Biochemical Pharmacology, Vol. 37, No. 5, pp. 897–903, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

THE *IN VITRO* AND *IN VIVO* ANTI-RETROVIRUS ACTIVITY, AND INTRACELLULAR METABOLISM OF 3′-AZIDO-2′,3′-DIDEOXYTHYMIDINE AND 2′,3′-DIDEOXYCYTIDINE ARE HIGHLY DEPENDENT ON THE CELL SPECIES

JAN BALZARINI,* RUDI PAUWELS,* MASANORI BABA,* PIET HERDEWIJN,* ERIK DE CLERCQ,* SAMUEL BRODER† and DAVID G. JOHNS‡

* Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium, and † Clinical Oncology Program and ‡ Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, U.S.A.

(*Received* 14 *May* 1987; *accepted* 30 *August* 1987)

Abstract—Cell lines derived from different species show striking differences in their sensitivity to the cytostatic and anti-retrovirus activity, as well as the intracellular metabolism, of 3′-azido-2′,3′-dideoxythymidine (AzddThd) and 2′,3′-dideoxycytidine (ddCyd). AzddThd and ddCyd are considerably more cytostatic to human (i.e. Raji, Molt/4F, ATH8) cell lines than murine (i.e. L1210) cells. The intracellular levels of AzddThd 5′-triphosphate and ddCyd 5′-triphosphate formed do not seem related to the cytostatic effects achieved by these compounds. In human lymphoid (ATH8, Molt/4F) and caprine ovary (Tahr) cells AzddThd accumulates as its 5′-monophosphate (AzddTMP), whereas in murine leukemia (L1210) cells it is readily metabolized to the 5′-triphosphate (AzddTTP). The rapid conversion of AzddThd to AzddTTP in murine cells may explain why AzddThd has a pronounced activity against Moloney murine sarcoma virus (MSV)-induced transformation of murine C3H cells *in vitro* and MSV-induced tumor development in newborn NMRI mice *in vivo*. In contrast, ddCyd has not much activity in these murine assay systems, and this may seem related to the poor conversion of ddCyd to its 5′-triphosphate in murine cells. In human cells, however, ddCyd is more extensively phosphorylated to its 5′-triphosphate than in murine cells. When [^{3}H]AzddThd and [^{3}H]ddCyd were compared for their metabolism in ATH8 and Molt/4F cells, little [^{3}H]AzddTTP was formed even after a 48-hr incubation period, whereas under the same conditions substantial levels of [^{3}H]ddCTP built up gradually. Thus, much higher ddCTP than AzddTTP levels were achieved in human lymphoid cells, an observation that may be particularly relevant from a therapeutic viewpoint.

Acquired immunodeficiency syndrome (AIDS) was first recognized as a new disease entity in 1981 [1–3]. A few years later, the etiologic agent of this disease was shown to be a retrovirus recently designated as human immunodeficiency virus (HIV) [4–7]. One of the first drugs reported to have potent *in vitro* activity against HIV was 3′-azido-2′,3′-dideoxythymidine (AzddThd), a thymidine analogue in which the 3′-hydroxylgroup of the 2′-deoxyribose moiety is replaced by an azido ($-N_3$) group (Fig. 1) [8]. AzddThd proved effective *in vitro* against the replication of HIV in the human T4 lymphocyte lines ATH8, H9, MT4 as well as peripheral mononuclear blood lymphocytes [8–11]. The drug also results in both clinical and immunological improvement when given to AIDS patients [12–16]. AzddThd anabolism to the 5′-mono-, 5′-di- and 5′-triphosphate is similar in uninfected and HIV-infected H9 cells [17]. The thymidine kinase from H9 cells efficiently converts AzddThd to its 5′-monophosphate (AzddTMP), while the thymidylate kinase (dTMP-K) further phosphorylates AzddTMP to AzddTDP. The apparent K_m value of AzddTMP for dTMP-K is only 2-fold greater than that of dTMP, whereas the maximal phosphorylation rate of AzddTMP is 0.3% of the rate noted for dTMP [17]. These data are consistent with the observations of Furman *et al.* [17] that the intracellular levels of AzddTMP are much higher than the AzddThd 5′-di- and 5′-triphosphate levels. Several laboratories have reported that AzddTTP selectively inhibits HIV reverse transcriptase [17–19]. In fact, AzddTTP inhibits the HIV reverse transcriptase about 100–300-fold more efficiently than the cellular DNA polymerase α [17, 19, 20].

