[CANCER RESEARCH 43, 1847–1850, April 1983]
0008-5472/83/0043-0000$02.00

S-Adenosylhomocysteine and S-Adenosylhomocysteine Hydrolase in Various Tissues of Mice Given Injections of 9-β-D-Arabinofuranosyladenine[1]

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ABSTRACT

The S-adenosylhomocysteine (AdoHcy) hydrolase (EC 3.3.1.1) activity and the metabolism of AdoHcy were investigated in various tissues of mice given a single injection or repetitive injections of 9-β-D-arabinofuranosyladenine (ara-A) with and without the adenosine deaminase inhibitor, 2′-deoxycoformycin (dCF). A single injection of ara-A (50 mg/kg) rapidly inactivated AdoHcy hydrolase in several organs (liver, kidney, spleen, lung, heart, skeletal muscle, and brain). Then, the enzyme activity in these tissues gradually recovered. This process, termed reactivation of AdoHcy hydrolase, was not sensitive to cycloheximide but was partly inhibited by dCF. In the absence of dCF, nearly no increase in AdoHcy content in the tissues was observed, whereas a single injection of ara-A plus dCF induced a small, transient increase in AdoHcy content of most tissues. Repetitive injections of ara-A (without dCF) caused a moderate increase in the AdoHcy level of tissues, whereas repetitive injections of the drug combination ara-A plus dCF resulted in a massive accumulation of AdoHcy in liver and kidney and, to a lesser degree, in other tissues. A moderate increase in S-adenosyl-L-methionine was observed in some tissues. These metabolic effects were associated with a rapid inactivation of AdoHcy hydrolase, but a fraction of the enzyme activity (about 8% in liver) was not or only slowly inactivated. AdoHcy accumulated in serum of mice receiving this treatment. Treatment of mice with dCF alone for up to 10 hr induced no increase in AdoHcy content of the tissues.

INTRODUCTION

The biological effects of ara-A,[3] an antiviral and antitumor agent (2), are enhanced by adenosine deaminase inhibitors, like dCF (4), which block the conversion of ara-A to its inactive metabolite, 9-β-D-arabinofuranosylhypoxanthine (2, 4). ara-A irreversibly inactivates isolated AdoHcy hydrolase (3, 5, 8, 10), inactivates AdoHcy hydrolase, and thereby blocks the degradation of AdoHcy in intact cells (6, 9, 13, 18). These data suggest that some effects of ara-A stem from accumulation of the endogenous transmethylase inhibitor, AdoHcy (1).

We have reported previously the effect of ara-A on AdoHcy metabolism and AdoHcy hydrolase in isolated rat hepatocytes and some cultured cells (6, 7). ara-A induces a massive accumulation of AdoHcy, especially in hepatocytes, and the effect is enhanced by dCF. Furthermore, when the cells exposed to ara-A are suspended in a medium containing adenosine deaminase, the AdoHcy hydrolase activity progressively recovers. This process, termed reactivation of AdoHcy hydrolase, is inhibited by dCF (7).

The present paper describes the effect of ara-A, administered alone or in combination with dCF, on the amount of AdoHcy and AdoHcy hydrolase activity in various tissues of mice.

MATERIALS AND METHODS

Materials. Sources of most chemicals have been given in previous publications (6, 7). Pelliguard LC-18 (40 μm) was obtained from Supelco, Inc., Bellefonte, Pa., and male albino NMRI mice (28 to 34 g) were from Statens Institutt for Folkehelse (SIF), Oslo, Norway.

Treatment of Animals. One of the following 4 protocols was used for the i.p. injections with ara-A and dCF given mice. (*a*) A single injection of ara-A (50 mg/kg) was given alone. (*b*) A single injection of the drug combination ara-A (50 mg/kg) and dCF (0.16 mg/kg) was given. (*c*) Repetitive injections of ara-A at intervals of 1 hr were given. The first injection was 50 mg/kg followed by 25 mg/kg each hr. This regimen is referred to as continuous treatment with ara-A. (*d*) This regimen was described under *c*, except that each injection was supplemented with dCF (0.16 mg/kg) and is referred to as continuous treatment with ara-A plus dCF.

