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DPC 817: a Cytidine Nucleoside Analog with Activity against Zidovudine- and Lamivudine-Resistant Viral Variants

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Highly active antiretroviral therapy (HAART) is the standard treatment for infection with the human immunodeficiency virus (HIV). HAART regimens consist of protease inhibitors or nonnucleoside reverse transcriptase inhibitors combined with two or more nucleoside reverse transcriptase inhibitors (NRTIs). DPC 817, 2',3'-dideoxy-2',3'-dideoxy-5-fluorocytidine (PSI 5582 D-D4FC) is a potent inhibitor of HIV type 1 replication in vitro. Importantly, DPC 817 retains activity against isolates harboring mutations in the reverse transcriptase gene that confer resistance to lamivudine (3TC) and zidovudine (AZT), which are frequent components of initial HAART regimens. DPC 817 combines this favorable resistance profile with rapid uptake and conversion to the active metabolite DPC 817-triphosphate, which has an intracellular half-life of 13 to 17 h. Pharmacokinetics in the rhesus monkey suggest low clearance of parent DPC 817 and a plasma half-life longer than that of either AZT or 3TC. Together, these properties suggest that DPC 817 may be useful as a component of HAART regimens in individuals with resistance to older NRTI agents.

Nucleoside analogs are a class of inhibitors of the human immunodeficiency virus (HIV) reverse transcriptase (RT) that, once bioconverted to their active triphosphate forms, act as alternative substrates and subsequently as chain terminators (20). The nucleoside RT inhibitors (NRTIs) were the first, and for many years the only, therapeutic options available for treatment of HIV disease prior to the discovery of potent inhibitors of the viral protease. Today, NRTIs still serve as critical components of therapy, in combination with either protease inhibitors or nonnucleoside RT inhibitors (NNRTIs) (4, 5).

The clinically useful NRTIs, while acting by similar overall mechanisms, possess significant differences within the group, since they represent two distinct subclasses, the pyrimidines (zidovudine [AZT], zalcitabine [ddC], stavudine [d4T], and lamivudine [3TC]) and the purines (didanosine [ddI], tenofovir, and abacavir), which is a guanosine analog. There are second-generation analogs ([(-)]-FTC (cobicistat), DAPD (amdoxovir), and [(-)]-D(TC) with reportedly improved properties in clinical trials.

The potency of the various NRTIs spans a huge range, both for individual compounds assessed in a variety of ways and among the seven approved drugs. There is also little apparent correlation between in vitro potency and the recommended daily dosage. Unlike the protease inhibitors and NNRTIs, whose efficacy is related to the ratio of the trough level of free drug to the protein binding-adjusted in vitro potency, the relationship between total exposure and efficacy for NRTIs is complicated by the fact that they must enter the target cell and be phosphorylated to the triphosphate level in order to inhibit the target enzyme. Cellular uptake, the rate of phosphorylation, the ratio of phosphorylated NRTI to the naturally occurring triphosphate substrate, and the intracellular half-life of phosphorylated NRTI will thus determine the overall potency of this class of antivirals (24). Studies to date of approved and experimental inhibitors show a large range in uptake efficiencies and intracellular residence times of nucleoside triphosphate (NRTI-TP) (11, 12, 17, 22, 25, 32).

