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3′-Azido-3′-Deoxythymidine Resistance Suppressed by a Mutation Conferring Human Immunodeficiency Virus Type 1 Resistance to Nonnucleoside Reverse Transcriptase Inhibitors

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Nonnucleoside reverse transcriptase (NNRT) inhibitors {R82913; (+)-*S*-4,5,6,7-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)-imidazo[4,5,1-jk][1,4]-benzodiazepin-2(1*H*)-thione; Cl-TIBO; and BI-RG-587, nevirapine} were used to select resistant human immunodeficiency virus type 1 (HIV-1) variants by passage in cell cultures of wild-type or 3′-azido-3′-deoxythymidine (zidovudine; AZT)-resistant strains. Similar to other NNRT inhibitors, Cl-TIBO induced a single mutation (Y181 to C) in reverse transcriptase (RT) that accounted for the resistance. BI-RG-587 induced a different mutation (V106→A) in AZT resistance backgrounds. A series of viable HIV-1 variants was constructed by site-directed mutagenesis of the RT, which harbored multiple drug resistance mutations, including Y181 to C. HIV-1 that was coresistant to NNRT inhibitors and 2′,3′-dideoxyinosine resulted when a 2′,3′-dideoxyinosine resistance mutation (L74 to V) was also present in RT. By contrast, however, the Y181 to C mutation in an AZT resistance background significantly suppressed resistance to AZT, while it conferred resistance to NNRT inhibitors. However, the V106→A substitution did not cause suppression of preexisting AZT resistance. Since certain combinations of nucleoside analogs and NNRT inhibitors might result in the development of coresistance, careful analysis of clinical isolates obtained during combination therapy will be needed to determine the potential significance of these observations.

Recent efforts in the search for new drugs that can be used to treat human immunodeficiency virus (HIV) disease have resulted in the identification of several families of nonnucleoside inhibitors active specifically against HIV type 1 (HIV-1) (1, 9, 21, 23, 27). Although these are diverse in structure (e.g., derivatives of benzodiazepines [TIBO compounds], dipyridodiazepinones, pyridinones, and piperazines), they all seem to share a common mechanism of action, mediated through selective binding to HIV-1 reverse transcriptase (RT) (1, 4, 5, 7, 9, 13, 21, 31, 32). Biochemical and genetic studies have demonstrated that amino acid residues Y181 and Y188 are critical for the anti-HIV-1 activities of these compounds (4, 6, 29). Furthermore, recently published X-ray structural data confirm that one of these inhibitors, BI-RG-587 (nevirapine), binds in a pocket which includes these residues (12). This is close to the putative polymerase catalytic site of RT but is probably distinct from the nucleotide binding region (12). Surprisingly, nonnucleoside RT (NNRT)-resistant variants are rapidly selected in cell culture in the presence of these compounds, and there appears to be significant cross-resistance between each class of inhibitor (22, 26). Consistent with these observations, genetic analysis of resistant HIV-1 variants selected with BI-RG-587 or pyridinone derivatives revealed a common mutation in RT (Y181 to C), although a second substitution at codon 103 was also observed with a pyridinone-resistant virus (22, 26). These data also confirmed the importance of Y181 in RT for NNRT inhibitor binding.

Prolonged clinical use of HIV inhibitors such as zidovudine (3′-azido-3′-deoxythymidine; AZT) has led to the identification and characterization of drug-resistant HIV strains (3, 16, 30). Decreases in AZT susceptibility are caused by a set of five specific amino acid substitutions in RT, at codons 41, 67, 70, 215, and 219 (11, 18). These mutations seem to occur in an ordered manner, resulting in a stepwise increase in resistance as substitutions accumulate (2, 11). Introduction of the five substitutions by mutagenesis into a wild-type virus background, singly and in various combinations, has confirmed that each contributes to AZT resistance (11, 17, 18). Four or five mutations (at codons 67, 70, 215, 41, and/or 219) are required to produce highly resistant virus (50% inhibitory concentration [IC_{50}], >1 μM), whereas lesser combinations can result in partially resistant variants (IC_{50}, >0.1 μM <1 μM) (11, 17, 18). These AZT-resistant strains are cross-resistant only to nucleoside analogs containing a 3′-azido group, not to other nucleoside analogs such as 2′,3′-dideoxyinosine (ddI) or the new group of nonnucleoside RT inhibitors (1, 9, 14, 25).

