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Metabolism of 9-β-D-Xylofuranosyladenine by the Chinese Hamster Ovary Cell

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SUMMARY

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The uptake, phosphorylation, and biological half-life of the purine nucleoside analogue 9-β-D-xylofuranosyladenine (xyl-A) was studied in wild-type Chinese hamster ovary cells and in nucleoside kinase-deficient mutants. It was found that [^{3}H]xyl-A and [^{3}H]adenosine were readily phosphorylated to the triphosphate level in both the wild-type and deoxycytidine kinase (EC 2.7.1.74)-deficient mutant, but neither of these adenine nucleosides was phosphorylated by the adenosine kinase (EC 2.7.1.20)-deficient cells. The reproductive capacity of wild-type and deoxycytidine kinase deficient cells was inhibited 50% by 3 and 4 μM xyl-A, respectively, whereas cells deficient in adenosine kinase were resistant to 100 μM xyl-A. Cellular uptake of xyl-A into the wild-type cells was followed through 6 hr of incubation. Values for the apparent K_m and V_{max} of this uptake process were 43.9 μM and 118.7 nmoles/min/10^9 cells, respectively. The major intracellular metabolite of xyl-A, the 5′-triphosphate xyl-ATP, accumulated to a 3.6-fold higher concentration than xyl-ADP, with very little xyl-AMP detected. The biological half-life of xyl-ATP was 5.1 hr, significantly longer than the congener analogue, 9-β-D-arabinofuranosyladenine 5′-triphosphate, in the same cell line. These results demonstrate in a single cell line that xyl-A does not produce cytotoxicity as a free nucleoside; phosphorylation to the nucleoside 5′-triphosphate, an activating pathway initiated by adenosine kinase, is required for activity of the compound.

INTRODUCTION

The adenine nucleoside analog, xyl-A,[4] produces inhibitory effects on a variety of metabolic reactions which result in the loss of viability of mammalian cells in culture. These actions, studied in several cell types, include the inhibition of methylation of RNA (1, 2), a decrease in the phosphorylation of non-histone chromosomal proteins (3), disruption of the synthesis of 5-phosphoribosyl-1-pyrophosphate (4), and inhibition of nucleic acid synthesis (5, 6). The nature of the processes affected suggests that the inhibitory action of xyl-A may be elicited after phosphorylation to the 5′-triphosphate, xyl-ATP, which may act directly as an analogue of ATP.

Recent efforts of this laboratory have been directed toward a biochemical evaluation of the mechanism of cytotoxicity of xyl-A to CHO cells. It was shown that, after a brief incubation with xyl-A, cells destined to lose reproductive capacity had accumulated high intracellular concentrations of xyl-ATP and experienced a rapid onset of inhibition of RNA and DNA synthesis. During these events, intracellular pool levels of 5-phosphoribosyl-1-pyrophosphate, ribonucleoside 5′-triphosphates, and deoxyribonucleoside 5′-triphosphates were not significantly different from those of control cells. These results indicated that the mechanism of toxicity of xyl-A was

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[4] The abbreviations used are: xyl-A, 9-β-D-xylofuranosyladenine; xyl-ATP, -ADP, and -AMP, 9-β-D-xylofuranosyladenine 5′-triphosphate, diphosphate, and monophosphate, respectively; CHO cell, Chinese hamster ovary cell; EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine; CHO-LA cells, CHO cells obtained from Dr. R. A. Tobey (Los Alamos, N. M.); ribavirin, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide; ara-C, 1-β-D-arabinofuranosylcytosine; HPLC, high-pressure liquid chromatography; AK$^-$, adenosine kinase-deficient; dCK$^-$, deoxycytidine kinase-deficient; ara-ATP, 9-β-D-arabinofuranosyladenine 5′-triphosphate; ara-A, 9-β-D-arabinofuranosyladenine; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosyl-L-methionine.

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associated with the inhibition of nucleic acid synthesis by xyl-ATP (7).

