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Metabolism and activities of 3′-azido-2′,3′-dideoxythymidine and 2′,3′-didehydro-2′,3′-dideoxythymidine in herpesvirus thymidine kinase transduced T-lymphocytes

Richard R. Drake [a,*], Robyn McMasters [a], Stephanie Krisa [a], Steven D. Hume [a], Tammy M. Rechtin [a], Robert L. Saylors [c], Yawen Chiang [d], Rangaswamy Govindarajan [b], Nikhil C. Munshi [b]

[a] *Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, 4301 W. Markham Little Rock, AR 72205, USA*

[b] *Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA*

[c] *Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR 72205 USA*

[d] *Genetic Therapy Incorporated, Gaithersberg, MD, USA*

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Abstract

T-lymphocytes transduced with the conditionally toxic herpesvirus thymidine kinase gene (HSV-1 TK) are increasingly becoming important tools in genetic therapy approaches for treating viral infections and cancers. Therefore, the effects of different antiviral nucleoside drugs on the growth inhibition of parental and HSV-1 TK-transduced human T-lymphocyte cell lines (H9 and CEM TK^-) were examined. As expected, both transduced cell lines were most sensitive to growth inhibition by ganciclovir (GCV). While the presence of HSV-1 TK did not potentiate 3′-azido-2′,3′-dideoxythymidine (AZT) growth inhibition of H9 cells containing cellular TK; transduction of HSV-1 TK into the cellular TK-deficient CEM cells (CEM TK^-) restored sensitivity to AZT. In both transduced cell lines, an HSV-1 TK-dependent growth inhibition with 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T) was observed and a K_m of 143 μM for d4T and HSV-1 TK was determined. Metabolic labeling analysis showed that drug metabolism correlated with the observed effects on cell growth. The effects of HIV-1 replication in the CEM TK^- cell lines in the presence of AZT or d4T was evaluated. CEM TK^- cells are largely resistant to AZT or d4T inhibition of HIV-1 replication, however, transduction of HSV-1 TK into the CEM TK^- cells completely restored AZT and d4T inhibition of HIV-1 replication. These studies confirm the requirement for a thymidine kinase activity for the anti-HIV activities of d4T and suggest that AZT, but not d4T, could be potentially administered to patients receiving HSV-1 TK-transduced lymphocytes. © 1997 Elsevier Science B.V.

Keywords: Herpes simplex virus type 1 thymidine kinase; Antiviral nucleoside drugs; Gene therapy; T-lymphocytes

* Corresponding author. Tel.: +1 501 6865420; fax: +1 501 6868169.

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1. Introduction

Viral vectors containing herpes simplex virus type 1 thymidine kinases (HSV-1 TK) with co-administration of ganciclovir (GCV) are currently being evaluated in clinical gene therapy trials involving T-cell replacement therapies (Munshi et al., 1997; Bordignon and Bonini, 1995; Riddell et al., 1992) and for the treatment of many types of cancers (reviewed in Tiberghien, 1994; Culver and Blaese, 1994). A similar approach has also been used to inhibit HIV-1 replication in $CD4^+$ lymphoid cells by introducing an HIV-inducible HSV-1 TK gene, representing a potential ablative approach for treating HIV (Brady et al., 1994; Caruso and Klatzmann, 1992; Venkatesh et al., 1990). Another proposed approach is to incorporate HSV-1 TK into loss-of-function, live-attentuated HIV-1 to use as a safer HIV-1 vaccine for protective immunization (Smith et al., 1996). The rationale for these approaches is based on the ability of HSV-1 TK to selectively phosphorylate GCV to its monophosphate with subsequent formation of GCV-triphosphate by cellular kinases (Moolten, 1986; Tiberghien, 1994). As a result, only cells expressing HSV-1 TK will metabolize GCV to its toxic triphosphate metabolite for incorporation into DNA and inhibition of cell replication.

Because of the many current and future potential clinical uses of HSV-1 TK-transduced T-cells, antiviral drug sensitivities in parental and HSV-1 TK-transduced human T-lymphocyte cell lines were examined. Growth inhibition comparisons between TK-transduced and control T-lymphocyte cell lines were carried out with the drugs GCV, acyclovir (ACV), 3′-azido-2′,3′-dideoxythymidine (AZT), 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T), 2′,3′-dideoxycytidine (ddC) and hydroxyurea (HU). Additionally, the effect of HSV-TK-transduction on HIV replication in the presence of d4T or AZT was investigated. Our investigation showed that transduction of the CEM TK^- cells with HSV-1 TK allows recovery of AZT and d4T metabolism, including inhibition of HIV-1 replication with implications for AZT and d4T therapy in patients receiving HSV-TK-transduced cells.

