Modulation of the Metabolism of β-L-(−)-2′,3′-Dideoxy-3′-thiacytidine by Thymidine, Fludarabine, and Nitrobenzylthioinosine

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β-L-(−)-2′,3′-Dideoxy-3′-thiacytidine (3TC) is a cytosine nucleoside analog that potently inhibits the replication of human and duck hepatitis B viruses and human immunodeficiency virus through the activity of its 5′-triphosphate ester metabolite. The present study examined the intracellular decay of 3TC 5′-phosphates and tested strategies for modulating the cellular content of those nucleotides in primary cultures of duck hepatocytes and in human hepatoma 2.2.15 cells and CCRF-CEM T lymphoblasts. Inhibition by deoxycytidine of the 5′-phosphorylation of 3TC in duck hepatocytes confirmed that, as in mammalian cells, deoxycytidine kinase catalyzed 3TC activation. The 5′-mono-, 5′-di-, and 5′-triphosphates of 3TC underwent monoexponential elimination from duck hepatocytes and 2.2.15 cells (half-lives, 3.6 to 8.0 h). Thymidine and fludarabine, which are agents that enhance the activity of deoxycytidine kinase, were tested in strategies for increasing the cellular content of 3TC 5′-phosphates. Coordinate treatment of cells with 3TC and thymidine (50 μM) increased the content of 3TC 5′-monophosphate in duck hepatocytes and the content of 3TC 5′-di- and 5′-triphosphates in 2.2.15 cells, but enhancement of 3TC 5′-phosphate levels in CCRF-CEM cells required a higher thymidine concentration (100 μM). Fludarabine (5 μM) did not affect the contents of 3TC 5′-di- and 5′-triphosphates in duck hepatocytes, but modestly increased the contents of those nucleotides in 2.2.15 cells and CCRF-CEM cells. Nitrobenzylthioinosine (NBMPR), an inhibitor of the es facilitated diffusion nucleoside transporter, reduced the level of entry of 3TC into 2.2.15 cells and abolished inward fluxes of thymidine, adenosine, and deoxycytidine. In 2.2.15 cells and CCRF-CEM cells, NBMPR reduced the formation of 3TC 5′-di- and 5′-triphosphates and reversed the thymidine- and fludarabine-induced increases in the formation of those nucleotides. NBMPR protected against the cytotoxicity of 3TC in CCRF-CEM cells, whereas thymidine potentiated that toxicity, apparently by enhancing the formation of 3TC 5′-triphosphate. Taken together, these results indicate that deoxycytidine kinase and the es nucleoside transporter are targets for manipulation of the metabolism and activity of 3TC.

β-L-(−)-2′,3′-Dideoxy-3′-thiacytidine (3TC) is a cytosine nucleoside analog that potently inhibits the replication of human immunodeficiency virus (HIV) (11, 30), as well as human hepatitis B virus (HBV) and duck hepatitis B virus (DHBV) (9, 12). Inside mammalian cells, 3TC is sequentially phosphorylated by deoxycytidine kinase (9, 17, 33) and pyrimidine nucleotide kinases to form 3TC 5′-triphosphate (3TC-TP), which inhibits viral reverse transcriptase and DNA polymerase activities (9, 18, 32). 3TC-TP is incorporated into HIV proviral DNA, resulting in chain termination (18); similarly, as a chain terminator, the 5′-triphosphate metabolite inhibits both the reverse transcriptase and the DNA polymerase activities of the DHBV polymerase (32). The low cellular toxicity of 3TC (10, 31, 35) has been attributed to a weak interaction of 3TC-TP with cellular DNA polymerases (9, 18). 3TC has been tested in phase I/II clinical studies with HIV-infected patients (30, 37) and is currently under evaluation in studies with HBV-infected patients.

Several studies have shown that the efficacies of cytosine nucleoside drugs that are phosphorylated by deoxycytidine kinase may be increased through the use of agents that enhance the activity of that enzyme. The enhancement by thymidine of the anabolism of cytosine nucleosides is apparently a consequence of the formation of dTTP, which inhibits ribonucleotide reductase and consequently depletes dCTP, a feedback inhibitor of deoxycytidine kinase (4). For example, thymidine was used to increase the activities of cytarabine (1-β-D-arabinofuranosylcytosine) (6), 2′,2′-difluoro-2′-deoxycytidine (dFdC) (19, 20), and 2′,3′-dideoxycytidine (dDC) (4). In an alternative tactic, fludarabine (9-β-D-arabinofuranosyl-2-fluoroadenine) potentiated the activity of cytarabine in vitro (16) and in vivo (15) through the activity of fludarabine 5′-triphosphate (FaraATP). The mechanism of potentiation by FaraATP was complex, because it not only directly activated deoxycytidine kinase but it also inhibited ribonucleotide reductase, reducing the feedback inhibition of deoxycytidine kinase by dCTP (16).