The present studies have used CHO cell lines bearing specific nucleoside kinase deficiencies to investigate the route of phosphorylation of xyl-A. The kinetics and quantitation of intracellular accumulation of xylosyl nucleotides and the cellular degradation of xyl-ATP have been determined. These studies demonstrate in the intact CHO cell that adenosine kinase is responsible for the initial phosphorylation of xyl-A and that cytotoxic effects are correlated with the intracellular accumulation of xyl-ATP. A preliminary report of these findings has been presented (8).

MATERIALS AND METHODS

Materials. The compound xyl-A (NSC-7359) and the adenosine deaminase inhibitor EHNA (9) were provided by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md.). ^{3}H-Labeled xyl-A (95 mCi/mmole), prepared by ICN Pharmaceuticals (Irvine, Calif.), was purified to greater than 99% by high-pressure liquid chromatographic methods, as previously described (7). [5-^{3}H]Deoxycytidine (11 Ci/mmol) and [8-^{3}H]adenosine (23 Ci/mmole) were obtained from ICN Pharmaceuticals. All other chemicals were reagent grade.

Cell cultures. Metabolism studies were performed with CHO-LA cells adapted to suspension culture. Drug-resistant lines were derived from CHO cells originally obtained from Dr. P. N. Rao of the Department of Developmental Therapeutics, The University of Texas System Cancer Center. All long-term cultures were maintained attached to 25-cm^2 plastic flasks (Corning Glass Works, Corning, N. Y.) in McCoy's 5a medium supplemented with 10% fetal bovine serum (Grand Island Biological Company, Grand Island, N. Y.) without antibiotics. For suspension cultures, CHO-LA cells were trypsinized and suspended into McCoy's 5a medium modified for suspension culture. Because of its low adenosine deaminase activity, horse serum (20% final concentration, from Grand Island Biological Company) was used to supplement the medium in these cultures.

Drug-resistant cell lines. Conventional methods (10) were utilized for mutagenesis and subsequent isolation of drug-resistant CHO cell clones. Cell line Rb^{R-1} (Table 1) was selected with 200 μM ribavirin and found to be deficient in adenosine kinase. A deoxycytidine kinase-deficient clone (aC^{R-7}) was selected with 10 μM ara-C. Both of these cell lines are stable and were maintained in the absence of drugs.

TABLE 1

Enzyme deficiencies of variant CHO cell lines

CHO cell lines selected for two enzyme deficiencies by conventional procedures were assayed for specific activity of nucleoside kinases as described under Materials and Methods.

Cell line	Selective agent	Specific activity[a]	
		AK	dCK
CHO	—	1.12	0.023
Rb^{R-1} (AK$^-$)	Ribavirin	< 0.01	0.029
aC^{R-7} (dCK$^-$)	ara-C	1.19	0.005

[a] Specific activity expressed as nanomoles of product formed per minute per milligram of protein.

Enzyme determinations. Adenosine kinase activity was measured by the method of Hershfield *et al.* (11). Incubation mixtures for deoxycytidine kinase contained 5 mM ATP (neutralized), 50 μM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 25 μM [8-^{3}H]deoxycytidine (4 Ci/mmol), and cell extract in a final volume of 100 μl. At 10-min intervals, 10-μl samples were removed, applied to DE81 paper circles (Whatman Inc., Clifton, N. J.) that were washed three times with 1 mM ammonium formate, once with H_2O, and once with ethanol, dried, and placed in vials containing 1 ml of 1 N HCl. After 10 min, 11 ml of Aquasol (New England Nuclear Corporation, Boston, Mass.) were added and the solution was mixed and counted. Protein was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.), a dye-binding procedure (12), using bovine serum albumin as standard. Specific activities are expressed as nanomoles of product formed per minute per milligram of protein.

