Suppression of Replication of Multidrug-Resistant HIV Type 1 Variants by Combinations of Thymidylate Synthase Inhibitors with Zidovudine or Stavudine

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ABSTRACT

The replication of recombinant multidrug-resistant HIV-1 clones modeled on clinically derived resistant HIV-1 strains from patients receiving long-term combination therapy with zidovudine (AZT) plus 2',3'-dideoxythymidine was found to regain sensitivity to AZT and stavudine (D4T) as a consequence of a pharmacologically induced decrease in de novo dTMP synthesis. The host-cell system used was phytohemagglutinin-stimulated peripheral blood mononuclear cells; dTMP and dTTP depletion were induced by single exposures to a low level of the thymidylate synthase inhibitor 5-fluorouracil alone or in combination with AZT or D4T, even at drug levels severalfold higher than those used in the viral inhibition studies. These studies may provide useful information for the potential clinical use of AZT/5-FU or D4T/5-FU combinations for the prevention or reversal of multidrug resistance associated with long-term dideoxynucleoside combination therapy.

The development of drug resistance is one of the major obstacles to successful single-agent chemotherapy of HIV-1. The high replication rate of the virus, together with the low reverse transcription fidelity of HIV reverse transcriptase (RT) and its lack of DNA polymerase-associated 3'-5'-exonucleolytic proofreading capacity (Roberts et al., 1988), leads to a high frequency of uncorrected deoxynucleotide insertion errors and the continuous generation of HIV-1 mutants, including drug-resistant mutants; as a consequence, drug monotherapy is now little used, having been replaced by a variety of combination regimens, usually consisting of two 2',3'-dideoxynucleoside (ddN) agents with complementary resistance spectra [e.g., 3'-azido-2',3'-dideoxythymidine (AZT) and β-L-2',3'-dideoxy-3'-thiacytidine (3TC); Larder et al., 1995] plus an agent acting at an independent site of the viral replication cycle, typically an HIV-1 protease inhibitor.

We and others recently postulated, however, that sensitivity to individual ddNs [all of which act as their 2',3'-dideoxynucleoside-5'-triphosphates (ddNTPs)] could be restored at least in part to ddN-resistant mutant strains through the selective depletion of the corresponding physiological dNTP (Lori et al., 1997; Johns and Gao, 1998). It has long been known that the anti-HIV activity of ddNs as RT inhibitors and viral DNA chain terminators does not depend on the absolute level of the ddNTP generated intracellularly but rather depends on the ratio of the concentration of the latter to that of the competing 2',3'-dideoxynucleoside-5'-triphosphate (dNTP) [i.e., 5'-triphasphate of 2',3'-dideoxythymidine (ddC) (ddATP)/dATP, 2',3'-dideoxyadenosine-5'-triphosphate (ddATP)/dATP, 5'-triphasphate of AZT (AZTTP)/dATP, and so on]. Thus, a pharmacologically induced decrease in the level of the corresponding host-cell-derived dNTP can, in principle, have as great an antiviral effect as an increase in the ddNTP concentration. More importantly, because HIV-1,

ABBREVIATIONS: RT, reverse transcriptase; ddN, 2',3'-dideoxynucleoside; AZT, 3'-azido-2',3'-dideoxythymidine, zidovudine; D4T, 2',3'-dideoxy-2',3'-dideoxythymidine, stavudine; ddG, 2',3'-dideoxyguanosine; 3TC, β-L-2',3'-dideoxy-3'-thiacytidine; ddNTP, 2',3'-dideoxynucleoside-5'-triphosphate; dNTP, 2',3'-dideoxy-5'-triphosphate; dC, 2',3'-dideoxythymidine; ddCTP, 5'-triphosphate of 2',3'-dideoxythymidine; ddATP, 2',3'-dideoxyadenosine-5'-triphosphate; AZTTP, 5'-triphosphate of AZT; ddI, 2',3'-dideoxyinosine; 3-FU, 5-fluorouracil; FUdR, 2'-deoxy-5-fluorouridine; PHA, phytohemagglutinin; PBM, peripheral blood mononuclear; TCID50, 50% tissue culture-infective doses.
whether wild-type or drug-resistant mutant, has an absolute requirement for host-cell dNTPs, successful replication of mutant virus is as susceptible to inhibition through dNTP depletion as is replication of wild-type virus.

