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BRIEF REPORT

Accumulation of Multiple Mutations In Vivo Confers Cross-Resistance to New and Existing Integrase Inhibitors

Wendy W. Zhang,[1,2] Peter K. Cheung,[2] Natalia Oliveira,[2] Marjorie A. Robbins,[2,a] P. Richard Harrigan,[1] and Aniqa Shahid[2]

[1]University of British Columbia, and [2]British Columbia Centre for Excellence in HIV/AIDS, Vancouver, Canada

Bictegravir (BIC) and cabotegravir (CAB) are the latest available HIV integrase inhibitors in clinical trials. The combination of major integrase inhibitor substitutions G140S/Q148H has been shown to confer high-level resistance to the approved integrase inhibitors raltegravir (RAL) and elvitegravir (EVG) but not necessarily dolutegravir (DTG). We assayed recombinant viruses made from patient-derived RNA extracts for resistance phenotype for a panel of viruses containing G140S/Q148H with additional accessory substitutions. The accumulation of multiple integrase substitutions confers high-level resistance to all 5 integrase inhibitors. There is extensive cross-resistance between DTG, BIC, and CAB (r = 0.96–0.97).

Keywords. integrase inhibitors; bictegravir, cabotegravir; dolutegravir; integrase resistance testing; phenotype.

Integrase inhibitors are the most recent class of antiretroviral drugs approved for treatment of human immunodeficiency virus (HIV) infections. Raltegravir (RAL), approved for use in Canada in 2009, elvitegravir (EVG), approved in 2012, and dolutegravir (DTG), approved in 2014, are the 3 integrase inhibitors currently most widely in clinical use. New integrase inhibitors, bictegravir (BIC) and cabotegravir (CAB), in late-phase clinical trials have shown good efficacy and tolerability [1, 2]. Resistance to these integrase inhibitors could present a potential barrier to effective treatment.

Mutations in integrase that confer resistance to RAL and EVG have been well documented, with many of the combinations of mutations having a lesser effect on phenotypic resistance to DTG than either RAL or EVG [3]. In the VIKING trials, integrase resistance mutations in RAL and EVG-experienced patients failing treatment lead to decreases in DTG efficacy. In particular, the combination of Q148 substitutions and 2 additional substitutions were common in patients failing RAL and EVG and led to a decreased response to DTG [4, 5]. Recent in vitro studies have shown that the combination G140S/Q148H with or without additional substitutions can affect BIC and CAB in addition to DTG efficacy. BIC tends to exhibit smaller fold change phenotypic resistance and CAB exhibits higher fold changes than DTG, although BIC, CAB, and DTG were not directly compared in these studies [6, 7]. Currently, little is known about the extent to which known integrase inhibitor resistance mutations compromise the effect of BIC and CAB and the amount of cross-resistance between the new and existing integrase inhibitors.

To study the impact of G140S/Q148H with 1 or more additional substitutions on integrase inhibitor susceptibility, we assayed the in vitro phenotypic susceptibility of patient-derived recombinant viruses to RAL, EVG, DTG, BIC, and CAB. We also assessed cross-resistance between DTG and these integrase inhibitors.

METHODS

Cells, Viruses, and Integrase Inhibitors

CEM-GXR cells and the HIV-1 pNL4.3 plasmid with integrase gene deleted (Δ*int*) were kindly provided by Dr Mark Brockman (Simon Fraser University, Burnaby, Canada) [8]. MT4-LTR-EGFP cells were kindly provided by Dr Theresa Pattery (Janssen Diagnostics, Beerse, Belgium). The drugs RAL, EVG, DTG, and CAB were purchased from Selleck Chemicals (Houston, TX). BIC was synthesized by the Centre for Organic Synthesis, University of British Columbia (Vancouver, Canada).

Recombinant Virus Construction

The British Columbia Centre for Excellence in HIV/AIDS (BCCfE) collected patient plasma samples as part of routine clinical testing. Patient samples that were tested for integrase genotype containing a variety of combinations of major, minor, and accessory mutations based on current Stanford HIV Drug Resistance Database defined mutations were selected for recombinant virus construction [9]. Ethical approval was granted by the University of British Columbia Providence Health Care Research Ethics Board (protocol H14-03423). Samples were extracted using the NucliSens easyMag Extractor (bioMérieux, Saint-Laurent, Canada). Oligogenome amplification was performed on viral extracts by dilution in diethyl pyrocarbonate-treated water to approximately 500 viral copies/mL in order to achieve primarily single-copy amplification. Diluted extracts were polymerase chain reaction (PCR) amplified in 12 parallel reactions using the Transcriptor One-step RT-PCR kit (Roche, Basel, Switzerland) for reverse transcription and first-round PCR. A second round of PCR amplification was performed on each reaction using integrase-specific primers IN4155F

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[a]Current affiliation: BC Children's Hospital Research Institute, Vancouver, BC, Canada.