The sites of cellular entry of nucleosides may also be targets for modulation of nucleoside drug activity. In animal cells, the cellular uptake of physiological nucleosides and many nucleoside analogs is mediated by an array of nucleoside-specific membrane transport processes, which include both facilitated diffusion and Na⁺-dependent, concentrative processes (8, 27). Nitrobenzylthioinosine (NBMPR) is a tightly bound, inhibitory ligand of the es facilitated diffusion nucleoside transporter (21, 29). Potent inhibition by NBMPR of the widely expressed es nucleoside transport process is the basis of tactics that have used NBMPR to manipulate the efficacies of several cytotoxic...
nucleoside drugs (1, 2, 14, 23, 29). In the present study and elsewhere (16a), we have shown that the membrane permeation of 3TC is partly mediated by the ex nucleoside transport, providing a rationale for testing NBMPR in the modulation of 3TC activity.

This report describes the effects of thymidine and fludarabine on the formation of the 5'-triphosphate ester metabolite of 3TC in primary cultures of hepatocytes from DHBV-infected Pekin ducks, in the human hepatoma 2.2.15 cell line that harbors replicating HBV, and in the human T-lymphoblastoid cell line CCRF-CEM. We also report the effects of NBMPR on 3TC metabolism and cytotoxicity in human cells.

MATERIALS AND METHODS

Chemicals. [methyl-3H]thymidine, [5-3H(N)]3TC (custom labelled), [5-3H]deoxyadenosine, [2,8-3H]adenosine, H2O, and [U-14C]sucrose were from Moravek Biochemicals Inc., Brea, Calif. 3TC was provided by Glaxo Group Research, Greenford, United Kingdom. 3TC-TP was synthesized by John S. Wilson, Department of Medical Microbiology and Immunology, University of Alberta. NBMPR was a gift from Alan R. P. Paterson, Department of Pharmacology, University of Alberta. Other nucleosides and nucleotides, [3,4,5-(dimethylthiolo-furan-2-yl)]-5'-methylthio-L-thymidine (MTT), and Geneticin (antibiotic G418) were from Sigma Chemical Co., St. Louis, Mo.

Cells. Primary cultures of hepatocytes from DHBV-infected Pekin ducks were plated (2.5 × 106 cells) in Nunc Multidishts (Gibco) in 400 µl of L-15 medium (Gibco) containing 5% bovine serum (FBS), 50 IU of penicillin G per ml, and 10 µg of streptomycin sulfate per ml. The cultures were incubated at 37°C in a humidified atmosphere of air and were used 1 day after plating, at which time the plating medium was replaced with fresh medium containing or lacking test nucleosides.

Human hepatoma 2.2.15 cells, obtained from Mary Ann Sells and George Acs, The Mount Sinai Hospital, New York, were maintained as stock cultures in 75-cm2 flasks in Eagle minimal essential medium (Gibco) containing 4% Geneticin and 2% FBS at 37°C in a humidified atmosphere of 5% CO2 in air and were subcultured at weekly intervals by dilution to concentrations that ensured exponential growth. For metabolism experiments, 2.2.15 cells were plated in Nunc Multidish four-well plates (5 × 106 cells in 1 ml per well) in culture medium containing 5 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; pH 7.4) and were used just before the monolayers had become confluent. For nucleoside transport experiments, 2.2.15 cells were plated in Falcon Primaria 35-mm plastic dishes (106 cells in 2 ml per dish) and were used when the monolayers were confluent.

Stocks of human T-lymphoblastoid CCRF-CEM cells were maintained in RPMI 1640 medium (Gibco) containing 10% FBS at 37°C in a humidified atmosphere of CO2 in air. The cell suspensions were diluted with fresh medium every 3 to 4 days to maintain exponential growth. For metabolism experiments, CCRF-CEM cell cultures contained 2 × 106 cells in 1 ml of medium to which 2 mM (final) HEPES (pH 7.4) was added, with or without test nucleosides.