In an attempt to combat AZT resistance, one strategy has been to switch individuals from AZT therapy to ddI therapy. Unfortunately, this sometimes results in ddI-susceptible virus becoming less susceptible to the drug (30). In some instances, however, virus that was initially AZT resistant appears to regain susceptibility to AZT during ddI therapy (30). A novel mutation in RT (L74 to V) accounts for the increased resistance to ddI and, remarkably, is able to "silence" or suppress the effects of AZT resistance mutations (30). In view of these observations, the possibility that HIV-1 acquires coresistance to nucleoside analogs and NNRT inhibitors was investigated. Such studies should indicate the potential clinical utility of combining these classes of inhibitors, with the hope that they will suppress the emergence of resistant variants. Two approaches to address this question were taken. First, resistance to NNRT inhibitors was induced by cell culture selection starting with preexisting AZT resistant strains. Second, HIV-1 variants were created by site-directed mutagenesis with various combinations of AZT, ddI, and NNRT inhibitor resistance mutations in RT. The susceptibilities of these viruses to

nucleoside analogs and NNRT inhibitors were then determined.

MATERIALS AND METHODS

Cells and virus. The human T-lymphoblastoid cell line MT-2 (10) was used to propagate HIV-1 for in vitro selection of resistant virus and for electroporation experiments. MT-2 cells were maintained in RPMI 1640 medium containing 10% (vol/vol) fetal calf serum plus antibiotics. Polybrene (2 μg/ml) was included for HIV-1 growth and drug resistance selection. HT4LacZ-1 cells (human $CD4^+$ HeLa cells) (24) were used in cell inhibitor susceptibility assays (14). These cells were maintained in Dulbecco modified Eagle medium containing 10% (vol/vol) fetal calf serum plus antibiotics. The HIV-1 strains used in this study were the wild-type virus HXB2 derived from the infectious molecular clone pHXB2-D (8) and a number of previously described variants constructed from pHXB2-D by site-directed mutagenesis of specific nucleotides in RT (11, 17, 18, 30). HIV-1 stocks were stored as cell-free culture supernatants at −70°C.

Selection of NNRT inhibitor-resistant HIV-1 in MT-2 cells. MT-2 cells (2×10^6) were infected with cell-free HIV-1 at low multiplicity (less than 0.1 50% tissue culture infective dose per cell) and were exposed to inhibitor initially at subinhibitory concentrations as described previously for the selection of AZT-resistant strains (15). The virus passaged in these experiments was wild-type HXB2 or the highly AZT-resistant variant HIVRTMC (mutations in RT were as follows: Asp-67 to Asn, Lys-70 to Arg, Thr-215 to Phe, Lys-219 to Gln). The NNRT inhibitor (+)-*S*-4,5,6,7-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)-imidazo[4,5,1-jk][1,4]-benzodiazepin-2(1*H*)-thione (R82913; Cl-TIBO) was purchased from Pharmatech International. The concentration of Cl-TIBO added to the culture medium during each passage for HXB2 and HIVRTMC is indicated in Table 1. The concentration used at each passage was empirically derived to allow maximum replication of each virus in the presence of inhibitor. The concentration of BI-RG-587 used in selection experiments is indicated in the legend to Table 2.