2. Materials and methods

2.1. Reagents and cell lines

Media, serum and supplemental reagents were obtained from Gibco-BRL. ddC, AZT, HU and d4T were from Sigma; clinical grade GCV (Synthex) and ACV (Glaxo Wellcome) were purchased from the UAMS hospital pharmacy. All radiolabeled nucleosides were purchased from Moravek. H9 cells were purchased from ATCC and were mycoplasma free. The parental CEM T-lymphocyte cell lines and HIV-1 strain were obtained from the NIH AIDS Resource and Reference Reagent Program, Division of AIDS, NIAID, NIH: CEM TK^- cells from Dr Dennis A. Carson; CEM SS cells from Dr Peter Nara; H9 cells and HIV-1 strain IIIB from Dr Robert Gallo.

2.2. Transduction and selection of T-lymphocyte cell lines

H9 cells were transduced with a retroviral vector, G1Tk1SvNa.7 (Genetic Therapy) containing HSV-1 TK as previously described (Munshi et al., 1997). The H9-TK^+ cells described in this report were initially selected with G418 (800 μg/ml) for 2 weeks. Pools of HSV-1 TK expressing cells were further selected by plating 1000 cells/well in 24-well plates. After 2 days, duplicate cultures were prepared into another 24-well plate containing 150 μM GCV. After 4 days, cell pools with growth inhibition of 80% or greater were identified. This process was repeated four times using decreasing GCV concentrations (100, 50, 25 and 5 μM, respectively) until the final H9-TK^+ cell pool utilized in this report was isolated. CEM TK^- cells were similarly transduced and selected, but only enriched through the 25 μM GCV step. Prior to the transduction and cell studies, the CEM TK^- cells were grown in the presence of 100 μM 5-bromo-deoxyuridine for 2 weeks and for 2 weeks in the presence of 100 μM AZT. These cells were cultured for at least 2 weeks in the absence of either drug prior to growth inhibition and HIV-1 experiments. In addition to evaluating sensitivity to GCV, the expression of HSV-1

TK or neo in these cell lines was confirmed by Western blot analysis using a rabbit anti-HSV-1 TK antibody and/or by PCR amplification of genomic DNA (Munshi et al., 1997). The expression of CD4 in the drug selected CEM TK^-/TK^+ cell line (99% expression) was confirmed by flow cytometry analysis and compared with the parental CEM TK^- cells (97% expression). There was also no significant difference in growth rates between parental and transduced cell lines.

2.3. HSV-1 thymidine kinase assays

HSV-1 TK was expressed in *E. coli* and purified by DEAE-cellulose chromatography as previously described (Rechtin et al., 1995). For K_m determination, a filter binding assay for HSV-1 TK was used as previously described (Rechtin et al., 1995) with the following reaction conditions: 1.5 μg HSV-1 TK partially purified by DEAE-cellulose, 40 mM sodium phosphate, 1 mM ATP, 5 mM $MgCl_2$, 40 mM KCl and varying concentrations (10–200 μM) of [^{3}H]d4T were incubated for 15 min at 37°C.

2.4. Growth inhibition and drug removal assays

All cell lines were grown in RPMI 1640 supplemented with 10% fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin and 2 mM glutamine (all from Gibco-BRL). A standard growth inhibition assay consisted of 1.5×10^5 cells in 5 ml media with or without the indicated drug concentration. After 5 days, total cell numbers were determined using a Coulter cell counter or trypan blue exclusion assay. For drug removal assays after 1 or 3 days incubation, cells were transfered to a 15 ml tube, 4 ml of media was added to each flask, this media was pooled with the removed cells and the cells were pelleted by centrifugation. Supernatant was aspirated and the cell pellets were rinsed with 5 ml fresh media. Following centrifugation, the cells were resuspended in 5 ml fresh media and cultured for a cumulative total of 5 days.

2.5. Metabolic labeling

For GCV, 5 μCi of [8-^{3}H]GCV (17.6 Ci/mmol; 18 μM final) was added to 1×10^6 cells and incubated for 24 h. Cells were pelleted, rinsed three times with phosphate buffered saline and extracted in 1 ml ice-cold 70% methanol. Extracts were concentrated to 10 μl and aliquots were separated on PEI-cellulose thin layer chromatography plates developed in 1 M LiCl. For AZT and d4T, 6 μCi of [^{3}H]AZT (18.9 Ci/mmol; 30 μM final) or 6 μCi [methyl-^{3}H]d4T (26.5 Ci/mmol; 30 μM final) were used. Nucleotide extracts were separated on PEI-cellulose thin layer chromatography plates developed in 0.5 M LiCl. Each resulting lane was excised and divided into 1 cm fractions for scintillation counting. For analysis of the products of HSV-1 TK incubation with [^{3}H]d4T, an FPLC-purified HSV-1 TK preparation was used as previously described (Rechtin et al., 1995). Products were separated and quantitated by the thin layer chromatography system described above.

