Biochemical Pharmacology. Vol. 28. pp. 201–206.

0006-2952/79/0115-0201 $02.00/0

# COMPARISON OF THE ACTIVITY OF 2′-DEOXYCOFORMYCIN AND ERYTHRO-9-(2-HYDROXY-3-NONYL)ADENINE *IN VIVO**

WILLIAM PLUNKETT, LILLIE ALEXANDER, SHERRI CHUBB and TI LI LOO
Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030, U.S.A.

(*Received* 24 *February* 1978; *accepted* 16 *May* 1978)

**Abstract**—The inhibition of P388 cell deamination of arabinosyladenine (ara-A) *in vivo* by the adenosine deaminase inhibitors 2′-deoxycoformycin (dCF) and erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) and their subsequent effects on ara-A metabolism were determined and compared. A single i.p. injection of EHNA (3 mg/kg, 10.9 μmoles/kg) initially inhibited ara-A deamination *in vivo* by 96 per cent with recovery to 50 per cent of control values within 30 min. In comparison, dCF (0.2 mg/kg, 0.75 μmole/kg) inhibition of ara-A deamination was initially low (4 per cent), but maximized (96 per cent) after 15 min. This inhibition was sustained for 2 hr and did not recover to 50 per cent of control values until after 10 hr. Injected alone, the $T_{\frac{1}{2}}$ of ara-A in the peritoneal ascitic fluid was less than 1 min, but was increased to 7 min when injected with EHNA and to 12 min when injected 15 min after dCF. The rate of efflux of ara-A and its metabolites from the peritoneal cavity ($T_{\frac{1}{2}}$ = 15–18 min) was not affected significantly by either deaminase inhibitor. Cellulat ara-ATP concentrations were elevated and the extent and duration of inhibition of DNA synthetic capacity were increased identically in cells of mice treated with ara-A and either deaminase inhibitor as compared with those treated with ara-A alone. Sustained deaminase inhibition after intraperitoneal concentrations of ara-A had been diminished by otherwise normal disposition did not augment the biochemically demonstrable activity of ara-A. Therefore, it appears that maintenance of the initial high concentrations of ara-A is the primary function of a deaminase inhibitor in increasing the therapeutic efficacy of this analog.

The recent availability of EHNA† [1] and dCF [2], two potent inhibitors of adenosine deaminase, has prompted a variety of studies that have demonstrated the potential therapeutic usefulness of these compounds. Coadministration of these deaminase inhibitors with adenosine deaminase-sensitive "normal" nucleosides, such as adenosine or 2′-deoxyadenosine [3, 4], or nucleoside analogs, such as ara-A [5, 6], xyl-A [7, 8] or cordycepin [5], greatly increases the *in vitro* cytotoxicity of the latter compounds. The antiviral activity of ara-A *in vitro* is similarly enhanced in the presence of an adenosine deaminase inhibitor [9–11], and the antitumor activity of ara-A [5, 12–15], xyl-A [16] and cordycepin [17] is increased greatly by coadministration with either EHNA or dCF. Furthermore, in the presence of adenosine, inhibitors of adenosine deaminase depress the blastogenic response of human lymphocytes [18–23] and monocyte maturation [24] *in vitro*. Both dCF and EHNA may be effective alone as immunosuppressive agents *in vivo* [25, 26]. Studies on the kinetics of inhibition of partially purified adenosine deaminase have indicated that the binding of each inhibitor appears to be competitive with the substrate, but that the rates of association and dissociation of EHNA are more rapid than those of the more potent and tightly bound dCF [27].

The purpose of the present study was to determine and compare the extent and duration of inhibition of adenosine deaminase evoked by therapeutically useful levels of each inhibitor *in vivo*, and to observe and compare the metabolism of ara-A by P388 lymphocytic leukemia *in vivo*, alone and in the presence of EHNA and dCF.

## MATERIALS AND METHODS

*Materials.* [2,8-$^3$H]arabinofuranosyladenine (11 Ci/m-mole) was obtained through the NCI from the Stanford Research Institute. Although designated as being labeled in the 8-position, incubation in 0.3 N KOH at 37° for 18 hr, followed by neutralization, evaporation and redissolving, resulted in no change in the specific activity of the compound. Similar treatment of [8-$^3$H]adenosine resulted in a 60 per cent decrease in specific activity. Therefore, it was concluded that only a very minor portion of the tritium was attached at C-8. All [$^3$H]ara-A was evaporated to dryness and recrystallized from water before use. Its purity was greater than 99 per cent as determined by thin-layer chromatography. [Methyl-$^3$H]thymidine was obtained from Schwarz/Mann Corp. (Orangeburg, NY). Its radiochemical purity was determined to be greater than 99 per cent by

*This work has been supported by USPHS Grants CA 14528 and CA 16672, and NCI contract CM-53773.

