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Expression of Human Mitochondrial Thymidine Kinase in *Escherichia coli*: Correlation between the Enzymatic Activity of Pyrimidine Nucleoside Analogues and Their Inhibitory Effect on Bacterial Growth

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ABSTRACT. Mitochondrial thymidine kinase (TK2) phosphorylates pyrimidine nucleosides to monophosphates and is expressed constitutively through the cell cycle in all cells. Because of the overlap of its substrate specificity with that of the cytosolic thymidine kinase (TK1) and deoxycytidine kinase (dCK), it has been difficult to determine the role of TK2 in activating nucleosides used in chemotherapy. In this report, we described the construction of a recombinant *Escherichia coli* strain which could be used to test if TK2 activity is limiting for the toxicity of nucleosides. Enzymes of bacterial origin which are involved in thymidine and deoxyuridine anabolism and catabolism were eliminated, and the cDNA for human TK2 was introduced. In the crude extract of the engineered *E. coli*, the level of thymidine kinase was, after induction of TK2 expression, several hundred fold higher than in the control strain. Several pharmacologically interesting nucleoside analogues, including 3′-azidothymidine, 2′,3′-didehydro-2′,3′-dideoxythymidine, and 2′,3′-dideoxy-β-L-3′-thiacytidine, were tested for their effects on the growth of this recombinant strain. For a comparison, the phosphorylation of these compounds was determined with purified recombinant TK1, TK2, and dCK. A correlation was observed between the phosphorylation of several of these compounds by TK2 and their effects on bacterial growth. These results demonstrate that activation of growth-inhibiting pyrimidine nucleosides can be catalyzed by TK2, and together with recombinant *E. coli* strains expressing other cellular nucleoside kinases, this whole-cell bacterial system may serve as a tool to predict the efficacy and side effects of chemotherapeutic nucleosides. BIOCHEM PHARMACOL **59**;12:1583–1588, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. TK2; *E. coli* engineering; pyrimidine nucleoside analogues; growth inhibition; phosphorylation; drug development

In mammalian cells, deoxynucleoside salvage supplements the *de novo* synthesis of DNA precursors by phosphorylating deoxynucleosides to produce deoxyribonucleoside monophosphates, which can be further activated to triphosphates. Two forms of TK§ are involved in this pathway, one in the cytosol (TK1) and the other in mitochondria (TK2), and TK1 and TK2 differ in their primary sequences, substrate specificities, and regulation through the cell cycle [1]. Phylogenetic analysis indicates that TK2 belongs to the family of deoxynucleoside kinases from many species of the herpesvirus family, to which the cytosolic dCK also belongs, whereas TK1 shows homology to the thymidine kinases from *Escherichia coli* and poxviruses [2]. In agreement with this classification, TK2 also phosphorylates cytosine-containing nucleosides and thus, like many members of the herpes kinase family, is a pyrimidine deoxyribonucleoside kinase. TK2 is constitutively expressed in resting cells as well as in proliferative tissues, while TK1 is present only in S-phase cells [1]. There have been extensive studies focused on nucleoside analogues designed to be activated by TK1 or dCK in order to subsequently inhibit tumor cells or viral DNA synthesis. However, the role of TK2 in these processes is unclear, since TK2 activity is masked by TK1 and dCK activity *in vivo*.

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§ *Abbreviations:* 3TC, β-L-2′,3′-dideoxy-3′-thiacytidine; 5FdC, 5-fluoro-2′-deoxycytidine; 5FdU, 5-fluoro-2′-deoxyuridine; AraC, 1-β-D-arabinofuranosylcytosine; AraT, 1-β-D-arabinofuranosylthymine; AraU, 1-β-D-arabinofuranosyluracil; AZT, 3′-azido-2′,3′-dideoxythymidine; D4T, 2′,3′-didehydro-2′,3′-dideoxythymidine; dCK, deoxycytidine kinase; dFdC, 2′,2′-difluoro-2′-deoxycytidine; dFdU, 2′,2′-difluoro-2′-deoxyuridine; dUrd, 2′-deoxyuridine; FIAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil; FMAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil; IPTG, isopropyl-β-D-thiogalactopyranoside; Thd, thymidine; TK, thymidine kinase; TK1, cytosolic thymidine kinase; TK2, mitochondrial thymidine kinase; and Urd, uridine.