2.6. Inhibition of HIV-1 replication

For each CEM cell line tested, cells (5×10^5) were infected with 18 ng (p24 equivalents) of HIV-1 strain IIIB and grown as described above. On days 2 and 5 post-infection, cells were pelleted by centrifugation; fresh media and drug were added. On day 8, HIV-1 reverse transcriptase assays were done on culture supernatant using a filter binding assay, [α-^{35}S]TTP and poly-A/poly-dT as previously described (Dubay et al., 1992). For quantitation of HIV-1 p24 antigen, an HIV-1 p24-antigen plate (Coulter) was used per manufacturers instructions. Syncytium formation after 8 days of infection was quantitated as the number of syncytia per 10 000 cells.

3. Results

3.1. Growth inhibition of parental and HSV-1 TK-transduced T-lymphocyte cell lines by nucleoside drugs

The human T-lymphocyte cell line, H9, was transduced with HSV1-TK gene by the retroviral

vector G1Tk1SvNa.7 and H9-TK$^+$ cells were selected as described in Section 2 (for this report, TK$^+$ refers to any cell transduced with HSV-1 TK). The antiviral nucleoside drugs GCV, ACV, AZT, d4T and ddC were added in a range of concentrations to parental and HSV-1 TK-transduced H9 cells and total cell numbers were determined after a 5 day exposure. Drug concentrations required to inhibit H9 control and H9-TK$^+$ cell growth by 50% (CD_{50}) are presented in Table 1. Of the drugs tested, GCV, ACV and d4T displayed greater growth inhibition of the H9-TK$^+$ cells compared to control cells. Although the difference in determined CD_{50} for d4T inhibition in both cell lines was not that large, statistical analysis of the different concentrations tested resulted in a $P < 0.05$. The H9-TK$^+$ cells were most sensitive to growth inhibition by GCV (CD_{50} 0.1 μM), while the next most effective nucleoside inhibitors (ACV) required over 300-fold higher concentrations to achieve the same effect. HSV-1 TK transduction did not affect the growth inhibitory action of AZT and ddC (CD_{50} was similar in H9 and H9-TK$^+$ cells). Similar experiments were performed with the ribonucleotide reductase inhibitor, hydroxyurea (HU), to screen for any metabolic differences in nucleotide metabolism which could have resulted from the transduction and selection of the H9-TK$^+$ cell line. Parental and H9-TK$^+$ cells were both equally sensitive to growth inhibition by HU (Table 1).

Table 1
Growth inhibition by nucleoside drugs in parental and TK-transduced H9 cells

Compound	CD_{50} (μM)[a]	
	H9	H9-TK$^+$
GCV	75 ± 3	0.1 ± 0.01
AZT	35 ± 0.9	40 ± 2
ACV	>500	32 ± 2
d4T	125 ± 13	90 ± 6
ddC	15 ± 0.6	23 ± 1
Hydroxyurea	0.3 ± 0.03	0.3 ± 0.03

[a] CD_{50}, concentration of drug required to inhibit cell growth by 50%. Data are representative of at least three determinations.

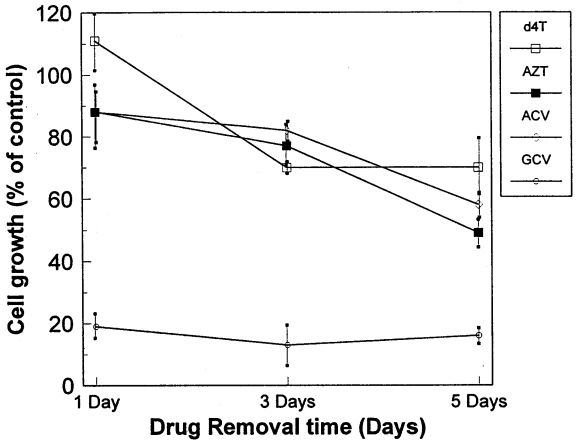

Fig. 1. Time and drug removal effects on growth inhibition by nucleoside drugs. H9-TK$^+$ cells were grown for 1, 3 or 5 days in the presence of the following four drugs at the indicated concentrations: 40 μM d4T; 40 μM AZT; 30 μM ACV; GCV 10 μM. Cell numbers were determined after 5 days using a Coulter counter. Numbers are normalized to minus drug control cultures and are representative of at least triplicate determinations.