Assessment of susceptibility to inhibitors by plaque reduction assay. The susceptibility of HIV-1 to inhibitors was determined by plaque reduction (foci of multinucleated giant cells) formed in monolayers of HT4LacZ-1 cells as described previously (14). Appropriate dilutions of cell-free virus from MT-2 cell cultures were used to infect HT4LacZ-1 cell monolayers in 24-well plates (inhibitor-free virus control wells contained about 100 to 150 plaques). When virus supernatants from in vitro resistance selection experiments was used for susceptibility analysis, the inoculum was removed after a 1-h adsorption period (at 37°C) prior to the addition of fresh culture medium containing various amounts of inhibitor. This was to eliminate carryover of inhibitor from MT-2 cell cultures. Susceptibility tests were performed between one and four times for each virus. Variations in IC_{50}s in duplicate assays were minimal (generally less than twofold).

Nucleotide sequence analysis of RT. DNA was extracted from HIV-infected MT-2 cells, and the complete 1.7-kb RT-coding region of Cl-TIBO-passaged HXB2 was amplified by polymerase chain reaction (PCR) as described previously (18). RT was ligated with the M13 vector mptac18.1 to enable expression of active enzyme in *Escherichia coli*. Clones expressing active RT were sequenced by the dideoxynucleotide chain termination method (28). The complete nucleotide sequences of five RT clones were determined from HXB2 virus passaged in Cl-TIBO by using a set of specific oligonucleotide primers (18). The viruses selected with BI-RG-587 and HIVRTMC selected with Cl-TIBO were analyzed by direct sequencing of PCR products as described previously (31a). Fragments of RT were generated using the oligonucleotide primers A and NE1, as detailed elsewhere (17).

Construction of RT mutants. Mutations were created in the RT-coding region by site-directed mutagenesis with synthetic oligonucleotides as described previously (19). A number of different HIV-1 RT constructs in the vector mptac18.1 (expressing active enzyme) were used as initial targets for mutagenesis. These included wild-type RT derived from pHXB2-D and previously constructed mutants containing AZT or ddI resistance mutations (11, 17, 18, 30).

Preparation and assay of RT. Crude extracts of recombinant RT were prepared by infecting *E. coli* TG-1 at a high multiplicity with M13 bacteriophage clones. After induction with isopropyl-β-D-thiogalactopyranoside, cells were lysed and RT was obtained by fractionation with a high salt concentration as described previously (20). RT activity was routinely assayed by using poly(rA) · oligo(dT) as the primer-template and [^{3}H]TTP (5 μM and 10 μCi/ml, respectively); in inhibition studies with Cl-TIBO, however, poly (rC) · oligo(dG) and [^{3}H]dGTP (2 μM and 22 μCi/ml, respectively) were used. The poly(rC) · oligo(dG) primer-template was used for inhibition studies because this has been shown to be a more sensitive assay system for measurement of HIV-1 inhibition by Cl-TIBO (23).

Construction of HIV-1 variants by recombination. The RT-coding region obtained from M13 clones was transferred into the HXB2 genetic background by homologous recombination with the RT-deleted proviral clone pHIVΔRTB stEII as described previously (11, 30). RT variants created by site-directed mutagenesis (as outlined above) were produced as replicative-form M13 DNA, digested with *Eco*RI and *Hin*dIII to release the 1.7-kb RT fragment, and mixed with pHIVΔRTBstEII linearized with *Bst*EII. This DNA mixture was used to transfect MT-2 cells by electroporation as described previously (19). Cells were maintained in RPMI 1640 medium containing 10% (vol/vol) fetal calf serum plus antibiotics, and virus stocks were prepared from cell-free supernatants at about 14 days posttransfection.

Detection of Tyr-181 to Cys mutation by selective PCR. Selective PCR was used to discriminate wild-type virus (Tyr-181) from mutant virus (Cys-181) at RT codon 181 in samples of DNA from virus-infected MT-2 cells. DNA was extracted and selective PCR was performed as described previously for AZT resistance mutations (17). The selective oligonucleotide primers 181W (5′-CAAATCATCCATGTA TTGTT-3′) and 181M (5′-CAAATCATCCATGTATTGTC-3′), with variations at the 3′ positions, were used in PCR assays. They were paired with the common primer A (5′-TTCCCATTAGTCCTATT-3′). PCR conditions were identical to those described previously for detection of the AZT resistance mutations at codon 215 (3, 17). DNA products were analyzed by electrophoresis in 1.5% agarose-Tris-borate-EDTA gels. The size of the specific PCR band was 563 bp.