RESULTS

Intracellular decay of 3TC 5'-phosphates. To evaluate the stability of 3TC phosphate esters in cells, we determined the t½,5 for the intracellular decay of those nucleotides. In these experiments, cells were loaded with [3H]3TC, and nucleotide concentrations were measured in cell extracts at graded time intervals following removal of drug from the cultures. Figure 1 presents the time courses of the elimination of 3TC 5'-phosphates, which were monophasic in primary cultures of duck hepatocytes and in 2.2.15 cells. The t½,5 for the decay of 3TC phosphate esters were similar in the two cell types, ranging from 3.6 to 8.0 h.

Effects of thymidine and fludarabine on the formation of 3TC 5'-phosphates in duck hepatocytes. We tested a rationale for the enhancement of cellular 3TC 5'-phosphate concentrations by using thymidine or fludarabine to enhance the activity of deoxycytidine kinase. Thymidine (50 µM) increased the levels of 3TC 5'-monophosphate in the duck hepatocyte cultures twofold. Modest increases in 3TC 5'-diphosphate (3TC-TP) were observed after thymidine treatment, but those changes did not reach statistical significance, and thymidine did not affect 3TC-TP concentrations in the duck hepatocyte cultures. After treatment with fludarabine (2.5 or 5.0 µM), none of the 3TC 5'-
phosphate concentrations differed significantly from control values.

Effects of thymidine and fludarabine on the formation of 3TC 5'-phosphates in 2.2.15 cells and CCRF-CEM cells. In 2.2.15 cells, 20 and 50 μM thymidine significantly increased the concentrations of 3TC-DP and 3TC-TP, so that the nucleotide levels were 1.6- and 1.7-fold higher than controls values, respectively, after treatment of the cells with 50 μM thymidine (Fig. 2). In contrast, the cellular content of 3TC phosphate esters in CCRF-CEM cells did not change in the presence of either 20 or 50 μM thymidine (data not shown). When 100 μM thymidine was tested, the concentrations of 3TC-DP and 3TC-TP were increased 1.6- and 1.4-fold, respectively, over those of controls in 2.2.15 cells, and the concentrations of 3TC-MP and 3TC-TP were increased 1.5- and 1.3-fold, respectively, in CCRF-CEM cells (Fig. 2). Treatment with fludarabine (5 μM) also increased 3TC-DP and 3TC-TP concentrations significantly in both cell types (Fig. 2).

Membrane transport of nucleosides in 2.2.15 cells. The time courses of the cellular uptake of 3TC and of several physiological nucleosides were measured in monolayer cultures of 2.2.15 cells. These experiments used assay procedures that measured initial rates of nucleoside uptake, which are measures of inward fluxes of nucleosides in these cells, and were aimed at determining the sensitivity of nucleoside fluxes to NBMPR. At permeant concentrations of 100 μM, the inward flux of 3TC was fourfold lower than that of thymidine (Table 1). When nucleoside fluxes were measured in the presence of 1 μM NBMPR, a potent inhibitor of the es nucleoside transport process in mammalian cells, inward fluxes of thymidine were virtually abolished, whereas 3TC fluxes were reduced to about 61% of control values (Table 1). In separate experiments (data not shown), inward fluxes of adenosine and deoxycytidine were also abolished in the presence of NBMPR, and fluxes of adenosine did not change when Na + ions were replaced by choline in permeant solutions.

Inhibition by NBMPR of 3TC 5'-phosphate formation and of the effects of thymidine and fludarabine. When NBMPR (1 μM) was tested as a modulator of the cellular accumulation of 3TC and its metabolites, the concentrations of 3TC-DP and 3TC-TP were significantly reduced (to 50 to 60% of control values) in 2.2.15 cells (Fig. 2). As well, NBMPR (1 μM) induced a modest reduction (70 to 90% of control values; P = 0.08) in the levels of 3TC-DP and 3TC-TP in CCRF-CEM cells (Fig. 2), which express only the es nucleoside transporter (5). The use of thymidine-NBMPR or fludarabine-NBMPR combinations significantly reduced 3TC phosphate concentrations to below control values in both cell types, reversing the enhancement of 3TC phosphate levels that occurred in the presence of thymidine or fludarabine alone (Fig. 2).