†Abbreviations: EHNA, erythro-9-(2-hydroxy-3-nonyl)-adenine; ara-A, 9-β-D-arabinofuranosyladenine; ara-ATP, 9-β-D-arabinofuranosyl-adenine 5′-triphosphate; cordycepin, 3′-deoxyadenosine; dCF, 2′-deoxycoformycin or (R)-3-(2-deoxy-β-D-erythro-pentofuranosyl)-3,6,7,8-tetrahydroimidazo [4,5-*d*] [1,3]diazepin-8-ol; DSC, DNA synthetic capacity; PCA, perchloric acid.

thin-layer chromatography in isobutyric acid–15 N $NH_4OH$–$H_2O$ (66:1:33) and was used as received. Ara-A, EHNA and dCF were all obtained through the NCI. Some samples of EHNA were generously provided by Dr. G. Elion, Wellcome Research Laboratories, Research Triangle Park, NC. The concentrations of the solutions of ara-A ($\varepsilon$ 257.5 nm in 0.1 N HCl = $12.7 \times 10^3$) [28], dCF ($\varepsilon$ 282 nm in $H_2O$ = $8.0 \times 10^3$) [2] and EHNA ($\varepsilon$ 260 nm in 0.01 N HCl = $16.0 \times 10^3$) were determined by spectrophotometry at the wavelengths of the absorption maxima.

*Tumors and mice.* P388 lymphocytic leukemia was provided by I. Wodinsky of A. D. Little, Inc. Camridge, MA, and maintained in female DBA/2 mice by weekly i.p. inoculation of $10^6$ cells. All determinations of *in vivo* deaminase activity were performed 5 days after i.p. inoculation of $10^6$ P388 cells into female C57BL/6 × DBA/2 ($BDF_1$) mice averaging 20 g in weight.

*Deaminase assay.* The deaminase activity of cells in the peritoneal cavity was determined by an *in situ* assay at various times after i.p. injection of either deaminase inhibitor. This functional *in vivo* determination of deaminase activity was designed to bypass uncertainties introduced in the assay of deaminase activity during aspiration, washing, and homogenization of cells after treatment with diffusable deaminase inhibitors. Tumor-bearing mice were injected with saline or a deaminase inhibitor at zero time. At the indicated times thereafter, the mice were injected i.p. with 50 mg [$^3$H]ara-A/kg (sp. act. $1.1 \times 10^7$ dis./min/$\mu$mole), and after 5 min were killed by cervical dislocation. The abdominal wall was opened rapidly and the ascitic fluid aspirated after each of two lavages with 1.5 ml of ice-cold saline. Two ml of the combined aspirates was brought immediately to 0.4 N PCA by the addition of 10 N PCA during constant stirring. After chilling for 30 min in an ice bath, the suspension was centrifuged and the sediment was re-extracted with 0.5 ml of 0.4 N PCA. The combined PCA-soluble extracts were neutralized with KOH, and the resulting precipitate ($KClO_4$) was removed by centrifugation. The supernatant fractions were reduced in volume to 0.50 ml on a Buchler Evapo-mix (Buchler Instruments, Fort Lee, NJ) and a 0.10-ml portion was counted with 0.90 ml of 0.01 N HCl in 11 ml PCS (Amersham/Searle Corp. Arlington Heights, Ill) to determine the amount of injected radioactivity extracted from the peritoneum. To 25 $\mu$l of the concentrated supernatant fluid were added ara-A and ara-Hx (each 25 nmoles in 5 $\mu$l) as chromatographic markers; 10 $\mu$l of the mixture was spotted on avicel F thin layer chromatography plates (Analtech Inc., Newark, DE) and chromatographed in 94.5 ml of water-saturated 1-butanol and 5.5 ml of 15 N $NH_4OH$; areas on the thin-layer plates containing the nucleosides were visualized under u.v. (254 nm), and scraped into scintillation vials containing 1.0 ml of 0.01 N HCl. After agitation for 1 hr on a reciprocal shaker, 11 ml PCS (Amersham/Searle) was added and the radioactivity was determined in a Packard Tricarb liquid scintillation spectrometer, model 2650. The dis./min, determined at an average counting efficiency of 35–37 per cent, were computed by the instrument with the aid of a preprogrammed external standard quench curve. Deamination of ara-A was calculated as a percentage of the total activity associated with ara-Hx and was expressed as a percentage of the control deamination, which ranged between 97 and 99 per cent.