3.2. Effect of drug exposure time on growth inhibition

Previous studies have shown that only 1 day GCV exposure is sufficient to kill HSV-1 TK-transduced cells in 5 days (Munshi et al., 1997). To examine the relative rates of metabolism and effectiveness of various other antiviral agents, another series of experiments were undertaken in which H9-TK$^+$ cells were exposed to GCV, ACV, AZT and d4T for 1, 3 or 5 days. The resulting growth inhibition was determined after 5 days as shown in Fig. 1. Only one day of treatment with GCV at 10 μM, was necessary to achieve cell death analogous to 5 days' treatment. For d4T (40 μM) and ACV (30 μM), little effect was observed after 1 day of treatment, while 3 days of treatment was essentially the same as 5 days. Only in AZT (40 μM) treated cells was there a correlation between treatment duration and growth inhibition.

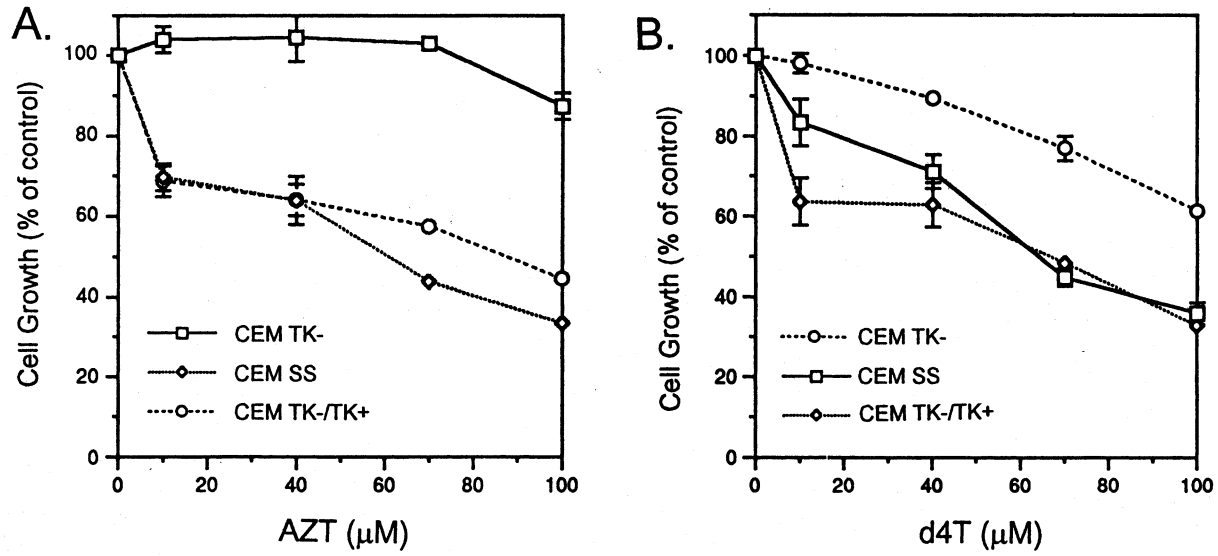

Fig. 2. Comparative inhibition of CEM cell line growth with AZT and d4T. CEM TK^-, CEM TK^-/TK^+ and CEM SS cells were grown for 5 days in the presence of (A) AZT or (B) d4T at the indicated concentrations. Cell numbers were determined using a Coulter counter and are representative of at least triplicate determinations.

3.3. 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T) as a substrate for HSV-1 TK

These growth inhibition results suggested that d4T was a substrate for HSV-1 TK, a finding not previously reported. To verify this enzymatically, [^{3}H]d4T and ATP were incubated with purified HSV-1 TK for 30 min in vitro. The separation of products by thin layer chromatography revealed complete conversion of [^{3}H]d4T to [^{3}H]d4TMP. No detectable diphosphate was formed via HSV-1 TK's thymidylate kinase activity. Using a filter-binding assay for HSV-1 TK (Rechtin et al., 1995), the K_m for d4T was determined to be 143 μM with a V_{max} of 7.1 nmol/min per mg. A similar K_m for d4T of 138 μM had been previously determined for a cellular thymidine kinase purified from H9 cells (Marongiu et al., 1990).