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TK2 was first purified to homogeneity from leukemic spleen [3], and its complete coding sequence has been recently cloned and expressed [4, and *]. Kinetic studies showed that TK2 is able to phosphorylate many nucleoside analogues, such as AZT and FIAU, which are also good substrates for TK1 and are clinically applied to patients with cancer and viral infections [3, 5]. There is evidence that TK2 might be involved in cellular, especially mitochondrial, toxicity caused by certain compounds, e.g. FIAU [5, 6]. It was therefore important to establish an *in vivo* system which allows examination of TK2-dependent toxicity caused by nucleoside analogues.

Cell culture is usually the first choice for drug-testing investigations. In the case of TK2, however, the overall nucleoside metabolism in the cell needs to be considered, e.g. the mitochondrial location of this enzyme, the low level of its expression, and the existence of the more active TK1 and dCK in the cytosol. All these factors contribute to the difficulty in constructing a cell line for screening drugs specifically activated by TK2. It was therefore necessary to construct a cell system where TK2 was the only deoxynucleoside kinase expressed and where the same cells lacking TK2 could be used as control.

Previously, we reported on a bacterial system in which human dCK or TK1 were expressed in *E. coli*, and several cytostatic and antiviral nucleoside analogues showed selective inhibition of bacterial growth [7]. In the present report, we constructed an *E. coli* strain expressing human TK2. Similarly to the strain expressing human TK1, bacterial enzymes involved in dUrd anabolism and catabolism were mutated, and the cDNA for human TK2 was introduced. The expression of TK2 in the bacterial culture was examined, and several cytostatic and antiviral pyrimidine analogues, e.g. D4T and 3TC, were tested. For several of the compounds tested in this study, a good correlation was observed between their inhibitory effects on the bacteria and the activity with purified TK2. Phosphorylation of the involved analogues with TK1, TK2, and dCK, all purified from recombinant sources, is also described. Combined with the strains expressing human TK1 and dCK, this whole-cell bacterial model system may be applied to test activities of nucleoside analogues with different cellular nucleoside kinases in an easy and rapid way and thereby provide information for future drug design.

MATERIALS AND METHODS

Bacterial Strains and Growth Media

The bacterial strains used in this study were all derivatives of *E. coli* K12 and are listed in Table 1. As rich medium, Luria broth (LB) was used [8]. The minimal medium was AB medium [9] supplemented with 0.2% glucose, 0.2% vitamin-free casamino acids, and 1 μg/mL thiamine. When required, tryptophan was added at 50 μg/mL and uridine at 20 μg/mL (82 μM). Antibiotics were used in the following final concentrations: ampicillin, 100 μg/mL; tetracycline, 10 μg/mL; and kanamycin, 30 μg/mL.

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TABLE 1. Bacterial strains used in this study

Strain	Genotype	Source or reference
SØ5218*	MC1061 *cdd*::Tn*10*/pTrcHUMdCK	[7]
SØ5286†	*thi lacZ rpsL* Δ(*deoCABD*) *zjj*-202::Tn*10 udp*::Tn5 *tdk-1*	[7]
SØ5288	SØ5286/pTrcHUMTK1	[7]
SØ5292	SØ5286/pTrc99-A	[7]
SØ5338	SØ5286/pTrcHUMTK2	Present Work

*The cytidine deaminase gene *cdd* was inactivated by insertion of transposon Tn*10* before the plasmid bearing the cDNA for human dCK was introduced.

†The thymidine phosphorylase gene *deoA*, the uridine phosphorylase gene *udp*, and the thymidine kinase gene *tdk-1* were inactivated by mutations.

Nucleoside Analogues

Most nucleoside analogues used in this study were purchased from Sigma. FIAU and FMAU were synthesized and provided by J. Fox at the Memorial Sloan Kettering Cancer Institute, U.S.A. 3TC was provided by Medivir AB, and dFdC and dFdU were from Eli Lilly & Co.

