Biochemical Pharmacology, Vol. 58, pp. 1603–1608, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00246-4

Cross-resistance of Dideoxycytidine-Resistant Cell Lines to Azidothymidine

Ram P. Agarwal, Wei Wang, Peggy Yo, Tieran Han and Marilyn Fernandez*
Department of Medicine, University of Miami School of Medicine, Miami, FL 33101, U.S.A.

ABSTRACT. 2′,3′-Dideoxycytidine (ddC) and azidothymidine (AZT) inhibit HIV-1 replication and currently are used in AIDS therapy. Long-term use of the drugs is associated with the selection of drug-resistant HIV strains, thus limiting their effectiveness. Another mechanism, associated with their altered metabolism in host cells, also can cause "cellular" drug resistance. Human lymphocytic H9 cell lines (H9-ddC0.5w and H9-ddC5.0w) selected for ddC resistance by exposure to 0.5 and 5.0 μM ddC were found to be cross-resistant to AZT. Compared with controls, the thymidine kinase (TK) activities in H9-ddC0.5w and H9-ddC5.0w cells were 56.7 and 51.4% (with thymidine as a substrate) and 50.3 and 42% (with AZT as a substrate). Consequently the cellular incorporation of AZT and thymidine (24-hr incubation) also was reduced to 51.3 and 70.0% in H9-ddC0.5w cells and to 12.1 and 17.3% in H9-ddC5.0w cells. A 3-hr incubation with 25 μM AZT and ddC decreased their cellular incorporation to 50.5 and 76.15% in H9-ddC0.5w cells and to 12.95 and 47.8% in H9-ddC5.0w cells compared with H9 cells. Thus, the change in AZT accumulation did not correlate exactly with the decrease in TK activity and far exceeded the effect on ddC accumulation. Evidence is presented that ddC, in addition to deoxycytidine kinase, affected TK1 activity. The involvement of multidrug resistance proteins in the mechanism of the resistance was ruled out by the failure of trifluoperazine and verapamil to alter cellular accumulations of AZT, ddC, daunorubicin, and rhodamine-123. Development of cellular ddC and AZT cross-resistance may affect the therapeutic efficacy of these antiviral agents. BIOCHEM PHARMACOL **58**;10: 1603–1608, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. 2′,3′-dideoxycytidine; azidothymidine; cellular resistance; ddC–AZT cross-resistance; reverse transcriptase inhibitors

Among the nucleoside reverse transcriptase inhibitors approved for the treatment of AIDS† patients with HIV infection, AZT and ddC have played a key role in antiviral therapy [1]. Mutations of HIV-1 reverse transcriptase and host cells following long-term treatment with these drugs, however, led to the development of drug resistance, diminishing their therapeutic benefits [2–5]. To become therapeutically active, these drugs must be phosphorylated by host cell kinases to their active metabolites, 5′-triphosphates, which then inhibit viral reverse transcriptase and mitochondrial DNA synthesis [6]. Decreased synthesis and cellular retention of nucleoside triphosphates have been associated with drug resistance to nucleoside analogs in cancer and antiviral therapy [4, 5, 7, 8]. Whereas a large number of studies have been reported on the mutation of HIV-reverse transcriptase and resistance to antiviral drugs, interest in exploring whether host cellular factors are responsible for resistance to antiviral agents has begun somewhat more recently [2–5, 9]. To gain more insight into the phenomenon of host cell resistance, we have produced cell lines, H9-ddC0.5w and H9-ddC5.0w, by *in vitro* exposure of a human lymphocytic cell line (H9) to 0.5 and 5.0 μM ddC. Even though different pathways metabolize ddC and AZT, these sublines also displayed strong collateral resistance to AZT, an analog of TdR. Magnani *et al.* [4] have isolated a ddC-resistant subline of the human monoblastic cell line U937. However, this subline was not examined for AZT resistance. An AZT-resistant cell line also has been isolated, but following exposure to AZT rather than ddC [5]. This is the first report, to our knowledge, demonstrating AZT cross-resistance in a cell line isolated for ddC resistance. A preliminary report has been presented elsewhere [10].

* Corresponding author: Dr. Ram P. Agarwal, Department of Medicine (M-862), University of Miami School of Medicine, 7084A RMSB, 1600 NW 10 Avenue, Miami, FL 33101. Tel. (305) 243-4817; FAX (305) 243-3181; E-mail: ragarwal@med.miami.edu

† *Abbreviations*: AIDS, acquired immune deficiency syndrome; ara-T, 1-β-D-arabinosylthymine; AZT, 3′-azido-3′-deoxythymidine (azidothymidine); AZTMP, AZTDP, AZTTP, mono-, di-, and triphosphates of AZT; CR, cytidine; dCK, deoxycytidine kinase; dCR, 2′-deoxycytidine; ddC, 2′,3′-dideoxycytidine; HIV, human immunodeficiency virus; MDR, multidrug resistance; TK, thymidine kinase; and TdR, thymidine.