Resistance is a major issue for the NRTIs, as is true for all classes of HIV drugs. Resistance arises when levels of the drug in plasma (or intracellular levels of active drug) are insufficient to cause complete suppression of virus quasispecies (10, 15, 16, 34, 35). Although there are differences in the primary mutational event associated with resistance to each agent, there are certain key mutations that cause fairly broad cross-resistance. The M184V mutation in HIV type 1 (HIV-1) RT causes high-
level resistance to 3TC and (−)-FTC and weak cross-resistance to abacavir, ddC, dOTC, and ddI (13, 28). Mutations after prolonged AZT therapy, including those at positions 41, 67, 70, 210, 215, and 219, frequently also cause loss of sensitivity to d4T (28). More problematic for future therapy is the emergence of multi-NRTI-resistant strains, including the Q151M-containing complex of mutations, insertions of double serine after residue 69, G333D or -E substitution, and several other multiple mutants that have arisen in patients whose second or third NRTI-containing regimen has failed (28). Recently, the prevalence and characteristics of multi-NRTI-resistant HIV among European patients receiving various combinations of nucleoside analogs was examined (33). This study found that the frequency of multi-nucleoside analog-resistant strains was low at present (1.9%) but that AZT- and 3TC-related mutations without Q151M or 69 insertions occurred at a high rate in isolates from patients with and without experience with these drugs. The overall prevalence of NRTI resistance mutations in isolates from drug-naive patients was 18%. Although the approval of potent NNRTIs, such as efavirenz, and the hope for second-generation NNRTIs and protease inhibitors offer options for viable second and third regimens, these new drugs will likely be used in a background of NRTIs as part of the combination regimen. Thus, there is a need for true second-generation nucleosides that address the resistance liabilities that the first-generation compounds have and that their use (and misuse) for over a decade has caused.

Our criteria for a true second-generation NRTI focused on the resistance profile and the need to provide suppression of mutant variants likely to be present in NRTI-experienced patients, i.e., salvage therapy. These mutant variants will include viruses with substitutions at positions 41, 65, 70, 74, 210, 215, 219, 151, and 184 and insertions at position 69. Although, as indicated above, there is no clear predictive relationship between potency and total daily dose for NRTIs, it is likely that demonstration of adequate levels of administered nucleoside in plasma, adequate levels of NRTI-TP in the cells of interest, and long intracellular half-life of this activated form may allow low doses and infrequent dosing regimens. We examined a number of pyrimidine and purine nucleoside analogs in in vitro antiviral assays aimed at identifying analogs with the ability to inhibit AZT- and 3TC-resistant viral variants. DPC 817 is a cytidine analog which combines potency against wild-type, AZT-resistant, and 3TC-resistant variants with a half-life consistent with once-daily or twice-daily dosing.

**MATERIALS AND METHODS**

**Synthesis of DPC 817.** The published syntheses of DPC 817 (2′,3′-dideoxy-2′,3′-dideoxy-5-fluorocytidine; PSI 5582) are based on the glycosylation of the trimethylsilylated derivative of fluorocytosine (2) with either a glycal derived from D-xylose (7) or a protected dideoxy 2-phenylselenyl ribofuranose (29). For the large-scale synthesis of DPC 817, the trimethylsilylated derivative of flucytosine (2) is coupled with a protected d-ribose (1) to give 5-fluorocytidine (3) after deprotection. Selective protection of the 5′ hydroxyl and reaction with an acid halide gives the nucleoside 4, which after reductive elimination and deprotection gives DPC 817 (Fig. 1).

**Other antiretrovirals.** Efavirenz (Sustiva; DMP 266) was synthesized at DuPont Pharmaceuticals. AZT, ddI, and d4T were purchased commercially (Sigma Chemical Co., St. Louis, Mo.). Abacavir and 3TC were supplied by R. F. Schinazi's laboratory. DPC 817-triphosphate was prepared by TriLink, Inc. 3TC- and ddC-triphosphates were purchased from Moravek Biochemicals, Brea, Calif. All NRTIs were stored as dimethyl sulfoxide solutions at −20°C.

**FIG. 1.** Synthesis of DPC 817.
Measurement of inhibition of viral and cellular polymerases. The ability of the triphosphorylated form of DPC 817 to inhibit purified HIV-1 RT (purified from New England Nuclear) or DNA polymerases α and γ was determined using a poly(rI)·oligo(dC)12-18 template-primer as a substrate and monitoring the incorporation of radiolabeled dCTP by separation of radiolabeled oligomers by ion-exchange membranes (8, 9). Reactions were carried out in the presence and absence of nucleoside analog in a total volume of 50 μl at 37°C for 45 min. The reaction mixture contained 0.14 nM HIV-1 RT, 0.125 μg of poly(rI)·oligo (dC)12-18, and 5 μM dCTP (0.5 μCi of [3H]dCTP) in 50 mM Tris HCl (pH 8.2) with 80 mM KCl, 12 mM MgCl₂, 1 mM dithiothreitol, 50 μM EGTA, 1 mg of bovine serum albumin/ml, and 0.01% Triton X-100. Newly elongated template-primer mixture was captured by filtration on Millipore DE 96-well filter plates containing DEAE filter disks and subsequent measurement of radioactivity.