Plasmid Constructions and Expression

Plasmid pTrc99-A (Pharmacia) was used as the cloning and expression vector. The coding sequence of human TK2 was amplified by polymerase chain reaction using pET14bHuTK2 [4] as the template. The 5′ sense primer, 5′CAGCC**CCATGG***C*ATCAGTGATCTG, contained an *Nco* I site (bold) including the ATG start codon (italics), and the 3′ reverse complement primer, 5′GCAGCC**GGATCC***T*ATGGGCAATGC, had a *Bam* HI site (bold) overlapping with the stop codon (italics). As it turned out that the amplified TK2 coding sequence contained an internal *Nco* I site, its cloning into the pTrc99-A expression vector had to be done in two steps. The amplified DNA was digested with *Nco* I and *Bam* HI, yielding two fragments, a 537-bp *Nco* I/*Nco* I fragment containing the 5′ end of the TK2 cDNA and a 118-bp *Nco* I/*Bam* HI fragment harboring the 3′ end with the stop codon. First, the 118-bp fragment was cloned into *Nco* I/*Bam* HI-digested vector, yielding pTrc3′TK2. Subsequently, the 537-bp fragment was cloned into the unique *Nco* I site of pTrc3′TK2, yielding pTrcHUMTK2 containing the entire TK2 coding region. The orientation of the last *Nco* I fragment was confirmed by restriction endonuclease mapping. In this construction, TK2 cDNA was transcribed from the vector-borne IPTG-inducible *trc* promoter, since the *lacI*^{q} gene encoding the *lac* repressor was also expressed from pTrc99-A. Translation of TK2 was initiated from the *lacZ* ribosomal binding site located 6-bp upstream of the *Nco* I cloning site. Plasmid pTrcHUMTK2 was transformed into SØ5286, yielding SØ5338 (Table 1). SØ5286 is unable to catabolize Thd and dUrd due to

TABLE 2. TK and dCK activity in bacterial extracts from recombinant *E. coli* expressing human TK2 and dCK

Strain	Concentration of IPTG (mM)	TK activity (pmol/min/mg protein)	dCK activity (pmol/min/mg protein)
SØ5292	0	2.4 ± 0.5	2.8 ± 0.2
	0.1	2.0 ± 0.3	2.3 ± 0.6
	1	1.9 ± 0.3	2.5 ± 0.6
SØ5338	0	101 ± 33	63 ± 15
	0.1	918 ± 33	564 ± 19
	1	1812 ± 109	1094 ± 22
SØ5218	0	154 ± 19	25 ± 8
	0.1	236 ± 19	652 ± 56
	1	207 ± 8	869 ± 39

mutational inactivation of thymidine phosphorylase (*deo*A) and uridine phosphorylase (*udp*). In addition, SØ5286 carries the *tdk-1* mutation inactivating endogenous *E. coli* thymidine kinase (Table 1) [10].

Determination of TK and dCK Activities in Bacterial Extracts

Crude extracts of bacterial cultures were prepared as follows: bacterial overnight cultures were diluted with fresh media containing 0.1 mM IPTG, to give a final A_{600} of 0.01. The new cultures were grown with shaking at 37° for 3 hr. The bacteria were collected by centrifuging at 5000 × *g* for 20 min and the pellets were re-suspended in buffer A (50 mM Tris, pH 7.6, 1 mM EDTA, 50 mM NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 0.5% Nonidet P-40, 2 mM dithiothreitol, and 1 mg/mL lysozyme). The samples were incubated at room temperature for 30 min and after centrifugation (15000 × *g* for 20 min) the supernatants were used for enzyme assay. The assays were performed as described previously [11]. The protein content in the extracts was determined with the Bio-Rad protein assay, with BSA as standard.

Inhibitory Effects of Nucleoside Analogues on Bacterial Growth

A fresh overnight culture, prepared by inoculating a single colony from a Petri dish into 10 mL minimal media containing antibiotics, was diluted into fresh minimal media supplemented with antibiotics and 0.1 mM IPTG to achieve an initial A_{600} of 0.01. These inoculated media was subsequently divided into tubes, 1 mL in each, and 20 μL analogue solution was added to each tube. The tubes were cultured at 37° with shaking for 3–4 hr, until the control tube, to which no analogue was added, reached an A_{600} of about 0.6. The A_{600} of all tubes were measured, and the relative growth of each sample was calculated by comparing its A_{600} to that of the control tubes, which was set as 100%.

Phosphorylation of Nucleoside Analogues with Purified Nucleoside Kinases

The activity of nucleoside analogues with nucleoside kinases was examined with the phosphoryl transfer assay as described previously [5]. The enzymes were purified from recombinant bacteria [4, 5, 12], and the ATP concentration was 100 μM in all assays.