Received 12 August 1998; accepted 22 April 1999.

MATERIALS AND METHODS

Materials

[*methyl*-^{3}H]AZT (11.7 Ci/mmol), [*methyl*-^{3}H]TdR (63 Ci/mmol), and [5,6-^{3}H]ddC (19.3 Ci/mmol) were purchased from Moravek Biochemicals. All other chemicals were

obtained from the Sigma Chemical Co. PE SIL G/UV TLC plates and DE81 paper discs were purchased from Whatman.

Cells

H9 cells were maintained in culture in RPMI 1640 medium as described earlier [11]. The ddC resistance was induced by exposing the cells to 0.5 and 5.0 μM ddC. Once the resistance was induced, the cells were washed and maintained in drug-free medium. These cells, referred to as H9-ddC0.5w and H9-ddC5.0w cells, had reduced dCK activities (44.1 and 46.3%) and reduced ddC anabolism.

AZT, TdR, and ddC Metabolism

Metabolism of AZT, TdR, and ddC was studied by incubating triplicate cell suspensions (0.5 to1.0 x 10^6 cells/mL) with [*methyl*-^{3}H]AZT, [*methyl*-^{3}H]TdR, or [^{3}H]ddC (25 μM; 1–4 μCi/mL) for 3 or 24 hr at 37°. The cells were collected by centrifugation (400 *g*, 20 min), washed twice with PBS, and extracted overnight at −20° with 0.5 to 1.0 mL of 65% (v/v) methanol. The methanol "soluble" fractions were collected by centrifugation (450 *g*) in a refrigerated centrifuge. Aliquots of the "soluble" fractions were counted directly or analyzed by HPLC (Bio-Rad) equipped with a Partisil-10-SAX column (4.5 × 250 mm). The elution was performed at a flow rate of 1.5 mL/min with a gradient of solution A (5 mM KH_2PO_4) and solution B (0.75 M KH_2PO_4). The eluate was monitored at 254 nm, and 1.5-mL samples were collected and counted for radioactivity.

Since the HPLC system failed to separate nucleosides (AZT and ddC) from their monophosphates, they were separated by TLC on a PE SIL G/UV TLC plate. The plate was developed with a mobile phase consisting of methanol: chloroform (1:4). The nucleosides and nucleotides (R_f values of 0.75 and 0.06) were visualized by UV light, cut out, and counted for radioactivity.

The methanol "insoluble" fractions were washed once with ice-cold 65% methanol, dissolved in NaOH, and counted for radioactivity.

TK Activity

To determine TK activity, exponentially growing cells were suspended in an extraction buffer containing 50 mM Tris–HCl (pH 7.6), 2 mM dithiothreitol, 5 mM benzamide, 0.5 mM phenylmethylsulfonyl fluoride, 20% (v/v) glycerol, and 0.5% (w/v) Nonidet P-40. Cell-free extracts were prepared by four cycles of freeze–thawing, and the supernatants were analyzed for TK activities. The reaction mixture (0.2 mL) contained 37.6 mM Tris–HCl (pH 7.5), 3.8 mM $MgCl_2$, 7.5 mM NaF, 1.5 mM dithiothreitol, 3.7 mM ATP, 1 μmol creatine phosphate, 1–5 units creatine kinase, and 25 μM tritiated TdR or AZT (2 μCi/mL), to which was added the cell supernatant. After incubation at 37° in a water bath, 20-μL aliquots were removed at 0–60 min, applied to DE81 discs (Whatman), and washed once with 1 mM ammonium formate and once with water. The discs were placed in scintillation vials, and the nucleotides were extracted in 1.0 mL of 0.1 N HCl containing 0.1 M KCl and counted for radioactivity. Protein concentrations were determined using the Bio-Rad protein assay.

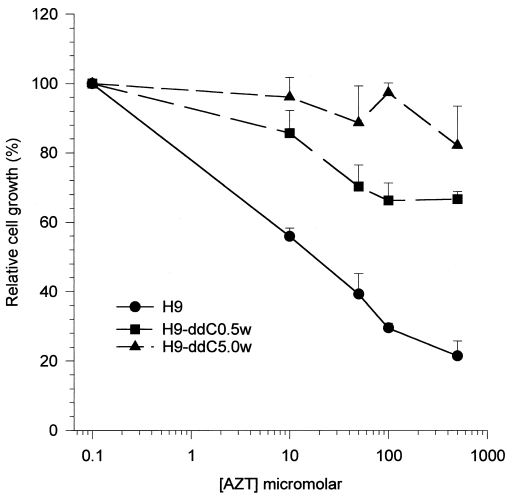

FIG. 1. Effect of AZT on the growth of H9, H9-ddC0.5w, and H9-ddC5.0w cells. Triplicate cell suspensions (2–4 ×10^4 cells/mL) were incubated with various concentrations of AZT. Following 72 hr of incubation at 37°, the trypan blue-excluding cells were counted. Values are means ± SEM of triplicate determinations.

