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EFFECT OF 2′-DEOXYCOFORMYCIN ON THE INHIBITION OF DEOXYRIBONUCLEIC ACID SYNTHESIS BY 9-β-D-ARABINOFURANOSYLADENINE 5′-TRIPHOSPHATE*

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Abstract—A biochemical basis for the increased cytotoxicity of 9-β-D-arabinofuranosyladenine (ara-A) in the presence of 2′-deoxycoformycin (dCF), a potent inhibitor of adenosine deaminase, has been investigated in CHO cells. Ten micromolar dCF was not toxic to this cell line, and it effected a 7-fold increase in the cytotoxicity of 50 μM ara-A during a 24-hr incubation. In the absence of dCF, CHO cells deaminated more than 85% of the initial amount of ara-A within 3 hr after drug addition. 9-β-D-Arabinofuranosyladenine 5′-triphosphate (ara-ATP) accumulated in these cells for only 6 hr to 150 μM, after which time the cellular ara-ATP concentration declined. At the conclusion of the incubation, no ara-ATP was detectable in the cells. In contrast, the presence of dCF maintained high levels of ara-A extracellularly and allowed a continuous intracellular accumulation of ara-ATP. It was demonstrated that the presence of dCF did not alter the relationship between the intracellular ara-ATP concentration and cellular DNA synthetic capacity (DSC). A concentration of 2.5 μM ara-ATP, accumulated during a 75-min drug incubation, was necessary to decrease DSC by 50%. The accumulation of more than 400 μM ara-ATP did not affect the intracellular concentrations of the four deoxyribonucleoside triphosphates. Furthermore, after a 3-hr incubation with ara-A alone or in the presence of dCF, DSC was inhibited more than 95% but increased substantially within 6 hr following drug washout. Recovery of DSC to more than 25% of the initial value corresponded to a decline in intracellular ara-ATP concentration to less than 35 μM. Thus, the presence of dCF can increase the duration of inhibition of DSC by allowing greater intracellular accumulation of ara-ATP, resulting in greater cell death.

9-β-D-Arabinofuranosyladenine (ara-A)§ is a purine nucleoside analog that has been clinically useful in the treatment of certain herpes virus infections [1, 2] and acute lymphoblastic leukemia [3]. However, the effectiveness of ara-A is severely limited by its rapid deamination to the relatively inactive compound, 9-β-D-arabinofuranosylhypoxanthine (ara-Hx), by the enzyme adenosine deaminase (ADA) [4, 5]. Co-administration of ara-A with an inhibitor of ADA, such as *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) [6] or 2′-deoxycoformycin (dCF) [7], synergistically increases it cytotoxicity *in vitro* [8, 9] and its activity against transplantable mouse tumors [8, 10–12]. Although both EHNA and dCF are potent inhibitors of ADA, the inhibition produced by dCF is of longer duration *in vitro* [13] and *in vivo* [14, 15]. The increased efficacy of ara-A in the presence of an inhibitor of ADA has prompted interest in clinical investigations using this drug combination [16].

Though the precise mechanism by which ara-A exerts its cytotoxic effects is not clear, it appears that the nucleoside analog must first be phosphorylated to its 5′-triphosphate, 9-β-D-arabinofuranosyladenine 5′-triphosphate (ara-ATP). Ara-ATP is a potent competitive inhibitor of DNA polymerase [17, 18], can inhibit ribonucleotide reductase [19], and is incorporated into DNA [20–22]. It is presently thought that the major effect contributing to the cytotoxicity of ara-A is the inhibition of DNA synthesis via ara-ATP [23].

Investigations into the mechanism by which deaminase inhibitors increase the potency of ara-A have demonstrated that higher ara-ATP concentrations are achieved in cells treated with ara-A in the presence of a deaminase inhibitor than in cells treated with ara-A alone, both *in vitro* [10] and *in vivo* [15, 24]. We have shown previously that the

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§ Abbreviations: ara-A, 9-β-D-arabinofuranosyladenine; ara-AMP, 9-β-D-arabinofuranosyladenine 5′-monophosphate; ara-ATP, 9-β-D-arabinofuranosyladenine 5′-triphosphate; ara-C, 1-β-D-arabinofuranosylcytosine; ara-Hx, 9-β-D-arabinofuranosylhypoxanthine; ADA, adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4); CHO cells, Chinese hamster ovary cells; dCF, (*R*)-3-(2-deoxy-β-D-*erythro*-pentafuranosyl) - 3,6,7,8 - tetrahydroimidazo-[4,5-*d*][1,3]diazepin-8-ol, 2′-deoxycoformycin; dNTP, deoxyribonucleoside triphosphate; dThd, thymidine; DSC, DNA synthetic capacity; HPLC, high pressure liquid chromatography; EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine; and PBS, phosphate-buffered saline.