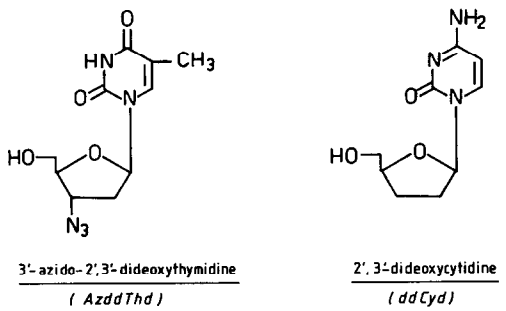

Fig. 1. Structural formulae of 3′-azido-2′,3′-dideoxythymidine (AzddThd) and 2′,3′-dideoxycytidine (ddCyd).

Recently, Mitsuya and Broder [21, 22] and Balzarini *et al.* [10, 23] described the potent and selective anti-HIV activity of a number of 2′,3′-dideoxyribosides, 2′,3′-dideoxycytidine (ddCyd) and 2′,3′-didehydro-2′,3′-dideoxycytidine (ddeCyd) being the

most potent inhibitors of HIV replication in ATH8 and H9 cells. Cooney *et al.* [24] found that there were no significant qualitative or quantitative differences between uninfected and HIV-infected ATH8 cells in their ability to phosphorylate ddCyd. Various other lymphoid and non-lymphoid cell lines were also shown to phosphorylate ddCyd, albeit to a varying extent [24]. Definitive proof was provided by Balzarini *et al.* [10, 16, 23] and Starnes and Cheng [25] that ddCyd is phosphorylated by cellular dCyd kinase. The apparent K_m of dCyd kinase for ddCyd is 180–200 μM, that is 60-fold greater than its K_m for the natural substrate dCyd. As has been shown for AzddThd 5'-triphosphate (AzddTTP), ddCyd 5'-triphosphate (ddCTP) is considered to be the active intracellular metabolite and it was demonstrated by Hao *et al.* [26] to have high affinity for HIV reverse transcriptase.

In this paper, we report on the cell species-dependence of the metabolism of AzddThd and ddCyd, and the differences in the rate of phosphorylation of AzddThd and ddCyd by human cells. Our findings warn against the premature extrapolation of conclusions drawn from metabolic data obtained for AzddThd and ddCyd in murine (or other animal) cell lines to human cells, and provide deeper insight in the metabolism of the compounds in human cells, which might be of critical importance from a therapeutic viewpoint.

MATERIALS AND METHODS

Cells. The origin and growth characteristics of the murine (L1210/0, L1210/BdUrd, L1210/araC) and human (Molt/4F, Raji and ATH8) cell lines used in our study have been described elsewhere [27–29].

Viruses. Human immunodeficiency virus (HIV) was obtained from the culture supernatant of a H9 cell line persistently infected with human T-cell lymphotropic virus type III_b [7]. Moloney murine sarcoma virus (MSV) was prepared from tumors induced by *in vivo* infection of 3-day-old NMRI mice [30].

Compounds. 2',3'-Dideoxycytidine (ddCyd) and 2',3'-dideoxythymidine (ddThd) were obtained from Pharmacia-PL-Biochemicals (Piscataway, NJ, U.S.A.). Previous published methods were used to synthesize the following nucleosides: 2',3'-didehydro-2',3'-dideoxycytidine (ddeCyd) [31], 2',3'-didehydro-2',3'-dideoxythymidine (ddeThd) [32] and 3'-azido-2',3'-dideoxythymidine (AzddThd) [32]. The other reagents used were of the highest quality available.

