ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2002, p. 2254–2256 Vol. 46, No. 7
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.7.2254–2256.2002

Pressure of Zidovudine Accelerates the Reversion of Lamivudine Resistance-Conferring M184V Mutation in the Reverse Transcriptase of Human Immunodeficiency Virus Type 1

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Received 6 November 2001/Returned for modification 24 January 2002/Accepted 8 April 2002

We cultured lamivudine-resistant human immunodeficiency virus type 1 (HIV-1) variants over an extended period of time in the presence of zidovudine and observed a premature reversion of the resistance-conferring M184V mutation. These data suggest that the presence of ZDV amplifies differences in replication capacity between wild-type HIV-1 and the mutant variant.

The emergence of mutations that confer resistance to antiviral drugs used to treat infection with the human immunodeficiency virus type 1 (HIV-1) can severely compromise success in therapy. However, certain amino acid substitutions can sometimes increase susceptibility to other drugs in a given regimen. Various facets of these complex phenotypes have previously been described (2, 4, 6, 7, 11, 13, 14, 15). A prominent example is the M184V mutation in the reverse transcriptase (RT) gene of HIV-1. This mutation confers high-level resistance to lamivudine (3TC) and is also associated with resensitization of viruses that contain zidovudine (ZDV) resistance-conferring mutations (7), despite the fact that both drugs are nucleoside analogue RT inhibitors. The 184V mutation alone was shown to cause a two- to threefold increase in susceptibility to ZDV (12, 15).

Drug hypersusceptibility and the resensitization of resistant viruses are phenomena of potential clinical importance. However, these phenotypes may occur only transiently, as previously shown in the context of ZDV-3TC combination therapy (8). Cell culture experiments revealed that the presence of only one drug, i.e., ZDV, and the absence of 3TC drug pressure resulted in the loss of 3TC resistance in formerly dually resistant isolates (10). The new phenotype coincided with the reversion of the M184V mutation to wild type. Whether the loss of this mutation is solely attributable to a replication disadvantage of the mutant variant or whether increased susceptibility to ZDV exerts additional pressure that facilitates reversion to wild type is unknown.

To address this issue, we cultured M184V-containing viruses either in the absence of drugs or in the presence of increasing concentrations of ZDV. We used two clinical HIV-1 isolates, designated 3350-184V and 4246-184V and, for comparison, we also employed another 184V-containing construct that was generated by site-directed mutagenesis (HXB-2D-184V). The presence of M184V was confirmed by automated sequencing of the RT region comprising residues 39 to 244 by using the protocol and software provided by the supplier (Visible Genetics, Inc., Toronto, Ontario, Canada). Other known resistance-associated mutations were not identified. The mutant variants are associated with a two- to threefold increase in susceptibility to ZDV, based on both RT and p24 measurements (data not shown). These differences have been consistently measured not only in MT-4 cells (the present study) but also in $CD4^+$ HeLa cells (12) and peripheral blood mononuclear cells (15).

(This work was performed by K.D. in partial fulfillment of the requirements for a Ph.D. degree, Faculty of Graduate Studies and Research, McGill University, Montreal, Quebec, Canada.)

We initially used the clinical isolate 3350-184V and cultured this virus over a period of 20 weeks in MT-4 cells under different conditions, as specified in Table 1. Samples were analyzed after 5, 10, 15, and 20 weeks with regard to phenotypic susceptibility to 3TC (Table 1). The 50% infectious dose (IC_{50}) values were determined on the basis of RT activity measurements as previously described (15). Prior to these measurements, culture supernatants were equilibrated, and frozen cell pellets were used to extract proviral DNA for genotypic analysis. We observed a decrease in IC_{50} values for 3TC at week 20, when the virus was grown in the absence of drug. The presence of ZDV significantly accelerated this decrease. Increased susceptibilities to lamivudine are seen, in dose-dependent fashion, after ca. 10 weeks in culture.