biologic half-life of ara-ATP is not affected by the inhibition of ADA, but that dCF enhances the accumulation of ara-ATP from ara-A by maintaining high extracellular levels of ara-A as a substrate for subsequent cellular penetration and phosphorylation [25]. The experiments presented here extend these results by evaluating the enhanced toxicity of ara-A to CHO cells in the presence of dCF with regard to the effect of the ADA inhibitor on the cellular deamination and phosphorylation of ara-A. In addition, the effect of dCF on the relationship between the intracellular ara-ATP concentration and DNA synthetic capacity has been investigated to determine a biochemical basis for the enhancement of ara-A cytotoxicity by the deaminase inhibitor.

MATERIALS AND METHODS

Materials. All chemicals used were reagent grade. dCF, produced by Parke, Davis & Co. (Detroit, MI), and ara-A, a product of Pfanstiehl Laboratories (Waukegan, IL), were obtained through the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute. [2-^{3}H]Ara-A (19.8 Ci/mmole), [*methyl*-^{3}H]dThd (10 Ci/mmole) and [2-^{14}C]dThd (62 mCi/mmole) were purchased from ICN Pharmaceuticals, Inc. (Irvine, CA). The [^{3}H]ara-A was more than 98.0% pure as determined by thin-layer chromatography and high pressure liquid chromatography (HPLC). After recrystallization from H_2O, the purity was greater than 99.0%.

Cell culture methods. CHO cells were maintained in monolayer cultures in McCoy's modified 5a medium and supplemented with either 20% horse serum or 10% fetal calf serum (Grand Island Biological Co., Grand Island, NY). All experiments used exponentially growing cells that were detached with 0.05% trypsin and resuspended in McCoy's modified 5a medium for suspension culture supplemented with 20% horse serum. No detectable deamination of ara-A occurred in the culture medium containing horse serum. Cell number and cell volume were determined using a model ZBI electronic particle counter (Coulter Electronics, Inc., Hialeah, FL) equipped with a cell-sizing instrument (model C-1000) which was calibrated with latex beads 10.0 μm in diameter. The reproductive viability of the CHO cells was determined by cloning. Cells were harvested from the drug-containing medium and resuspended in fresh medium. After appropriate dilution, 1 ml of cell suspension was added to 4 ml of warm medium (containing either fetal calf or newborn calf serum) in 60 mm petri dishes in triplicate and incubated for 7 days, at which time the macroscopic colonies were counted.

Measurement of ara-A deamination. CHO cells were incubated with [^{3}H]ara-A in the absence or presence of 10 μM dCF. All cultures containing dCF were preincubated for at least 20 min with the deaminase inhibitor before the addition of ara-A to ensure complete enzyme inhibition, as suggested by the results of Agarwal *et al.* [26]. At various intervals, samples containing at least 1×10^6 cells were centrifuged, and the nucleosides in the medium were extracted with 0.4 N $HClO_4$ and neutralized with KOH [21]. A portion of the extract was added to non-radioactive ara-A and ara-Hx standards, placed on Avicel F thin-layer chromatography plates (Analtech, Inc., Newark, DE), and chromatographed in NH_4OH–H_2O saturated *n*-butanol (5.5:94.5) [27]. The areas containing ara-A or ara-Hx were detected under u.v. light (254 nm) and scraped into scintillation vials; the nucleosides were eluted with 0.01 N HCl for 1 hr. Eleven milliliters of Aquasol scintillation fluid (New England Nuclear Corp., Boston, MA) was added to each vial before counting in a Packard Tri-Carb scintillation spectrometer, model 2650. Quenching was automatically corrected with an external standard; counting efficiency for ^{3}H was 35%.