Determination of drug resistance. Resistance was determined by allowing clone formation in the presence of drug. Exponentially growing cells of each line were diluted and plated into 60-mm culture dishes at densities sufficient to provide growth of 50–200 colonies per dish. McCoy's 5a medium supplemented with 10% fetal calf serum and varying concentrations of xyl-A (1–100 μM) were added to each dish. After 6 days of incubation at 37° in a humid 5% CO_2:95% air atmosphere, the colonies were stained with 1% cystal violet and colonies of greater than 50 cells were recorded. Cloning efficiency of the cell lines incubated in drug-free medium were: CHO-LA, 68%; Rb^{R-1}, 50%; and aC^{R-7}, 63%.

HPLC. A Waters Associates (Milford, Mass.) ALC-204 high-pressure liquid chromatograph equipped with two Model 6000A pumps, a Model 660 solvent programmer, and a column of Partisil-10 SAX anion-exchange resin (25 cm × 4.6 mm, Whatman Associates) were used to separate cellular nucleotide components. Nucleotides were quantitated by UV absorbance at 254 nm with Waters' Model 440 absorbance detector. Chromatograms were recorded and peak areas were integrated by using Waters' Model 730 Data Module programmed with predetermined calibration curves for each nucleotide component. Quantitation of xyl-ATP was conducted by absorbance at 254 nm using the same extinction coefficient as that for ATP (14.5×10^3), which was used as standard. [^{3}H]xyl-ATP was also measured by liquid scintillation using the specific activity of the chromatographically pure [^{3}H]xyl-A as reference. Variation between these two methods for determining intracellular xyl-ATP concentration was less than 10%.

Two solvent systems were used to separate xylosyl nucleotides from endogenous nucleotide components. Solvent System 1, for rapidly measuring nucleoside 5′-triphosphates, [^{3}H]dCTP and xyl-ATP (7), employed a concave gradient (Line 7 on the 660 programmer) from 0.30 M $NH_4H_2PO_4$ (pH 3.7) to 0.75 M $NH_4H_2PO_4$ (pH 3.7) at 2 ml/min, over 30 min. Solvent System 2, for determining the intracellular levels of the nucleoside mono-, di-, and triphosphates, used a linear gradient (Line 6 on the 660 programmer) from 0.005 M $NH_4H_2PO_4$ (pH 2.8)

to 0.75 M $NH_4H_2PO_4$ (pH 3.7) at 2 ml/min, over 40 min (13).

Phosphorylation of nucleosides by mutant cell lines. The wild-type, AK^-, and dCK^- cells were assessed for their ability to phosphorylate xyl-A, adenosine, and deoxycytidine in the following manner. Exponentially growing cells attached to 25-cm^2 culture flasks were incubated with 10 μM concentration of [^{3}H]xyl-A, (7.98×10^4 cpm/nmole), [^{3}H]adenosine, (3.48×10^4 cpm/nmole), or [^{3}H]deoxycytidine (2.08×10^6 cpm/nmole) for 1 hr. The cells were then washed twice with cold (4°) phosphate-buffered saline[5] to remove the labeled precursors, and nucleotides were extracted with $HClO_4$ as described elsewhere (7). For determining cell number, a parallel culture of each cell line was trypsinized and counted in an electronic particle counter (Model ZBI; Coulter Electronics, Hialeah, Fla.). The accumulation of radioactivity into the ATP, dCTP, and xyl-ATP pools from the respective nucleoside precursors was determined after fractionation of the acid-soluble extract by HPLC and by recovering the portion of the column eluate which corresponded to each compound for liquid scintillation counting.

Phosphorylation of xyl-A by wild-type cells and determination of the biological half-life of xyl-ATP. Exponentially growing CHO-LA cells in suspension culture were incubated with five concentrations of xyl-A plus 10 μM EHNA, a nontoxic concentration of the adenosine deaminase inhibitor (7). At 30-min intervals after drug addition, a portion of the cell suspension was removed for cell count and size determination (Model C-1000 channelyzer; Coulter Electronics) and $HClO_4$ extraction. The acid-soluble supernatant from a known volume of cells was analyzed by HPLC for xyl-ATP. The intracellular concentration was computed by dividing the number of nanomoles of xyl-ATP by a divisor that included a factor for the cell volume and cell number.