The drugs were dissolved in 0.9% sodium chloride solution. The animals were put to death by decapitation 30 min or 1 hr after the (last) injection. The liver, kidney, spleen, lung, heart, skeletal muscle, and brain were rapidly removed and placed in liquid nitrogen. Blood was collected and left at room temperature for 5 min, and serum was deproteinized by perchloric acid.

Preparation of Tissue Extracts. Each organ was divided into 2 parts, one of which was homogenized (1/7, w/v) for 1 min at 0° in 80 mM potassium phosphate buffer, pH 7.0, containing 2 mM dithiothreitol, 20% glycerol, 0.5% Triton X-100, and 3 mM DL-homocysteine, using an Ultra-Túrrax homogenizer. The suitability of this buffer for the preservation of AdoHcy hydrolase activity in cellular extract has been documented (6).

The other part of the organ (used for determination of purines) was homogenized in 0.8 N perchloric acid (1/3, w/v) for about 1 min at 0°, using an Ultra-Túrrax homogenizer. Precipitated protein was removed by centrifugation of the extract (6). Part of the perchloric acid extract was neutralized to pH 6 to 7 by the addition of 1.08 M KOH/0.9 M $KHCO_3$. The neutralized solution was left at 0° for 15 min, and the precipitated potassium perchlorate was removed by centrifugation.

Assay for AdoHcy Hydrolase Activity. This was carried out by a radiochemical method described elsewhere (16). The assay mixture used for the determination of the synthetic reaction contained 200 μM [8-^{14}C]adenosine and 3 mM DL-homocysteine in 80 mM potassium phosphate buffer, pH 7.0, containing 80 mM KCl, 0.2% bovine serum albumin, 10 mM 2-mercaptoethanol, and 1 μM dCF. The temperature was 37°.

Determination of AdoMet and AdoHcy in Tissues. AdoMet and AdoHcy were determined in perchloric acid extracts by high-performance liquid chromatography using a Partisil SCX column, a Spectra-Physics SP 8000B liquid chromatograph, and a Model 160 Beckman UV detector, as described previously (6, 7). The mobile phases were 50 mM (AdoHcy) or 600 mM ammonium formate, pH 3.5. AdoHcy was also determined in neutralized perchloric acid extracts by a modification of a published

[1] Supported by grants from the Norwegian Society for Fighting Cancer and from the Norwegian Research Council for Science and the Humanities (Thyra og Erik V. Eneberg's testamentariske gave til kreftforskning).

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[3] The abbreviations used are: ara-A, 9-β-D-arabinofuranosyladenine; dCF, 2′-deoxycoformycin; AdoHcy, S-adenosyl-L-homocysteine; AdoMet, S-adenosyl-L-methionine; MI, methylation index.

Received July 6, 1982; accepted January 4, 1983.

method (7) based on high-performance liquid chromatography using a 3-μm Hypersil octadecylsilane column (0.5 × 10 cm) and the same solvent delivery system and detector as above. The column was equipped with a guard column (2.5 cm) packed with Pelliguard, which was separated from the analytical column by a tiny mesh. This reduced the number of theoretical plates of the column by less than 10% but greatly improved the mechanical stability of the column, allowing the elution of the column at a flow rate of 3 ml/min, corresponding to a back-pressure of 3900 psi. The mobile phase was 4.2% methanol in 10 mM potassium phosphate buffer (pH 6.0) or 10 mM sodium acetate buffer (pH 4.5). AdoHcy, ara-A, and adenosine eluted from the column at increasing retention times in the order mentioned and were separated from interfering compounds in the extracts in less than 5 min. In extracts from spleen and serum, a UV-absorbing compound coeluted with AdoHcy, but this material was separated from AdoHcy by cation-exchange chromatography.