RESULTS

Selection of HIV-1 variants resistant to Cl-TIBO. The wild-type HIV-1 strain HXB2 and the AZT-resistant mutant HIVRTMC were passaged separately in MT-2 cells in the presence of increasing Cl-TIBO concentrations (Table 1).

TABLE 1. Passage of HIV-1 strains in Cl-TIBO[a]

Passage no.	HXB2			HIV RTMC		
	Cl-TIBO concn (μM)	IC_{50} (μM)		Cl-TIBO concn (μM)	IC_{50} (μM)	
		Cl-TIBO	AZT		Cl-TIBO	AZT
0		0.11	0.016		0.16	1.5
1	0.5	0.25		1	0.5	2.1
2	0.5	0.5		1	1.4	0.9
3	0.5	4.2		1	1.8	0.8
4	1	13.3		5	3.5	0.28
5	10	14	0.025	5	8.8	0.09

[a] After each passage, virus was recovered in MT-2 cell culture supernatants and was assessed for its susceptibility to Cl-TIBO and AZT by a plaque reduction assay in HT4LacZ-1 cell monolayers. HXB2 is wild-type drug-susceptible virus, and HIVRTMC is a highly AZT resistant variant derived from an HIV-1 infectious molecular clone.

TABLE 3. Inhibition of mutant RT activities by Cl-TIBO[a]

HIV-1 RT genotype	RT activity (% of wild type)	Cl-TIBO inhibition (IC_{50} [μM])
Wild type	100	0.4
Y181C	88	10
L74V	79	0.7
L74V Y181C	100	9
L74V T215Y	52	0.4
L74V T215Y Y181C	100	7
M41L T215Y Y181C	54	ND
D67N K70R T215F K219Q	50	0.7
D67N K70R T215F K219Q Y181C	53	7

[a] RT activity in *E. coli* lysates infected with M13 clones was determined by using poly(rA) · oligo(dT) as the primer-template and [^{3}H]TTP as the substrate. Inhibition of RT activity by Cl-TIBO was assessed with poly(rC) · oligo(dG) as the primer-template and [^{3}H]dGTP as the substrate. ND, not done.

Cell-free virus recovered after each passage was assessed for its susceptibility to Cl-TIBO and AZT. By the fifth passage, viruses derived from HXB2 and HIVRTMC were substantially resistant to Cl-TIBO, with IC_{50}s being >50-fold those for the parental viruses (Table 1). During passage in Cl-TIBO, HXB2 remained fully susceptible to AZT; however, the initially AZT-resistant variant HIVRTMC appeared to become considerably less resistant to this inhibitor (Table 1).

Selection of HIV-1 variants resistant to BI-RG-587. Passage of wild-type HIV-1 in BI-RG-587 causes rapid selection of resistant virus (25). To investigate the possibility of coresistance between AZT and BI-RG-587, we passaged the AZT-resistant mutants HIVRTMC and HXB41L/215Y (RT mutations, Met-41→Leu and Thr-215→Tyr) in MT-2 cells with BI-RG-587. By passage 3, the resulting viruses were both resistant to BI-RG-587, with IC_{50}s about 100-fold higher than those of parental viruses (Table 2). It is interesting to note that both BI-RG-587-resistant isolates retained their original resistance to AZT (Table 2).

Genetic analysis of Cl-TIBO-resistant virus. In order to determine the genetic changes induced by Cl-TIBO selection, the entire RT-coding region was obtained by PCR amplification of DNA from MT-2 cells infected with passage five HXB2 virus. This was inserted into the M13 vector mptac18.1, and RTs from five clones able to express active enzyme in *E. coli* were sequenced. The only mutation common to all RT clones was a single nucleotide change (TAT to TGT) at codon 181. This conferred the predicted amino acid substitution of Tyr to Cys at this position. In order to determine whether this mutation was selected during passage of HIVRTMC in Cl-TIBO, selective PCR analysis was performed with DNA from cells infected with passage five virus. Diagnostic oligonucleotide primers were used to distinguish the observed mutation at codon 181 (see Materials and Methods). This analysis revealed that a mixed population of virus, both wild type and those with a mutation at codon 181, existed after this duration of exposure to Cl-TIBO (data not shown). This was confirmed by direct sequence analysis of PCR products generated from the RT region of the virus. In addition, it was found that a mixed population existed at codon 100 of Leu (wild type) and Ile and at codon 106 of Val (wild type) and Ala.