The biological half-life of xyl-ATP was determined in suspension culture by incubating exponentially growing cells with 10 μM xyl-A plus 10 μM EHNA for 30 min. The cells were then removed from the drug-containing medium by centrifugation, and resuspended in drug-free medium. At intervals thereafter, a portion of the culture was removed and extracted with $HClO_4$, and the intracellular concentration of xyl-ATP was determined as described above. The biological half-life of xyl-ATP was determined by exponential regression analysis.

RESULTS

Toxicity of xyl-A to kinase-deficient cells. The effect of continuous incubation with xyl-A on wild-type, AK^-, and dCK^- CHO cell reproductive capacity is shown in Fig. 1. The wild-type CHO cell, which readily accumulates high concentrations of xyl-ATP (7), was extremely susceptible to low levels of this toxic nucleoside (ID_{50} = 3 μM). In a separate experiment (not shown), the wild-type cells were unable to form clones in 3 μM xyl-A when horse serum was present. Because horse serum is noted for its lack of adenosine deaminase activity, this shift in the dose-response curve suggests that some of the xyl-A used in the experiments presented in Fig. 1 was deaminated by adenosine deaminase in fetal calf serum. The

[5] Composition: 8.1 g of NaCl, 0.22 g of KCl, 1.14 g of Na_2HPO_4 per liter of water, pH 7.4.

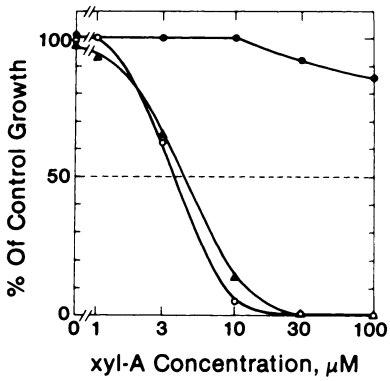

FIG. 1. *Effect of xyl-A on the clonability of wild-type, AK^-, and dCK^- cell lines*

Cells of each strain were incubated continuously with the indicated doses of xyl-A for 6 days. ○, Wild-type CHO; ▲, dCK^-; ●, AK^-.

AK^- cell line was very resistant to xyl-A, having a cloning efficiency that was only slightly reduced when the concentration of xyl-A in the medium was 100 μM. The dCK^- cell line had a sensitivity to xyl-A similar to the wild-type CHO with an ID_{50} of 4 μM.

Phosphorylation of nucleosides by CHO cell lines. The adenosine kinase and deoxycytidine kinase activity in cell extracts of wild-type, AK^-, and dCK^- cells is presented in Table 1. The wild-type parent CHO cell had enzyme specific activities of 1.12 nmoles/min/mg of protein for adenosine kinase and 0.023 nmole/min/mg of protein for deoxycytidine kinase. Rb^{R-1}, an AK^- cell selected with ribavirin, had negligible adenosine kinase activity, but normal deoxycytidine kinase activity. Conversely, aC^{R-7}, selected for the dCK^- phenotype, had a normal adenosine kinase specific activity, but significantly lower deoxycytidine kinase activity.

The ability of wild-type, AK^-, and dCK^- cells to accumulate [^{3}H]adenosine, [^{3}H]deoxycytidine, and [^{3}H]xyl-A into the respective cellular pools of ATP, dCTP, and xyl-ATP is shown in Table 2. When a 10 μM exogenous concentration of each labeled nucleoside was incubated with attached wild-type cells for 1 hr, 1221 nmoles of [^{3}H]adenosine per 10^9 cells was found in the acid-soluble pool of ATP while 10.9 nmoles of [^{3}H]deoxycytidine per 10^9 cells was found in the dCTP pool. In the AK^- cells, an amount of [^{3}H]deoxycytidine comparable with that of

TABLE 2

Anabolism of nucleosides by CHO kinase-deficient mutants

Cells of each line were incubated with the indicated ^{3}H-labeled nucleoside (10 μM) in the presence of 10 μM EHNA. After 1-hr incubation at 37°, an acid-soluble extract of the cells was obtained and assayed for labeled nucleoside triphosphates by using HPLC Solvent System 1 (Materials and Methods). Kinase activity is expressed in nanomoles of labeled nucleoside accumulated in the respective nucleoside triphosphate pool of 10^9 cells in 1 hr.