Radiochemicals. [2',3'-^{3}H]ddCyd (specific radioactivity 11 Ci/mmol) was obtained from Moravek Biochemicals (Brea, CA). [5'-^{3}H]AzddThd (specific radioactivity 2.44 Ci/mmol) was synthesized by J. Hill and G. A. Freeman from the Wellcome Research Laboratories (Research Triangle Park, NC).

Cytostatic assays. Cytostatic effects of the compounds were assessed by measuring the inhibition of cell proliferation. The experimental procedures have been described previously [27, 28]. Briefly, L1210/0, L1210/BdUrd, L1210/araC, Raji and Molt/4F cells were suspended in growth medium and added to microplate wells (200 μl) at a density of 5–7.5 $\times$ 10^4 cells/well in the presence of varying concentrations of the test compounds. The cells were then allowed to proliferate for 48–72 hr at 37°. At the end of the incubation period, the total cell number was counted in a Coulter counter. C3H cells were seeded at 10^4 cells/well and after their attachment to the wells varying concentrations of the compounds were added. The cells were allowed to proliferate for 72 hr, trypsinized and counted in a Coulter counter. The ID_{50} was defined as the concentration of compound that reduced the number of viable cells by 50%.

Metabolism of [2',3'-^{3}H]*dideoxycytidine and* [5'-^{3}H]*3'-azido-2',3'-dideoxythymidine in murine leukemia* L1210, *human lymphoblast Molt/4F and human lymphocyte ATH8 cells.* The L1210, ATH8 or Molt/4F cells (seeded at 2 $\times$ 10^5 cells/ml) were incubated with 5 μM of [2',3'-^{3}H]ddCyd or [5'-^{3}H]AzddThd for 5, 24 or 48 hr. At each time point, the cell number was determined, and the cells were centrifuged at 300 *g* for 10 min, washed three times with cold phosphate-buffered saline, whereafter 0.5 ml of cold TCA 10% was added to the cell precipitate. TCA-insoluble material was precipitated by centrifugation at 12,000 *g*, and the supernatants were subsequently neutralized with tri-*n*-octylamine in freon.

HPLC analysis of the neutralized cell extracts was carried out using a radial compression column of Partisil-SAX equilibrated and developed with 0.01 M ammonium phosphate, pH 3.6 for 15 min, followed by a linear gradient to 0.6 M ammonium phosphate, pH 3.8 over the next 25 min, and finally by a 5 min isocratic elution with 0.6 M ammonium phosphate, pH 3.8 [24].

The elution times were as follows: ddCyd, 3–4 min; ddCMP, 4–6 min; ddCDP, 32–35 min; ddCTP, 44–48 min; AzddThd, 3–5 min; AzddTMP, 11–13 min; AzddTDP, 30–32 min; AzddTTP, 46–48 min. The different fractions of the eluate were assayed for radioactivity in a toluene-based scintillant.

Anti-HIV assay in ATH8 cells. The procedure to measure anti-HIV activity in ATH8 cells has been described previously [10]. After 6–7 days incubation of HIV- or mock-infected ATH8 cells with appropriate concentrations of the test compounds, the number of viable cells was determined in a blood cell counting chamber after trypan blue staining. The 50% effective dose (ED_{50}) was defined as the concentration of compound that conferred a 50% protection (based on cell viability) of HIV-infected cells. The 50%-inhibitory dose (ID_{50}) was defined as the concentration of compound that reduced viability of mock-infected cells by 50%.

In vitro *transformation of murine C3H embryo fibroblasts by Moloney murine sarcoma virus.* Murine C3H embryo fibroblasts were seeded into 2.3 cm^2 wells of Costar Tissue Culture Cluster plates (Costar Broadway, Cambridge, MA) at 50,000 cells per ml and grown to confluency. Cell cultures were then infected by 150 foci-forming units of Moloney murine sarcoma virus (MSV) during 90 min, whereafter the medium was replaced by 1 ml fresh culture medium containing different con-

Table 1. Cytostatic effects of 2',3'-dideoxynucleoside analogues on murine and human cell lines

Compound	ID_{50}* (μM)						
	C3H	L1210/0	L1210/BdUrd	L1210/araC	Raji	Molt/4F	ATH8†
AzddThd	63	936	>2000	1600	74	63	40
ddeThd	183	32	>800	>800	561	567	110
ddThd	>800	>800	>800	>800	>800	>800	>2000
ddeCyd	336	195	288	>800	25	19	30
ddCyd	>800	118	163	>800	25	22	35

* 50% inhibitory dose, or dose required to inhibit the C3H, L1210, Raji and Molt/4F cell proliferation by 50% or ATH8 cell viability by 50%.