Modulation by thymidine and NBMPR of the cytotoxicity of 3TC in CCRF-CEM cells. To determine whether the changes in 3TC-TTP concentrations induced by thymidine or NBMPR in CCRF-CEM cells were accompanied by changes in the cyto-
TP (9, 18) suggests that the moderate cytotoxicity of 3TC is attributable to its inhibition of mammalian DNA polymerases. Similarly, weak inhibition of deoxycytidine kinase (9, 18) and HBV DNA polymerase (32) by 3TC-TP supports the view that the 5'-triphosphate metabolite of 3TC accounts for the antiviral activity of that agent. The intracellular concentrations of 3TC, with or without the modulating agents, were measured in a 4-day MTT assay in the presence of graded concentrations of 3TC in the absence (C) or presence of 10 μM thymidine (T), 100 μM thymidine (T) and then (ii) for 17 h (2.2.15 cells) or 4 h (CCRF-CEM cells) in media containing 10 μM [3H]3TC in the absence (C, F, FN) or presence of 100 μM thymidine (T) and/or 1 μM NBMPR (CN, TN, and FN). Cultures in which fludarabine was tested did not contain that agent during the second incubation interval. Extracts of cells were analyzed by thin-layer radiochromatography to obtain the intracellular nucleotide concentrations presented here. Each value is the mean ± standard error of the mean of four to 12 replicate measurements. *, significantly different from control (P < 0.05).

**DISCUSSION**

Potent inhibition of the activities of HIV reverse transcriptase (9, 18) and HBV DNA polymerase (32) by 3TC-TP supports the view that the 5'-triphosphate metabolite of 3TC accounts for the antiviral activity of that agent. Similarly, weak inhibition of mammalian DNA polymerases α and β by 3TC-TP (9, 18) suggests that the moderate cytotoxicity of 3TC is also attributable to that nucleotide metabolite. In the present study, we measured the intracellular stabilities of 5'-phosphates of 3TC and tested tactics aimed at increasing the intracellular concentrations of these metabolites. The values of the t_{1/2} of 3TC-TP reported herein (4.9 to 5.6 h) are lower than those determined in human peripheral blood lymphocytes (10.5 to 15.5 h) (7), but indicate that 3TC-TP is more stable than the 5'-phosphates of ddC, dFdC, or cytarabine, which decayed in a monophasic fashion in cultured or fresh leukemic lymphoblasts with t_{1/2}s of 2.6, 3.3, and 1.8 to 4.0 h, respectively (19, 20, 36). The t_{1/2} of 3TC-TP in 2.2.15 cells determined in the present study (5.6 h) also exceeded that of the 5-fluoro derivative, (−)-cis-2',3'-dideoxy-5-fluoro-3'-thiacytidine 5'-triphosphate, which decayed with a t_{1/2} of 2.4 h in 2.2.15 cells (26).

With a view to testing the enhancement of 3TC anabolism through thymidine-induced increases in deoxycytidine kinase activity in duck hepatocytes, we first sought evidence for the involvement of deoxycytidine kinase in 3TC metabolism in those cells. When 3TC anabolism was measured in the presence of deoxycytidine, the formation of 3TC 5'-phosphates was abolished (data not shown), suggesting that deoxycytidine kinase was involved in the phosphorylation of 3TC in the duck cell line.

**TABLE 1. Inhibition by NBMPR of inward fluxes of 3TC and thymidine in 2.2.15 cells**

<table>
<thead>
<tr>
<th>Flux assay condition</th>
<th>Inward flux of permeant (pmol/s/μl cell water)</th>
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<tbody>
<tr>
<td>[3H]3TC (100 μM)</td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td>[3H]3TC (100 μM) + NBMPR (1 μM)</td>
<td>0.46 ± 0.10</td>
</tr>
<tr>
<td>[3H]Thymidine (100 μM)</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>[3H]Thymidine (100 μM) + NBMPR (1 μM)</td>
<td>0.017 ± 0.052</td>
</tr>
</tbody>
</table>

a Inward fluxes were measured as initial rates of permeant uptake by fitting second-order polynomial equations to progress curve data, as described in Materials and Methods. Values are means ± standard errors of the means of at least five determinations.

b Cells were exposed to radiolabelled permeant and NBMPR simultaneously.