Drug Sensitivity Assays

AZT cytotoxicity assays were performed in triplicate by seeding 2–4 × 10^4 cells/mL in 24-well plates (Costar). The appropriate concentrations of AZT were added, and the trypan blue-excluding cells were counted after 72 hr of incubation at 37°. The IC_{50} values were calculated from the growth curves.

Evaluation of the MDR Phenotype

The presence of MDR was evaluated by flow cytometry using rhodamine-123 or daunorubicin in the presence and absence of the MDR inhibitor verapamil, as described by Krishan *et al.* [12]. Briefly, the cell samples (1 × 10^6 cells/mL) were incubated with rhodamine-123 or daunorubicin hydrochloride (1 μg/mL) in the presence and absence of verapamil (10 μg/mL) at 37° for 15–30 min. Flow cytometric analysis was performed using a Coulter flow cytometer.

RESULTS

Growth Inhibitory Effect of AZT

The effect of AZT on the growth of H9 and ddC-resistant cells is shown in Fig. 1. The IC_{50} values of AZT estimated

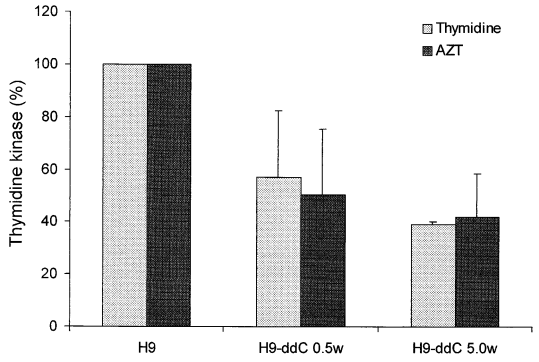

FIG. 2. Comparative TK activity in H9, H9-ddC0.5w, and H9-ddC5.0w cells with TdR and AZT as substrates. The TK activities in H9 cells were 335 ± 5 (N = 7) and 474 ± 148 (N = 4) pmol/mg protein per min with TdR and AZT as substrates, respectively. Other values are means ± range of 2 experiments.

from these curves were 18 μM for H9 cells and > 500 μM for ddC-resistant cells. Thus, the ddC-resistant cells were > 27.7-fold less sensitive to AZT.

TK Activity in Cell Extracts

TK activities of H9-ddC0.5w and H9-ddC5.0w cells with TdR as a substrate were 56.7 and 51.4% of that in control H9 cells (335 ± 5 pmol/mg protein per min). The activities with AZT as a substrate also were reduced to 50.3% in H9-ddC0.5w and 42% in H9-ddC5.0w cells (Fig. 2). However, the differences in the K_m values of either TdR (1.1, 2.3, and 5.0 μM) or AZT (4.7, 5.2, and 7.1 μM) among the cell lines were insignificant.

TdR and AZT Incorporation in the Cellular Pool

The distribution of the incorporated TdR and AZT in methanol "soluble" and "insoluble" fractions of H9, H9-ddC0.5w, and H9-ddC5.0w cells following 24 hr of incubation with 25 μM TdR and AZT is shown in Fig. 3. The majority of TdR (55.0 to 79.3%) was incorporated into DNA (insoluble fraction), whereas the majority (>97%) of AZT remained in the "soluble" fraction. The total AZT incorporation in H9-ddC0.5w and H9-ddC5.0w cells (206.9 and 49.1 pmol/10^6 cells) was 51.3 and 12.1% of the incorporation in H9 cells (403.5 pmol/10^6 cells). The total cellular incorporation of TdR was reduced to 70.0 and 17.3% in H9-ddC0.5w and H9-ddC5.0w cells, respectively.