To date, this latter concept has been tested only with the combination hydroxyurea/2',3'-dideoxyinosine (ddI) (Lori et al., 1997). The principle is, however, applicable to all ddNs, and the recent availability of a related series of recombinant HIV-1 clones incorporating mutations in the polymerase domain of RT associated with resistance to multiple ddNs (AZT, d4T, ddC, ddI, 2',3'-dideoxyguanosine (ddG)) (Shirasaka et al., 1995) has given us the opportunity to examine this concept experimentally with the two widely used dideoxynucleoside-based agents AZT and 2',3'-didehydro-2',3'-dideoxynucleoside ( stavudine; d4T). The prototype agents selected to deplete dTTP (the competing dNTP for AZTTP and d4TTP) were the thymidine synthase inhibitors 5-fluorouracil (5-FU) and its deoxyx nucleoside, 2'-deoxy-5-fluorouridine (FdUr), compounds that act on the de novo biosynthetic pathway for thymidylate (Santi et al., 1974).

**Experimental Procedures**

**Materials.** All chemicals used were of reagent grade. 5-FU, FdUr, and phytohemagglutinin (PHA) were obtained from Sigma Chemical Co. (St. Louis, MO). AZT and d4T were supplied by Dr. Karl Flora (Developmental Therapeutics Program, National Cancer Institute). Recombinant interleukin-2 was purchased from R&D Systems (Minneapolis, MN). Radioimmunoassay kits of p24 Gag protein were purchased from DuPont (Boston, MA). Sequenase enzyme (2.0 version) was obtained from United States Biochemical Corp. (Cleveland, OH). Oligonucleotides used as template primers were purchased from Genosys Biotechnologies, Inc. (Woodlands, TX).

**Cells.** Peripheral blood mononuclear (PBM) cells were isolated from heparinized venous blood of healthy donors and were incubated with PHA (10 μg/ml) in RPMI 1640 medium supplemented with 15% heat-inactivated fetal calf serum, 15 U/ml recombinant interleukin-2, 4 mM l-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin for 48 h.

**Multidrug-Resistant Infectious Clones.** Earlier studies (Shirasaka et al., 1993; Shafer et al., 1994) showed that patients receiving long-term anti-HIV therapy with AZT/ddC or AZT/ddI developed multidrug-resistant strains of the virus with five common mutations at codons 62, 75, 77, 116, and 151 in the pol gene. On nucleotide sequencing at 7, 16, 21, 27, and 38 months after the start of therapy, it was found that the mutations had developed in the order Q151M (16 months), F77L/F116Y (27 months), and A62V/V75I (38 months) (Shirasaka et al., 1995).

The procedure was used in the present study for constructing the infectious clones with these mutations that has previously been described in detail (Shirasaka et al., 1995). Briefly, a plasmid was constructed by ligating the HpaI/XbaI fragment of pHXB2RIP7 (a gift from Dr. Marvin Reitz, Jr.) to SmaI/XbaI-digested pSVK3 (Pharmacia, Piscataway, NJ). Cloning sites (XmaI and NheI) were generated by introducing four mutations at codons 14, 15, 267, and 268 into the ApaI/SalI fragment by site-directed mutagenesis, generating the plasmid p5SUM9. To generate recombinant infectious clones, mutations of interest were introduced into the XmaI/NheI fragment by site-directed mutagenesis, and the fragment was transferred to p5SUM9, generating the one-, three-, and five-mutation clones p5SUM9, p5SUM12, and p5SUM13, respectively. DNA (10 μg) from each molecular clone was transfected into COS-7 cells by the calcium phosphate method. Infectious virions were harvested at 48 h and propagated in H9 cells for 10 to 14 days, generating HIV-1Q151M, HIV-1F77L/F116Y/Q151M, and HIV-1A62V,V75I,F116Y,Q151M. The culture supernatants were stored at −70°C until use. Determination of the nucleotide sequence of these infectious clones confirmed that each had the intended mutations. The clone containing a single mutation (Q151M) showed a 10-fold reduction in sensitivity to AZT, ddC, ddI, and d4T, whereas the clones incorporating three and five mutations showed a high level of insensitivity to all five ddNs (Shirasaka et al., 1995).

In addition to the recombinant clones, an HIV-1 clinical strain, ERS104, isolated as previously described from a patient before receiving any antiviral therapy (Shirasaka et al., 1993), was examined in these studies for comparison of its drug sensitivity to that seen with the mutagenesis-derived clones.