RESULTS

Activity of TK and dCK in Crude Extracts from the Recombinant Bacteria

To ascertain the expression of human TK2 in the engineered *E. coli*, crude extracts from strain SØ5338, containing the plasmid-borne coding sequence of human TK2 and the control strain SØ5292 (Table 1), were assayed for TK and dCK activity (Table 2). Both strains were devoid of endogenous *E. coli* TK activity due to the *tdk-1* mutation. Since TK2 overlaps in its substrate specificity with dCK, the TK and dCK activities of strain SØ5218 (Table 1), harboring the coding sequence of human dCK on a plasmid [7], were also determined. As shown in Table 2, the activities of both the TK and dCK of the control strain SØ5292 were very low, regardless of the IPTG concentration, and may represent background values in this assay. SØ5338 had TK levels 40-, 460-, and 950-fold higher than those of SØ5292 with IPTG at 0, 0.1, and 1 mM, respectively. In all three cases, the dCK level in SØ5338 was about 60% of that for TK, in accordance with the activity of TK2 with dCyd and Thd as substrates [13]. Increasing the IPTG concentration from 0.1 mM to 1 mM only doubled the TK level in SØ5338. Since we observed that the growth of SØ5338 in the presence of 1 mM IPTG was significantly slower than with IPTG at 0.1 mM or zero, we employed 0.1 mM IPTG in all subsequent growth experiments. The TK level in SØ5218 was independent of the presence of IPTG and represented the activity of the TK of *E. coli* origin.

Effect of Pyrimidine Analogues on the Growth of SØ5338

Several important antiviral and cytostatic nucleoside analogues were tested with SØ5338 (Table 3). At the concentration of 0.1 μM, 5FdU (U1) inhibited the growth of SØ5338 only slightly more than the growth of the control strain (Table 3). Among other Thd/dUrd analogues, dFdU (U2), FIAU (U3), FMAU (U4), and AZT (U7) showed different levels of selective inhibition varying between 29%

TABLE 3. Relative growth of recombinant *E. coli* expressing human TK2 in the presence of antiviral and cytostatic dUrd and dCyd analogues

Compound*	SØ5292 (TK2$^-$)	SØ5338 (TK2$^+$)
U1 5FdU (0.1 μM)	73%	61%
U2 dFdU	114%	60%
U3 FIAU	107%	71%
U4 FMAU	109%	46%
U5 AraU	110%	99%
U6 AraT	110%	113%
U7 AZT	85%	42%
U8 D4T	95%	96%
C1 5FdC	58%	64%
C2 dFdC	100%	29%
C3 AraC	110%	94%
C4 3TC	97%	100%

Values are the means of results from two duplicate experiments. Cultures in the absence of any nucleoside were set to 100%.

*The compounds were all tested at 20 μM, unless otherwise specified, in the presence of 0.1 mM IPTG and 82 μM (20 μg/mL) Urd.

to 58%, while AraU (U5), AraT (U6), and D4T (U8) had no effect on SØ5338. Of the four dCyd analogues tested with SØ5338, only dFdC (C2) had a selective inhibitory effect. Both SØ5338 and SØ5292 were inhibited by 5FdC (C1), while none of the arabinosyl nucleosides displayed any significant effect towards SØ5338.

EC_{50} Values of Inhibitory Nucleoside Analogues with SØ5338

For dFdU (U2), AZT (U7), FIAU (U3), FMAU (U4), and dFdC (C2), which showed selective inhibition of SØ5338, the growth curves of this strain with different concentrations of drugs were determined, as exemplified by the curve with dFdC (Fig. 1). EC_{50} values were calculated and listed in Table 4. The inhibiting efficiencies of these compounds

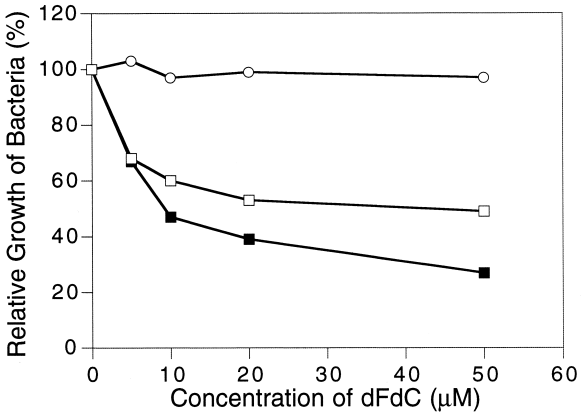

FIG. 1. Sensitivity to dFdC of *E. coli* expressing human TK2 in the presence of 82 μM dUrd. The growth of SØ5338 in the absence of IPTG (□), the growth of SØ5218 in the presence of 0.1 mM IPTG (■), and the growth of the control strain SØ5292 (○).