Measurement of antiviral activity. The ability of DPC 817 to inhibit HIV replication in immortalized human T-cell lines and activated peripheral blood mononuclear cells (PBMCs) in tissue culture was assessed using five different assay systems. In yield reduction assays, the quantity of infectious virus present after a 3-day acute infection was determined by plaque assay (30). In a second system, newly synthesized viral RNA produced in a 3-day acute infection was captured on streptavidin plates and detected by derivatized oligonucleotide hybridization (1). In p24 assays, viral p24 antigen produced in a 3-day acute infection (NLA-3) or a 7-day infection (LAI) was detected by enzyme-linked immunosorbent assay (ELISA) (18). In RT assays, virus obtained from the cell supernatant on day 6 after infection was quantitated by an RT assay using tRNA, oligo(dT)12-18 as a template-primer (2). In a fifth system, recombinant viruses incorporating clinically derived protease and RT genes that had been PCR amplified from patients’ plasma virus were assayed in a reporter cell line as described by Hertogs et al. (Antivirogram) (14) and conducted at Virco Laboratories, Viroc NV, Belgium. Viruses representing non-clade B isolates were subtyped by a combination of sequencing and a heteroduplex mobility shift assay on regions of the Gag and Env genes (M-P. De Bethune, K. Hertogs, L. Heyndrickx, J. Vingerhoets, K. Fransen, H. Azijn, L. Michiels, W. Janssens, A. Scholliers, B. Larder, S. Bloor, R. Pauwels, and G. Van der Groen, Proc. Third Heyndrickx, J. Vingerhoets, K. Fransen, H. Azijn, L. Michiels, W. Janssens, A. Scholliers, B. Larder, S. Bloor, R. Pauwels, and G. Van der Groen, Proc. Third

Cytotoxicity determination. Cells were incubated with DPC 817 for 3 (MT-2 cells), 4 (Vero cells), 6 (PBMCs), or 7 (Huh7, 293, and MDBK cells) days. Cell viability was determined by measurement of the reduction of the formazan dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, which is catalyzed by mitochondrial succinate dehydrogenase (1, 30).

Protein binding. Equilibrium dialysis and liquid chromatography-mass spectrometry (LC-MS) detection were used to determine the percent free drug present in human serum using pooled human serum and concentrations of 2 or 10 μM DPC 817.

Determination of intracellular DPC 817-TP and half-life measurement. The apparent half-life of the active triphosphosphate form of DPC 817 (DPC 817-TP) was determined in a functional assay and by physical separation and detection. In the functional assay, MT-2 cells were incubated with either 6.5 μM DPC 817 for 2 h (to mimic a transient peak plasma level likely with oral dosing) or 1.3 μM DPC 817 for 24 h (to mimic the concentration present when 90% inhibition of viral replication was observed in tissue culture). Following this drug exposure, residual drug was removed from the extracellular medium by extensive washing of the cells by cycles of centrifugation and resuspension in tissue culture medium. Finally, the cells were plated in 24-well dishes, and HIV-1 (RF) was added at various times. Viral p24 antigen produced in 3 days of infection was quantitated by ELISA (NEN kit). The percent inhibition relative to cultures mock treated by ELISA (NEN kit). The percent inhibition relative to cultures mock treated

<table>
<thead>
<tr>
<th>Polymerase</th>
<th>DPC 817-TP</th>
<th>ddC-TP</th>
<th>3TC-TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV RT</td>
<td>0.14 ± 0.007</td>
<td>ND</td>
<td>0.57 ± 0.05 (n = 3)</td>
</tr>
<tr>
<td>DNA polymerase β</td>
<td>1.20 ± 0.08</td>
<td>0.25 ± 0.05</td>
<td>10.3 ± 1.68 (n = 2)</td>
</tr>
<tr>
<td>DNA polymerase γ</td>
<td>0.74 ± 0.07</td>
<td>0.07 ± 0.03</td>
<td>43.1 ± 6.53 (n = 5)</td>
</tr>
</tbody>
</table>

* ND, not done.