**Determination of Anti-HIV-1 Activity.** PHA-stimulated PBM cells were plated onto 24-well tissue culture plates at a density of 1 × 10⁶ cells/well. Drugs were added in 2 ml of supplemented RPMI medium. After incubation for 24 h, cells were exposed to 5 × 10⁻⁶ M 50% tissue culture-infective doses (TCID₅₀) of each strain per well, and half of the culture medium was replaced with fresh culture medium containing the same concentrations of drugs on day 4 postinfection. On day 8, the medium was harvested, and the amount of p24 protein was determined through radioimmunoassay.

**Thymidylate Synthase Assays.** The effect of FdUr on thymidylate synthase activity (dUMP → dTMP) in PHA/PBM cells was assayed by the procedure of Dolnick and Cheng (1977). PBM cells (48 h after PHA stimulation) were incubated with FdUr over a range of concentrations or with a fixed concentration of FdUr (0.2 μM) over a range of exposure time periods as indicated in the figure legends. Cells were harvested, frozen, thawed, and sonicated, and the conversion of [³²P]-dUMP (0.18 mCi/μmol) to [³²P]-dTMP by the cell-free extracts was determined as described previously (Dolnick and Cheng, 1977). One unit of thymidylate synthase activity is defined as the amount of enzyme required to form 1 nmol of dTMP/min/ml at 37°C under our assay conditions.

**Analysis of Intracellular dTTP Pools in Cells Exposed to FdUr.** Intracellular dTTP pools were quantified as described previously (Sherman and Fyfe, 1989; Gao et al., 1994a). The Sequenase reaction mixture contained 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 5 mM dithiothreitol, 0.25 μM template primer, and 2.5 μM [³²P]dATP (15 Ci/mmol).

**Analysis of D4T Phosphates in Cells Exposed to D4T plus Low-Level FdUr.** Intracellular levels of D4T monophosphates, diphosphates, and triphosphates after exposure of D4T-treated PHA/PBM cells to 0.05, 0.20, and 0.80 μM FdUr were determined by HPLC as described previously (Ahuwalia et al., 1996).

**Results**

**Effect of 5-FU on Inhibition by AZT and D4T of Replication of Multidrug-Resistant Variants and of HIV-1 Clinical Isolate.** As shown in Fig. 1, in the absence of 5-FU, only the control (wild-type) clone showed typical susceptibility to AZT inhibition in the PHA/PBM test system (IC₅₀ = 11 nM), with HIV-1Q151M being partially susceptible (IC₅₀ = 36 nM). In the presence of 1 μM 5-FU, the activity of AZT against replication of the wild-type clone and of HIV-1Q151M increased by 5- and 8-fold, respectively. Both fully resistant strains, HIV-1F77L,F116Y,Q151M and HIV-1A62V,V75I,F116Y,Q151M became partially AZT susceptible, with 50% inhibitory concentrations of 47 and 50 μM AZT, respectively (Fig. 1).

For D4T, only the control (wild-type) and the five-mutation clone HIV-1A62V,V75I,F116Y,Q151M were examined, and the results for two normal PBM cell donors, A and B, are plotted separately (Fig. 2). As with AZT, the five-mutation clone became partially susceptible to the drug, with IC₅₀ values of 0.2 μM for D4T for both donors in the presence of 1 μM 5-FU (a 9.5-fold increase in susceptibility for donor A and an 11.5-fold increase for donor B).
Similar enhancements of AZT and D4T activity were observed with the clinical HIV-1 isolate ERS104_pre and were dose dependent over the range of 0.5 to 2.0 μM 5-FU (Table 1). In uninfected PHA/PBM cells, the low concentrations of 5-FU used in these studies did not elicit detectable cytotoxicity over a 6-day incubation period, with inhibition of host-cell replication not being noted until the 5-FU concentration reached 20 μM (data not shown).

Similar results were obtained with the 2'-deoxynucleoside of 5-FU, FUdR. In the presence of FUdR (0.2 μM), the inhibitory activity of AZT against the clinical isolate increased by 6-fold, with no significant cytotoxicity detectable in uninfected cells (data not shown).