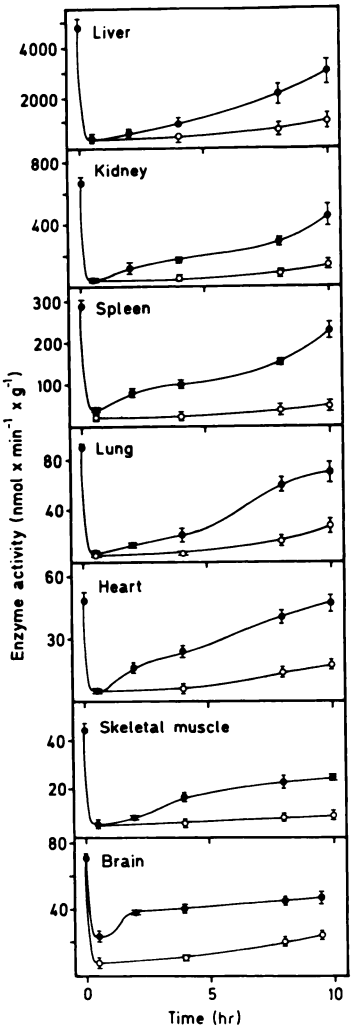

Chart 1. AdoHcy hydrolase activity in tissues of mice treated with ara-A or ara-A plus dCF. Mice were treated with a single i.p. injection of ara-A (●) or ara-A plus dCF (○). *Points*, mean of 3 to 8 determinations; *bars*, S.E.

RESULTS

AdoHcy Hydrolase Activity in Tissues of Mice Receiving a Single Injection of ara-A. AdoHcy hydrolase activity decreased rapidly to 7 to 10% of pretreatment values in various organs (liver, kidney, spleen, lung, heart, and skeletal muscle) of mice receiving a single injection of ara-A. The decrease in enzyme activity was somewhat less pronounced in brain (Chart 1). When ara-A was injected alone (without dCF), the AdoHcy hydrolase activity gradually recovered in all tissues investigated (Chart 1). The increase in enzyme activity was not inhibited by cycloheximide (50 mg/kg) injected i.p. 30 min before ara-A or repetitive 50-mg/kg injections each hr. This suggests that the increase in enzyme activity is not dependent on protein synthesis. It is suggested that AdoHcy hydrolase inactivated by ara-A is reactivated in several tissues *in vivo*, as shown previously for isolated rat hepatocytes (7). The reactivation proceeds at about equal rates in all tissues examined, and 50 to 90% of the enzyme activity is recovered within 8 hr (Chart 1).

Reactivation of AdoHcy hydrolase was partly inhibited when dCF was injected together with ara-A (Chart 1). Injection of dCF (0.16 mg/kg) 4 hr after the animals were treated with ara-A inhibited further reactivation of AdoHcy hydrolase in the tissues investigated (liver, kidney, spleen, lung, heart, and skeletal muscle) (data not shown). This finding shows that the effect of dCF is not related to inhibition of ara-A breakdown occurring immediately after injection of ara-A.

Tissue Level of AdoHcy and AdoMet. The content of AdoHcy and AdoMet in various tissues of mice not exposed to ara-A is shown in Table 1. Only a slight increase in AdoHcy level in tissues was observed in mice receiving a single injection of ara-A, but the effect of ara-A on the AdoHcy level was enhanced when this agent was given in combination with dCF, as shown for mouse liver in Chart 2. Similar data were obtained from the other tissues (kidney, spleen, lung, heart, and skeletal muscle).

AdoHcy Hydrolase Activity in Tissues of Mice Receiving Repetitive Injections of ara-A. In mice given injections of ara-A (without dCF) each hr, the AdoHcy hydrolase activity in various

Table 1

AdoHcy, AdoMet content, and methylation index (MI) in various tissues of mice receiving continuous treatment with ara-A plus dCF and of mice receiving no treatment

Mice were given i.p. injections of ara-A plus dCF at 1-hr intervals for 8 hr.

	Nontreated animals			Treated animals		
Tissue	AdoHcy (nmol/g)	AdoMet (nmol/g)	MI[a]	AdoHcy (nmol/g)	AdoMet (nmol/g)	MI
Liver	28.4 ± 2.8[b]	126 ± 18	4.4	427 ± 56	181 ± 36	0.42
Kidney	9.7 ± 1.2	132 ± 23	13.6	622 ± 94	198 ± 32	0.32
Spleen	2.7 ± 0.5	50.7 ± 12	18.8	83.2 ± 2.5	123 ± 9.7	1.5
Lung	6.2 ± 0.9	55.9 ± 6.6	9.0	48.0 ± 2.9	54.0 ± 5.8	1.1
Heart	1.6 ± 0.5	62.8 ± 16	39.2	36.7 ± 4.9	77.2 ± 10	2.1
Skeletal muscle	1.9 ± 0.2	37.6 ± 8.9	19.8	37.9 ± 8.6	45.1 ± 8.0	1.2
Brain	1.6 ± 0.3	34.0 ± 4.5	21.3	28.6 ± 3.4	76.0 ± 2.8	2.7

[a] MI, amount of AdoMet divided by the amount of AdoHcy.
[b] Mean ± S.E. for 3 to 8 determinations.