Quantitation of ara-ATP. After the appropriate incubation period with ara-A in the absence or presence of dCF, cells were harvested and extracted with $HClO_4$ as described previously [28]. The nucleotides in the neutralized acid-soluble fraction were analyzed by HPLC using a Waters Associates (Milford, MA) ALC-204 high pressure liquid chromatograph equipped with two model 6000A pumps, a model 660 gradient programmer and a column (250 × 4 mm) containing Partisil-10 SAX anion exchange resin (Whatman, Inc., Clifton, NJ). Samples of 0.05 to 1.0×10^7 cell equivalents were injected by means of the U6K-LC injection system. Nucleotides were eluted from the column initially with 50% 0.005 M $NH_4H_2PO_4$, pH 2.8, and 50% 0.750 M $NH_4H_2PO_4$, pH 3.7. The buffer concentration was increased to 100% 0.75 M $NH_4H_2PO_4$, pH 3.7, using a concave gradient (curve no. 8 on the solvent programmer) over a period of 30 min. Eluted compounds were detected by their absorbance at 254 nm by the model 440 detector and quantitated with a CDS-111 electronic integrator (Varian Associates, Palo Alto, CA). Peak areas were converted to nmole amounts using predetermined calibration curves. In addition, the [^{3}H]ara-ATP eluate was fractionated at 0.5-min intervals and collected into scintillation vials containing 0.3 ml H_2O and 11 ml Aquasol, and the radioactivity in each vial was determined by liquid scintillation counting. Knowledge of the amount of nucleotide extracted from a known number of cells of a mean volume determined at the time of sampling permitted the calculation of the concentration of each nucleotide in the cells.

Incorporation of [^{3}H]*dThd or* [^{14}C]*dThd into DNA.* To determine the amount of DNA synthesis at various times during drug exposure, CHO cells were incubated with [^{3}H]dThd for 15 min. In experiments in which [^{3}H]ara-A was used to allow accurate quantitation of less than 1.0 μM ara-ATP, [^{14}C]dThd was employed as the DNA precursor for measurement of DSC. Following the incubation with ^{3}H- or ^{14}C-labeled dThd, the cells were diluted, washed with PBS, and extracted with $HClO_4$ as described [28]. The acid-insoluble pellet was washed with 5.0 ml of 0.4 N $HClO_4$ and then resuspended in 1.5 ml of 0.001 N NaOH. Two drops of 1 N NaOH were added to completely solubilize the pellet. One ml of this fraction was added to a scintillation vial containing 11 ml Aquasol and the radioactivity was measured by liquid scintillation counting. The counting efficiency for ^{14}C was 89%.

Quantitation of intracellular deoxyribonucleoside

triphosphates (dNTP). CHO cells (2.0 to 3.0 × 10^7 cells per sample) were diluted, harvested by centrifugation, washed twice with ice-cold PBS, extracted with 0.4 N $HClO_4$, and neutralized as described previously [28]. After the ribonucleotides were destroyed by periodate oxidation [29], the dNTP were analyzed by HPLC. Samples (1.0 to 3.0 × 10^7 cell equivalents) were loaded onto an anion-exchange column, and periodate oxidation products, (nucleoside mono- and diphosphates) were eluted isocratically with 75% 0.005 M $NH_4H_2PO_4$ and 25% 0.75 M $NH_4H_2PO_4$ at a flow rate of 3 ml/min. After 20 min a concave gradient (curve no. 9 on the solvent programmer) which increased the buffer concentration to 95% 0.75 M $NH_4H_2PO_4$ was run over a period of 30 min. dCTP, dTTP and dGTP were detected and quantitated by their absorbance at 254 nm. Since ara-ATP was resistant to periodate oxidation and coeluted with dATP, the eluted fractions containing these compounds were collected, dephosphorylated, and the resulting ara-A and dAdo were easily separated by reverse phase HPLC. The column eluate containing ara-ATP and dATP was combined with 2 nmoles GTP, added as an internal standard, and the nucleotides were adsorbed onto activated charcoal, which was washed free of ammonium phosphate with H_2O. The triphosphates were then eluted with a mixture of CH_3CH_2OH–NH_4OH–H_2O (65:2:33), reduced to dryness, and dissolved in 1 ml of Tris buffer (50 mM Tris-HCl, pH 8.5, and 5 mM $MgCl_2$). One microliter of alkaline phosphatase (20 units/ml) was added and dephosphorylation proceeded for 30 min at 37°. The reaction was terminated by heating in a boiling water bath. The resulting nucleosides were separated by HPLC on a C_{18} μBondapak column (Waters Associates), using isocratic elution with 10% CH_3OH at a flow rate of 2 ml/min. Recovery of nucleotides from charcoal was greater than 80%, as determined by the recovery of a known amount of [^{3}H]dATP after adsorption onto the charcoal in control experiments. The quantitation of deoxyadenosine in each sample was corrected for the amount of the total sample recovered, as determined by the recovery of guanosine from the dephosphorylation of GTP.