**Effect of Low-Level FUdR on Thymidylate Synthase Activity and on dTTP Pools in PHA/PBM Cells.** On examining the time course of inhibition of thymidylate synthase by 0.2 μM FUdR, we noted a striking decline in the rate of de novo dTMP formation at the earliest time point measured (30 min), with the rate continuing to decrease until reaching a minimum (8% of the control rate of 0.43 nmol dTMP/min/ml) at 3 h (Fig. 3). Substantial recovery was noted over the period of 6 to 24 h. A sharp FUdR-induced decline in dTTP pools was also noted, with the latter also reaching a minimum (40% of control) at 3 h, followed by a recovery period similar in duration to that seen for de novo dTMP formation (Fig. 3).

**Effects of FUdR/D4T Combinations on D4T Phosphorylation.** PHA/PBM cells were exposed to 5 μM 3H-labeled D4T in the presence of FUdR over the concentration range of 0 to 0.80 μM, and D4TTP and dTTP pool sizes were determined as previously described in Experimental Procedures. The intracellular ratio of D4TTP/dTTP increased from 0.07 in the absence of FUdR to 2.62 in the presence of FUdR (0.80 μM) as a consequence of an increase of up to 6-fold in D4TTP levels and a corresponding drop (4-fold) in dTTP pool size (Table 2).

**Effect of Schedule of 5-FU Exposure on AZT-Induced Inhibition of HIV-1 Replication.** We next examined the mode of action of 5-FU in combination with AZT. Schedules were designed to vary only in the time period of 5-FU exposure, with 5 nM AZT being continuously present throughout the experiments. The HIV-1 clinical isolate strain ERS104_pre and PHA/PBM cells were used for these studies. As shown in Fig. 4, the presence of 5 nM AZT alone caused 26% inhibition of viral p24 protein production on 9-day exposure, whereas 1
μM 5-FU alone caused 23% inhibition. A slight variation in these values was seen with clinical isolates from two other donors (data not shown). Comparing 5-FU exposure time and antiviral effect, schedule D (5-FU present from day 1, the day of virus infection, to day 5, 4 days after infection), produced the most profound inhibition compared with the no-5-FU control. We also noted that the short 5-FU exposure of schedule A (preincubation of cells with 5-FU for 12 h) inhibited p24 production by 64%.

These data suggest that the simultaneous presence of 5-FU and AZT before or during reverse transcription is of critical importance in suppression of HIV-1 replication.

Discussion
ddNs are unique among anti-HIV agents in that their antiviral activity depends not only on their own pharmacological activity but also on the intracellular level of the corresponding host-cell-generated physiological dNTP. In in vitro studies, we and others have taken advantage of this property to enhance the activity of the purine-based ddNs ddI and 2'-β-fluoro-2',3'-dideoxyadenosine by depleting the pool size of the corresponding deoxynucleotide, dATP, through the use of ribonucleotide reductase inhibitors, particularly hydroxyurea (Gao et al., 1994b, 1995a, 1998; Lori et al., 1994; Malley et al., 1994). Clinical studies of the combination ddI/

TABLE 1
Enhancement by 5-FU of AZT and D4T activities against replication of HIV-1 clinical isolate in PHA/PBM cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IC_{50} in culture</th>
<th>nM</th>
<th>fold</th>
<th>nM</th>
<th>fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>No 5-FU</td>
<td>7.1 ± 1.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 μM 5-FU</td>
<td>1.1 ± 0.3</td>
<td>6</td>
<td></td>
<td>60.2 ± 5.4</td>
<td>2</td>
</tr>
<tr>
<td>1.0 μM 5-FU</td>
<td>0.5 ± 0.1</td>
<td>14</td>
<td></td>
<td>16.9 ± 1.5</td>
<td>7</td>
</tr>
<tr>
<td>2.0 μM 5-FU</td>
<td>0.2 ± 0.04</td>
<td>36</td>
<td></td>
<td>1.4 ± 0.2</td>
<td>79</td>
</tr>
</tbody>
</table>

TABLE 2
Effects of FUdR/D4T combinations on D4T phosphorylation and on dTTP pool sizes in PHA/PBM cells