Cell line	Nucleoside substrate		
	Adenosine	Deoxycytidine	xyl-A
CHO	1221	10.9	1930
Rb^{R-1} (AK^-)	6[a]	17.8	5
aC^{R-7} (dCK^-)	1211	0.3	1465

[a] Lower limit of detection; no radioactivity detected above background counts.

the wild-type cell accumulated in the dCTP pool, while no radioactivity derived from [^{3}H]adenosine became associated with the ATP. The dCK$^-$ cell line accumulated an equivalent amount of [^{3}H]ATP when incubated with [^{3}H]adenosine, but very little radioactivity was found in that portion of the chromatogram corresponding to the elution time for dCTP after incubation with [^{3}H]deoxycytidine. The radioactivity associated with the labeled acid-insoluble material of wild-type and AK$^-$ cells incubated with [^{3}H]deoxycytidine was very similar, whereas that accumulated by dCK$^-$ cells was 10-fold lower (not shown).

The phosphorylation of [^{3}H]xyl-A is also shown in Table 2. Wild-type CHO cells and the dCK$^-$ mutant accumulated 1930 and 1465 nmoles of [^{3}H]xyl-ATP per 10^9 cells, respectively, in 1 hr, but the AK$^-$ cell line failed to accumulate xyl-ATP to any measurable extent. By using HPLC Solvent System 2, we were able to determine the relative amount of xyl-A nucleotides formed in the wild-type cell compared with the AK$^-$ cell. The *open rectangles* in Fig. 2 illustrate the distribution of radioactivity among the three levels of nucleoside phosphorylation after separation of a wild-type cell extract by HPLC. Radioactive compounds eluting at 15.5 and 32.0 min have specific activities of 5.7×10^4 and 8.8×10^4 cpm/nmole, which correspond to xyl-ADP and xyl-ATP, respectively. These compare well with the specific activity of exogenous xyl-A, which was 8.0×10^4 cpm/nmole. The value of intracellular xyl-ATP/xyl-ADP favored the triphosphate (3.6:1); very little [^{3}H]xyl-AMP (6 min) was detected. The radioactivity derived from [^{3}H]xyl-A associated with ADP and ATP in wild-type cells may have arisen from salvage of [^{3}H]hypoxanthine liberated after deamination of [^{3}H]xyl-AMP and subsequent dephosphorylation and phosphorolysis by purine-nucleoside phosphorylase (EC 2.4.2.1). [^{3}H]Xylosylhypoxanthine may have been generated to a much smaller extent, due to incomplete inactivation of adenosine deaminase (EC 3.5.4.4) by EHNA. The latter possibility provides an explanation for the presence of small amounts of ^{3}H associated only with the adenine nucleotides in AK$^-$ cells after incubation with [^{3}H]xyl-A (Fig. 2).

The *solid rectangles* superimposed upon the *open rectangles* in Fig. 2 represent the distribution of radioactivity in the nucleotides of the AK$^-$ cell line incubated with [^{3}H]xyl-A for 1 hr. No UV absorbance of the cell extract from AK$^-$ cells is presented to promote clarity, but the retention times of endogenous nucleotides were identical in both chromatograms. No radioactivity above background was observed in either the xyl-ADP or the xyl-ATP region of the chromatogram. Assuming a maximum of 30 cpm in the xyl-ATP fraction, the amount equivalent to background, the maximal amount of [^{3}H]xyl-ATP which could be present is 0.6 nmoles/10^9 cells.