RESULTS

The effect of dCF on the cytotoxicity of ara-A was studied, as illustrated in Fig. 1. CHO cells were incubated for 24 hr with 50 μM ara-A in the absence or presence of 10 μM dCF. The deaminase inhibitor alone produced no decrease in cell viability, but it enhanced the cytotoxicity of ara-A more than 7-fold. The effect of dCF on the deamination of ara-A and on the extent of accumulation of ara-ATP was also investigated during this experiment. When CHO cells were incubated with ara-A alone, more than 85% of the nucleoside analog was deaminated during the first 3 hr of incubation (Fig. 2A). Measurement of the corresponding intracellular ara-ATP concentrations demonstrated that the amount of ara-ATP in the cells increased during the first 6 hr after drug addition to a maximum concentration of 154 μM, but this level of the triphosphate decreased to less than 50 μM by 12 hr, when more than 92% of the

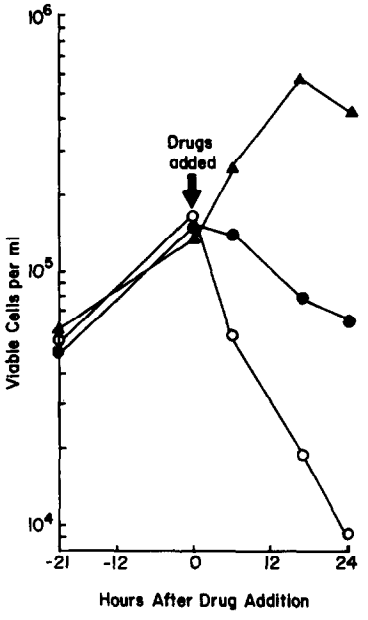

Fig. 1. Effect of dCF on the cytotoxicity of ara-A. CHO cells were incubated with either no drug (▲), 50 μM ara-A (●), or 50 μM ara-A + 10 μM dCF (○) for 24 hr. Cell viability was determined at the indicated intervals by a cloning assay.

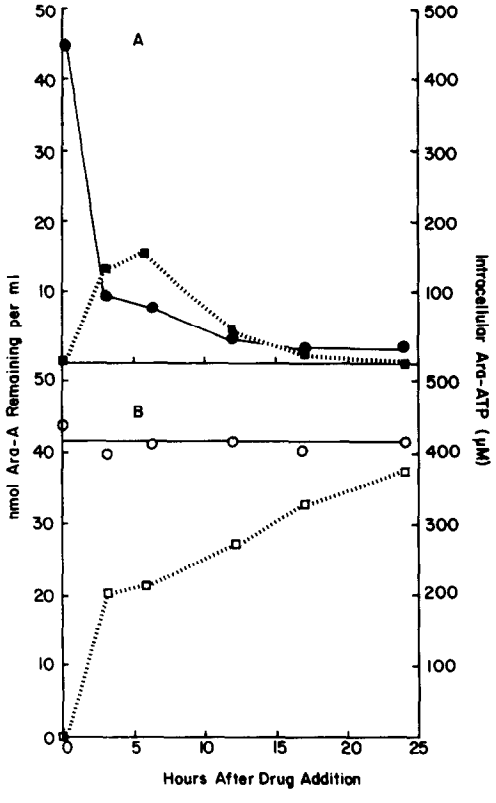

Fig. 2. Deamination and phosphorylation of ara-A by CHO cells. Cells were incubated with [^{3}H]ara-A (sp. act. 2.51 × 10^7 dpm/μmole) at an initial concentration of 50 μM in the absence (A) or presence (B) of 10 μM dCF. Deamination of ara-A (●, ○) and accumulation of ara-ATP (■, □) were determined at the indicated intervals as described in Materials and Methods.

initial amount of ara-A had been deaminated. Considering that ara-ATP has a biological half-life of 1.7 hr in this cell line [25], it is reasonable to expect the intracellular concentration of ara-ATP to decline when the exogenous supply of ara-A has been substantially depleted. In contrast, the presence of 10 μM dCF completely inhibited the deamination of ara-A for the duration of the experiment and enhanced the intracellular accumulation of ara-ATP (Fig. 2B). After 3 hr of incubation with ara-A in the presence of dCF, the amount of ara-ATP accumulated was nearly twice the maximum level attained from incubation with ara-A alone. Ara-ATP continuously accumulated intracellularly from ara-A when ADA was inhibited, achieving a concentration of nearly 371 μM by 24 hr. Thus, the inclusion of dCF maintained high extracellular levels of ara-A for subsequent cellular penetration and phosphorylation and, although ara-ATP is rapidly catabolized intracellularly [25], there was a net accumulation of the triphosphate for the duration of the experiment.