RESULTS AND DISCUSSION

Potency and selectivity against HIV-1 RT. DPC 817-TP was assessed for its ability to inhibit HIV RT and DNA polymerases γ and β in primer extension assays using a poly(rI)·oligo(dC) template-primer system (Table 1). The ability of DPC 817-TP to inhibit dCTP incorporation was compared to those of 3TC-TP and ddC-TP. The mode of inhibition was established, and as expected, DPC 817-TP was competitive with respect to dCTP. In kinetic studies of RT inhibition, the K<sub>m</sub> for dCTP was established at 7 μM and the K<sub>i</sub> for DPC 817-TP was 0.1 μM, which yields a favorable K<sub>m</sub>-to-K<sub>i</sub> ratio. The K<sub>i</sub> was also determined for the M184V-containing RT. In these studies, the K<sub>i</sub> for DPC 817-TP was 0.3 μM, whereas for 3TC-TP was 85 μM. Attainment of ≥0.3 μM levels of intracellular DPC 817-TP in cells should thus allow for potent inhibition of the RT by DPC 817, including RT from viruses carrying the M184V mutation, which are highly resistant to 3TC.

DPC 817-TP shows some selectivity for inhibition of HIV RT relative to cellular polymerases (Table 1). DPC 817-TP shows eightfold selectivity for inhibition of the viral RT versus the cellular repair enzyme DNA polymerase β. A 5.3-fold increase in IC50<sub>α</sub> was demonstrated for the mitochondrial DNA polymerase γ. Considering the desired level of intracellular triphosphate of ~0.3 μM, there is the potential for inhibition of the γ polymerase within the mitochondria. However, it has been shown for the nucleoside analog ddC that inhibition of mitochondrial function is related to the ability of intact mitochondria to take up the triphosphorylated form of ddC, as opposed to passive transport of the uncharged ddC into mitochondria, followed by phosphorylation by mitochondrial ki-
TABLE 2. Antiviral activity of DPC 817 against laboratory and clinical isolates

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cells</th>
<th>Assay</th>
<th>IC(_{50}) ((\mu)M)</th>
<th>IC(_{90}) ((\mu)M)</th>
<th>No. of replicates on isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>MT-2</td>
<td>RNA</td>
<td>1.2 ± 0.5</td>
<td>0.4 ± 0.05</td>
<td>9</td>
</tr>
<tr>
<td>RF</td>
<td>MT-2</td>
<td>Yield</td>
<td>0.4 ± 0.5</td>
<td>0.6 ± 0.3</td>
<td>1</td>
</tr>
<tr>
<td>HXB2</td>
<td>MT-4</td>
<td>P24</td>
<td>1.1 ± 0.4</td>
<td>1.5 ± 0.9</td>
<td>2</td>
</tr>
<tr>
<td>LAI</td>
<td>PBMCs</td>
<td>RT</td>
<td>0.8</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td>Thal H9666</td>
<td>MT-2</td>
<td>Yield</td>
<td>0.8 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>1</td>
</tr>
<tr>
<td>HIV-2</td>
<td>MT-2</td>
<td>Yield</td>
<td>0.8 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>1</td>
</tr>
</tbody>
</table>

Clinical isolates
A/A, D.E, or G  0.6 ± 0.3  7
B/B or A        0.5 ± 0.3  7
C/C             0.4                      2
D/D or F        0.4 ± 0.2  5
F/F             0.6  1
H/H             1.1 ± 0.9  3
Not determined/  0.4 ± 0.1  4
C or D          Group O 0.9  1

\(a\) Recombinant clinical isolates from different clades (subtype in GAG/ENV).

