individuals. Clinical studies should be performed to address these possibilities.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01299-18.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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