3.4. Growth inhibition in transduced and non-transduced CEM TK^- cell lines

To better define the contribution of HSV-1 TK and cellular TK for metabolism of d4T and AZT, CEM TK^- a T-lymphocyte cell line lacking a cellular TK activity was transduced with G1Tk1SvNa.7 retrovirus, selected in G418 and enriched for GCV sensitivity as described for the H9 cells. The resulting CEM TK^-/TK^+ cell line had an IC_{50} of 25 μM for GCV. Another CEM cell line, CEM-SS which has a functional cellular TK, was used as an additional control. As shown in Fig. 2, the effects of increasing concentrations of AZT and d4T were determined in the CEM TK^-, CEM TK^-/TK^+ and CEM SS cell lines. Whereas AZT had little growth inhibitory effect on the CEM TK^- cells, AZT-induced growth inhibition was restored in the HSV-1 TK-transduced CEM TK^-/TK^+ cells to a level similar to the CEM SS cells. The growth inhibitory effect of d4T was diminished in the CEM TK^- cells, but potentiated approximately two-fold in the CEM TK^-/TK^+ and CEM SS cells.

3.5. Metabolic labeling with radiolabeled GCV, d4T and AZT

Metabolic labeling was done with [^{3}H]GCV, [^{3}H]d4T or [^{3}H]AZT in the two H9 and three CEM cell lines. The primary metabolites detected for AZT and d4T were the nucleotide monophosphates of each drug, while both [^{3}H]GCVMP and

Table 2
Levels of drug metabolites after metabolic labeling with [^{3}H]GCV, [^{3}H]d4T or [^{3}H]AZT

Cell line	[^{3}H]GCVMP	[^{3}H]GCVTP	[^{3}H]d4TMP	[^{3}H]AZTMP
H9	2.2	1.5	3.3	186
H9-TK$^+$	15	38	11	224
CEM TK$^-$	3.7	1.1	56	2.0
CEM TK$^-$/TK$^+$	17	45	95	118
CEM SS	5.8	1.8	135	130

Values expressed as pmol/10^6 cells. Data are the mean of two to four independent experiments.

[^{3}H]GCVTP were detected for GCV labeling (see Table 2). Both [^{3}H]GCVMP and [^{3}H]GCVTP were found predominantly in the HSV-TK-transduced cell lines. Interestingly, [^{3}H]AZT labeling led to only minor increases in [^{3}H]AZT-5′-monophosphate (AZTMP) levels in the H9-TK$^+$ cells compared to parental H9 cells. As predicted from the growth inhibition curves, only trace AZT metabolites were detected in the CEM TK$^-$ cells, while the CEM TK$^-$/TK$^+$ cells had markedly increased levels of AZTMP levels that were comparable to the CEM SS cells. For [^{3}H]d4T, two to three fold increases in [^{3}H]d4TMP levels have been consistently observed in the H9-TK$^+$ cells as compared to parental H9 cells. However, unlike the observation for AZT metabolites, significant [^{3}H]d4TMP levels were observed in the CEM TK$^-$ cells. A 1.8-fold higher level of [^{3}H]d4TMP was detected in the CEM TK$^-$/TK$^+$ cells compared with CEM TK$^-$ cells.

3.6. HIV − 1 replication in CEM TK$^-$ cells transduced with HSV-1 TK

The effects on inhibition of HIV-1 replication by AZT and d4T were determined in the three CEM cell lines at concentrations ranging from 0.01 to 25 μM. As shown in Fig. 3, inhibition of HIV-1 replication by AZT was restored in the CEM TK$^-$/TK$^+$ cells compared to the CEM TK$^-$ cells as assayed by inhibition of reverse transcriptase activity (Dubay et al., 1992). A 100-fold increase in d4T inhibition of HIV-1 replication in the CEM TK$^-$/TK$^+$ cells versus CEM TK$^-$ cells was detected. A previous study had determined EC_{50} values for AZT and d4T inhibition of HIV − 1 replication in CEM cells to be 0.01 and 0.05 μM, respectively (Sergheraert et al., 1993), values consistent with those observed in the CEM TK$^-$/TK$^+$ and CEM SS cells. Analysis of HIV-1 p24 antigen levels in these cultures gave similar results and a corresponding decrease in syncytium formation was observed in CEM TK$^-$/TK$^+$ and CEM SS cells treated with AZT and d4T (data not shown). As the HSV-TK$^+$ cells lines were selected for GCV sensitivity to growth inhibition, inhibition of HIV replication by GCV was not evaluated due to the inability to differentiate GCV-mediated cytotoxicity from any effects of HIV-1 infection. To summarize, the expression of HSV-1 TK in the CEM TK$^-$ cells completely restored AZT and d4T inhibition of HIV-1 replication.