The ability of DPC 817 to inhibit the replication of laboratory and clinical isolates of HIV was determined by several methods (Table 2). The assays were performed using established T-cell lines (MT-2 and MT-4) and PBMCs. As shown in Table 2, DPC 817 inhibits virus replication 90% at concentrations of 1 mM in mouse bone marrow cells, conditions under which ddC produces detectable mitochondrial dysfunction (P. J. Ciaccio, B. D. Car, S. A. Wert, and S. P. Adams, unpublished data).

Antiviral activity against laboratory strains and clinical isolates of HIV-1. The ability of DPC 817 to inhibit the replication of laboratory and clinical isolates of HIV was determined by several methods (Table 2). The assays were performed using established T-cell lines (MT-2 and MT-4) and PBMCs. As shown in Table 2, DPC 817 inhibits virus replication 90% at concentrations of 1 mM in mouse bone marrow cells, conditions under which ddC produces detectable mitochondrial dysfunction (P. J. Ciaccio, B. D. Car, S. A. Wert, and S. P. Adams, unpublished data).

Cytotoxicity of DPC 817. As a class, the NRTIs are considered to be more cytotoxic than other classes of anti-HIV agents. For this reason, we have conducted additional cytotoxicity measurements beyond the limited assessment used with other antivirals (27). The concentration yielding 50% toxicity in various cells is indicated in Table 3, where it can be seen that DPC 817 does not show significant cytotoxicity against a range of cell lines at concentrations less than 30 \(\mu\)M.

Recently, mitochondrial toxicity has been proposed as a mechanism to explain the relatively high degree of toxicity of the NRTI class (19, 26, 31). The evidence for this association was summarized by Sommadossi (31) as follows: “The underlying mechanisms for these toxicities is certainly multifactorial, and a delayed mitochondrial toxicity has been proposed to be partly responsible for nucleoside related adverse effects. Previous studies on ddC-induced peripheral neuropathy and AZT-induced myopathy indicated a preferential depletion of mitochondrial DNA content in drug-treated cells, which could subsequently damage mitochondrial functions in target organs.” Sommadossi has further shown that 2’-deoxy-2’-F-beta-D-arabino-furanosyl-5-iouracil (FIAU) is incorporated into mitochondrial DNA in HepG2 cells, which led to marked mitochondrial dysfunction despite the fact that little inhibition of mitochondrial DNA synthesis could be measured. Mitochondrial toxicity caused by NRTIs has been explored using a variety of measurements, including the effects of compounds on lactic acid production by HepG2 cells; mitochondrial morphology, including cristae appearance; and determination of mitochondrial DNA content in treated cells using hybridization with mitochondria-specific probes. In the case of ddC, Chen and Cheng (6) have shown that ddC must first be phosphorylated by cytosolic kinases prior to (active) transport into mitochondria and incorporation into mitochondrial DNA by the mitochondrial \(\gamma\) polymerase. Other studies have shown that ddCDP-choline (formed from ddCTP via phosphocholine cytidylyltransferase) may be the toxin responsible for the mitochondrial effects of ddC (26). Thus, the extent to which a given NRTI causes mitochondrial damage is likely the result of how well the active metabolite(s) is taken up by mitochondria and how profoundly the triphosphate metabolites inhibit the \(\gamma\) polymerase enzyme. DPC 817 has shown no effect on mitochondrial function as assessed by lactate production, rhodamine 123 cell fluorescence, or proliferation of freshly isolated mouse bone marrow cells at concentrations up to 1 mM, whereas ddC showed toxicities consistent with previous findings in the literature (6, 19, 24, 31). In HepG2 cells, DPC 817 caused toxicity that was intermediate between those of 3TC and ddC (Ciaccio et al., unpublished).