Uptake kinetics of xyl-A in the CHO cell. The kinetics of uptake of xyl-A into exponentially growing wild-type CHO cells was studied. Cells in suspension were incubated with concentrations of xyl-A from 1 μM to 100 μM for up to 6 hr. At ½-hour intervals, a portion of the cell suspension was removed, extracted with $HClO_4$, and analyzed for intracellular xyl-ATP concentration by using HPLC Solvent System 1. The accumulation of xyl-ATP through 2 hr of incubation is shown in Fig. 3. A double-reciprocal kinetic plot of the xyl-A uptake through 30

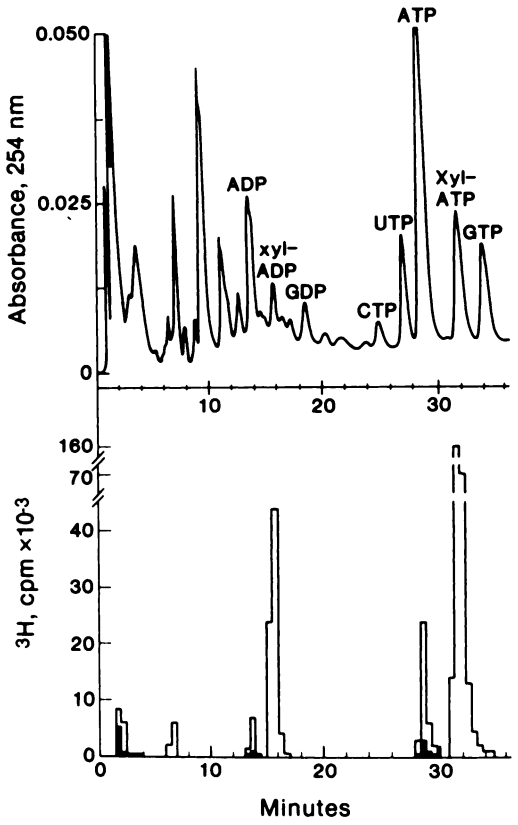

FIG. 2. *Accumulation of labeled nucleotides in wild-type and adenosine kinase-deficient cells from [^{3}H]xyl-A*

Cells of each line were incubated with 10 μM [^{3}H]xyl-A plus 10 μM EHNA for 1 hr prior to extraction with $HClO_4$. The nucleotides in the acid-soluble material of 1.2×10^7 wild-type cells were separated by using HPLC Solvent System 2 (Materials and Methods) with the UV absorbance presented in the upper portion of the figure and the radioactive fractions represented as the *open rectangles* below. The acid-soluble nucleotides from a comparable number of cell equivalents of an adenosine kinase-deficient line were also separated by HPLC with the radioactive fractions (*solid rectangles*) superimposed upon the *open rectangles* in the lower portion of the figure.

min produced an apparent K_m value of 43.9 μM and an apparent V_{max} of 118.7 nmoles/min/10^9 cells. The kinetic data for xyl-A was also plotted according to the procedure described by Eisenthal and Cornish-Bowden (14) (not shown). The apparent K_m value of 28 μM and apparent V_{max} value of 74 nmoles/min/10^9 cells approximate the values obtained from the double-reciprocal plot. The contributions of transport and nonmediated permeation of nucleosides to over-all uptake and subsequent phosphorylation have been critically reviewed (15, 16).

Biological half-life of xyl-ATP. Information concerning the biochemical fate of xyl-ATP was obtained by a determination of the biological half-life of the drug. Exponentially growing CHO cells in suspension were incubated with 10 μM xyl-A for 30 min, which afforded an intracellular accumulation of xyl-ATP in excess of 500 μM. Cells were removed from medium containing xyl-A, resuspended in drug-free medium, and xyl-ATP concentrations were determined as described under Materials and Methods. The intracellular xyl-ATP concentrations determined during this washout procedure are illustrated in Fig. 4. xyl-ATP disappeared from the cells with first-order decay kinetics over a period of 38 hr with a half-life of 5.1 hr. This half-life was much longer than the 1.7