![FIG. 2. Modulation of the formation of 3TC 5'-phosphates in 2.2.15 cells and CCRF-CEM cells. (Upper panels) 2.2.15 cells were incubated (i) for 4 h in media without test agents (control; C) or supplemented with 20 μM thymidine (20T) or 50 μM thymidine (50T) and then (ii) for a further 4-h interval in media containing 10 μM [3H]3TC in the absence (control; C) or presence of 20 or 50 μM thymidine. (Center and lower panels) 2.2.15 cells or CCRF-CEM cells were incubated (i) for 4 h in media without test agents (controls; C) or containing 1 μM NBMPR (CN), 100 μM thymidine (T), 100 μM thymidine plus 1 μM NBMPR (TN), 5 μM fludarabine (F), or 5 μM fludarabine plus 1 μM NBMPR (FN) and then (ii) for 17 h (2.2.15 cells) or 4 h (CCRF-CEM cells) in media containing 10 μM [3H]3TC in the absence (C, F, FN) or presence of 100 μM thymidine (T and TN) and/or 1 μM NBMPR (CN, TN, and FN). Cultures in which fludarabine was tested did not contain that agent during the second incubation interval. Extracts of cells were analyzed by thin-layer radiochromatography to obtain the intracellular nucleotide concentrations presented here. Each value is the mean ± standard error of the mean of 4 to 12 replicate measurements. *, significantly different from control (P < 0.05).](http://aac.asm.org/)

![FIG. 3. Modulation of the cytotoxicity of 3TC in CCRF-CEM cells. The cells were incubated in microwell plates in media containing graded concentrations of 3TC in the absence (●) or presence of 10 μM thymidine (●) or 1 μM NBMPR (●). After 4 days, cell viability was determined in the MTT assay. Control cultures contained no test agents, 10 μM thymidine, or 1 μM NBMPR. NBMPR alone reduced cell numbers by less than 7%, whereas thymidine alone reduced cell numbers by 23%. The data presented here are means ± standard errors of the means of three to six experiments, with six replicate measurements in each experiment.](http://aac.asm.org/)
cells, as has been demonstrated in several mammalian cell types (9, 17, 33), although that result did not exclude the participation of other enzymes in the activation of 3TC. The effects of thymidine on 3TC metabolism in duck hepatocytes were slight, however, because the only significant change was an increase in the concentration of 3TC-MP in the presence of 50 μM thymidine. The lack of a significant effect of thymidine on 3TC-DP and 3TC-TP levels may be related to the apparently rate-limiting conversion of 3TC-MP to higher phosphates in the duck cells, reflected in the levels of 3TC-DP that were consistently lower than those of the other 3TC 5'-phosphates measured in these experiments (data not shown). Thus, an increase in the activity of deoxycytidine kinase, which was apparently not rate limiting in the formation of 3TC phosphates in duck hepatocytes, would not necessarily enhance the levels of 3TC-DP and 3TC-TP.

In contrast, the cellular content of 3TC 5'-phosphates was significantly increased in 2.2.15 cells by thymidine at all concentrations tested (20 to 100 μM) and in CCRF-CEM cells treated with 100 μM thymidine. The differential effects of low (20 to 50 μM) concentrations of thymidine in CCRF-CEM and 2.2.15 cells indicate that a cell-type selectivity could be achieved in the use of thymidine as a modulator of 3TC metabolism in the human cell types. In both CCRF-CEM cells and 2.2.15 cells, 3TC-MP levels were lower than 3TC-DP and 3TC-TP levels, suggesting that the formation of 3TC-MP by deoxycytidine kinase was a rate-limiting step in the activation of 3TC and supporting the view that deoxycytidine kinase is the ultimate target of thymidine modulation in those cells. Furthermore, the cytotoxicity of 3TC in CCRF-CEM cells was enhanced (Fig. 3) when the cells were exposed to 3TC and thymidine for a time period that yielded approximately the exposure (thymidine concentration-time product) that increased 3TC-TP concentrations (Fig. 2). That result is consistent with the idea that 3TC-TP is a cytotoxic metabolite of 3TC.