† As monitored by the trypan blue dye exclusion method (see Refs. [8, 10]).

centrations of the test compounds. After 6 days, the transformation of the cell cultures was examined microscopically.

Tumor formation in NMRI mice inoculated intramuscularly with Moloney murine sarcoma virus shortly after birth. NMRI mice were infected at 2–3 days after birth with Moloney murine sarcoma virus (day 0). AzddThd and ddCyd were administered intraperitoneally (i.p.) at a dose of 125, 25, 5 and 1 mg/kg/day, starting at day −1 and continued till day +19. Tumor development, and mortality associated therewith, were recorded daily. There were 10 mice per group.

RESULTS

Cytostatic effects of 2',3'-dideoxynucleoside analogues on murine and human cell lines

The pyrimidine 2',3'-dideoxynucleoside analogues ddThd, ddeThd, AzddThd, ddCyd and ddeCyd were evaluated for their inhibitory effects on the proliferation of murine (embryo fibroblast C3H and leukemia L1210/0) and human (B-lymphoblast Raji, T-lymphoblast Molt/4F and T-lymphocyte ATH8) cells. A thymidine (dThd) kinase-deficient and a deoxycytidine (dCyd) kinase-deficient L1210 cell line (designated L1210/BdUrd and L1210/araC, respectively) were also included in this study (Table 1).

Except for the inhibitory effect of ddeThd on L1210/0 cell proliferation and ddeThd and AzddThd on C3H cell growth, all pyrimidine 2',3'-dideoxynucleoside analogues were markedly more cytostatic for the human Raji, Molt/4F and ATH8 cells than for the murine C3H and L1210 cells, the difference in cytostatic action being 4- to 10-fold for ddCyd and ddeCyd, and 15- to 28-fold for AzddThd. No significant inhibitory effect on either murine or human cell proliferation was noted with ddThd even at a concentration as high as 800 μM. ddCyd and ddeCyd were not inhibitory to L1210/araC cell proliferation; they were equally inhibitory to L1210/BdUrd and L1210/0 cells. In contrast, AzddThd and ddeThd were less inhibitory to both the dThd kinase-deficient and dCyd kinase-deficient L1210 cells than their parent L1210/0 counterparts (Table 1).

Differential metabolism of [5'-^{3}H]AzddThd and [2',3'-^{3}H]ddCyd in murine (L1210) and human (ATH8 and Molt/4F) cells

The metabolism of [5'-^{3}H]AzddThd and [2',3'-^{3}H]ddCyd was measured in murine (L1210) and human (ATH8 and Molt/4F) cells upon incubation with 5 μM of the radiolabeled compound for 5, 24 and 48 hr (Table 2). AzddThd was extensively metabolized to its 5'-mono-, 5'-di- and 5'-triphosphate in L1210 cells. Upon incubation with

Table 2. Phosphorylation of [^{3}H]AzddThd and [^{3}H]ddCyd in murine leukemia L1210, human lymphocyte ATH8 and human lymphoblast Molt/4F cells

Metabolite	Intracellular concentration of phosphorylated product* (μM)								
	L1210			ATH8			Molt/4F		
	5 hr	24 hr	48 hr	5 hr	24 hr	48 hr	5 hr	24 hr	48 hr
AzddTMP	5.0	7.5	3.5	10.3	9.9	3.1	143	131	31
AzddTDP	3.1	4.4	2.5	0.3	0.15	0.3	0.8	0.5	0.2
AzddTTP	6.0	6.4	3.6	0.2	0.1	<0.1	0.6	0.4	0.2
ddCMP†	112	21	11	4.5	13	5.3	3.8	5.6	11
ddCDP	1.3	1.3	0.65	0.7	2.7	2.9	19	71	117
ddCTP	1.7	1.2	0.75	0.5	2.0	2.7	2.6	12	17

* Initial concentration of [^{3}H]AzddThd or [^{3}H]ddCyd in culture medium: 5 μM.