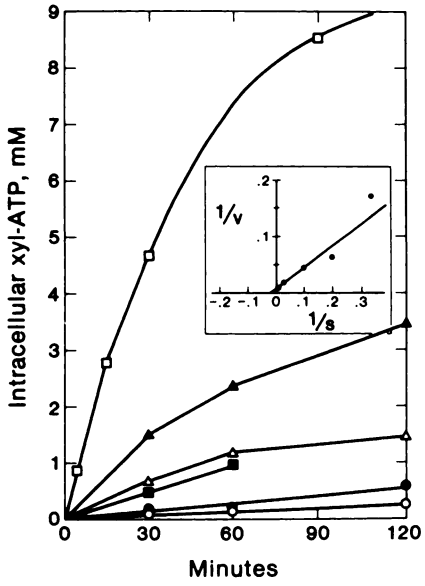

FIG. 3. *Uptake of xyl-A by wild-type CHO cells*

CHO cells in suspension were incubated with various concentrations of xyl-A plus EHNA (10 μM). At the times indicated, a portion of the cell suspension was extracted with $HClO_4$ and analyzed for xyl-ATP content by using HPLC Solvent System 1 (Materials and Methods). The velocity of xyl-ATP accumulation during the linear phase of uptake (through 30 min) was plotted on a double-reciprocal plot (*inset*). The point corresponding to 1 μM, though not shown, was used in the calculation of the kinetic parameters. The exogenous xyl-A concentrations shown are: ○, 1 μM; ●, 3 μM; ■, 5 μM; △, 10 μM; ▲, 33 μM; □, 100 μM.

hr reported for the disappearance of ara-ATP in the same cell line (17). Since the biological half-life of ara-ATP is independent of the presence of EHNA (17), it is reasonable to assume that the half-life of xyl-ATP would likewise be unaffected.

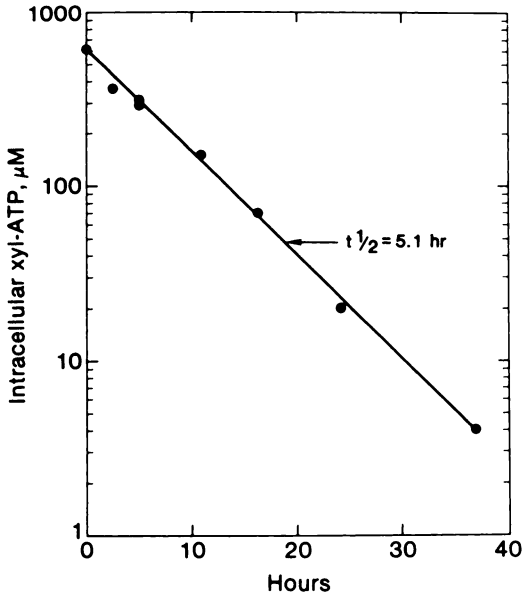

FIG. 4. *Biological half-life of xyl-ATP*

CHO cells were incubated with 10 μM EHNA for 30 min before drug removal. At the indicated times, 1–2 × 10^6 cells were extracted with $HClO_4$ and the xyl-ATP concentration was determined by HPLC Solvent System 1 (Materials and Methods).