*Effects of ara-A plus EHNA or dCF on DNA synthetic capacity.* In the experiment described in Table 1, female $BDF_1$ mice, weighing 19–21 g, were inoculated i.p. with $1 \times 10^6$ P388 cells. After 5 days, groups of three mice were injected i.p. with either saline, 50 mg ara-A/kg alone or with 3.0 mg EHNA/kg or with 0.2 mg dCF/kg followed in 15 min by an injection of 50 mg ara-A/kg. Seven min after injection of the ara-A, each mouse was injected s.c. on the back with 50 $\mu$Ci [methyl-$^3$H]thymidine (sp. act. 6.0 Ci/m-mole). After an additional 23 min, the mice were killed and the ascitic fluid was processed as described above. In the experiment shown in Fig. 6, the DNA synthetic capacity was determined 30 min after [$^3$H]thymidine injection at the indicated times. The PCA-soluble material was assayed for ara-ATP content and is described below. The specific activity of [$^3$H]thymidine incorporated into the PCA-insoluble material was determined as follows. After two washes in 5 ml of 0.4 N PCA, the PCA-insoluble sediment was resuspended in 1 ml of 0.4 N PCA and incubated for 15 min at 90°. After cooling and removal of hot PCA-insoluble material by centrifugation, the deoxyribose content of the supernatant fluid was determined by the method of Burton [29] using deoxyadenosine as a standard. The radioactivity in a 1-ml portion of each colorimetric reaction mixture was determined by liquid scintillation counting. The DNA synthetic capacity (DSC) of the P388 cells in the ascitic aspirate was defined as the dis./min incorporated/$\mu$mole of deoxyribose and expressed as a percentage of the control value which was $3.25 \times 10^5$ dis./min/$\mu$mole deoxyribose in Table 1 and $1.69 \times 10^5$ dis./min/$\mu$mole of deoxyribose in Fig. 6. Control experiments demonstrated that after an initial 5-min lag, incorporation of [$^3$H]thymidine into PCA-insoluble material after s.c. injection was linear over 40 min. This is in contrast to the rapid uptake and subsequent plateau of incorporation 10 min after i.p. injection. Greater than 98 per cent of the incorporated activity remained PCA-insoluble after an 18-hr incubation in 0.3 N KOH.

*Effects of EHNA and dCF on ara-ATP concentrations in P388 cells.* The effect of each deaminase inhibitor on the ara-ATP concentrations in the P388 cells 30 min after injection of 50 mg ara-A/kg, injected i.p. alone or simultaneously with 3.0 mg EHNA/kg or 15 min after i.p. injection of 0.2 mg dCF/kg, was determined. The PCA-soluble extracts prepared as described above from the mice used in one thymidine incorporation experiment (Table 1) were analyzed for ara-ATP by the following high-pressure liquid chromatographic procedure. A Waters Associates (Milford, MA) ALC 204 high-pressure liquid chromatograph equipped with two model 6000A pumps, model 660 gradient programmer and a column of Partisil-10 SAX anion-exchange resin (25 cm × 4.6 mm, Whatman, Inc., Clifton, NJ) was employed to fractionate nucleotide pools as described by Rose and Brockman [30]. Samples ($0.5–2 \times 10^7$ cell equivalents) were injected by means of the U6K-LC injection

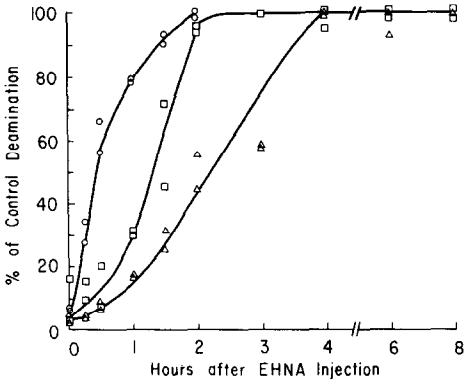


Fig. 1. Inhibition of ara-A deamination by EHNA. Mice bearing P388 cells were injected i.p. with 3 mg EHNA/kg (○), 10 mg EHNA/kg (□), or 30 mg EHNA/kg (△) at zero time. Deaminase activity was determined at the indicated times as described in Materials and Methods. Controls deaminated 96.3 per cent of the injected ara-A.

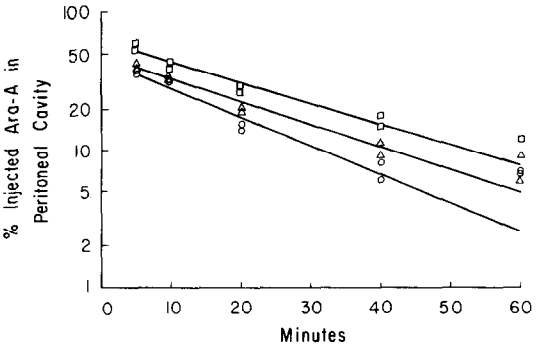


Fig. 3. Effect of EHNA and dCF on the efflux of ara-A from the peritoneal cavity of mice bearing P388 cells. Mice bearing P388 cells were injected i.p. with either 50 mg [$^3$H]ara-A/kg (sp. act. 1.1 × $10^7$ dis./min/μmole) alone (○) or with 3 mg EHNA/kg (□) or 15 min after 0.2 mg dCF/kg (△) at zero time. The amount of $^3$H in the ascitic fluid was determined at the indicated times as described in Materials and Methods.

system and eluted with a linear gradient (40 min) from 0.005 M $NH_4H_2PO_4$ (pH 2.8) to 0.750 M $NH_4H_2PO_4$ (pH 3.7) at a flow rate of 2 ml/min. The eluted compounds were detected at 254 nm by the model 440 detector and quantitated using a CDS-111 electronic integrator (Varian Associates, Walnut Creek, CA). Compounds were identified by comparison with the retention times of known standards. Ara-ATP in cell extracts was quantitated by comparison with the area of known amounts of ara-ATP (purchased from P-L Biochemicals, Milwaukee, Wis.), added to, and fractionated with extracts from untreated cells.