Antiviral potency against recombinant mutant variants of HIV-1. To characterize the antiviral potency of DPC 817 further, a panel of recombinant viruses with selected mutations in the RT gene was utilized. Two types of studies were conducted. In the first study, recombinant mutant HIV-1 variants containing RT mutations associated with resistance to one or more NRTIs were constructed by site-directed mutagenesis in the LAI or NL4-3 background and assessed in MT-2 or MT-4 cells via p24 antigen production. The IC\(_{50}\), and in some cases the IC\(_{90}\), was reported; for the purposes of comparison, an IC\(_{90}\) can be estimated by multiplying the IC\(_{50}\) by a factor of 5.0 (see Materials and Methods). In a second study, recombinant viruses were constructed in the HXB2 background using the protease and RT genes from plasma virus and were assessed as described by Hertogs et al. (14). The IC\(_{50}\) was measured. Table 4 and Fig. 2 show the results of resistance profiling using site-directed and clinical isolate chimeric viruses for DPC 817.

TABLE 3. Cytotoxicity of DPC 817 in various cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>No. of days in culture</th>
<th>TC(_{50}) ((\mu)M)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT-2</td>
<td>3</td>
<td>&gt;206</td>
</tr>
<tr>
<td>293</td>
<td>7</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Huh7</td>
<td>7</td>
<td>&gt;30</td>
</tr>
<tr>
<td>MDBC</td>
<td>7</td>
<td>&gt;30</td>
</tr>
<tr>
<td>PBMC</td>
<td>7</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Vero</td>
<td>4</td>
<td>&gt;100</td>
</tr>
<tr>
<td>CEM</td>
<td>4</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

\(^a\) TC\(_{50}\), 50% toxic concentration.
and other NRTIs. The IC_{50} and IC_{90} were compared to those of isogenic wild-type viruses to determine the fold resistance. As Table 4 shows, DPC 817 maintains potency against many different types of mutant HIV, including those with multiple substitutions in RT associated with resistance to AZT and/or 3TC, such as the mutations M184V, M41L, D67N, K70R, T215Y, and K219Q. Virus containing the point mutation K65R showed the highest degree of resistance (seven- to ninefold) of the site-directed mutants examined. K65R has been observed in tissue culture in response to selective pressure exerted by the guanosine analog 1-beta-D-2,6-diaminopurine dioxolane (2) and in some but not all in vitro selection experiments with DPC 817 (J. T. Hammond, C. K. Chu, R. F. Schinazi, U. Parikh, E. Arnold, S. Serafinos, and J. W. Mellors, Proc. Fifth Int. Workshop Drug Resist. Treat. Strateg., abstr. 2328, 2001). Interestingly, the composition and stereochemistry of both the base and the sugar appeared to play a role in the resistance and cross-resistance observed with molecules related to DPC 817 (Hammond et al., Proc. Fifth Int. Workshop Drug Resist. Treat. Strateg.). However, K65R is rarely observed in patients for whom any of the currently available nucleoside-containing NRTI-resistant variants will be possible. Figure 2 also shows that ddI and d4T share the property of maintained potency against 3TC- and AZT-resistant viruses with DPC 817, with median IC_{50} of 2.09 and 1.62 μM, respectively, corresponding to IC_{90} of 10.5 and 5.8 μM, and resistance values of <three-fold. The clinical significance of small shifts in virus susceptibility has not been firmly established.

### Plasma protein binding of DPC 817

The free fraction present in human serum was determined by equilibrium dialysis and LC-MS detection of DPC 817. As with other nucleoside analogs useful in the treatment of HIV disease, DPC 817 is scarcely bound by plasma proteins; the free fraction in human serum was determined to be 96%.

### Intracellular half-life of DPC 817-TP

Two approaches were taken to assess the intracellular half-life of the active metabolite of DPC 817, DPC 817-TP. In the first approach, direct measurement of DPC 817-TP in human PBMCs was carried out. Pooled phytohemagglutinin-activated PBMCs from multiple donors were used in all experiments.