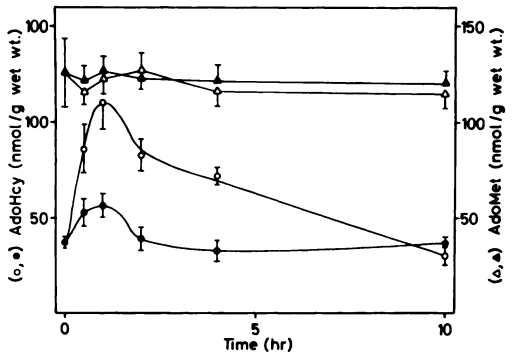

Chart 2. AdoHcy and AdoMet content in the liver of mice treated with ara-A or ara-A plus dCF. Mice were treated with a single i.p. injection of ara-A (●, ▲) or ara-A plus dCF (○, △). *Points*, mean of 3 to 8 determinations; *bars*, S.E.

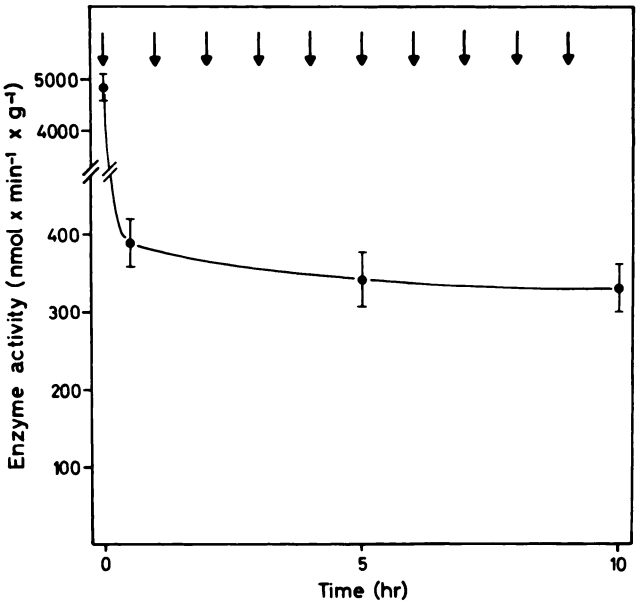

Chart 3. Time course for the inactivation of AdoHcy hydrolase in the liver of mice continuously treated with ara-A plus dCF. Mice received injections each hr with ara-A plus dCF (↓). *Points*, mean of 4 to 8 determinations; *bars*, S.E.

tissues (liver, kidney, spleen, lung, heart, and skeletal muscle) decreased rapidly to 8 to 12% of the pretreatment values (Chart 1) and then remained nearly constant for at least 8 hr (data not shown). When the mice were treated with ara-A in combination with dCF, the residual activity was slowly inactivated, as shown for liver in Chart 3. In the spleen, lung, heart, skeletal muscle, and brain, the residual enzyme activity gradually declined and approached undetectable levels within 10 hr (data not shown).

Tissue Level of AdoHcy and AdoMet. The AdoHcy content in tissues of mice continuously treated with ara-A for 8 hr increased only slightly (less than 150%) in liver, kidney, spleen, lung, heart, skeletal muscle, and brain (data not shown). Continuous treatment of mice with the drug combination ara-A plus dCF induced a massive accumulation of AdoHcy in these tissues, and AdoHcy reached particularly high levels in liver and kidney (Chart 4). In liver, kidney, spleen, and brain, a moderate increase in AdoMet content was observed (Chart 4). This may be explained by reduced consumption of AdoMet.

The MI is the [AdoMet]/[AdoHcy] ratio, which may reflect the ability of cells to carry out transmethylation reactions (1, 11, 15). The MI values for various tissues of mice receiving no treatment are shown in Table 1 and are compared with the corresponding values for tissues of mice treated with ara-A plus dCF for 8 hr. In liver and kidney, the MI was reduced below 1, *i.e.*, the cellular level of AdoHcy was higher than AdoMet.