## RESULTS

In vivo *comparison of the effects of EHNA and dCF on deaminase activity*. The extent and duration of deaminase inhibition by EHNA and dCF were characterized by determining the deamination of ara-A after i.p. injection into mice bearing P388 cells. As seen in Fig. 1, simultaneous injections of 3 mg EHNA/kg with ara-A produced 92 per cent of deamination. The extent of this inhibition was increased only slightly by 10 mg EHNA/kg and 30 mg EHNA/kg. It is unlikely that the lack of complete deaminase inhibition could be due to the lag time necessary to establish an effective inhibitor–enzyme interaction since injection of ara-A 15 min after EHNA resulted in less deaminase inhibitions at all EHNA concentrations. At 3 mg EHNA/kg, a dose which in combination with ara-A produced markedly increased therapeutic responses in tumor-bearing mice [4, 11], half the deaminase activity was recovered before 30 min and recovery was complete by 2 hr. Higher EHNA doses gave more prolonged inhibition of deaminase activity, but the recovery of deaminase activity after the different doses of EHNA occurred at different rates.

In contrast to EHNA, 0.2 mg dCF/kg injected simultaneously with ara-A inhibited only 4 per cent of deaminase activity (Fig. 2). This dose of dCF gave the same extent and duration of inhibition of DNA synthetic capacity [14] and approximately the therapeutic value in combination with ara-A as does 3 mg

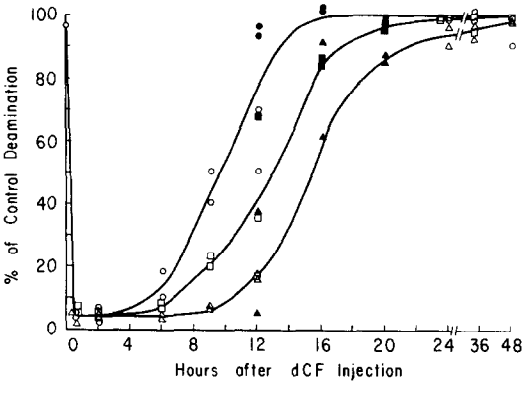


Fig. 2. Inhibition of ara-A deamination by dCF. Mice bearing P388 cells for 5 days were injected i.p. with 0.2 mg dCF/kg (circles), 0.6 mg dCF/kg (squares), or 2.0 mg dCF/kg (triangles) at zero time. Deaminase activity was determined at the indicated times as described in Materials and Methods. Filled and open symbols designate separate experiments, in which controls deaminated 96.5 and 98.2 per cent of the injected ara-A, respectively.

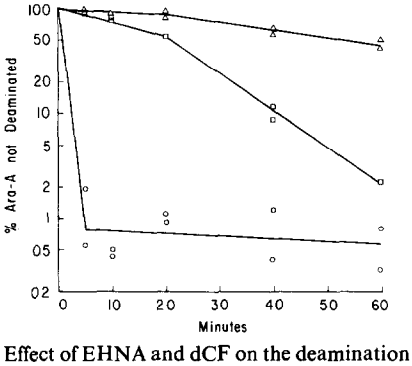


Fig. 4. Effect of EHNA and dCF on the deamination of ara-A *in vivo*. Mice bearing P388 cells were injected with 50 mg [$^3$H]ara-A/kg alone or in the presence of EHNA or dCF as described in Fig. 3. The amount of deamination was determined at the indicated times. Key: ara-A alone (○); ara-A plus EHNA (□); ara-A 15 min after dCF (△).

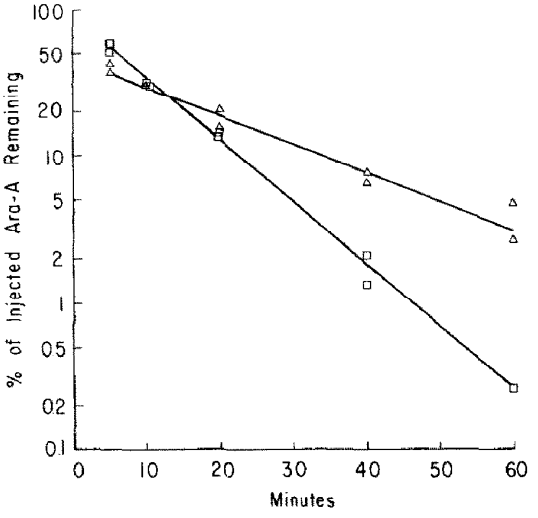


Fig. 5. Effect of EHNA and dCF on the removal of ara-A from the peritoneal cavity of mice bearing P388. The product of the percentage of ara-A and metabolites present in the extracts of ascitic fluid determined in Fig. 3 and the percentage of the $^3H$ associated with ara-A in those extracts determined from Fig. 4 is plotted at various times for mice injected i.p. with ara-A in the presence of EHNA (□) or dCF (△).