Preliminary experiments indicated that uptake and conversion of DPC 817 to the triphosphate were rapid (with a plateau at 12 to 24 h) and saturable with DPC 817 concentration, with a plateau above 10 μM DPC 817. To determine the half-life, PBMCs at 2 × 10^6/ml were incubated for 24 h with 5 or 10 μM DPC 817. The cells were then washed, resuspended in fresh tissue culture medium, and incubated at 37°C (the washout period). At various times, aliquots of cells were pelleted and frozen for subsequent methanol extraction. Figure 3 shows the concentration of DPC 817-TP measured during the washout phase.

Following the 24-h incubation, DPC 817-TP concentration-versus-time profiles exhibited a nearly monoexponential decline. The results are summarized in Table 5 and are compared to half-life values published for other anti-HIV nucleoside analogs.

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replication was measured after 3 days relative to cultures not exposed to DPC 817. Figure 4 shows that a brief exposure to 6.5 μM or a 24-h exposure to 1.3 μM DPC 817 yielded an initial ~90% inhibition of replication. Substantial inhibition was apparent when virus was added 12 h after the washout period, and measurable inhibition remained even after 24 h post-inhibitor washout, indicating that the active form of DPC 817 was still capable of incorporation into, and chain termination of, newly formed cDNA synthesized by the viral RT.

Taken together, the direct measurement and indirect functional assays for DPC 817-TP indicate a long intracellular half-life for the active metabolite. The long intracellular half-life suggests that once-daily or twice-daily dosing may be sufficient to maintain levels of intracellular DPC 817-TP that are in excess of those required for inhibition of the RT from both wild-type HIV-1 and mutant variants likely to be present in individuals exposed to AZT and/or 3TC. In contrast, the measured half-life for d4T-TP is only a few hours, and the level of the parent compound in the plasma is below the predicted IC₉₀ level based on in vitro studies (Table 5). Thus, although the loss in susceptibility to mutant variants is small for d4T (Fig. 2), the ability of d4T to be maintained in plasma and subsequently converted to the active metabolite is suboptimal (Table 5); the result may be a loss of efficacy against viruses with only subtle decreases in drug susceptibility. The intracellular half-life of ddA-TP formed from ddI is adequate; however, the plasma level of the parent compound and the intracellular level of ddA-TP are only a fraction of the level likely required for inhibition of NRTI-resistant mutant variants of HIV-1 RT (Table 5).

Comparison of i.v. pharmacokinetics for DPC 817, 3TC, and AZT in rhesus monkeys. Table 6 compares the published i.v. pharmacokinetic data for DPC 817 (21), 3TC (3), and AZT

FIG. 2. Cross-resistance profiles of nucleosides; susceptibility of recombinant viruses constructed from plasma virus of individuals exposed to one or more NRTI inhibitors (14). (A) Susceptibilities of a panel of seven isolates resistant to AZT and containing mutations at positions 41 and 215 plus three to six additional mutations, frequently D67N, L210W, T69D, and L214F. The susceptibility of the wild-type laboratory strain HXB2 was also determined. (B) Susceptibilities of a panel of five 3TC-resistant isolates containing the M184V mutation plus one or two additional mutations, including K65R, V75M, L74V, L214F, and R211G. The susceptibility of wild-type HXB2 was also determined. (C) Susceptibilities of a panel of 10 isolates resistant to AZT and 3TC and containing M41L, D67N, M184V, and T215Y plus three to nine additional mutations, frequently L210W, R211K, L214F, and K219N, -E, or -R. (D) Fold resistance (the IC₉₀ of the test strain divided by the IC₉₀ of the wild-type HXB2 reference strain) is shown for each dual-resistant isolate. Median IC₉₀ for the wild-type reference strain were as follows: AZT, 0.02 mM; 3TC, 0.52 mM; ddI, 1.4 μM; d4T, 0.94 μM; and DPC 817, 0.31 μM. Median values for fold resistance are indicated by horizontal lines; a fold resistance of 1.0 (no resistance) is indicated by the dashed line.
(23) in rhesus monkeys after a single i.v. dose. The systemic clearance (CL<sub>S</sub>) of DPC 817 was 0.43 liter/h/kg and was lower than the reported CL<sub>S</sub> values for 3TC (0.78 ± 0.12 liter/h/kg) and AZT (1.11 ± 0.4 liter/h/kg). The renal clearance values (CLR) for all three compounds exceed the glomerular filtration rate reported for rhesus monkeys (0.12 liter/h/g), suggesting active tubular secretion. The elimination half-life for DPC 817 (3.6 h) in monkeys is longer than the half-lives reported for 3TC (1.4 h) and AZT (1.1 h). These pharmacokinetic data suggest that DPC 817 has a lower CL<sub>S</sub> and a longer half-life than AZT or 3TC in rhesus monkeys.