TABLE 4. EC_{50} values of inhibitory dUrd and dCyd analogues with SØ5338

Compound	EC_{50} (μM)
U2 dFdU	10
U7 AZT	10
U3 FIAU	4
U4 FMAU	1
C2 dFdC	5

Values are the means of results from two duplicate experiments.

were as follows: FMAU (U4) > FIAU (U3) > dFdC (C2) > dFdU (U2) = AZT (U7).

Phosphorylation of Analogues with Recombinant TK1, TK2, and dCK

All the compounds involved in this study were examined as substrates for purified TK2, TK1, and dCK, and earlier results [3–5, 13] as well as experiments carried out in this study are summarized in Table 5. Purified TK2 phosphorylates these compounds in the order 5FdU > FMAU > FIAU > 5FdC > dFdU = dFdC = AraC > AraT > AraU, while D4T and 3TC were very poor substrates.

DISCUSSION

5-Fluorouracil was designed for use as an antineoplastic agent [14], and 5FdU is one of its metabolites *in vivo* [15]. In cells, 5FdU has several metabolic routes. It is mainly phosphorylated by TK1 and TK2 to form 5FdU monophosphate, which can bind tightly to thymidylate synthase and inhibit the enzyme, thus impeding the transformation of dUMP to dTMP [15, 16]. 5FdU can also be converted to 5-fluorouracil through a reversible reaction catalyzed by thymidine phosphorylase or, less efficiently, uridine phosphorylase [17]. Finally, 5FdU can be phosphorylated to the triphosphate level and be incorporated into DNA [15, 16]. In the mutant strain SØ5338, bacterial TK, thymidine phosphorylase, and uridine phosphorylase all were deactivated by mutations before the TK2 cDNA was introduced. In a previous study, we showed that 5FdU inhibited growth of an *E. coli* strain expressing human TK1, as well as its control strain, and the presence of Urd reversed the toxic effect on the latter but not on the former. This was most likely due to the fact that the phosphorylation of 5FdU occurring via uridine kinase was blocked by the addition of Urd [7]. In enzymatic assays, 5FdU is a better substrate for TK2 than Thd [4]. When 5FdU (U1) was tested with SØ5338 which expresses TK2, however, its inhibitory effect was much weaker than that on the strain expressing human TK1. The effect of 5FdU at 0.1 μM on SØ5338 and its control strain SØ5286 was barely distinguishable. Both strains were inhibited equally with an increased concentration of 5FdU. Furthermore, the sensitivity of SØ5338 was lower than that of strains expressing TK of bacterial origin

TABLE 5. Phosphorylation of nucleoside analogues with purified TK1, TK2, and dCK

Substrate	TK1	TK2	dCK
Thd	1 (2.1 ± 0.4)	1 (1 ± 0.12)	0.1 (272 ± 88)
dCyd	<0.001	0.67 (6.9 ± 0.2)	1 (6.3 ± 2.5)
U-1 5FdU	0.95* (2.2†)	2.3‡ (0.7†)	ND
U-2 dFdU	<0.001	0.6‡ (12)	ND
U-3 FIAU	0.76 (140 ± 38)§	1.3 (4 ± 0.6)§	ND
U-4 FMAU	0.48 (33 ± 1.5)§	1.7 (7 ± 0.2)§	ND
U-5 AraU	<0.001	0.19‡	<0.001*
U-6 AraT	<0.001*	0.5‡ (16)	<0.001
U-7 AZT	0.52 (0.6)†	0.04 (33)	<0.001*
U-8 D4T	0.07	0.01	<0.001
C-1 5FdC	<0.001*	0.9	0.2*
C-2 dFdC	<0.001	0.6 (16)	0.17 (4.3)
C-3 AraC	<0.001*	0.65 (820)	1.2*
C-4 3TC	<0.001	0.003	0.57 (3.5 ± 1.2)

Results were achieved by assays with ATP and nucleoside compounds both at 100 μM, unless indicated otherwise (ND = not determined). Values in bold are relative phosphorylation rates compared to the activities with Thd (for TK1 and TK2) or dCyd (for dCK), and values in parentheses are apparent K_m values in μM.

*Data from Eriksson *et al.*, Ref. [13].

†Data from Munch-Petersen *et al.*, Ref. [3]. The assays were carried out with 2.5 mM ATP and 10 μM nucleoside compounds.