EHNA/kg [12, 31]. However, greater than 95 per cent inhibition of deamination was seen when ara-A was injected 30 min after dCF. This result is consistent with the finding of Agarwal *et al.* [27] that dCF is slow to equilibrate with the enzyme in cell-free assays. As was the case with EHNA, the deaminase activity assayed in this manner was not inhibited completely by the doses of dCF employed. The duration of maximal deaminase inhibition was increased by higher doses of dCF, and the rate of recovery of deaminase activity was approximately the same for each dose.

*Influence of EHNA and dCF on ara-A disposition.* The following experiments were performed to determine the effect of deaminase inhibition on ara-A disposition and to compare the effects of each deaminase inhibitor. A therapeutic dose of [$^3H$]ara-A (50 mg/kg) was injected i.p. into mice bearing P388 tumor either alone, simultaneously with 3.0 mg EHNA/kg, or 15 min after injection of 0.2 mg dCF/kg. This scheduling of the deaminase inhibitors was used to insure maximal efficacy of each compound. Mice were killed at the indicated times, the peritoneal cavities were lavaged with ice-cold saline, and the amount of $^3H$ in the PCA-extracted ascitic fluid was determined. The difference between the total activity injected and that recovered, expressed as a percentage, was taken as an indication of the amount of ara-A and metabolites that had effluxed from the peritoneal compartment (Fig. 3). Under all conditions, the efflux was exponential for 60 min. Although the amount of activity recovered from the peritoneum of those mice injected with ara-A alone was less than that recovered in the presence of either deaminase inhibitor, the half-life of injected ara-A and metabolites in the peritoneum was not significantly different (15–18 min) in the presence of the deaminase inhibitors.

Analysis of these PCA-soluble extracts by thin-layer chromatography indicated that the radioactivity was associated with either ara-A or ara-Hx (Fig. 4). When expressed as the percentage of the injected ara-A present in the peritoneal wash that had not been deaminated, it is clear that when injected alone, the initial half-life of ara-A was less than a minute. In contrast, EHNA, and to a greater extent, dCF, inhibited the deamination of ara-A, although neither compound was capable of entirely blocking deamination.

Efflux of ara-A from the peritoneum and deamination of ara-A probably represent the major pathways for the disappearance of the nucleoside analog from the ascitic fluid. The summation of these two rates is approximately the rate at which the nucleoside analog is removed from the peritoneal cavity. The percentage of the injected ara-A remaining in the peritoneum at any given time may be estimated by multiplying the amount of ara-A and metabolites that remain in the peritoneum (Fig. 3) by the fraction of ara-A yet to be deaminated (Fig. 4). The calculated products are expressed in Fig. 5 as the per cent of the injected ara-A remaining in the peritoneum at various times after injection. As was shown in Fig. 4, when injected alone only a small quantity of ara-A remains after the first 5 min. Injected in the presence of either deaminase inhibitor however, the initial decrease in ara-A is substantially less and appears to decay exponentially at constant rates over the first 60 min with a half-life of 7 and 12 min for EHNA and dCF respectively. These calculations indicate that 1 hr after injection of dCF, then ara-A, about 3 per cent of the injected nucleoside was present intact in the peritoneum. This figure was reduced 10-fold to 0.3 per cent for ara-A plus EHNA.

*Effect of EHNA and dCF on cellular ara-ATP and inhibition of DNA synthetic capacity.* To determine the pharmacological and biochemical effects of deaminase inhibitors on the action of ara-A *in vivo*, 50 mg ara-A/kg was injected i.p. alone into mice bearing P388 tumor, or in combination with either 3 mg EHNA/kg or 15 min after injection of 0.2 mg dCF/kg. Thirty min after injection, cellular ara-ATP concentrations and the DNA synthetic capacity of P388 cells were determined. At these inhibitor concentrations and injection schedules, ara-A deamination would be inhibited to the same extent initially, but EHNA-treated cells would recover 60 per cent deaminase activity during the incubation (Fig. 1),

Table 1. Ara-ATP concentration and DNA synthetic capacity (DSC) of P388 cells 30 min after injection of 50 mg ara-A/kg alone or with either 3 mg EHNA/kg or 0.2 mg dCF/kg*

| Inhibitor | (nmoles Ara-ATP/ μmole deoxyribose) | % of Control DSC† |
|---|---|---|
| None | 217 ± 19‡ | 19.3 ± 2.9 |
| EHNA | 321 ± 15 | 8.3 ± 1.0 |
| dCF | 343 ± 19 | 6.7 ± 1.9 |

*EHNA was injected simultaneously with ara-A, whereas dCF was injected 15 min before ara-A.