Ara-ATP is a potent inhibitor of DNA synthesis both *in vitro* and *in vivo* [18, 30–33]. Because the action of dCF affected the amount of ara-ATP accumulated from ara-A (Fig. 2B), the accumulation of ara-ATP from various concentrations of ara-A in the absence or presence of dCF was determined (Fig. 3), and the resulting effects on DNA synthesis in the same cells were measured (Fig. 4). Figure 3 illustrates that less than 1 μM ara-ATP accumulated from 1 μM ara-A alone or in the presence of dCF. A 10-fold increase in the exogenous ara-A concentration effected a similar increase in the intracellular accumulation of ara-ATP. The inclusion of dCF increased the cellular ara-ATP concentration less than 2-fold at each concentration.

The effect of incubation of CHO cells with the various concentrations of ara-A described in Fig. 3 on DSC was also determined, as illustrated in Fig. 4. The incorporation of [^{14}C]dThd into acid-soluble material decreased from greater than 100% to less than 40% of the control value as the exogenous ara-A concentration increased from 0.1 to 10.0 μM. Incubation with 2.5 μM ara-A alone was required for the cells to accumulate sufficient ara-ATP to block DNA synthesis by 50% and, in the presence of dCF, 2.3 μM ara-A was required to produce a similar inhibition. The inclusion of dCF in the incubation affected DSC by less than 10%, which reflects the less than 2-fold increase in cellular ara-ATP concentration by the action of the deaminase inhibitor (Fig. 3). dCF alone had no effect on DSC.

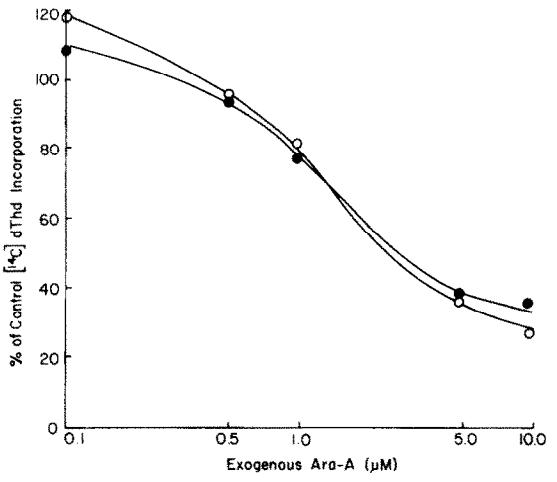

Fig. 4. Effect of ara-A on the DNA synthetic capacity of CHO cells. CHO cells were incubated with the indicated concentrations of ara-A in the absence (●) or presence (○) of 10 μM dCF as described in Fig. 3. The DNA synthetic capacity of the cells was quantitated after a pulse of [^{14}C]dThd (sp. act. 1.38×10^8 dpm/μmole) during the final 15 min of the incubation. The control incorporated 90 pmoles [^{14}C]dThd/10^7 cells in 15 min.

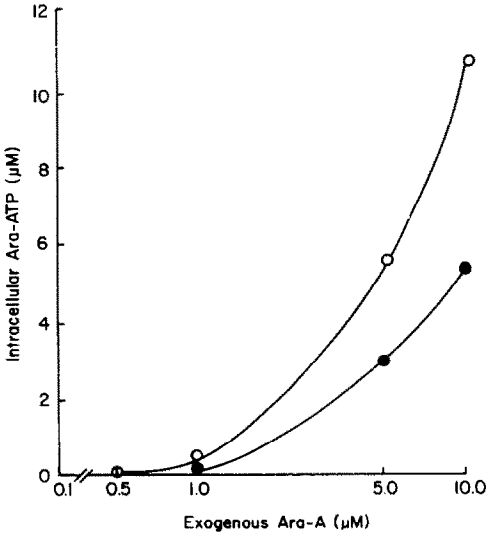

Fig. 3. Effect of dCF on the accumulation of ara-ATP from ara-A. Cells were incubated with the indicated concentrations of [^{3}H]ara-A (sp. act. 1.43×10^7 dpm/μmole) in the absence (●) or presence (○) of 10 μM dCF for 75 min. Nucleotides were extracted and ara-ATP was quantitated by HPLC as described in Materials and Methods.