<table>
<thead>
<tr>
<th>FUdR</th>
<th>D4TMP</th>
<th>D4TDP</th>
<th>D4TTP</th>
<th>SUM</th>
<th>dTTP</th>
<th>d4TTP/ddTTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>μM</td>
<td>pmol/10^6 cells</td>
<td></td>
<td>pmol/10^6 cells</td>
<td></td>
<td>pmol/10^6 cells</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.13 ± 0.01</td>
<td>(100%)</td>
<td>0.03 ± 0.01</td>
<td>(100%)</td>
<td>0.29 ± 0.03</td>
<td>(100%)</td>
</tr>
<tr>
<td>0.05</td>
<td>0.34 ± 0.02</td>
<td>(261%)</td>
<td>0.08 ± 0.01</td>
<td>(266%)</td>
<td>1.58 ± 0.12</td>
<td>(544%)</td>
</tr>
<tr>
<td>0.20</td>
<td>0.37 ± 0.03</td>
<td>(264%)</td>
<td>0.09 ± 0.01</td>
<td>(300%)</td>
<td>1.69 ± 0.15</td>
<td>(582%)</td>
</tr>
<tr>
<td>0.80</td>
<td>0.38 ± 0.03</td>
<td>(292%)</td>
<td>0.09 ± 0.01</td>
<td>(300%)</td>
<td>1.72 ± 0.18</td>
<td>(593%)</td>
</tr>
</tbody>
</table>

Fig. 3. Left, effect of FUdR on thymidylate synthase activity in PHA/PBM cells. Cells (5 × 10^7/assay) were incubated with FUdR over the concentration range of 0 to 5 μM for 20 h at 37°C (top left) or were exposed to 0.2 μM FUdR for different time periods, as indicated (bottom left). Cells were harvested and thymidylate synthase activity was determined as described in Experimental Procedures. The no-drug control values were 0.30 and 0.34 nmol of dTMP formed/min/ml for the dose dependence and time course experiments, respectively. Data represent means of three independent experiments with duplicate determinations in each experiment. Right, effect of FUdR on dTTP pool size in PHA/PBM cells. Fig. 4. Top right, cells (5 × 10^7/10 ml/assay) were incubated with FUdR over the concentration range of 0 to 2 μM for 3 h at 37°C. Cells were treated and dTTP pool sizes were determined as described in Experimental Procedures. The value for the no-drug control was 54 pmol/10^6 cells. Bottom right, PHA/ PBM cells were incubated with 0.2 μM FUdR for different time periods. Cells were treated, and dTTP pool sizes were determined. The value for the no-drug control was 57 pmol/10^6 cells. Values shown are mean ± S.D. for three experiments, with duplicate determinations in each experiment. Error bars are not visible for some values because they were smaller than the symbol.
hydroxyurea are now well advanced (Vila et al., 1996; Lori et al., 1997; Rutschmann et al., 1998).

In addition, we and others have previously demonstrated enhancement of the anti-HIV activity of the thymidine-based ddN D4T by the thymidylate synthase inhibitors FUdR and 5-FU (Gao et al., 1995b; Aihluwalia et al., 1996; Gong et al., 1996), as well as by other agents inhibiting dTTP biosynthesis, such as methotrexate and pyrazofurin (Aihluwalia et al., 1996). Medina et al. (1996) demonstrated that cell lines with elevated ability to phosphorylate thymidine to dTTP exhibit decreased susceptibility to the anti-HIV effect of AZT and that such AZT susceptibility can be fully restored by coadministration of FUdR or 5-FU. That such enhancement is not a nonspecific consequence of the cytotoxic effects of 5-FU was shown in control experiments by Gong et al. (1996), who demonstrated that 5-FU had no enhancing effect on the anti-HIV activity of the purine-based dideoxynucleoside ddI.