†The average DSC in three control mice was $3.25 \times 10^5$ dis./min/μmole of deoxyribose.

‡All values presented are the means ± S.E. of individual determinations on ascitic aspirates from three mice.

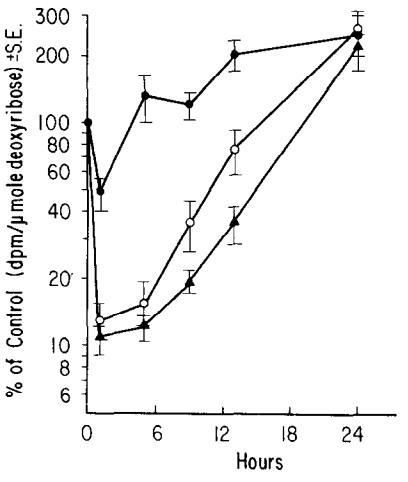


Fig. 6. Effects of ara-A alone or in the presence of either EHNA or dCF on the DNA synthetic capcity of P388 cells. Ara-A (50 mg/kg) was injected alone(●), simultaneously with EHNA (3 mg/kg) (▲) or 15 min after dCF (0.2 mg/kg) (○), and the DNA synthetic capacity was determined, as described in Materials and Methods, at the indicated times. Each point is the average of single determinations on six mice. The DNA synthetic capacity of controls was $1.69 \times 10^5$ dis./min/μmole of deoxyribose.

whereas the deaminase of dCF-treated cells would remain maximally inhibited (Fig. 2). The results are presented in Table 1. Significantly less ara-ATP accumulated in P388 cells when ara-A was injected alone than when injected with EHNA ($P < 0.03$) or dCF ($P < 0.02$). In addition, the DNA synthetic capacity of cells in the ascitic fluid from mice treated with ara-A combined with either deaminase inhibitor was significantly less than when ara-A was used alone ($P < 0.02$). However, there was no significant difference in either the amount of ara-ATP accumulated or in the DNA synthetic capacity of cells from mice treated with ara-A plus either EHNA or dCF.

The following experiment was performed to determine the duration of the inhibition of DNA synthetic capacity produced by these drug treatments in P388 cells. Mice bearing P388 tumor were injected as described above and the DNA synthetic capacity was determined at various times thereafter (Fig. 6). One hr after injection of ara-A alone, the DSC of P388 cells in the ascitic fluid was 50 per cent of the control. The DSC of these cells recovered to greater than control values by 5 hr and continued to exceed the control for the remainder of the experiment. In contrast, the DSC of cells from mice injected with ara-A and either EHNA or dCF was similarly reduced to less than 15 per cent of control values at 1 hr. This inhibition was sustained through 5 hr, after which recovery to control values ocurred sometime after 13 hr in both EHNA- and dCF-treated cells. Since the extent of inhibition of DNA synthesis is most likely a direct function of the cellular ara-ATP concentration, these data suggest that the different degrees of deaminase inhibition encountered throughout this incubation (Figs 1 and 2) had little effect on the rate of decay of the similar cellular ara-ATP concentrations present at 1 hr in cells treated with ara-A and either EHNA or dCF (Table 1). This interpretation is consistent with our findings that the half-life of ara-ATP in CHO cells incubated *in vitro* was not altered by the presence of either EHNA or dCF (D. S. Shewach and W. Plunkett, manuscript in preparation).

## DISCUSSION

The purpose of this study was to determine the extent and duration of inhibition of adenosine deaminase *in vivo* by therapeutic doses of EHNA and dCF that were effective in increasing the efficacy of the deaminase-sensitive nucleoside analog, ara-A. Furthermore, the effects of each deaminase inhibitor on the disposition and metabolism of ara-A were to be determined and compared. With regard to the former point, the findings of Agarwal *et al.* [27] concerning the activity of partially purified adenosine deaminase in the presence of each inhibitor have been predictive of the *in vivo* situation. Our results indicate that, *in vivo*, dCF is a more potent inhibitor of P388 adenosine deaminase than is EHNA. The inhibitory activity of EHNA appeared to be effective immediately upon injection (Fig. 1), whereas dCF required a time of incubation before attaining maximal inhibition (Fig. 2). Although the extent of deaminase inhibition caused by 3 mg EHNA/kg and 0.2 mg dCF/kg was similar and was not increased by 10-fold greater doses of either inhibitor, both the duration of maximal inhibition and the time before recovery to control deaminase levels were dose-dependent for each compound. The duration of deaminase inhibition produced by EHNA was relatively transient when compared to the greatly sustained inhibitory effect of dCF. Previous studies of dCF pharmacology and inhibition of adenosine deaminase activity in mice [13, 32, 33] and dogs [34] showed long-lasting deaminase inhibition after a single dose, even though much of the drug was excreted in 2–4 hr [32, 33]. Our findings are consistent with a rapid dissociation of the enzyme–EHNA complex and a much slower dissociation of the enzyme–dCF complex as predicted by the studies of Agarwal *et al.* [27]. However, the possibility exists, particularly in the case of the prolonged inhibition evoked by dCF, that the synthesis of new enzyme may contribute to the eventual recovery of deaminase activity.