† Because the retention time of ddCyd and ddCMP was almost similar, ddCMP levels could not be accurately measured in L1210 and ATH8 cells. Therefore, the values reflect the sum of the intracellular ddCyd and ddCMP levels.

AzddThd at an initial concentration of 5 μM, the intracellular AzddTTP levels measured after 5, 24 and 48 hr were 6, 6.4 and 3.6 μM, respectively. Comparable amounts of the 5'-mono- and 5'-diphosphate metabolites were also detected in the L1210 cells exposed to AzddThd. In contrast, when human ATH8 or Molt/4F cells were incubated with AzddThd, much higher intracellular levels of AzddTMP than of AzddTDP and AzddTTP were found. Maximum levels of AzddThd metabolites in ATH8 and Molt/4F cells were already reached after a 5-hr incubation. Upon longer incubation times (24 and 48 hr) the intracellular AzddTMP, AzddTDP and AzddTTP levels declined. The amounts of AzddTTP detected in ATH8 and Molt/4F cells were considerably lower (10- to 20-fold) than those detected in the murine L1210 cells.

Upon incubation of L1210 cells with [2',3'-^{3}H]ddCyd, maximum ddCTP levels were recorded after a 5-hr incubation. Thereafter, they slightly decreased. The ddCTP levels attained in L1210 cells were invariably 3- to 5-fold lower than the AzddTTP levels measured under identical experimental conditions (Table 2). In human ATH8 and Molt/4F cells ddCTP levels continued to increase during the 48-hr incubation period and reached peak levels which exceeded the levels attained by AzddTTP under the same conditions by 27- or 85-fold.

In vitro *and* in vivo *anti-retrovirus effects of AzddThd and ddCyd*

AzddThd inhibited the *in vitro* transformation of C3H mouse embryo fibroblasts by Moloney murine sarcoma virus (MSV) at a 50% effective dose of 0.06 μM, that is a concentration 400- to 600-fold lower than the ED_{50} of ddCyd and ddeCyd; ddeThd and ddThd inhibited C3H cell transformation by MSV at a concentration of 2.1 and 47 μM, respectively (Table 3). In contrast, ddCyd and ddeCyd proved 10- to 20-fold more effective than AzddThd and ddeThd in inhibiting HIV replication in ATH8 cells. The 50% effective doses of ddCyd and ddeCyd for inhibition of HIV replication in human ATH8 cells were 100- to 200-fold lower than the doses required to effect a 50% protection of murine C3H cells against transformation by MSV. *Vice versa*, AzddThd was active in the murine system at a 40-fold lower concentration than in the human system (Table 3).

The remarkable difference in efficiency of AzddThd and ddCyd to inhibit the *in vitro* replication

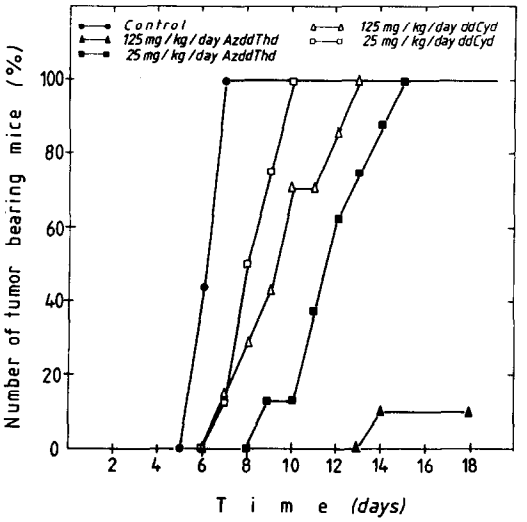

Fig. 2. Effect of AzddThd and ddCyd on tumor formation in NMRI mice inoculated with Moloney murine sarcoma virus (MSV) at the 3rd day after birth. The compounds were given daily intraperitoneally, starting one day prior to MSV infection, and continued up to 19 days after MSV infection.