4. Discussion

In this report, we have evaluated the effects of different antiviral drugs on growth inhibition and HIV-1 replication in several HSV-1 TK-transduced T-lymphocyte cell lines. Previous studies have shown that ACV is effectively inhibits HIV replication in T-lymphocyte cell lines transduced with HIV-inducible HSV-1 TK (Venkatesh et al., 1990; Caruso and Klatzmann, 1992). AZT has been reported previously by other investigators to be an effective substrate for HSV-1 TK (Munir et al., 1993; Mao et al., 1995). However, a study by Lowy et al. investigated the effects of AZT and ACV on HIV replication in cellular TK-deficient Jurkat cells transduced with HSV-1 TK (Lowy et al., 1994) and showed AZT to have no effect on HIV-replication, although ACV was effective. The

lack of an AZT effect in the HSV-1 TK-transduced cells in that study was attributed to AZT not being a substrate for HSV-1 TK and no metabolic labeling data was presented. The metabolic differences between the Jurkat cell studies and the CEM cell lines reported here are not clear. However, the metabolic labeling data presented in Table 2 clearly demonstrate effective metabolism of AZT by the CEM TK^-/TK^+ cells compared to CEM TK^- cells. AZT inhibition of HIV-1 replication was completely restored in CEMTK^-/TK^+ cells (Fig. 3).

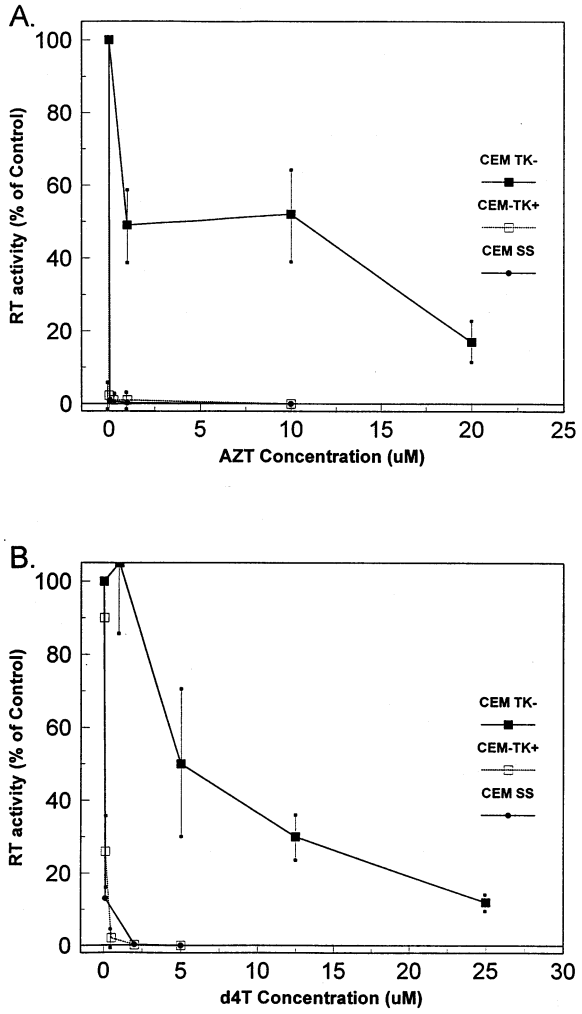

Fig. 3. Inhibition of HIV-1 replication by AZT and d4T in the CEM cell lines. CEM TK^-, CEM TK^-/TK^+ and CEM SS cells were infected with HIV-1 IIIB in the absence or presence of AZT (A) or d4T (B) at the indicated concentrations as described in Section 2. After 8 days, reverse transcriptase assays (Dubay et al., 1992) were carried out on cell free supernatants and results are plotted as percent of detected HIV-RT activity relative to activities in control cultures (100%) without any drug.

It has been demonstrated previously that AZTMP accumulates in H9 cells due to the low affinity of cellular thymidylate kinase for AZTMP (Furman et al., 1986). In studies comparing the toxicity of AZT in CEM cells and cellular TK-depleted CEM cells, decreased levels of AZTMP accumulation were found to be correlated with decreased cell death (Tornevik et al., 1995). Our results are consistent with these studies, but also demonstrate that HSV-1 TK can restore AZT metabolism in CEM TK^- cells. Interestingly, there appears to be a maximal sustainable level of AZTMP in the other cell lines (H9, H9-TK^+ and CEM SS). In the H9-TK^+ cells after AZT addition, there was not a significant additive increase in AZTMP levels and no effect on the inhibition of cell growth was observed compared to the parental H9 cells. Identification of the cellular components that maintain this AZTMP maxima may be useful in the ongoing evaluation of mechanisms that control AZT toxicity.