Correspondence: P. Richard Harrigan, PhD, Division of AIDS, Department of Medicine, University of British Columbia, Vancouver, British Columbia V6T 1Z2, Canada, (richard.harrigan@ubc.ca).

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(5′-GTACCAGCACACAAAGGAATTGGAG) and IN5219R (5′-CCTAGTGGGATGTGTACTTCTGAAC) creating an amplicon covering amino acids 1–288 of integrase. PCR amplicons were sequenced on an ABI 3730xl sequencer (Thermo Fisher Scientific, Waltham, MA) and analyzed using the in-house base-calling software ReCall (University of British Columbia, Vancouver, Canada) [10]. Amplicons that contained no inferred amino acid mixtures at 27 codons associated with HIV integrase exposure upon sequence analysis ([9]; Supplementary Table) were chosen for recombinant virus construction. Where multiple amplicons met the criteria, one was chosen arbitrarily. Recombinant viruses were generated by cotransfecting amplicons with linearized Δ*int*-pNL4.3 plasmid into CEM-GXR cells via electroporation using the Bio Rad Gene Pulser II, using a previously described method [8]. Recombinant viruses, generated as previously described, were grown in RPMI-1640 media plus 20% fetal bovine serum (R20+ media) and harvested at approximately 30% GFP. The virus sequences were confirmed to be identical to the amplicon sequences at 27 codons associated with HIV integrase exposure.

Phenotypic Susceptibility Assay and Data Analysis

Recombinant virus titers were determined by infecting MT4-LTR-EGFP cells in a 3-day assay and infectivity data collected using a Spectramax i3 Minimax 300 microplate reader (Molecular Devices, San Jose, CA). This microplate reader and cytometer captures images of each individual well of a 96-well plate and counts the number of infected fluorescent green fluorescent protein (GFP)-positive cells and noninfected GFP-negative cells. Titered volumes of recombinant viruses (such that 15%–30% GFP+ is reached on the third or fourth day postinfection) and MT4-LTR-EGFP cells were plated in triplicate with 8 concentrations ranging from no drug to 1000 nM (10-fold dilutions from 0.1 nM to 1000 nM with 3 nM and 45 nM included) of RAL, EVG, DTG, BIC, and CAB. Percent infection was measured using the Spectramax microplate reader.

Data Analysis

The 50% effective concentrations (EC_{50}) were calculated by fitting to a 4-parameter EC_{50} model using R with in-house scripts. Fold changes in EC_{50} of the virus relative to a NL4.3 control virus were calculated using mean EC_{50} (performed in triplicate). Median fold change was calculated from 1 or more independent experiments. Graphs and statistical analysis were done in R.

RESULTS

Eleven patient samples containing G140S/Q148H substitutions were identified from the BCCfE genotype database and 12 recombinant viruses with patient-derived integrase gene were constructed from clonal PCR products. The majority of the viruses tested were subtype B viruses (11/12) with 1 subtype C virus also included. Recombinant viruses were assayed for phenotypic susceptibility to integrase inhibitors (Table 1). Of the 12 viruses examined, 7 had only G140S/Q148H, 2 had the combination G140S/Q148H + T97A and 3 viruses had G140S/Q148H + T97A + L74M. Viruses with G140S/Q148H were highly resistant to RAL and EVG with fold change >100 but had relatively small changes in susceptibility to DTG, BIC, and CAB (median fold change 2.5 to 3.7) (Table 1). Viruses with an additional T97A substitution or additional T97A + L74M substitutions maintained high fold change to RAL and EVG (fold change >100) but were increasingly resistant to DTG, BIC, and CAB. Resistance to DTG, BIC, and CAB increased 3- to 20-fold with each additional substitution. Resistance to DTG increased to 28 (range 23–33) with T97A and to 345 (range 263–514) with the further addition of L74M. Similar increasing trends were observed with BIC and CAB: 9 (range 5.6–12) to 67 (range 55–88) for BIC and 74 (range 30–117) to 586 (range 249–866) for CAB. Fold change resistance to BIC was slightly lower than for DTG and CAB.

Correlation coefficients of log fold change values for DTG and the other 4 integrase inhibitors were calculated. Dolutegravir log fold change resistance values were very strongly correlated with BIC and CAB with correlation coefficients of 0.97 (slope 0.69) and 0.96 (slope 1.1), respectively (Figure 1A and 1B). DTG resistance, however, was poorly correlated with RAL and EVG resistance (data not shown).