TABLE 2. Passage of HIV-1 strains in BI-RG-587[a]

Passage no.	IC_{50} (μM)			
	HXB41L/215Y		HIVRTMC	
	BI-RG-587	AZT	BI-RG-587	AZT
0	0.05	0.7	0.06	1.5
3	17.8	1.1	5.6	1.4

[a] Virus was passaged three times in 1 μM BI-RG-587. Virus recovered in MT-2 cell culture supernatants after passage 3 was assessed for susceptibility to BI-RG-587 and AZT by plaque reduction assay in HT4LacZ-1 cell monolayers. Both virus strains were initially AZT resistant and were derived from infectious molecular clones.

Genetic analysis of BI-RG-587-resistant virus. DNA was extracted from MT-2 cells infected with each passage 3 virus and an RT fragment (around 800 bp) from both isolates was amplified by PCR. These fragments were sequenced directly in the regions including RT codons 40 to 108 and 170 to 225. At passage 3, both HIVRTMC and HXB41L/215Y had acquired a mutation of Val→Ala at codon 106. This change was present in each as an apparently pure population.

Analysis of HIV-1 RT clones containing multiple drug resistance mutations. I wished to investigate the activity of HIV-1 RT when various combinations of mutations were present, including the NNRT inhibitor mutation Y181 to C, with the changes conferring resistance to AZT and ddI. Therefore, site-directed mutagenesis was used to introduce changes into the RT-coding region cloned into an M13 construct able to express active enzyme in *E. coli*. Bacterial lysates made after infection with these clones were used in RT assays. In all cases, significant RT activity was detected, with at most a twofold variation, indicating that RT is able to accommodate multiple drug resistance mutations (Table 3).

The relative susceptibility of the expressed RT to Cl-TIBO was next tested by using bacterial lysates as the enzyme source and [^{3}H]dGTP plus poly(rC) · oligo(dG) to assess activity. The IC_{50} for wild-type HIV-1 RT was 0.4 μM, however, this increased to 10 μM with RT in which only the Y181 to C change was present. For all of the tested RT mutants that harbored the Y181 to C change plus other drug resistance mutations, similar elevations in the Cl-TIBO IC_{50} were observed (Table 3). This suggests that the presence or absence of Y181 to C solely dictates susceptibility to Cl-TIBO and that this is not influenced by nucleoside analog resistance mutations in RT.

Susceptibility of HIV-1 variants containing multiple drug resistance mutations to nucleoside analogs and NNRT inhibitors. Since the RT activities of clones containing multiple drug resistance mutations were not significantly different from wild-type RT activity, it was anticipated that viable virus could be propagated with these changes in RT. To

TABLE 4. Susceptibilities of viruses with L74 to V and Y181 to C mutations to nucleoside and nonnucleoside inhibitors

Virus	RT genotype			IC_{50} (μM)[a]			
	Leu-74	Tyr-181	Thr-215	AZT	ddI	Cl-TIBO	BI-RG-587
HXB2				0.01	1.4	0.11	0.06
HXB2(74V)	Val			0.02	11.2	0.16	0.05
HXB2(181C)		Cys		0.01	1.3	4.5	12.6
HXB2(74V/181C)	Val	Cys		0.02	8.9	4.0	4.0
HIVRTMF(74V)	Val		Tyr	0.02	10.0	0.1	0.05
HIVRTMF(74V/181C)	Val	Cys	Tyr	0.02	10.0	2.5	3.2