A distinct concentration dependent inhibition of cell growth in the HSV-TK^+ cell lines was observed at d4T concentrations below 0.1 mM (Table 1 and Fig. 2) and higher levels of [^{3}H]d4TMP were present in these cells compared to non-TK-transduced cells (Table 2). Unlike AZT, which is clearly metabolized by the cellular thymidine kinase (TK1) (Furman et al., 1986; Tornevik et al., 1995), there are conflicting studies for the role of this enzyme in d4T metabolism. One study determined a K_m of 138 μM for purified H9 TK1 (Marongiu et al., 1990) another reported a K_m of 142 μM for an enzyme from MT-4 cell extracts which was likely distinct from TK1 (Balzarini et al., 1989), while other studies reported no metabolism of d4T by thymidine kinase purified from human leukemic spleen (Munch-Petersen et al., 1991). In our study, the growth inhibition caused by d4T was clearly potentiated by the presence of HSV-1 TK, yet it also

caused inhibition of cell growth and HIV-1 replication in the CEM TK^- cells. Combined with the d4T metabolite profiles in Table 2, these results suggest that cellular TK1 will metabolize d4T, but there are alternative metabolic enzymes that will phosphorylate d4T in these cell lines. However, cellular TK1 does appears to be the primary enzyme for the most efficient d4T metabolism in the cell lines tested. Thus, this combination of cell lines will make an excellent system for more detailed analyses of the effects of AZT and d4T on dNTP pool sizes, drug/thymidine triphosphate ratios and mechanisms of cellular toxicity.

An initial evaluation of potential drug interactions with several human T-lymphocyte cell lines transduced with HSV-1 TK have been presented. As the clinical use of primary patient T-lymphocytes transduced with HSV-TK increases, it is likely that different populations of patients will require concurrent antiviral or anti-cancer therapies via T-lymphocyte infusions. In the H9 and CEM T-lymphocyte cell lines tested, it is clear that GCV possesses the requisite low cytoxicity and potent inhibitory properties for effective HSV-1 TK gene therapy. For ACV, HSV-1 TK dependence was observed, but at much higher concentrations and longer treatment durations, which is consistent with previous reports (Balzarini et al., 1993; Kuriyama et al., 1996). For d4T, the K_m of 143 μM for HSV-1 TK is much higher than the nanomolar levels of drug required to inhibit HIV replication in most cell lines, yet HSV-1 TK still proved effective at restoring d4T inhibition of HIV-1 replication in the CEM TK^-/TK^+ cell line. Based on previous results (Lowy et al., 1994) and those reported here, it seems plausible that primary HSV-1 TK-transduced lymphocytes could still be used in HIV-1 infected patients receiving ddC or AZT. However, we hypothesize this would not be the case for d4T due to the potentiated increase in metabolism and cell death in the HSV-TK cells. Similar drug studies using primary, HSV-1 TK-transduced human T-lymphocytes (Munshi et al., 1997) are currently in progress.

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References

Balzarini, J., Herdewijn, P., De Clercq, E., 1989. Differential patterns of intracellular metabolism of 2′,3′-didehydro-2′,3′-dideoxythymidine and 3′-azido-2′,3′-dideoxythymidine, two potent anti-human immunodeficiency virus compounds. J. Biol. Chem. 264, 6127–6133.

Balzarini, J., Bohman, C., De Clercq, E., 1993. Differential mechanism of cytostatic effect of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine, 9-(1,3-dihydroxy-2-propoxymethyl)guanine and other antiherpetic drugs on tumor cells transfected by the thymidine kinase gene of herpes simplex virus type 1 or type 2. J. Biol. Chem. 268, 6332–6337.

Bordignon, C., Bonini, C., 1995. Clinical protocol: Transfer of the HSV-TK gene into donor peripheral blood lymphocytes for in vivo modulation of donor anti-tumor immunity after allogeneic bone marrow transplantation. Hum. Gene Ther. 6, 813–819.

Brady, H., Miles, C., Pennington, D., Dzierzak, E., 1994. Specific ablation of human immunodeficiency virus tat-expressing cells by conditionally toxic retroviruses. Proc. Natl. Acad. Sci. USA 91, 365–369.

Caruso, M., Klatzmann, D., 1992. Selective killing of $CD4^+$ cells harboring a human immunodeficiency virus-inducible suicide gene prevents viral spread in an infected cell population. Proc. Natl. Acad. Sci. USA 89, 182–186.

Culver, K.W., Blaese, R.M., 1994. Gene therapy for cancer. Trends Genet. 10, 174–178.

Dubay, J.W., Roberts, S.J., Brody, B., Hunter, E., 1992. Mutations in the leucine zipper of the human immunodeficiency virus type I transmembrane glycoprotein affect fusion and infectivity. J. Virol. 66, 4748–4756.