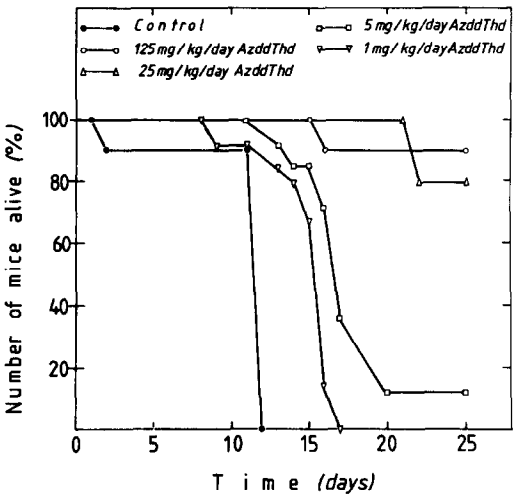

Fig. 3. Effect of AzddThd on the survival of NMRI mice inoculated with Moloney murine sarcoma virus (MSV) at the 3rd day after birth. The compound was given daily intraperitoneally, starting one day prior to MSV infection, and continued up to 19 days after MSV infection.

Table 3. Inhibitory effect of 2',3'-dideoxynucleoside analogues on MSV-induced transformation of murine C3H embryo fibroblasts and HIV-induced cytopathogenicity in ATH8 cells

Compound	ED_{50}* (μM) MSV-infected C3H cells	HIV-infected ATH8 cells
AzddThd	0.06	2.4
ddeThd	2.1	4.1
ddThd	47	101
ddeCyd	37	0.3
ddCyd	25	0.2

* 50% effective dose, or dose required to inhibit transformation of C3H or destruction of ATH8 by 50%.

of retroviruses in murine, as compared to human cells, was also reflected by our *in vivo* experiments in which NMRI mice were infected at 3 days after birth with MSV. Intramuscular inoculation of MSV in these mice resulted in tumor formation and mortality associated therewith. Treatment of the MSV-infected mice with AzddThd at 125 mg/kg/day almost completely prevented the induction of tumors (Fig. 2) and resulted in a 90% survival of the mice at day 25 (Fig. 3). When AzddThd was administered at 25 mg/kg/day, significant delay in tumor formation (Fig. 2) and considerable prolongation of the life-span of the mice (Fig. 3) was observed. In contrast, ddCyd treatment at 125 or 25 mg/kg/day only resulted in a slight delay of tumor formation (Fig. 2), without any significant effect on the survival rate of the mice (data not shown). Administration of ddCyd at 625 mg/kg/day resulted in severe toxicity (i.e. anemia) and early death of the mice (data not shown).

DISCUSSION

The cytostatic and anti-retrovirus activity, and the intracellular metabolism, of AzddThd and ddCyd have been measured in several cell lines from different species. Striking differences in drug potency and intracellular metabolism were observed among the different cell lines examined. AzddThd, ddCyd and ddeCyd were 5- to 15-fold more cytostatic to the human (Raji, Molt/4F and ATH8) cells than the murine (C3H and L1210) cells evaluated in this study (Table 1). The differences in the cytostatic action of AzddThd on L1210 and Molt/4F cells could not be attributed to differential affinities of AzddThd for murine and human dThd kinases, since AzddThd did not show a marked preference in this regard [K_i: 8.5 and 3.4 μM, respectively (data not shown)]. Nor could the higher toxicity of AzddThd for human cells be accounted for by increased formation of the 5'-triphosphate of AzddThd since the AzddTTP levels recorded in human ATH8 and Molt/4F cells were 10- to 60-fold lower than those attained in murine L1210 cells, while AzddThd was 15- to 20-fold more cytostatic for ATH8 and Molt/4F cells than for L1210 cells. Also, ddCyd proved equally toxic for ATH8 and Molt/4F cells, although its 5'-triphosphate reached 5- to 7-fold higher levels in Molt/4F cells than ATH8 cells. These observations may be particularly relevant from a chemotherapeutic viewpoint since the anti-retrovirus effects of ddCyd and AzddThd are mediated through the 5'-triphosphates of the drugs, and it thus appears that the absolute intracellular 5'-triphosphate levels may not be directly involved in the cytostatic effects of the compounds.