[a] Inhibitor susceptibility was determined by a plaque reduction assay in HT4LacZ-1 cells.

generate such virus variants, the RT mutants described above were mixed with the RT-deleted HXB2 molecular clone pHIVΔRTBstEII and were used to transfect MT-2 cells. Viable virus is created when recombination occurs between the clones to produce full-length infectious DNA with an intact RT-coding region. Infectious virus from transfections with all of the mutant RT clones was recovered. These variants all replicated in MT-2 cells to similar titers, and the presence of the expected mutations in each was confirmed by selective PCR (data not shown). Plaque reduction assays were performed with HIV-1 mutants in monolayers of the cell line HT4LacZ-1. Variants with combinations of mutations conferring resistance to NNRT inhibitors (Y181 to C) and to ddI (L74 to V) were first analyzed. The results of this analysis are given in Table 4. As expected, all variants with Y181 to C in RT were resistant to Cl-TIBO (IC_{50}s increased 25- to 40-fold) and cross-resistant to BI-RG-587 (IC_{50}s increased 60- to 210-fold compared with IC_{50}s for parental strains). All variants were equally susceptible to AZT; however, those with both the Y181 to C and L74 to V mutations in their RTs were coresistant to NNRT inhibitors and ddI.

Variants with combinations of mutations conferring resistance to NNRT inhibitors (Y181 to C) and AZT (M41 to L, T215 to Y and D67 to N, K70 to R, T215 to F, K219 to Q) were then analyzed. Again, the results of this analysis (Table 5) showed that those variants with the Y181 to C mutation in any genetic background were resistant to Cl-TIBO (IC_{50}s increased 18- to 25-fold) and cross-resistant to BI-RG-587 (IC_{50}s increased 112- to 200-fold compared with IC_{50}s for parental strains). As expected, all of these mutants were equally susceptible to ddI. Remarkably however, preexisting AZT-resistant strains showed a decrease in AZT resistance when the Y181 to C change was present. The AZT IC_{50} for virus mutant at codons 41 and 215 was initially 70-fold greater than that for the wild type. However, the variant that also had the Y181 to C mutation was almost equally as susceptible to AZT as the wild-type virus was (IC_{50}s 0.02 and 0.01 μM, respectively). Similarly, the highly AZT resistant strain HIVRTMC showed a dramatic decrease in resistance to AZT when the Y181 to C mutation was also present (the IC_{50} decreased from 1.5 to 0.05 μM). This observation was reminiscent of the behavior of HIVRTMC after serial passage in the presence of Cl-TIBO.

DISCUSSION

It is becoming accepted that the use of single agents to treat HIV disease will inevitably result in the appearance of drug-resistant virus strains. Although the clinical consequences of resistance are not yet clear, it would seem desirable to prevent resistant HIV from emerging during therapy. The use of drug combinations might be one way to achieve this, even with inhibitors directed at the same target, such as RT. For example, our previous work (30) highlighted AZT and ddI as a potentially beneficial combination, since the ddI resistance mutation (L74 to V) suppresses the effect of AZT resistance mutations. The present study was designed to gain insight into the potential use of nucleoside analogs combined with NNRT inhibitors in preventing drug resistance. I wished to determine whether multiple drug resistance mutations in RT were compatible with retention of enzyme activity and what effects these mutations have on the drug susceptibility profile of virus harboring such mutations.

Previous studies (22, 26) have shown that the Y181 to C RT mutation in a wild-type HIV-1 background is sufficient alone to confer resistance to NNRT inhibitors. In the present study, it was confirmed that exposure of wild-type HIV-1 to Cl-TIBO in cell culture also causes rapid selection of the same mutation at codon 181. The presence of this mutation in a variety of different genetic backgrounds did not cause significant detrimental effects on RT activity. Furthermore, when incorporated into a replication-competent HIV-1 clone, virus mutants harboring these multiple drug resistance mutations were viable in cell culture. The presence of the Y181 to C mutation in the context of AZT resistance or ddI resistance mutations always conferred both Cl-TIBO and BI-RG-587 resistance. This implies that these nucleoside analog resistance mutations have little influence on the codon 181 substitution. However, positive interactions can occur between amino acid substitutions in RT to boost the level of NNRT inhibitor resistance, as has been observed with pyridinone resistance in which codon 103 and 181 changes can occur together (22).