Furman, P.A., Fyfe, J.A., St. Clair, M.H., Weinhold, K., Rideout, J.L., Freeman, G.A., Lehrman, S.N., Bolognesi, D.P., Broder, S., Mitsuya, H., Barry, D.W., 1986. Phosphorylation of 3′-azido-3′-deoxythymidine and selective interaction of the 5′-triphosphate with human immunodeficiency virus reverse transcriptase. Proc. Nat. Acad. Sci. USA 83, 8333–8337.

Kuriyama, S., Nakatani, T., Masui, K., Sakamoto, T., Tominaga, K., Yoshikawa, M., Fukui, H., Ikenaka, K., Tsuji, T., 1996. Evaluation of prodrugs ability to induce effective ablation of cell transduced with viral thymidine kinase gene. Anticancer Res. 16, 2623–2628.

Lowy, I., Caruso, M., Goff, S., Klatzmann, D., 1994. Cellular thymidine kinase activity is required for the inhibition of HIV-1 replication by AZT in lymphocytes. Virology 200, 271–275.

Mao, F., Rechtin, T.M., Jones, R., Cantu, A., Anderson, S., Radominska, A., Moyer, M.P., Drake, R.R., 1995. Synthesis of a photoaffinity analog of 3′-azidothymidine, 5-azido-3′-azido-2′,3′-dideoxyuridine: Interaction with herpesvirus thymidine kinase and cellular enzymes. J. Biol. Chem. 270, 13660–13664.

Marongui, M.A., August, E.M., Prusoff, W.H., 1990. Effect of 3′-deoxythymidine-2′-ene (d4T) on nucleoside metabolism in H9 cells. Biochem. Pharmacol. 39, 1523–1528.

Moolten, F.L., 1986. Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: Paradigm for a prospective cancer control strategy. Cancer Res. 46, 5276–5281.

Munch-Petersen, B., Cloos, L., Tyrsted, G., Erikkson, S., 1991. Diverging substrate specificity of pure human thymidine kinases 1 and 2 against antiviral dideoxynucleosides. J. Biol. Chem. 266, 9032–9038.

Munir, K., French, D., Loeb, L., 1993. Thymidine kinase mutants obtained by random sequence selection. Proc. Natl. Acad. Sci. USA 90, 4012–4016.

Munshi, N.C., Govindarajan, R., Drake, R., Ding, L.M., Iyer, R., Saylors, R., Kornbluth, J., Marcus, S., Chiang, Y., Ennist, D., Kwak, L., Reynolds, C., Tricot, G., Barlogie, B., 1997. Thymidine kinase gene transduced human lymphocytes can be highly purified, remain fully functional and are killed with ganciclovir. Blood 89, 1334–1340.

Rechtin, T.M., Black, M.E., Mao, F., Lewis, M.L., Drake, R.R., 1995. Purification and photoaffinity labeling of herpes simplex virus type-1 thymidine kinase. J. Biol. Chem. 270, 7055–7060.

Riddell, S.R., Greenberg, P.D., Overel, R.W., Loughran, T.P., Gilbert, M.J., Lupton, S.D., Agosti, J., Scheeler, S., Coombs, R.W., Corey, L., 1992. Phase I study of cellular adoptive immunotherapy using genetically modified $CD8^+$ HIV-specific T cells for HIV seropositive patients undergoing allogeneic bone marrow transplant. Hum. Gene Ther. 2, 319–338.

Sergheraert, C., Pierlot, C., Tartar, A., Henin, Y., Lemaitre, M., 1993. Synthesis and anti-HIV evaluation of D4T and D4T 5′-monophosphate prodrugs. J. Med. Chem. 36, 826–830.

Smith, S.M., Markham, R.B., Jeang, K.-T., 1996. Conditional reduction of human immuodeficiency virus type 1 replication by a gain of herpes simplex virus 1 thymidine kinase function. Proc. Natl. Acad. Sci. USA 93, 7955–7960.

Tornevik, Y., Ullman, B., Balzarini, J., Wahren, B., Eriksson, S., 1995. Cytotoxicity of 3′-azido-3′-deoxythymidine correlates with 3′-azido-3′-deoxythymidine-5′-monophosphate (AZTMP) levels, whereas anti-human immunodeficiency virus (HIV) activity correlates with 3′-azido-3′- deoxythymidine-5′-triphosphate (AZTTP) levels in cultured CEM T-lymphoblastoid cells. Biochem. Pharmacol. 49, 829–837.

Tiberghien, P., 1994. Use of suicide genes in gene therapy. J. Leuk. Biol. 56, 203–209.

Venkatesh, L., Arens, M., Subramanian, T., Chinnadurai, G., 1990. Selective induction of toxicity to human cells expressing human immunodeficiency virus type 1 tat by a conditionally cytotoxic adenovirus vector. Proc. Natl. Acad. Sci. USA 87, 8746–8750.