AZT and ddC Metabolism

To avoid any changes in cellular conditions due to reduced levels of TK and dCK, the cellular accumulation and anabolism of AZT and ddC were examined for a 3-hr interval at the same time in the same cell cultures. Compared with H9 cells, the total cellular accumulation of AZT was decreased to 50.5 ± 19.6 and 12.95 ± 11.1%, and

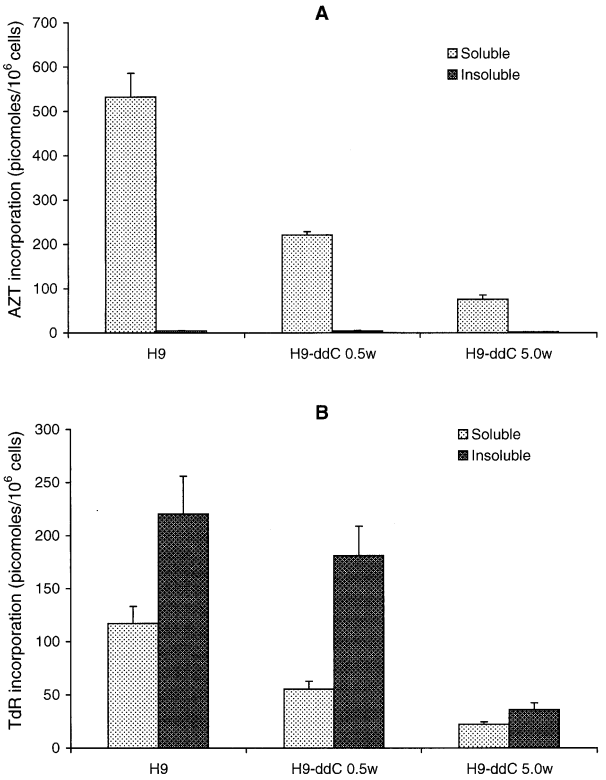

FIG. 3. Intracellular accumulation of AZT and TdR in the methanol "soluble" and "insoluble" fractions in H9, H9-ddC0.5w, and H9-ddC5.0w cells. Triplicate cell suspensions (0.5 to 1.0 × 10^6 cells/mL) were incubated with 25 μM [^{3}H]AZT or [^{3}H]TdR for 24 hr at 37°. The cells were harvested, washed, and extracted with 65% methanol. The methanol "soluble" and "insoluble" fractions were counted for radioactivity, and the amounts in picomoles/10^6 cells were calculated. The values of AZT incorporation in H9 cells represent means ± SD of 4 experiments, whereas other values are means ± range of 2 experiments.

the accumulation of ddC to 76.15 ± 28.5 and 47.8 ± 17.9% in H9-ddC0.5w and H9-ddC5.0w cells, respectively (Fig. 4). Thus, in ddC-resistant cells the AZT cellular concentrations were affected much more than the ddC concentrations.

Consistent with earlier studies from our and other laboratories [11, 13–15], analysis of the "soluble" fractions revealed three major metabolites of ddC: ddCMP (44.8 to 50.2%), ddCDP (21.4 to 31.6%), and ddCTP (2.1 to 9.9%). ddCDP-choline and/or ddCDP-ethanolamine [13], if formed, were not identified in the present system. AZT was metabolized predominantly to AZTMP (60.2 to 92.9%), with negligible metabolism to AZTDP (1.8 to 15.3%) and AZTTP (0.3 to 5.0%) (Table 1). The extent of AZT phosphorylation in H9-ddC0.5w and H9-ddC5.0w cells was significantly lower (22.8 and 0.39%) than the extent of ddC phosphorylation (70.7 and 19.3% of control). Poor catalytic activity of TMP kinase for AZTMP perhaps limits its further phosphorylation to AZTDP and AZTTP [11, 15]. In H9-ddC5.0w cells, the AZT-derived radioactivity in AZTDP and AZTTP was too low for quantitative evaluation.

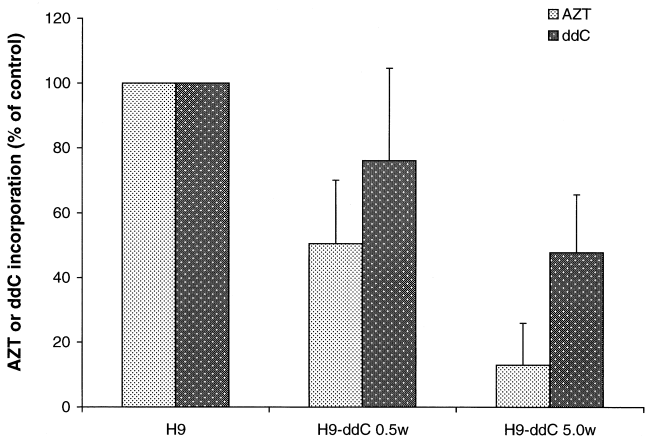

FIG. 4. Intracellular accumulation of AZT and ddC in H9, H9-ddC0.5w, and H9-ddC5.0w cells. Conditions were similar to those described in Fig. 3, except that TdR was replaced with ddC, and the incubation period was 3 hr. The experiments were performed at the same time in the same cell culture. The percent incorporation was calculated in each experiment, and the values are presented as means ± SD of 4 experiments. The values of 100% AZT and ddC-derived radioactivity in H9 cells were 5230 and 2780 cpm, respectively.