Because of the marked accumulation of AzddTMP in human ATH8 and Molt/4F cells, and the higher toxicity of AzddThd for human cells than for murine cells, we evaluated the role of thymidylate synthase (TS) in the inhibitory effect of AzddThd on human and murine cell proliferation. Indeed, thymidylate synthase represents a key enzyme in DNA synthesis since it is the only *de novo* source for the cells to synthesize thymidylate, and it has been demonstrated that inhibition of this enzyme may lead to potent inhibition of cell growth and DNA synthesis [33, 34]. AzddTMP was evaluated for its inhibitory effect against partially purified TS from L1210/0 and Molt/4F cells, and the inhibitory effect of AzddThd on TS in intact L1210/0 and Molt/4F cells was evaluated by measuring the effect of AzddThd on tritium release from [5-^{3}H]2'-deoxyuridine and [5-^{3}H]2'-deoxycytidine at different time points (up to 72 hours) during AzddThd incubation [35]. However, inhibition of thymidylate synthase does not seem to account for the toxic effects of AzddThd as assessed with both free enzyme extracts and intact L1210/0 and Molt/4F cells (data not shown). The biochemical reason(s) for the differential toxicities of AzddThd for human and murine cell lines need further investigation.

Furman *et al.* [17] recently demonstrated that, while only low levels of AzddTDP and AzddTTP are achieved, AzddTMP accumulates in human lymphocyte H9 cells, and, furthermore, AzddTMP acts as a potent alternative substrate-inhibitor of thymidylate kinase (dTMP-K). Our data obtained with ATH8 and Molt/4F cells are consistent with the observations of Furman *et al.* [17] for H9 cells. However,

Table 4. Ratios of intracellular levels of AzddTMP vs AzddTTP, ddCMP vs ddCTP and ddCTP vs AzddTTP in murine L1210, human ATH8 and Molt/4F cells upon incubation of the cells with either AzddThd (5 μM) or ddCyd (5 μM)

Ratio	L1210 5 hr	L1210 24 hr	L1210 48 hr	ATH8 5 hr	ATH8 24 hr	ATH8 48 hr	Molt/4F 5 hr	Molt/4F 24 hr	Molt/4F 48 hr
AzddTMP / AzddTTP	0.8	1.2	1.0	51	99	>31	238	327	155
ddCMP / ddCTP	—*	—	—	—	—	—	1.5	0.5	0.6
ddCTP / AzddTTP	0.3	0.2	0.2	2.5	20	>27	4.3	30	85

* Because the retention time of ddCyd and ddCMP was almost similar, ddCMP levels could not be accurately measured.

inhibition of dTMP-K by AzddTMP is not generally observed when the metabolism of AzddThd is compared in cells from different species. Indeed, we found important differences in the metabolism of AzddThd in murine leukemia (L1210) cells (Table 2), caprine ovary (Tahr) cells (data not shown), and human T-lymphoblast Molt/4F and T-lymphocyte (ATH8) cells (Table 2). While AzddTMP accumulated in the human lymphoid and caprine ovary cells (ratio AzddTMP/AzddTTP: 50–300), extensive conversion of AzddTMP to AzddTTP was noted in murine leukemia L1210 cells (ratio AzddTMP/AzddTTP: ~1.0) (Table 4). With ddCMP no accumulation in human cells was observed (Table 4). Interestingly, ddCDP levels were 6- to 7-fold higher than ddCTP levels in Molt/4F cells but not in ATH8 cells. The ddCTP levels were also invariably higher in Molt/4F cells than ATH8 cells irrespective of the time of incubation of the cells. These data indicate that the metabolism of ddCyd quantitatively differs for different human cell lines, and corresponds with the observations of Cooney *et al.* [24] who found that ddCMP, ddCDP and ddCTP levels varied up to 10- to 50-fold depending on the human cell type evaluated.