Discussion

In this study, we compared the phenotypic profiles of HIV integrase inhibitors on a panel of patient-derived viruses containing

Table 1. Fold Change in EC_{50} of Recombinant Viruses With G140S and Q148H Mutations and Additional Mutations for RAL, EVG, DTG, BIC, and CAB

Mutations	G140S + Q148H	G140S + Q148H + T97A	G140S + Q148H + T97A + L74M
No. of patients	6	2	3
No. of viruses	7	2	3
Fold change in EC_{50}, median (range)			
RAL	>100 (>50–>100)	>100 (>50–>100)	>100 (>50–>100)
EVG	>100 (>100–>100)	>100 (>100–>100)	>100 (>100–>100)
DTG	3.0 (1.9–8.2)	28 (23–33)	345 (263–514)
BIC	2.5 (1.6–3.2)	9 (5.6–12)	67 (55–88)
CAB	3.7 (1.8–5.8)	74 (30–117)	586 (249–866)

Abbreviations: BIC, bictegravir; CAB, cabotegravir; EC_{50}, 50% effective concentration; EVG, elvitegravir; DTG, dolutegravir; RAL, raltegravir.

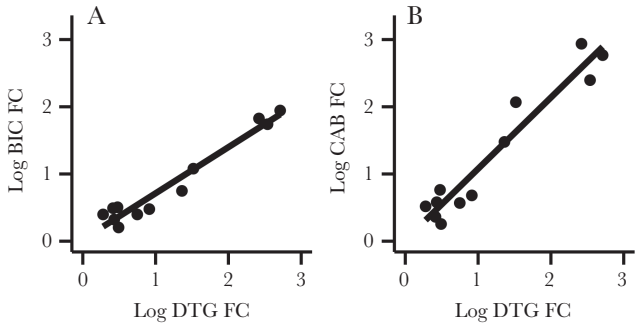

Figure 1. Correlation between log bictegravir (BIC), cabotegravir (CAB), and dolutegravir (DTG) fold change (FC) values for the same sample. *A*, Log BIC FC versus log DTG FC; correlation (*r* value) 0.97 and slope 0.69. *B*, Log CAB FC versus log DTG FC; correlation (*r* value) 0.96 and slope 1.1.

G140S/Q148H substitutions, particularly in combination with additional minor or accessory substitutions. The Q148 pathway of resistance is one of the major pathways to developing high-level integrase inhibitor resistance. Q148 substitutions are commonly seen in patients failing RAL and EVG and have even been observed in a case of CAB failure [1]. Similar to reports elsewhere, we observed that these variants had greatly reduced RAL and EVG susceptibility, exceeding the limits of our analysis [11]. Viruses containing only G140S/G148H had relatively low (2- to 4-fold) shifts in resistance to DTG, BIC, and CAB. However, small shifts in fold change may have important clinical effects for these next-generation integrase inhibitors, as seen in the VIKING-2 and VIKING-3 studies where even 2-fold to 10-fold changes in susceptibility had an impact on clinical outcomes [4, 5].

We observed very substantial decreases in susceptibility to DTG, BIC, and CAB when the substitutions T97A and L74M were combined with the major substitutions G140S/Q148H. With at least 2 additional substitutions, Q148 substitutions can confer clinically relevant resistance to DTG and can decrease in vitro susceptibility to BIC [4–6]. The substitution T97A has been observed in patients on RAL and, in combination with G140S/Q148H, has been associated with 13-fold and >100-fold decreases in susceptibility to DTG and RAL, respectively [12, 13]. The substitution L74M is a polymorphic substitution found in up to 10% of patients and confers further increased resistance to RAL and EVG when combined with the N155H but combined alone with G140S/G148H does not affect DTG susceptibility [14]. Phenotypic resistance data have not been previously reported for the pattern G140S/Q148H + T97A + L74M with RAL, EVG, BIC, or CAB [3].

There is broad cross-resistance between RAL and EVG as they share some of the same resistance pathways and similar mechanisms of interaction with HIV integrase [15]. It is the only in vitro study so far to directly compare DTG, BIC, and CAB resistance. The highly RAL- and EVG-resistant virus variants in this study had slightly greater susceptibility to BIC than to DTG or CAB. However, the nearly colinear relationship between log fold change of DTG, BIC, and CAB, and the similarity of trough drug levels of the 3 drugs (1.1–2.6 μg/mL) suggests that it would not be feasible to use of any of these agents as salvage therapy after high-level integrase resistance develops.

In summary, the accumulation of substitutions T97A and L74M greatly decreases susceptibility to DTG, BIC, and CAB, leading to high-level resistance to all 5 integrase inhibitors. Additionally, there is extensive cross-resistance between DTG, BIC, and CAB. Considering the limited scope of this study, further studies to assess the effect of other reported integrase substitutions in various permutations on DTG, BIC, and CAB susceptibility will be needed.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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