Effect of Nucleosides on Cellular Accumulation of AZT

As shown in Table 2, the accumulation of AZT-derived radioactivity was lowered significantly to 3.3 and 21.3% by TdR and to 55.0 and 68.5% by nonradioactive AZT in H9 and H9-ddC0.5w cells, respectively. ara-T had little or no effect on AZT incorporation in either cell line. The AZT accumulation in the presence of CR, dCR, and ddC was 86.5, 81.5, and 76.9% in H9 cells and in H9-ddC0.5w cells 85.9, 76.3, and 100.8%, respectively. In H9-ddC5.0w cells the accumulation was very low and was not affected by any of these nucleosides.

Evaluation of MDR

To determine if MDR proteins played a role in the cross-resistance of ddC-resistant cells to AZT, drug retention studies were done using the fluorescent compounds rhodamine-123 and daunorubicin [12]. There were no differences in the fluorescent profiles between H9 and ddC-resistant cells when treated with the fluorochromes in the presence or absence of an MDR blocker, verapamil (data not shown). To further evaluate the role of MDR proteins, cellular accumulation of ddC and AZT was determined in the presence and absence of another MDR blocker, trifluoperazine [9]. As shown in Fig. 5, trifluoperazine also failed to alter the accumulation of the drugs significantly.

TABLE 1. Distribution of AZT and ddC in the nucleotide pool

	H9	H9-ddC0.5w	H9-ddC5.0w
	(cpm)		
AZT	1,050	960	20
AZTMP	19,900	4,390	50
AZTDP	390	170	15*
AZTTP	80	90	5*
ddC	620	690	630
ddCMP	3,860	3,110	890
ddCDP	2,410	1,720	430
ddCTP	760	150	40

Triplicate cell suspensions (0.5×10^6 cells/mL) were incubated with 25 μM [^{3}H]AZT or [^{3}H]ddC (4 μCi/mL). After incubation for 3 hr at 37°, the cells were harvested, washed, and extracted with 65% methanol. Aliquots from triplicate samples were pooled, dried, reconstituted, and analyzed by HPLC and TLC.

*Counts were too low for an accurate assessment.

TABLE 2. Effect of nucleosides on the cellular accumulation of radioactive AZT in H9, H9-ddC0.5w, and H9-ddC5.0w cells

	H9	H9-ddC0.5w	H9-ddC5.0w
Additions	Percent incorporation		
None	100	100	100
ddC (10 μM)	76.9	100.8	96.3
dCR (10 μM)	81.5	76.3	93.1
TdR (10 μM)	3.3	21.3	95.8
CR (10 μM)	86.5	85.9	105.8
AZT (25 μM)	55.0	68.5	99.6
ara-T (10 μM)	96.0	100.0	

Duplicate cell suspensions (0.5×10^6 cells/0.5 mL) were incubated with 25 μM [^{3}H]AZT (4 μCi/mL) with or without the nucleosides at indicated concentrations. The cells were harvested after 2 hr of incubation at 37°, washed twice with PBS, and extracted with 0.4 vol. of 5% ice-cold perchloric acid. The extracts were neutralized with KOH, and 0.2-mL fractions were counted for radioactivity. Average 100% counts in H9, H9-ddC0.5w, and H9-ddC5.0w cells represent 9500, 2130, and 240 cpm (variation < 10%), respectively. Abbreviations: ara-T, 1-β-D-arabinosylthymine; AZT, 3′-azido-3′-deoxythymidine (azidothymidine); CR, cytidine; dCR, 2′-deoxycytidine; ddC, 2′,3′-dideoxycytidine; and TdR, thymidine.

DISCUSSION

Our studies clearly demonstrated cross-resistance of ddC-resistant cells to AZT (a TdR analog). In fact, the AZT resistance far exceeded (>27.7-fold) the ddC resistance (5.4- to 15.7-fold) in ddC-resistant cells. This difference in response to AZT and ddC was consistent with the much lower cellular accumulation of AZT (50.5 and 12.95% of control) compared with the accumulation of ddC (76.2 and 47.8%) in H9-ddC0.5w and H9ddC-5.0w cells (Fig. 4). Thus, it appears that ddC is a far better inducer of AZT resistance than of ddC resistance.