Our data clearly indicate that the metabolism of AzddThd and ddCyd is highly dependent on the cell species. The peculiar metabolism of AzddThd by murine cells may explain why AzddThd (and also ddeThd) are potent inhibitors of the *in vitro* murine C3H fibroblast transformation by Moloney murine sarcoma virus, and, in this respect, superior to ddCyd. The metabolism of AzddThd by murine cells also bears on the observation that AzddThd causes such a dramatic inhibitory effect on MSV-induced tumor development in mice, and mortality associated therewith. These *in vivo* data are in agreement with those of Ruprecht *et al.* [36] who found that AzddThd treatment of mice infected with Rauscher murine leukemia virus prevented the development of splenomegaly and suppressed viremia if treatment was started soon after virus infection. Hence, the pronounced protective effects exhibited by AzddThd on retrovirus infection in murine cells most probably result from the high levels of AzddTTP formed in these cells. The anti-retrovirus activity of AzddThd thus seems to depend more on the species the host cells are derived of than the origin of the virus. In this respect, Dahlberg *et al.* [37] recently reported that AzddThd markedly inhibits the replication of Kirsten murine sarcoma virus with its associated murine leukemia amphotropic helper virus in mouse and rat cells, but is only moderately effective in human cells and virtually inactive in caprine ovary cells.

When the levels of AzddTTP and ddCTP were compared in the murine and human cell lines (Tables 2 and 4), remarkable differences were noted. The intracellular AzddTTP levels peaked at 5 hours after incubation of ATH8 and Molt/4F cells with AzddThd, and a decline of the intracellular AzddTTP levels was noted upon further incubation. In contrast, intracellular ddCTP levels continuously increased up to 48 hours after incubation of the cells with ddCyd. Due to the different pharmacological behavior of ddCyd and AzddThd, the ratio of the intracellular concentration of ddCTP to the intracellular concentration of AzddTTP increased considerably as a function of incubation time (Table 4). This was even more striking for Molt/4F than ATH8 cells. These findings may be quite important from a therapeutic viewpoint, as the decreasing levels of AzddTTP observed from 5 hours onwards and the increasing levels of ddCTP up at least 48 hours suggest a relatively short and long biological half-life for AzddThd and ddCyd, respectively. Consequently, one may infer that the anti-retrovirus effects of AzddThd in human cells would not last as long as those achieved by ddCyd. In fact, Mitsuya *et al.* [8] and Balzarini *et al.* (unpublished) observed a dramatic decrease in the anti-retrovirus activity of AzddThd, but not of ddCyd upon prolonged incubation (> 10 days) of HIV-infected ATH8 cells with these drugs. It seems imperative that these properties of AzddThd and ddCyd should also be investigated in AzddThd- and ddCyd-treated patients because they may have an important impact on delineating an appropriate treatment schedule for these drugs in the chemotherapy of AIDS.

Acknowledgements—HIV (HTLV-III$_B$) was kindly provided by Dr R. C. Gallo, NCI, NIH, Bethesda, MD, U.S.A. [5′-^{3}H]AzddThd was a generous gift from Dr W. Miller from the Wellcome Research Laboratories, Research Triangle Park, NC, U.S.A Dr. M. Baba is a recipient of a fellowship from the Japan Society for the Promotion of Science, 2-438. Dr. P. Herdewijn is a Research Associate of the Belgian "Nationaal Fonds voor Wetenschappelijk Onderzoek". These investigations were supported in part by grants from the Belgian "Fonds voor Geneeskundig Wetenschappelijk Onderzoek" (Project no. 3.0040.83), and the Belgian "Geconcerteerde Onderzoeksacties (Project no. 85/90-79). We thank Christiane Callebaut for her dedicated editorial help.

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