The effects of EHNA and dCF on the *in vivo* metabolism of ara-A were determined in order to assess how the two deaminase inhibitors, with such different inhibition kinetics, could give similar therapeutic results in combination with ara-A. The incomplete, but sustained, inhibition of adenosine deaminase by dCF (Fig. 2) resulted in a low but measurable rate of ara-A deamination in the ascitic fluid of the peritoneal cavity (Fig. 4). In contrast, the relatively transient deaminase inhibition of EHNA permitted a greater initial rate of ara-A deamination which increased throughout the incubation (Fig. 4). Neither EHNA nor dCF altered the apparent disposition of ara-A, as indicated by the similar rates of efflux of ara-A and its metabolites from the peritoneum (Fig. 3). The rate of efflux of each deaminase inhibitor remains to be determined.

For the 15 min after ara-A injection, the difference in ara-A concentrations in the peritoneal cavities of mice treated with ara-A plus deaminase inhibitors was nearly 100-fold that of mice receiving ara-A

alone (Fig. 5). This differential was maintained in dCF-treated mice, although the recovery of deaminase activity after EHNA injection was primarily responsible for the estimated 10-fold difference in ara-A concentration after 1 hr.

Thirty min after ara-A injection the cellular ara-ATP concentrations were similar in EHNA- and dCF-treated mice, but significantly higher than those treated with ara-A alone (Table 1). Increased accumulation of cellular ara-ATP after injection of mice bearing L1210 leukemia [30] or upon incubation of mouse fibroblasts [35] and of human red blood cells [36] with ara-A and a deaminase inhibitor has been detected. Consistent with the proposed mechanism of action of ara-A via ara-ATP acting as a competitive inhibitor of DNA polymerase [37, 38], cells with higher ara-ATP concentrations showed less DNA synthetic capacity than those with lower ara-ATP levels (Table 1).

We conclude that the functional role of each deaminase inhibitor in increasing the therapeutic activity of ara-A in tumor-bearing mice is to maintain high levels of ara-A in the peritoneal cavity as a substrate for subsequent cellular penetration and phosphorylation to ara-ATP. Ara-A and its metabolites pass from the peritoneal cavity of the mouse (Fig. 3) and are excreted rapidly in the urine [39], as is the case after i.v. administration in man [40]. Therefore, the success of experimental chemotherapy is critically dependent upon the inhibition of ara-A deamination at the time of ara-A injection. The presence of the deaminase inhibitors has little effect on either the disposition of ara-A (Fig. 3) or the initial accumulation of ara-ATP (Table 1). The inhibitors may not affect the degradation of cellular ara-ATP (Fig. 6), and do not display antitumor activity alone [5, 12, 13, 31]. Therefore, inhibition of adenosine deaminase after effective levels of ara-A have been depleted may give rise to nonspecific effects [41–43] and may be viewed as a potential source of host toxicity [18–26]. This suggests that the greatest therapeutic effect with the least host toxicity may be achieved if maximal deaminase inhibition is maintained in the tumor while the level of deaminase-sensitive nucleosides such as ara-A are at therapeutic levels in the host. Subsequent release of the deaminase inhibition should be affected as soon as possible after cessation of ara-A therapy.

Although it seems unlikely that the potency or duration of these deaminase inhibitors will take on different proportions in man, the possibility exists that either the inhibition of adenosine deaminase or other nonspecific actions of these compounds may give rise to previously unforseen toxicities. Thus, a decision on the choice of inhibitor to be used in combination chemotherapy trials with deaminase-sensitive nucleoside analogues must await completion of comparative studies of the actions and effects of these deaminase inhibitors in man.