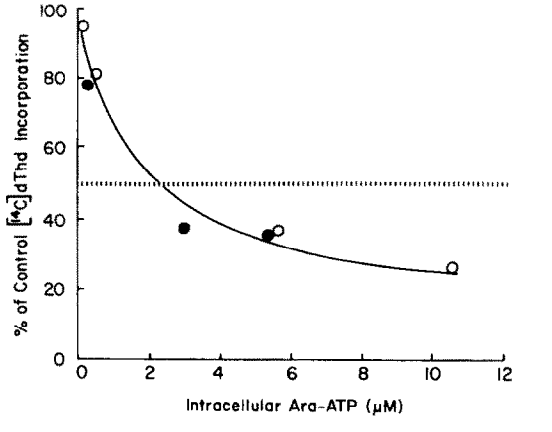

Fig. 5. Relationship between intracellular ara-ATP concentration and DNA synthetic capacity in CHO cells. Cells were incubated with various concentrations of ara-A in the absence (●) or presence (○) of dCF as described in Fig. 3. DSC and intracellular ara-ATP concentrations were determined as described in Figs. 3 and 4 respectively.

The findings from these two different analyses of the same experiment presented in Figs. 3 and 4 are combined in Fig. 5 to facilitate evaluation of the effect of cellular concentrations of ara-ATP, accumulated in the absence of presence of dCF, on DNA synthesis. The data in Fig. 5 demonstrate that a 10-fold increase in the cellular ara-ATP concentration, from 0.3 to 3.0 μM, was necessary to effect a 40% decrease in [^{14}C]dThd incorporation. This figure also illustrates that, for a given intracellular concentration of ara-ATP, the extent of inhibition of DSC was the same whether dCF was or was not present during the accumulation of ara-ATP from ara-A. The cellular concentration of ara-ATP necessary to reduce [^{14}C]dThd incorporation to 50% of the initial uninhibited value was 2.5 μM.

It has been demonstrated that ara-ATP is a competitive inhibitor of purified DNA polymerase α, competing with dATP for the active site on the enzyme [30–32]. Assuming that ara-ATP inhibits DSC in whole cells through competitive inhibition of the replicative polymerase, then the effect of ara-ATP on DSC as shown in Fig. 5 was actually due to the relative concentrations of the polymerase inhibitor, ara-ATP, and the normal substrate, dATP. Thus, it is important to determine the intracellular dATP concentration when evaluating the effect of the accumulation of ara-ATP on DSC. Deoxyribonucleoside triphosphate levels in CHO cells were quantitated in an experiment in which the cells were incubated with 200 μM ara-A in the presence of 10 μM dCF for 5 hr (Table 1). During the intracellular accumulation of 412 μM ara-ATP, there was no significant difference between cellular dATP concentrations in the drug-treated or untreated control cells ($P > 0.50$ according to Student's t-test for the difference between two means). The levels of dCTP, dTTP and dGTP after ara-A incubation also were similar to their corresponding levels in control cells. Although the dTTP levels appear to be elevated after ara-A exposure, the results of similar determinations in experiments to determine the effect of ara-A on the specific activity of [^{3}H]dTTP pools indicated that ara-ATP accumulation did not affect the intracellular dTTP concentration. In addition, the specific activities of the [^{3}H]dTTP pools in these experiments were the same in control and ara-A-treated cells (2.16×10^9 dpm/μmole and 1.75×10^9 dpm/μmole respectivley). The exposure of CHO cells to 10 μM dCF alone had no effect on the intracellular dNTP concentrations. Thus, the amount of ara-ATP (2.5 μM) that inhibited DSC by 50% was approximately one-half of the cellular dATP concentration.