DISCUSSION

The anabolic and catabolic routes of metabolism of the antineoplastic nucleoside analogue xyl-A were studied in the CHO cell and in two mutant cell lines deficient in adenosine kinase and deoxycytidine kinase activity. It was found that the AK^- cell was resistant to the toxic effect of xyl-A (Fig. 1) because of its inability to phosphorylate this nucleoside (Table 2; Fig. 2). Two significant conclusions may be drawn from these data. First, xyl-A must be phosphorylated to produce its characteristic toxicity. Both the wild-type CHO cell and the dCK^- mutant accumulated xylosyl nucleotides, and both were restricted in their reproductive ability; the AK^- cell was resistant to 100 μM xyl-A. 2′-Deoxyadenosine and ara-A are known to produce a nucleotide-independent toxicity by inhibiting *S*-adenosylhomocysteinase (EC 3.3.1.1.), facilitating the intracellular accumulation of SAH, which inhibits various methylation reactions in the cell (18–20). A CHO cell mutant resistant to high levels of adenosine has been described that is not resistant to low levels of xyl-A (21). This is in agreement with our findings that xyl-A does not produce toxicity as a free nucleoside. A recent report concerning the mechanism of "suicide inactivation" of *S*-adenosylhomocysteinase implicated a steric requirement of a β-3′ hydroxyl group for keto formation with the resultant *trans* elimination of adenine from 2′-deoxyadenosine (22). Consistent with the hypothesis that a β-configuration of the 3′ hydroxyl group of adenine nucleosides is required for the inactivation of *S*-adenylhomocysteinase and because xyl-A does not produce cytotoxicity as a free nucleoside (Fig. 1; Table 2), it is likely that xyl-A does not function significantly as an inhibitor of *S*-adenosylhomocysteinase. The steric configuration of the 3′ hydroxyl group may not be the only determinant of inhibition potential, since modifications of the purine base can also induce higher intracellular concentrations of SAH (23). Furthermore, the lack of toxicity to cells unable to phosphorylate xyl-A also suggests that the observed inhibition of nuclear RNA methylation by xyl-A (1) is unlikely to result from a mechanism involving the inhibition of *S*-adenosylhomocysteinase with subsequent interference in SAM-mediated methylation reactions by accumulated SAH (24). Rather, the observed inhibitory effects of xyl-A on the incorporation of methionine into SAM (2) and the accompanying inhibition of nuclear RNA methylation (1) are more likely to result from the inhibition of SAM formation by the phosphorylated metabolite, xyl-ATP.

The second conclusion is that xyl-A is phosphorylated by adenosine kinase. Both the wild-type cell and the mutant lacking deoxycytidine kinase phosphorylated xyl-A, yet the mutant lacking adenosine kinase was unable to phosphorylate the nucleoside. Because the ratio of xyl-ATP to the other phosphorylated forms of xyl-A was high, it was reasoned that the toxicity of xyl-A in the CHO cell was primarily produced by xyl-ATP. The compound xyl-ATP was also found to be the major metabolite of xyl-A in the TA3 ascites tumor (25). The relative contribution of transport and subsequent phosphorylation to the uptake of nucleosides has been the topic of extensive experimentation and mathematical modeling (15, 16). There is considerable evidence that uptake

studies, such as that presented in Fig. 3, primarily reflect the *in situ* activity of the nucleoside kinase as opposed to the transport enzyme. Therefore, the apparent K_m for uptake of xyl-A by the CHO cell, 43.9 μM, may be a reasonable estimate of the substrate activity of xyl-A for CHO adenosine kinase in intact cells. Purified adenosine kinase from rabbit liver has an 86 μM K_m for xyl-A (26).

The biological half-life of xyl-ATP in the CHO cell, 5.1 hr (Fig. 4), is much longer than that of the congener nucleotide analogue, ara-ATP, in the same cell line (17). Although xyl-ATP might function only as an analogue of ATP, whereas ara-ATP may also act as a dATP analogue, the long biological half-life of xyl-ATP probably is a complex function of its inability to substitute for ATP in a multitude of cellular reactions and the high efficiency with which degradation products are rephosphorylated. The α-configuration of the 3′ hydroxyl moiety of xyl-ATP makes it most unlikely that the analogue triphosphate would be incorporated into the internal phosphodiester linkages of nucleic acids. However, a role as a possible terminator of RNA chains or of the RNA primers required for nascent DNA synthesis is in accord with the accumulated evidence. A chain-terminating inhibitor acting on both RNA and DNA that accumulates to high cellular concentrations and disappears slowly might be expected to produce sustained inhibition of these processes. Such an inhibition, characterized by a lack of recovery over a period of two cell cycle passages, has been observed (8) and will be the subject of a subsequent publication.

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