## REFERENCES


1. H. J. Schaeffer and C. F. Schwender, *J. med. Chem.* **17**, 6 (1974).
2. P. W. K. Woo, H. W. Dion, S. M. Lang, L. F. Dahl and L. J. Durham, *J. heterocyclic Chem.* **11**, 641 (1974).
3. L. Lapi and S. S. Cohen, *Biochem. Pharmac.* **26**, 71 (1977).
4. J. K. Lowe, B. Gowans and L. Brox, *Cancer Res.* **37**, 3013 (1977).
5. W. Plunkett and S. S. Cohen, *Cancer Res.* **35**, 1547 (1975).
6. C. E. Cass and T. H. Au-Yeung, *Cancer Res.* **36**, 1486 (1976).
7. W. Plunkett and S. S. Cohen, *Proc. Am. Ass. Cancer Res.* **16**, 27 (1975).
8. W. Plunkett, *Adv. Chromat.* **16**, 211 (1978).
9. Y. Bryson, J. D. Connor, L. Sweetman, S. Carey, M. A. Stuckey and R. Buchanan, *Antimicrob. Agents Chemother.* **6**, 98 (1974).
10. B. W. Williams and A. M. Lerner, *J. infect. Dis.* **131**, 673 (1975).
11. M. G. Falcon and B. R. Jones, *J. gen. Virol.* **36**, 199 (1977).
12. F. M. Schabel, Jr., M. W. Trader and W. R. Laster, Jr., *Proc. Am. Ass. Cancer Res.* **17**, 46 (1976).
13. G. A. LePage, L. S. Worth and A. P. Kimball, *Cancer Res.* **36**, 1481 (1976).
14. W. Plunkett, L. Alexander and T. L. Loo, *Proc. Am. Ass. Cancer Res.* **18**, 58 (1977).
15. S. H. Lee, N. Caron and A. P. Kimball, *Cancer Res.* **37**, 1953 (1977).
16. R. H. Adamson, D. W. Zaharevitz and D. G. Johns, *Pharmacology* **15**, 84 (1977).
17. D. G. Johns and R. H. Adamson, *Biochem. Pharmac.* **25**, 1441 (1976).
18. I. N. Fox, E. C. Keystone, D. D. Gladman, M. Moore and D. Cance, *Immun. Commun.* **4**, 419 (1975).
19. G. Wolberg, T. P. Zimmerman, K. Himestro, M. Winston and L.-C. Chu, *Science, N.Y.* **187**, 957 (1975).
20. F. F. Snyder, J. Mendelsohn and J. E. Seegmiller, *J. clin. Invest.* **58**, 654 (1976).
21. D. A. Carson and J. E. Seegmiller, *J. clin. Invest.* **57**, 274 (1976).
22. T. Hovi, J. F. Smyth, A. C. Allison and S. C. Williams, *Clin. exp. Immun.* **23**, 395 (1976).
23. K. R. Harrap and R. M. Paine, *Adv. Enzyme Regulat.* **15**, 169 (1977).
24. D. Fischer, M. B. Van der Weyden, R. Snyderman and W. N. Kelley, *J. clin. Invest.* **58**, 399 (1976).
25. C. T. Lum, D. E. R. Sutherland and J. S. Najarian, *New Eng. J. Med.* **296**, 819 (1977).
26. M. M. Chassin, M. A. Chirigos, D. G. Johns and R. H. Adamson, *New Engl. J. Med.* **296**, 1232 (1977).
27. R. P. Agarwal, T. Spector and R. E. Parks, Jr., *Biochem. Pharmac.* **26**, 359 (1977).
28. S. S. Cohen, *Prog. nucleic Acid Res. molec. Biol.* **5**, 1 (1966).
29. K. Burton, *Biochem. J.* **62**, 315 (1956).
30. L. M. Rose and R. W. Brockman, *J. Chromatog.* **133**, 335 (1977).
31. F. M. Schabel, Jr., *Open Memo*, Birmingham, AL, Aug. 27, 1976.
32. W. R. McConnell, *Proc. Am. Ass. Cancer Res.* **18**, 42 (1977).
33. W. J. Suling, L. S. Rice and W. M. Shannon, *Proc. Am. Ass. Cancer Res.* **18**, 42 (1977).
34. M. M. Chassin, R. H. Adamson and D. G. Johns, *Proc. Am. Ass. Cancer Res.* **18**, 147 (1977).
35. W. Plunkett and S. S. Cohen, *Ann. N.Y. Acad. Sci.* **284**, 91 (1977).
36. T. Chang and A. J. Glazko, *Res. Commun. Chem. Path. Pharmac.* **14**, 127 (1976).
37. J. J. Furth and S. S. Cohen, *Cancer Res.* **28**, 2061 (1968).
38. N. R. Cozzarelli, *A. Rev. Biochem.* **46**, 641 (1977).
39. G. A. LePage, Y. T. Lin, R. E. Orth and J. A. Gottlieb, *Cancer Res.* **32**, 2441 (1972).
40. G. A. LePage, A. Khaliq and J. A. Gottlieb, *Drug Metab. Dispos.* **1**, 756 (1973).
41. M. Debatisse and G. Buttin, *J. Cell Biol.* **70**, 348a (1976).
42. R. P. Agawal and R. E. Parks, Jr., *Biochem. Phar.* **26**, 663 (1977).
43. J. F. Henderson, L. Brox, G. Zombar, D. Hunting and C. A. Lomax, *Biochem. Pharmac.* **26**, 1967 (1977).