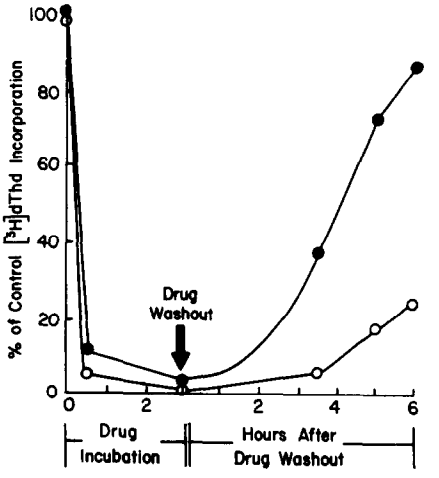

Fig. 6. Recovery of DSC after ara-A incubation. Cells were incubated with 500 μM ara-A in the absence (●) or presence (○) of 10 μM dCF for 3 hr, at which time the cells were harvested and resuspended in drug-free medium. DSC was quantitated based on the amount of [^{3}H]dThd (sp. act. 2.92×10^8 dpm/μmole) incorporated into acid-insoluble cellular material after 30 min. The control incorporated 417 pmoles [^{3}H]dThd/10^7 cells in 15 min.

Considering that the inhibition of purified DNA polymerase α by ara-ATP was competitive and thus reversible, it was of interest to determine whether the inhibition of DSC in whole cells occurred by a similar mechanism. Thus, the possibility that the inhibition of DSC was reversible after exposure to ara-A was investigated. CHO cells were incubated with 500 μM ara-A in the absence or presence of 10 μM dCF for 3 hr, in order to rapidly accumulate ara-ATP intracellularly to levels that were sufficiently high to inhibit DSC by more than 90% ($\geq$30 μM). After the incubation period, the cells were harvested and resuspended in drug-free medium. DSC was determined both during and after drug treatment, as illustrated in Fig. 6. Within 30 min after drug addition, DSC was inhibited by at least 90%, whether dCF was or was not present, and remained below that level for the duration of the incubation. Ara-ATP had accumulated to 139 μM and 286 μM from ara-A in the absence or presence

Table 1. Effect of ara-ATP accumulation on the intracellular dNTP pools*

Drug concentration	Cellular nucleotide concentration (μM)				
	dCTP	dTTP	dATP	dGTP	ara-ATP
No drug	31.3	12.4	5.4	0.7	
200 μM ara-A + 10 μM dCF	37.7	21.2	5.9	0.5	412
10 μM dCF	31.7	15.8	7.0	0.7	

* Cells were incubated with 200 μM ara-A in the absence or presence of 10 μM dCF for 5 hr, at which time the dNTP concentrations were determined as described in Materials and Methods. Each value is the mean of three determinations.

of dCF, respectively, after the 3-hr incubation. Within 3.5 hr after drug washout, a dramatic increase in [^{3}H]dThd incorporation was observed in the cells exposed to ara-A alone, which continuously increased to 87% of the initial uninhibited level by 6 hr after drug washout. In the absence of exogenous ara-A, the intracellular ara-ATP concentration had decreased to 33 μM within 3.5 hr after drug washout and declined further to 15 μM at 6 hr. The cells incubated with ara-A and dCF also exhibited a recovery of DSC after drug washout, but the increase in [^{3}H]dThd incorporation was much less than that observed in the cells exposed to ara-A alone and, correspondingly, the intracellular ara-ATP concentration was considerably higher at each time point. After drug washout, DSC increased slowly to 26% of the initial uninhibited value, when the intracellular ara-ATP concentration was 24 μM. Thus, the data illustrate that the inhibition of DSC by ara-ATP was reversible when the intracellular ara-ATP concentration declined to less than 35 μM.

DISCUSSION

In this study, the metabolism of ara-A in the absence or presence of dCF, a potent inhibitor of ADA, was examined. It has been reported that dCF enhances the cytotoxicity of ara-A to several cell lines in culture [9], including the CHO cell line in our laboratory (Fig. 1), presumably via the action of its 5′-triphosphate, ara-ATP, on DNA synthesis. The effect of dCF on the deamination of ara-A, on the accumulation of ara-ATP, and on DSC was investigated as discussed in this paper in order to determine a biochemical basis for the observed increase in the cytotoxicity of ara-A in the presence of this deaminase inhibitor.