Less well recognized, however, is the potential therapeutic usefulness of these combinations in suppressing the replication of mutant viruses, including drug-resistant mutants such as those in the present study. All HIV-1 mutants, like wild-type virus, have an absolute requirement for the host-cell-generated deoxynucleosides essential for viral DNA replication; the importance of this requirement is illustrated by the enzyme kinetic studies of ddI-resistant virus by Martin and coworkers and of the multidrug-resistant clones used here (Martin et al., 1993; Ueno et al., 1995). These previous studies have shown that with the RT of drug-resistant mutants, little or no change is seen in the Km values and in the catalytic efficiencies (Ki/Km ratios) for the physiological dNTPs (i.e., dATP, dCTP, dGTP, and dTTP). Resistance appears instead to be associated with amino acid substitutions that decrease the RT affinities of the corresponding ddNTPs. In the ddI-resistant mutant L74V, for example, Martin and colleagues found a 5-fold increased Ki value for ddATP; Ueno and coworkers observed that with the single-mutation RT Q151M, the Ki value for AZTTP increased 3.5-fold, whereas with the five-mutation RT E62V/75I/77L/116Y/151, the increase was 62-fold over that seen with RT from a wild-type clone. A parallel loss in ddNTP “substrate affinities” (i.e., ddNTP Km values, an index of ability to incorporate in and chain-terminating HIV-1 DNA) was also observed. Thus, resistance to ddNs arises because although RT catalytic efficiency (i.e., ability to use physiological dNTPs for reverse transcription) is little impaired, the ability of ddNTPs to inhibit RT and to compete with dNTPs for chain insertion is substantially decreased. Indeed, it would appear from first principles that retention of a substantial measure of catalytic efficiency in drug-resistant mutants must necessarily be the case; mutants whose RT lacks the essential capacity to support a critical level of viral replication could (and probably do) arise but would be unable to survive more than marginally because of their inability to compete effectively with other members of the HIV-1 quasispecies for the dNTPs required for replication (for detailed discussions of the correlations between these enzyme kinetic studies of the RT affinities of dNTPs and ddNTPs and the three-dimensional structure of wild-type and mutant polymerase, see Ueno et al. (1995) and also extensive investigations by Boyer et al. (1994)).

Assuming, therefore, that ddNTPs and dNTPs behave kinetically as competing substrates (until chain termination of the former occurs, after which inhibition by ddNTPs is irreversible and noncompetitive), a logical approach to restoration of complete or partial sensitivity to ddNTPs in drug-resistant mutants is to lower the concentration of the corresponding physiological nucleotide by pharmacological or other means. When the Km change is relatively small (3.5-fold for AZTTP in the Q151M mutant; Ueno et al., 1995), the results from the present study indicate that sensitivity can be restored to that seen with the wild-type clone by a single exposure to 5-FU, with the consequent fall in dTMP synthesis and thus in dTTP levels (Fig. 3 and Table 2). Contributing to the effect in this case would be a corresponding increase in the efficiency of AZT or D4T phosphorylation (Table 2) because the salvage enzyme thymidine kinase (responsible for the first step in AZT and D4T activation) is under feedback regulation by dTTP (Bresnick and Karjala, 1964). In the case of a major loss in ddNTP inhibitor and “substrate” activity (Ki and Km, respectively), substantial, although incomplete, restoration of AZT or D4T sensitivity in HIV-1 A62V, V75I, F77L, F116Y, Q151M is observed after a single 5-FU exposure (Figs. 1 and 2). These in vitro observations of a single drug exposure in a model system may, however, underestimate the potential recovery of sensitivity because in a clinical situation, re-
peated cycles of AZT or D4T with an inhibitor of dTTP synthesis would be used, analogous to the ddI/hydroxyurea clinical protocols currently in use.

The general applicability of this method of resensitizing ddN-resistant HIV-1 mutants should be emphasized. The three multidrug-resistant clones studied here were selected because of our extensive prior knowledge of the biological characteristics of this closely related series and because these constructs were based on typical HIV-1 mutants from patients who had received dual (AZT/ddC or AZT/ddI) ddN therapy for long periods. There is no fundamental reason, however, why the reduction in dTMP and dTTP pools would not be effective in the resensitization of other clinically significant AZT- or D4T-resistant mutants. In addition, this strategy could be applied to mutants resistant to any of the anti-HIV ddNs, providing the appropriate dNTP is susceptible to pharmacological depletion (e.g., the resensitization of ddN-resistant mutants through dATP depletion; Lori et al., 1997).

It is of interest that the proviral DNA replication process is susceptible to a relatively brief reduction in dNTP levels, whereas host-cell genomic DNA synthesis appears to be little affected (as indicated by the absence of host-cell toxicity at levels of 5-FU or FdUdR sufficient to cause a profound, if transient, decrease in dTTP pool size). As is well known, DNA polymerases α and δ, the enzymes primarily responsible for chromosomal DNA synthesis, exhibit significant ability to discriminate between physiological dNTPs and non-physiological ddNTPs, whereas the viral polymerase, with its lack of effective proofreading capacity and resultant relatively high error rate, is more susceptible to misinsertion of AZTTP or d4TTP for dTTP. Because RT has no associated 3′-5′ exonuclease activity, once the latter substitutions occur, progression of HIV DNA synthesis, unlike that of human cell genomic DNA synthesis, is irreversibly blocked, and the return of dTTP pools to normal levels cannot then bring about the resumption of HIV DNA chain extension.

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References