As expected, the presence of 0.1 μM 3TC alone resulted in maintenance of 3TC resistance at the latest time point tested, i.e., week 20. However, this concentration of 3TC was insufficient to prevent the premature loss of 3TC resistance in the additional presence of 0.1 μM ZDV (Table 1). Maintenance of resistance to 3TC in the presence of 0.1 μM ZDV required concentrations of 3TC higher than 0.1 μM. We further demonstrated that the premature loss of resistance to 3TC is a specific effect, one that is attributable to the specific interaction between ZDV and the M184V mutation. As additional controls, neither the presence of nevirapine nor the presence of stavudine caused an early reduction in 3TC IC_{50} values. More-

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TABLE 1. Summary of IC_{50} values for 3TC for strain 3350-184V grown in the presence or absence of various drugs

Virus/drug (concn [μM])	3TC IC_{50} (μM)[a] at (wk of sample collection):			
	Wk 5	Wk 10	Wk 15	Wk 20
3350-184V/no drug	R	R	R	20.4
3350-184V/stavudine (0.25)	R	R	R	15.5
3350-184V/nevirapine (0.1)	R	R	R	14.9
3350-184V/ZDV (0.01)	R	R	R	19.5
3350-184V/ZDV (0.1)	R	5.6	3.0	1.5
3350-184V/ZDV (0.5)	R	2.6	1.9	1.0
3350-184V/ZDV (0.1) and 3TC (0.1)	R	6.0	3.5	1.9
3350-184V/ZDV (0.1) and 3TC (0.25)	R	R	R	R
3350-184V/ZDV (0.1) and 3TC (0.5)	R	R	R	R
3350-184V/3TC (0.1)	R	R	R	R

[a] R, high-level resistance (IC_{50} > 100 μM). Values are means of results of at least three independent experiments.

over, we obtained nearly identical results with three distinct 184V-containing viruses that were generated by different methods, i.e., site-directed mutagenesis (HXB-2D-184V), in vivo selection (3350-184V), and in vitro selection (4246-184V) (data not shown). The fact that an accelerated increase in susceptibility to 3TC was seen with both clinical isolates and the cloned construct shows that this change occurs independently of a background of minority wild-type species.

Genotypic analysis revealed that the 184V mutation was present at week 5 when concentrations of 0.01, 0.1, and 0.5 μM ZDV were used in the cultures (Table 2). At week 10, 184V was still present when the virus had been grown in the presence of 0.01 μM ZDV. In contrast, mixtures of 184V and 184M were seen in the presence of 0.1 μM ZDV, and complete reversion to 184M was seen with 0.5 μM ZDV. The presence of 3TC prevented the reversion to wild type, when the drug was used at sufficiently high concentrations. The M184V mutation was maintained when viruses were grown in the presence of 0.1 μM ZDV and >0.1 μM 3TC. Thus, the genotypic analysis is consistent with the phenotypic data and shows a premature reversion to the wild-type codon in a dose-dependent fashion. It appeared that the reversion of 184V to wild type coincided with a decrease in susceptibility to ZDV (data not shown). However, we cannot exclude that other genetic alterations outside the sequenced RT region might have occurred under constant pressure of ZDV. For instance, the RT-associated mutation G333E and other changes outside the RT gene have previously been linked to resistance to nucleoside analogue RT inhibitors (5, 9). It is beyond the scope of the present study to address this problem, which merits further detailed investigation.

TABLE 2. Genotypes of strain 3350-184V after growth in various concentrations of ZDV

Virus/drug (concn [μM])	Genotype at wk:		
	5	10	15
3350-184V/no drug	184V	184V	184V
3350-184V/ZDV (0.01)	184V	184V	184V
3350-184V/ZDV (0.1)	184V	184V/M	184M
3350-184V/ZDV (0.5)	184V	184M	184M

There may exist a correlation between hypersusceptibility to certain drugs and improved virological suppression (11); however, the loss of important amino acid substitutions that actually cause such increased drug susceptibilities may completely reverse potentially beneficial effects. Our data show that the presence of ZDV significantly accelerates the reversion of the 184V mutation to wild type. Our findings show for the first time that pharmacological drug pressure can force the loss of resistance-conferring mutations in HIV-1 RT. Previous studies have shown that the mutant variant replicates more slowly than wild-type HIV-1 (1), and it appears that the presence of ZDV amplifies these differences in viral replication capacity. We have recently demonstrated that the recombinant M184V-containing enzyme can block the phosphorolytic excision of incorporated 3TC-monophosphate and diminishes rates of unblocking of ZDV-terminated primer strands (3). The184V-containing enzyme has also been associated with slight reductions in the rates of polymerization and processive DNA synthesis (1). These biochemical data may help to explain the accelerated outgrowth of wild-type HIV-1 in the presence of ZDV. The approach taken here could also help to identify specific mutational patterns in more complex clinical isolates that are implicated in drug hypersusceptibility (2, 6, 11, 13, 14).

This research was supported by grants to M.G. and M.A.W. from the Canadian Institutes of Health Research.

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