The enhanced cytotoxicity of ara-A when dCF was present (Fig. 1) was due to a greater cellular exposure of ara-A through inhibition of its deamination (Fig. 2), resulting in greater intracellular accumulation of ara-ATP (Figs. 2 and 3). The presence of dCF enhanced the cellular ara-ATP accumulation no more than 2-fold when the drug incubation period did not exceed 6 hr, a result observed in other similar experiments in which cells were incubated with ara-A at concentrations ranging from 10 to 200 μM. This finding may reflect the affinity of the membrane-dependent uptake system [34] as well as the relatively low affinities of the phosphorylating enzymes [35–37]. The importance of dCF in allowing greater accumulation of ara-ATP intracellularly was more marked when cells were incubated with ara-A for more than 6 hr, as illustrated in Fig. 2, in which the inclusion of the deaminase inhibitor allowed a constant increase in the intracellular concentration of ara-ATP for at least 24 hr. In contrast, the cells incubated with ara-A alone could not maintain high levels of ara-ATP after 6 hr due to severe depletion of the exogenous supply of ara-A.

The presence of dCF did not alter the quantitative relationship between the cellular ara-ATP concentration and its inhibitory effect on DSC (Fig. 5). The inhibitor alone had no effect on cytotoxicity, cellular half-life of ara-ATP [25], or on DSC. Thus, the function of dCF in potentiating the cytotoxicity of ara-A was to allow accumulation of higher intracellular concentrations of ara-ATP through inhibition of the deamination of ara-A.

It was estimated that 2.5 μM ara-ATP would produce a 50% inhibition of DSC in the absence or presence of dCF. Similarly it has been reported that, in human lymphoblastoid cells, 3 μM ara-ATP inhibits DSC by 50% [28]. Several investigators have demonstrated that the inhibition of DNA polymerase α by ara-ATP is competitive with respect to dATP and non-competitive with respect to other deoxyribonucleotides. The published K_i values range from 0.3 to 8.0 μM [18, 30–32]. Although the kinetics of inhibition of purified DNA polymerase α, the proposed replicative polymerase [38], cannot be compared directly to the inhibition of DNA synthesis in intact cells, it would appear that the complex replication process in CHO cells is as sensitive to ara-ATP as is the more simplified purified enzyme system. In addition, it was demonstrated that the accumulation of 412 μM ara-ATP had no effect on the intracellular dNTP concentrations, indicating that the inhibition of DSC during ara-A incubation is not an indirect effect due to the alteration of the cellular dNTP levels.

When CHO cells were incubated with 500 μM ara-A alone or in the presence of 10 μM dCF, potent inhibition of DSC was observed within 30 min. Following drug washout, a substantial increase in [^{3}H]dThd incorporation was observed in both cultures, although the onset of recovery of DSC occurred sooner in the cells incubated with ara-A alone. The increase in [^{3}H]dThd incorporation corresponded to a decrease in the intracellular ara-ATP concentration to less than 35 μM whether dCF was or was not present during the cellular incubation with ara-A. This result was observed in two other experiments (not shown here) in which the peak intracellular concentration of ara-ATP ranged from 129 to 254 μM. The ability to synthesize DNA after a cytotoxic dose of ara-A or ara-C has also been reported [33, 39], but the intracellular concentrations of the corresponding nucleoside triphosphates were not determined. The data presented here illustrated that the inhibition of DNA synthesis in CHO cells by ara-ATP was reversible and dependent upon the cellular concentration of the nucleotide analog, similar to the competitive action of the drug with DNA polymerase α *in vitro* [18, 30–32].

Although it is possible that recovery of DSC after ara-A incubation (Fig. 6) represented the resumption of DNA synthesis in the cell population that survived the drug exposure, incorporation of [^{3}H]dThd in the viable cells was not distinguishable from that in the non-viable cells. If ara-A exerts its cytotoxic action through inhibition of DNA synthesis by ara-ATP, then it would be important to determine whether DSC does or does not recover in the non-viable cells after ara-A exposure, since the cytotoxic event may occur during the recovery of DNA synthesis due to a variety of mechanisms. For example, it is known that ara-AMP is incorporated into DNA [20–22], and if the fraudulent nucleotide is recognized as such and is eventually removed by an excision–repair process during recovery of DSC, then lethal errors may be made in the newly synthesized DNA. Alter-

natively, a significant portion of incorporated arabinosylnucleotides may terminate nascent DNA and render it an unsuitable substrate for the addition of subsequent dNTPs and thus block recovery of DSC. In addition, if recovery of DSC occurs in the non-viable cell population, it is possible that an increasing duration of DSC inhibition is associated with greater cytotoxicity. Investigations designed to evaluate these possibilities are under study in our laboratory at the present time and will be the subject of separate publications.

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