AZTTP is believed to be the AZT metabolite responsible for cytotoxicity and antiviral activity. However, the K_i values of AZTTP for cellular DNA polymerases are much higher (70–230 μM) than the K_i values for the inhibition of viral reverse transcriptase (0.4 μM) [15]. Although the AZTTP concentrations in H9-ddC0.5w and H9 cells were similar, the former cell line was about 27-fold more resistant to AZT. This observation indicates that AZTTP levels are not involved directly in the cytotoxic effect of the drug. Therefore, it is likely that AZTMP, which was reduced to 12 and 0.3% of control in H9-ddC0.5w and H9-ddC5.0w cells, may be responsible for the reduced cytotoxicity of the

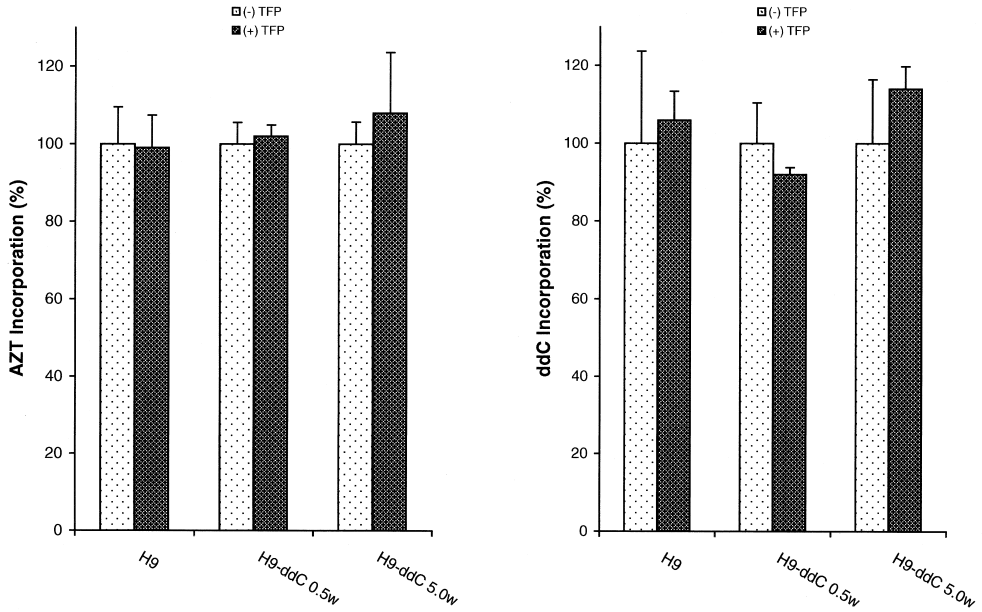

FIG. 5. Effect of trifluoroperazine (TFP) on intracellular accumulation of AZT and ddC. Triplicate cell suspensions (0.5×10^6 cells/mL) were preincubated for 20 min in the absence (−) or presence (+) of 1 μM TFP. Then radiolabeled AZT or ddC (25 μM) was added, and the incubation continued for 3 hr. The cells were extracted with 65% methanol, and fractions were counted for radioactivity. Percent incorporation was calculated in each experiment, and the values are presented as means ± SD of 3 experiments. The 100% counts were 9170, 1810, and 190 cpm for AZT and 1880, 1980, and 980 cpm for ddC in H9, H9-ddC0.5w, and H9-ddC5.0w cells, respectively.

drug. This hypothesis is consistent with a report implicating AZTTP in the antiviral activity of AZT and AZTMP in the cell growth-inhibitory effect [16]. It is possible that the use of ddC at low concentrations (e.g. 0.5 μM) may produce cellular conditions (as seen in H9-ddC0.5w cells) that will reduce AZTMP concentrations without affecting AZTTP concentrations. Such a situation may reduce the cytotoxic effect of AZT significantly without compromising the antiviral activity of the drug. This interesting hypothesis needs to be explored further.

Although the mechanism of cross-resistance is not clear, the lower anabolism of AZT to its nucleotides (as shown above) and their cellular retention could cause drug resistance. Antonelli *et al.* [9] have suggested the involvement of MDR proteins in the resistance to AZT in a CEM cell line (the CEM VBL100 subline) selected for vinblastine resistance. Failure of verapamil to alter rhodamine-123 and daunorubicin efflux and of trifluoperazine to increase AZT and ddC accumulation in our studies rules out the involvement of MDR proteins in the cellular retention and efflux of AZT nucleotides as a mechanism of AZT resistance. Thus, our observations clearly differ from those seen in CEM VBL100 cells. Intercellular variations between the two cell lines (H9 and CEM cells) used in these studies may have contributed to the observed differences. Another likely possibility is that ddC and vinblastine induced resistance by different mechanisms.

