Inhibition of Purified Recombinant Reverse Transcriptase from Wild-Type and Zidovudine-Resistant Clinical Isolates of Human Immunodeficiency Virus Type 1 by Zidovudine, Stavudine, and Lamivudine Triphosphates

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Cross-resistance between zidovudine, stavudine, and lamivudine was studied, using purified recombinant reverse transcriptase from a zidovudine-susceptible and -resistant pair of clinical isolates of human immunodeficiency virus type 1. The zidovudine-resistant isolate exhibited low-level cross-resistance to both stavudine and lamivudine in drug susceptibility assays. Enzyme from the resistant isolate demonstrated reduced inhibition by zidovudine triphosphate and stavudine triphosphate and, to a lesser extent, lamivudine triphosphate. These findings provide additional evidence at the viral and enzyme level for cross-resistance between zidovudine and stavudine, and they suggest a possible effect of zidovudine resistance on susceptibility to lamivudine.

Nucleoside analogue inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) form the cornerstone of antiretroviral therapy. When used in combination with protease inhibitors or nonnucleoside RT inhibitors or in triple-nucleoside combinations, the nucleoside analogues contribute to sustained suppression of HIV-1 replication and reductions in HIV-1–related morbidity and mortality. Despite these therapeutic advances, emergence of drug-resistant strains of HIV-1 remains a pressing problem and a significant cause of treatment failure.

Resistance to zidovudine emerges in a step-wise manner by the ordered accumulation of mutations in RT [1]. High-level resistance requires the combined presence of 4 or 5 of these mutations, leading to an ∼500-fold increase in the IC50 for zidovudine. By contrast, significant resistance to stavudine, the other approved thymidine analogue, has been more difficult to demonstrate [2]. Reports from our laboratory and others provide evidence that stavudine selects for the same resistance mutations as does zidovudine and that the presence of zidovudine resistance mutations confers cross-resistance to stavudine [3–5]. High-level resistance to lamivudine is conferred by the M184V mutation, but low-level resistance can be conferred by mutations at codons 44 and 118 [6].

Previous studies of zidovudine resistance generally have relied on RT derived from a laboratory-adapted isolate (Hxb2) molecularly engineered to carry one or more zidovudine resistance mutations. Such molecularly constructed viruses may differ significantly from viruses selected in response to drug pressure in vivo. In most cases, significant resistance to zidovudine triphosphate (TP) at the enzyme level has not been observed [7]. Because the expression of zidovudine resistance may vary in different HIV-1 genetic backgrounds, zidovudine-TP inhibition of purified RT derived from a pair of zidovudine-susceptible and -resistant clinical isolates was studied. In addition, inhibition of RT from these isolates by stavudine-TP and lamivudine-TP was studied, to determine the extent of cross-resistance to these nucleoside analogues at the enzyme level.

Materials and Methods

HIV-1 strains 18A and 18C are paired zidovudine-sensitive and -resistant isolates, respectively, that were obtained from the same patient before and after treatment with zidovudine monotherapy [8]. The isolates were provided by D. Richman (University of California–San Diego) through the National Institutes of Health AIDS Research and Reference Reagent Repository. Susceptibility of viruses expressing 18A and 18C RT was tested by a standard pe-
ripheral blood mononuclear cell (PBMC) assay [9] or by a recombinant virus assay (PhenoSense; ViroLogic) [10].

The entire RT-coding region of pol was amplified by nested polymerase chain reaction from PBMC infected with strains 18A or 18C in vitro and was cloned into the Escherichia coli expression plasmid pRT581 under control of the lacZ promoter [11]. Stop codons were inserted after nucleotide 4210 (corresponding to RT codon 560 in the HXB2 sequence) or after nucleotide 3853 (codon 441), to generate plasmids expressing p66 and p51 subunits, respectively. The RT coding sequence with a stop codon at nucleotide 3853 (codon 560 in the HXB2 sequence) or after nucleotide 3853 (codon 441) was then subcloned into pQE9 (Qiagen), to facilitate purification.

Nucleotide sequencing confirmed the presence of zidovudine resistance mutations 41L, 67N, 70R, 215Y, and 219Q in the RT-coding region of strains 18A and 18C RT had a 46.2-fold increase in IC50 for zidovudine, 2.8-fold for stavudine, and 4.5-fold for lamivudine, compared with control (figure 1). Recombinant viruses expressing 18A RT had a 46.2-fold increase in IC50 for zidovudine, 2.8-fold for stavudine, and 4.5-fold for lamivudine, compared with control (figure 1). Recombinant viruses expressing 18A RT had IC50 values for zidovudine, stavudine, and lamivudine that were 0.7-fold, 1.0-fold, and 1.5-fold those of the control, respectively (differences of >1.7-fold for stavudine and >2.5-fold for zidovudine and lamivudine are considered significant in this assay.)