Gandhi and Plunkett (16) have demonstrated that the sequential treatment of human leukemia K562 cells with fludarabine and cytarabine increased the activity of deoxycytidine kinase and enhanced the rate of cytarabine 5'-triphosphate accumulation (16). The activity of fludarabine has been attributed to both the direct and the indirect effects of FaraATP on deoxycytidine kinase (16); the indirect mechanism is thought to involve inhibition of ribonucleotide reductase in a series of events that parallel the effects of thymidine. Furthermore, the infusion of fludarabine before cytarabine in the treatment of acute myelogenous leukemia patients enhanced the accumulation of araCTP in circulating blasts (15). Those observations were the basis for the use of fludarabine as a modulator of 3TC metabolism in the present study. Fludarabine (5 μM) did not affect 3TC metabolism in duck hepatocytes, but it modestly increased the concentrations of 3TC-DP and 3TC-TP in 2.2.15 and CCRF-CEM cells. The magnitude of the fludarabine effects were probably limited by the concentration of fludarabine used (5 μM), which was chosen to be similar to the concentrations of fludarabine in plasma (2 to 3 μM) (22) associated with the clinically tolerable toxicities of that agent.

NBMPR, a potent inhibitor of the es facilitated diffusion nucleoside transport process (21, 29), has been used in experimental chemotherapy to modulate the cellular accumulation and retention of nucleoside analogs. NBMPR protected host tissues against potentially lethal doses of tubercidin (7-deazaadenosine) administered to mice in the experimental therapy of transplantable mouse neoplasms (23) and in the therapy of Schistosoma mansoni in a rodent model (14). Furthermore, NBMPR protected against the neurotoxicity of fludarabine in leukemic mice (1). In the sequential exposure of leukemia cells from patients with chronic lymphocytic leukemia to 2-chlorodeoxyadenosine and NBMPR, the transport inhibitor was a retentive agent that reduced the transporter-mediated efflux of 2-chlorodeoxyadenosine from cells (2). The effects of NBMPR in protection and retention tactics appear to be determined by the cell type-specific expression of one or more nucleoside transport processes that differ in sensitivity to the transport inhibitor (27).

In the present study, we determined the NBMPR sensitivity of fluxes of 3TC and of thymidine, adenosine, and deoxycytidine in 2.2.15 cells to establish a rationale for the use of NBMPR as a modulator of 3TC activity. The effects of Na+ depletion and of NBMPR on fluxes of the physiological nucleosides were measured in the 2.2.15 cells in order to recognize the expression of Na+-dependent and -independent nucleoside transporters, for which permeant selectivities and inhibitor sensitivities are known (8). These studies showed that in 2.2.15 cells, Na+-dependent nucleoside transporters were apparently not expressed and that mediated entry of nucleosides was attributable only to the es nucleoside transporter. Thus, partial inhibition by NBMPR of 3TC fluxes in 2.2.15 cells (Table 1) demonstrated that cellular entry of 3TC was attributable both to the es nucleoside transport process and to simple diffusion. A similar observation was reported for the membrane permeation of (–)-cis-2',3'-dideoxy-5-fluoro-3'-thiacytidine in HepG2 cells (26), the parent cell line from which the HBV-transfected 2.2.15 cells were derived. In CCRF-CEM cells, which express only the es nucleoside transporter (8), inward fluxes of 3TC were attributable to both simple and facilitated diffusion (16a).

In the present study, 3TC phosphate accumulation was reduced in NBMPR-treated 2.2.15 cells and CCRF-CEM cells, apparently as a consequence of the inhibition by NBMPR of es-mediated 3TC influx. Furthermore, NBMPR protected against the cytotoxicity of 3TC in a 4-day assay in CCRF-CEM cells, apparently by reducing the cellular accumulation of 3TC and its metabolites. These results indicate that membrane transport is a determinant of the activity of 3TC. As well, NBMPR reversed the enhancement of 3TC phosphate formation by the es substrates thymidine and fludarabine (34) (Table 1), apparently by reducing cellular uptake of the modulating nucleosides.

In summary, this study has demonstrated that two independent biochemical determinants of 3TC activity are targets for the modulation of that activity. Modulation of deoxycytidine kinase activity by thymidine and fludarabine enhanced the metabolic activation of 3TC in a cell type-dependent manner, which may reflect cellular differences in the expression of deoxycytidine kinase. Manipulation of the activity of the es nucleoside transporter by NBMPR reduced the activation of both 3TC and the nucleoside modulators. This study has shown the feasibility of altering 3TC metabolism by these mechanisms; further experiments will be needed to demonstrate the use of these tactics in manipulating the antiviral activity of 3TC and to explore the potential of these modulators in combination therapy with 3TC.

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