The importance of these in vitro studies needs to be

TABLE 5. Susceptibility of HIV-1 containing AZT resistance mutations and the Y181 to C mutation to nucleoside and nonnucleoside inhibitors

Virus	RT genotype						IC_{50} (μM)[a]			
	Met-41	Asp-67	Lys-70	Thr-215	Lys-219	Tyr-181	AZT	ddI	Cl-TIBO	BI-RG-587
HXB2							0.01	1.4	0.11	0.06
HXB2(181C)						Cys	0.01	1.3	4.5	12.6
HXB2(41L/215Y)	Leu			Tyr			0.7	1.6	0.11	0.05
HXB2(41L/181C/215Y)	Leu			Tyr		Cys	0.02	0.6	2.8	10.0
RTMC		Asn	Arg	Phe	Gln		1.5	2.0	0.14	0.05
RTMC(181C)		Asn	Arg	Phe	Gln	Cys	0.05	1.4	2.5	5.6

[a] Inhibitor susceptibility was determined by plaque reduction assay in HT4LacZ-1 cells.

validated by studying clinical isolates from individuals who are receiving nucleoside analogs and NNRT inhibitors. However, the combined use of ddI and an NNRT inhibitor might ultimately result in HIV-1 coresistant to both drugs, and the findings presented here raise questions about the utility of such a combination to prevent resistance. In contrast, the results presented here suggest that the situation with combinations of AZT and certain NNRT inhibitors might be different, since induction of the Y181 to C mutation in an AZT-resistant background significantly suppressed resistance to AZT. Because this occurred when HIVRTMC was passaged with Cl-TIBO in culture, it might theoretically be possible to specifically convert preexisting AZT-resistant virus harbored by an individual to a susceptible phenotype by deliberately inducing codon 181-mediated NNRT inhibitor resistance.

It is likely that subtle mechanistic differences exist between the different classes of NNRT inhibitors, despite their apparent common mode of action. In this respect, we found that an RT mutation at codon 106 (Val→Ala) and not codon 181 was induced by passaging AZT-resistant HIV-1 strains in the presence of BI-RG-587. Unlike the situation where the 181 codon change was present, the resulting virus mutants were coresistant to AZT and BI-RG-587. Thus, although the change at codon 106 also confers BI-RG-587 resistance (24a), it does not appear to cause the suppression of AZT resistance observed with the codon 181 substitution. It was interesting that sequence analysis of HIVRTMC passaged with Cl-TIBO revealed the development of the Val→Ala substitution at codon 106 in addition to another change (Leu-100→Ile) that has also been associated with resistance to NNRT inhibitors (20a). In this case, the codon 181 mutation also present in this virus population may be dominant, since suppression of AZT resistance clearly occurred.

Clinical trials conducted to determine the value of combining AZT with an NNRT inhibitor will be of considerable interest. In individuals who have already received prolonged AZT monotherapy and harbor AZT-resistant strains, careful assessment of HIV susceptibility before and after the addition of an NNRT inhibitor should be performed to determine whether it is able to potentiate AZT sensitivity (via induction of the codon 181 change) and render the resulting virus susceptible again to AZT. It will also be interesting to observe the pattern of resistance in HIV isolates from individuals given combinations of AZT and an NNRT inhibitor without initially receiving AZT alone.

Further studies to determine the phenotypic resistance profiles of HIV-1 variants constructed with other combinations of drug resistance mutations should prove valuable. It might be the case that certain combinations render the RT sufficiently less active to impair the replication capacity of the virus. While the use of specific drug combinations might result in resistance to one inhibitor, perhaps investigators will discover combinations that will simultaneously force the virus to remain (or become) susceptible to a second drug. The potential clinical benefit of this situation would then await evaluation.

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