The anabolic pathway of AZT consists of its transport into cells and then sequential phosphorylation by cellular kinases. Since AZT enters cells by non-facilitated diffusion, its influx may not have a significant contribution to the development of resistance [11, 17]. The lower AZT anabolism, therefore, may have resulted from reduced TK activity in the resistant cells. The enzyme TK catalyzes the first step in the anabolic pathway of AZT. Activity of this enzyme was reduced markedly in the resistant cells. The lower enzymatic activity, however, was a result of reduced V_{max} values rather than substrate affinity, as demonstrated by insignificant changes in K_m values. Lower or absent dCK and TK activities have been implicated in drug resistance to dCR and TdR analogs, which are phosphorylated by these enzymes [5, 18]. Thus, our observations are consistent with this mechanism of resistance to ddC and AZT.

TK and dCK, which phosphorylate AZT and ddC, respectively, are two different enzymes. Therefore, reduction of TK activity upon exposure to ddC was unexpected. The enzymes TK and dCK are known to have broad and overlapping substrate activities, anabolizing a number of anticancer and antiviral deoxynucleosides including purine nucleoside analogs. They exist in different isoforms and subcellular fractions [19–22]. Although TK2 phosphorylates AZT to some extent, cytoplasmic TK1 is considered to be the major isozymic form responsible for AZT activation [21]. In contrast, ara-T is phosphorylated mainly by TK2 [23]. The ability of TdR to inhibit (and the failure of ara-T to inhibit) cellular accumulation of [^{3}H]AZT more strongly in H9 cells than in H9-ddC0.5w cells suggests that the major effect of ddC-induced resistance is on TK1. Reduction of [^{3}H]AZT accumulation by dCR and ddC (although to a lesser extent than by TdR) indicates that ddC may

have altered a dCK isozyme that, in addition to activating ddC, also activates TdR and AZT. Studies in progress in our laboratory may provide an answer to these questions.

Cross-resistance of HIV to reverse transcriptase inhibitors is a common obstacle in the chemotherapy of AIDS [3]. The results of our study and the reports of ddC and AZT cellular resistance from other laboratories [4, 5, 9] strongly suggest that host cell-mediated mechanisms of drug resistance can also apply to antiviral agents and therefore must be considered during antiviral therapy. In addition to understanding its mechanism, an important question is whether the observed cross-resistance of ddC and AZT reduces their antiviral activity or enhances their therapeutic activity by reducing toxicity. Our future studies will focus on this important question.

The authors appreciate the technical help of Ms. Ying Nie and Dr. D. Lou. This work was supported by the Department of Health and Human Services Grant NIAID AI 29155.