**Conclusions.** The NRTI DPC 817 combines significant potency against wild-type and AZT- and 3TC-resistant mutant variants with pharmacokinetics and intracellular-metabolism kinetics consistent with once- or twice-daily dosing. Provided adequate levels can be obtained in the blood of HIV-infected subjects, DPC 817 may offer additional versatility in the design of combination regimens for drug-experienced patients. Preliminary safety assessment studies have been conducted in rats and dogs to support single- and multiple-dose pharmacokinetic studies in humans. Such studies were recently initiated.

**TABLE 5. Intracellular half-lives of nucleoside triphosphates**

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Plasma level of parent compound (µM)</th>
<th>Intracellular half-life of triphosphate (h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPC 817</td>
<td>Unknown</td>
<td>12.6–16.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>AZT</td>
<td>6.7</td>
<td>5–13</td>
<td>25, package insert</td>
</tr>
<tr>
<td>3TC</td>
<td>6.6</td>
<td>23–49</td>
<td>22, 25, package insert</td>
</tr>
<tr>
<td>d4T</td>
<td>2.7</td>
<td>3.5</td>
<td>17</td>
</tr>
<tr>
<td>ddI</td>
<td>4.2</td>
<td>25–40</td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup> C<sub>max</sub> and half-life (t<sub>1/2</sub>) were as follows (mean ± standard deviation): incubation in 10 µM DPC 817, C<sub>max</sub> = 1.45 ± 0.2 µM and t<sub>1/2</sub> = 16.6 ± 4.8 h; incubation in 5 µM DPC 817, C<sub>max</sub> = 0.77 µM and t<sub>1/2</sub> = 12.6 h.

FIG. 3. Intracellular half-life of DPC 817-TP. Mitogen-activated PBMCs were incubated with DPC 817 for 24 h, and the intracellular DPC 817-TP remaining after the 24-h incubation was determined on aliquots of 10<sup>7</sup> cells. See Materials and Methods for additional details. ■, DPC 817 at 10 µM, experiment A; □, DPC 817 at 10 µM, experiment B; ●, DPC 817 at 5 µM, experiment A; △, DPC 817 at 5 µM, experiment B.

FIG. 4. Functional half-life of DPC 817-TP. MT-2 cells were incubated with 6.5 µM DPC 817 for 2 h (solid bars) or with 1.3 µM DPC 817 for 24 h (shaded bars), and the residual drug was removed by cycles of centrifugation and resuspension in fresh tissue culture medium minus DPC 817. The resuspended cells were plated and infected with HIV-1 (RF) at various times post-drug exposure. Following 3 days of infection, viral p24 antigen was quantitated in the cell supernatents by ELISA. The percent inhibition of replication was calculated relative to cultures to which no DPC 817 was added. The data shown are the mean plus standard deviation for three experiments.
TABLE 6. Mean pharmacokinetic data for rhesus monkeys after a single i.v. dose of DPC 817, 3TC, or AZT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>33</td>
</tr>
<tr>
<td>No. of monkeys</td>
<td>3</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>3.6</td>
</tr>
<tr>
<td>CLr (liter/h/kg)</td>
<td>0.43</td>
</tr>
<tr>
<td>CLr (liter/h/kg)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

* Published by Ma et al. (21)
* Published by Blaney et al. (31)
* Published by Qian et al. (23)
* Average of two monkeys.

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