In the absence of inhibitor, purified RT heterodimer derived from strains 18A and 18C showed similar specific activities ([5.19 ± 0.86] × 10^4 U mg protein^-1 and [4.31 ± 0.34] × 10^4 U mg protein^-1, respectively). The specific activities of these enzyme preparations was comparable to the specific activity of SF2 RT that lacked a His-tag ([4.01 ± 0.19] × 10^4 U mg protein^-1; 1 U of RT activity was defined as the amount of enzyme that catalyzed the incorporation of 1 pmole of thymidine monophosphate in 1 min). The K_M values for dTTP of 18A and 18C RT did not differ significantly (3.00 ± 0.33 and 3.63 ± 0.29 μM, respectively; P = .294). Likewise, V_max for dTTP incorporation was similar to that for 18A and 18C RT (10.21 ± 0.90 and 11.58 ± 0.33 pmol/min, respectively; P = .179).

As compared with wild-type (18A) enzyme, RT from strain 18C demonstrated a 9.5-fold increase in IC50 for zidovudine (P < .001) and a 10.1-fold increase in IC50 for stavudine (P = .004). The IC50 for lamivudine-TP of 18C RT increased 2.6-fold, compared with 18A RT (P = .001). Because recent reports suggest that pyrophosphorolysis contributes to the mechanism of zidovudine resistance, the effect of PPI on inhibition of RT activity by nucleoside analogue TPs was examined. The presence of PPI at 150 μM increased the IC50 of zidovudine-TP for 18A and 18C RT 2–4-fold (table 1). How-
Figure 1. Susceptibility of recombinant viruses expressing human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) from strains 18A (A–C) and 18C (D–F) to zidovudine (A and D), stavudine (B and E), and lamivudine (C and F). Recombinant viruses were tested by a recombinant virus assay, as described elsewhere [10]. Y-axes show percentage of inhibition of RT. Ref., Reference.
Stavudine triphosphate for cross-resistance between zidovudine and stavudine at the dide-resistant HIV-1 [4]. They also provide additional evidence clinical response to stavudine in patients who harbor zidovudine-resistant clinical isolates. Results also are consistent with results that demonstrate reduced inhibition of purified RT from zidovudine-sensitive (18A) and -resistant (18C) clinical isolates. These results are consistent with results that demonstrate reduced inhibition of purified RT derived from zidovudine-sensitive and -resistant strains 1A and 18C differences from those of other studies, which failed to find such differences with R1s derived from zidovudine-resistant Hxb2 variants [7]. It is possible that sequence variation at sites not known to be involved in zidovudine resistance partly accounts for these differences. For example, RT from strains 1A and 18C differs from Hxb2 RT at 13 codons not known to be related to zidovudine resistance (authors’ unpublished observations). It is also possible that differences in the methods used for preparing purified RT heterodimer could account for differences between our results and those observed in earlier studies.

Cross-resistance between zidovudine and stavudine in strain 18C was found by use of a standard PBMC-based drug susceptibility assay and a recombinant virus assay. In addition, recombinant RT heterodimer derived from strain 18C showed cross-resistance to zidovudine-TP and stavudine-TP, Wild-type and mutant enzymes had similar specific activities and similar $K_M$ and $V_{max}$ values for dTTP, suggesting that the observed cross-resistance at the enzyme level was not an artifact of the enzyme preparation. These results are consistent with those of other studies that demonstrate selection by stavudine of mutations associated with zidovudine resistance [3, 5]. These results also are consistent with results that demonstrate reduced clinical response to stavudine in patients who harbor zidovudine-resistant HIV-1 [4]. They also provide additional evidence for cross-resistance between zidovudine and stavudine at the enzyme level.

In conclusion, steady-state kinetic studies of purified recombinant RT heterodimer derived from paired wild-type and zidovudine-resistant clinical isolates showed inhibition kinetics for zidovudine-TP, stavudine-TP, and lamivudine-TP that mirrored the phenotype of viruses expressing these RTs. The observed viral and biochemical phenotypes support the concepts that high-level zidovudine resistance confers at least partial cross-resistance to other nucleoside analogues and that cross-resistance within the nucleoside analogue class of HIV-1 inhibitors is more general than previously believed.

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References

3. Johnson VA, Bassett RL, Koel JL, et al. Selection of zidovudine resistance...
mutations by zidovudine of stavudine-based regimens and relationship to subsequent virological response in ACTG 370 [abstract 57]. Antiviral Ther 2000; 5:82.