References

1. De Clercq E, Antiviral therapy for human immunodeficiency virus infections. *Clin Microbiol Rev* **8**: 200–239, 1995.
2. Gao Q, Gu Z, Hiscott J, Dionne G and Wainberg MA, Generation of drug-resistant variants of human immunodeficiency virus type 1 by *in vitro* passage in increasing concentrations of 2′,3′-dideoxycytidine and 2′,3′-dideoxy-3′-thiacytidine. *Antimicrob Agents Chemother* **37**: 130–133, 1993.
3. Larder BA, Kohli A, Bloor S, Kemp SD, Harrigan PR, Schooley RT, Lang JM, Pennington KN and St. Clair MH and The Protocol 34,225–02 Collaborative Group, Human immunodeficiency virus type 1 drug susceptibility during zidovudine (AZT) monotherapy compared with AZT plus 2′,3′-dideoxyinosine or AZT plus 2′,3′-dideoxycytidine combination therapy. *J Virol* **70**: 5922–5929, 1996.
4. Magnani M, Gazzanelli G, Brandi G, Casabianca A, Fraternale A, Chiarantini L and Rossi L, 2′,3′-Dideoxycytidine induced drug resistance in human cells. *Life Sci* **57**: 881–887, 1995.
5. Wu S, Liu X, Solorzano MM, Kwock R and Avramis VI, Development of zidovudine (AZT) resistance in Jurkat T cells is associated with decreased expression of the thymidine kinase (TK) gene and hypermethylation of the 5′ end of human TK gene. *J Acquir Immune Defic Syndr Hum Retrovirol* **8**: 1–9, 1995.
6. Martin JL, Brown CE, Matthews-Davis N and Reardon JE, Effect of antiviral nucleoside analogs on human DNA polymerases and mitochondrial DNA synthesis. *Antimicrob Agents Chemother* **38**: 2743–2749, 1994.
7. Rustum YM, Metabolism and intracellular retention of 1-β-D-arabinofuranosylcytosine as predictors of response of animal tumors. *Cancer Res* **38**: 543–549, 1978.
8. Plunkett W, Benjamin RS, Keating MI and Freireich EJ, Modulation of 9-β-D-arabinofuranosyladenine 5′-triphosphate and deoxyadenosine triphosphate in leukemia cells by 2′-deoxycoformycin during therapy with 9-β-D-arabinofuranosyladenine. *Cancer Res* **42**: 2092–2096, 1982.
9. Antonelli G, Turriziani O, Cianfriglia M, Riva E, Dong G, Fattorossi A and Dianzani F, Resistance of HIV-1 to AZT might also involve the cellular expression of multidrug resistance P-glycoprotein. *AIDS Res Hum Retroviruses* **8**: 1839–1844, 1992.
10. Agarwal RP, Wang W and Yo P, Characteristics of 2′,3′-dideoxycytidine (ddC) and arabinosylcytosine (ara-C) resistance in a human lymphocytic cell line. *Proc Am Assoc Cancer Res* **39**: 216, 1998.
11. Agarwal RP and Mian AM, Thymidine and zidovudine metabolism in chronically zidovudine-exposed cells *in vitro*. *Biochem Pharmacol* **42**: 905–911, 1991.
12. Krishan A, Sauerteig A, Andritsch I and Wellham L, Flow cytometric analysis of the multiple drug resistance phenotype. *Leukemia* **11**: 1138–1146, 1997.
13. Magnani M, Bradi G, Casabianca A, Fraternale A, Schiavano GF, Rossi L and Chiarantini L, 2′,3′-Dideoxycytidine metabolism in a new drug-resistant cell line. *Biochem J* **312**: 115–123, 1995.
14. Balzarini J, Pauwels R, Baba M, Herdewijn P, De Clercq E, Broder S and Johns D, The *in vitro* anti-retrovirus activity, and intracellular metabolism of 3′-azido-2′,3′-dideoxythymidine and 2′,3′-dideoxycytidine are highly dependent on the cell species. *Biochem Pharmacol* **37**: 897–903, 1988.
15. Furman PA, Fyfe JA, St. Clair MH, Weinhold K, Rideout JL, Freeman GA, Lehrman SN, Bolognesi DP, Broder S, Mitsuya H and Barry DW, Phosphorylation of 3′-azido-3′-deoxythymidine and selective interaction of the 5′-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc Natl Acad Sci USA* **83**: 8333–8337, 1986.
16. Tornevik Y, Ullman B, Balzarini J, Wahren B and Eriksson S, Cytotoxicity of 3′-azido-3′-deoxythymidine correlates with 3′-azidothymidine-5′-monophosphate (AZTMP) levels, whereas anti-human immunodeficiency virus (HIV) activity correlates with 3′-azidothymidine-5′-triphosphate (AZTTP) levels in cultured CEM T-lymphoblastoid cells. *Biochem Pharmacol* **49**: 829–837, 1995.
17. Zimmerman TP, Mahony WB and Prus KL, 3′-Azido-3′-deoxythymidine: An unusual nucleoside analogue that permeates the membrane of human erythrocytes and lymphocytes by non-facilitated diffusion. *J Biol Chem* **262**: 5748–5754, 1987.
18. Bhalla K, Nayak R and Grant S, Isolation and characterization of a deoxycytidine kinase-deficient human promyelocytic leukemic cell line highly resistant to 1-β-D-arabinofuranosylcytosine. *Cancer Res* **44**: 5029–5037, 1984.
19. Cheng YC, Domin B and Lee LS, Human deoxycytidine kinase: Purification and characterization of the cytoplasmic and mitochondrial isozymes derived from blast cells of acute myelocytic leukemia patients. *Biochim Biophys Acta* **481**: 481–492, 1977.
20. Carson DA, Wasson DB, Kaye J, Ullman B, Martin DW Jr, Robins RK and Montgomery JA, Deoxycytidine kinase-mediated toxicity of deoxyadenosine analogs toward malignant human lymphoblasts *in vitro* and toward murine L1210 leukemia *in vivo*. *Proc Natl Acad Sci USA* **77**: 6865–6869, 1980.
21. Eriksson S, Kierdaszuk B, Munch-Peterson B, Oberg B and Johansson NG, Comparison of the substrate specificities of human thymidine kinase 1 and 2 and deoxycytidine kinase toward antiviral and cytostatic nucleoside analogs. *Biochem Biophys Res Commun* **176**: 586–592, 1991.
22. Johansson M, Brismar S and Karlsson A, Human deoxycytidine kinase is located in the cell nucleus. *Proc Natl Acad Sci USA* **94**: 11941–11945, 1997.
23. Arner ESJ, Spasokoukotskaja T and Eriksson S, Selective assay for thymidine kinase 1 and 2 and deoxycytidine kinase and their activities in extracts from human cells and tissues. *Biochem Biophys Res Commun* **188**: 712–718, 1992.