‡Data from Wang *et al.*, Ref. [4].

§Data from Wang and Eriksson, Ref. [5].

(results not shown), although IPTG-induced SØ5338 showed higher TK activity (Table 2). We presently cannot find a satisfactory explanation for this observation, and it is possible that there are unknown metabolic routes for 5FdU activation in this cell system. The low sensitivity of SØ5338 to 5FdU may also be partially due to the low expression of TK2 in the bacteria compared to expression of TK1 in SØ5288 [7].

Both SØ5338 and SØ5292 were inhibited by 5FdC (C1), suggesting that in *E. coli* this compound might have different anabolic routes, possibly resembling the fate of 5FdU after the action of cytidine/deoxycytidine deaminase. None of the arabinosyl nucleosides displayed any significant effect towards SØ5338, although AraC, AraU, and AraT all serve as substrates for TK2 [4]. This appeared to be similar to the situation with *E. coli* expressing human dCK [7], which was also resistant to arabinosyl analogues. The insensitivity of SØ5338 to this group of analogues is most likely due to poor uptake in bacteria of compounds with this sugar moiety [18]. The cellular kinase responsible for phosphorylating D4T (U8) is unknown, but this compound is a good inhibitor of both TK1 and TK2 [3, 19], and it showed fair activity with TK1 in our enzymatic assay (Table 5). To our knowledge, this is the first observation where a pure preparation of TK1 has been able to phosphorylate D4T, and the result is in contrast to earlier studies with highly purified leukemic TK1 [3]. 3TC (C4) is an important anti-human immunodeficiency virus (HIV) drug, and its unnatural β-L-configuration inspired a new field of antiviral and antitumor drug development. Cellular dCK is the enzyme responsible for its activation in cells [20]. Both D4T and 3TC were not inhibitory to SØ5338.

The efficiency of the pyrimidine nucleoside analogues in selectively inhibiting the growth of SØ5338 is in the order FMAU > FIAU > dFdC > dFdU = AZT, while the phosphorylation rate of those compounds with purified recombinant TK2 is in the order 5FdU > FMAU > FIAU > 5FdC > dFdU = dFdC = AraC > AraT > AraU. For compounds found to be active in both systems, a good correlation was observed. FIAU (U3) is an effective compound against hepatitis B virus, but severe toxicity was found with patients treated with this drug [21], a possible reason for this being the activation in mitochondria. FMAU (U4) is one of its metabolites in cells. In enzymatic assays with purified TK1 and TK2, FMAU was a better substrate than FIAU for TK1, while both are phosphorylated by TK2 with similar efficiency [5]. When they were tested with recombinant *E. coli* expressing human TK1, 10% and 28% inhibition was observed with FIAU and FMAU, respectively, at 20 μM. In the present study, 20 μM FMAU showed 54% inhibition of SØ5338, and this effect was roughly 2-fold as high as the effect of FIAU at the same concentration. The former had an EC_{50} value 4-fold lower than the latter. dFdU (U2) is known to be a good substrate for TK2 [4], and dFdC (C1) has been used as an antitumor agent. In cells, dCK is the enzyme that initiates the activation of dFdC [22]. Although purified TK2 is able to phosphorylate dFdC to a substantial extent, no TK2-dependent toxicity has been described to this point. AZT (U7) has been extensively used for anti-HIV chemotherapy. Although TK1 is the main enzyme activating AZT, TK2 has also been found to phosphorylate this compound [23]. Generally, our observation is the first direct proof for a role of TK2 in activating growth-inhibiting pyrimidine nucleoside analogues.

A similar system expressing human dCK and TK1 was described earlier [7]. There also exist other reports in which *E. coli* was engineered to express viral TK for antiviral drug development [e.g. 24]. Because of the difference in nucleoside anabolism between *E. coli* and mammalian cells,

including the transmembrane transport of nucleosides, the bacterial systems have several limitations. Nevertheless, this work showed that selective inhibition of the growth of SØ5338 caused by a group of pyrimidine nucleoside analogues had a relatively good correlation to the phosphorylation of TK2 with those compounds. In combination with the strains expressing TK1 and dCK, this bacterial system may help in evaluating the activity of nucleosides and defining the differences in DNA precursor metabolism between *E. coli* and mammalian cells. Further work will evaluate if this model system can also be used to predict the mitochondrial toxicity of pyrimidine deoxynucleosides.

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