Injections of dCF alone into mice each hr for 10 hr induced no increase in either AdoHcy content or AdoMet content in tissues (data not shown).

AdoHcy in Serum. No or only trace amounts of AdoHcy were detected in sera from nontreated mice. Continuous treatment with ara-A plus dCF induced a progressive increase in AdoHcy content in serum. This increase leveled off after about 2 hr and then the concentration of AdoHcy approached 30 μM (Chart 5).

DISCUSSION

ara-A inactivates AdoHcy hydrolase *in vivo* in mice. The rapid inactivation is followed by a progressive increase in AdoHcy hydrolase activity (Chart 1). The recovery of enzyme activity is not blocked by an inhibitor of protein synthesis, like cycloheximide (17), suggesting that the process is not dependent on new synthesis of enzyme. These data suggest that reactivation of AdoHcy hydrolase described previously in isolated rat hepatocytes (7) operates in several tissues in whole mice. The rate of

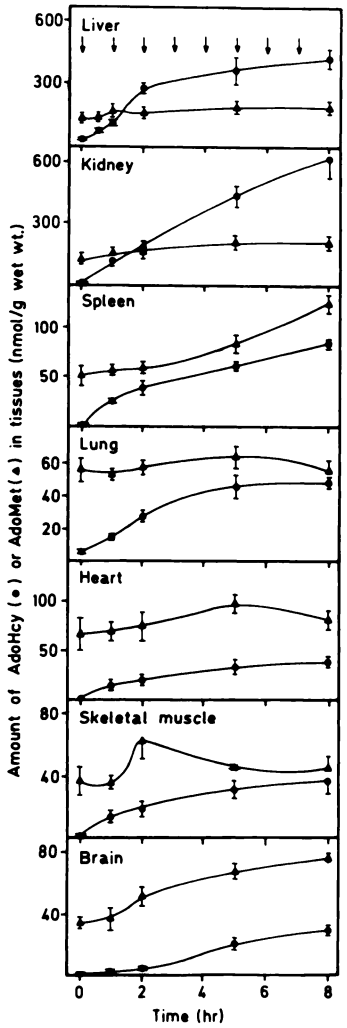

Chart 4. AdoHcy and AdoMet content in tissues of mice continuously treated with ara-A plus dCF. Mice were given injections each hr with ara-A plus dCF (↓). *Points*, mean of 4 to 8 determinations; *bars*, S.E.

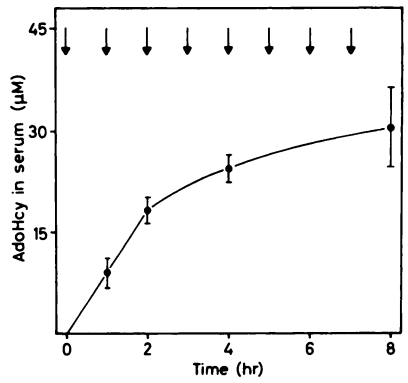

Chart 5. AdoHcy in serum of mice continuously treated with ara-A plus dCF. Mice were treated as described in the legend to Chart 4. *Points*, mean of 4 to 8 determinations; *bars*, S.E.

the reactivation in various tissues shows small variations from one tissue to another (Chart 1) and equals the rate of reactivation in isolated rat hepatocytes (7). It seems reasonable to suggest that reactivation of the ara-A/AdoHcy hydrolase complex is mediated by cellular mechanism(s) widely distributed in various tissues (Chart 1) and cell types (7, 13).

Reactivation of AdoHcy hydrolase in various tissues in whole mice is inhibited by dCF (Chart 1). Similar results were obtained with isolated rat hepatocytes (7). The effect of dCF is probably related to inhibition of nucleoside catabolism, *i.e.*, deamination of ara-A, adenosine, or 2′-deoxyadenosine. The observation that dCF induces a very slow inactivation of adoHcy hydrolase in tissues of mice given injections of dCF alone[4] indicates that dCF may exert its effect by mechanism(s) not involving ara-A.

A fraction of AdoHcy hydrolase activity (about 10%) in tissues of mice receiving continuous treatment with ara-A is not inactivated (Charts 1 and 3). Residual AdoHcy hydrolase activity has been demonstrated previously with hepatocytes and some cultured cells exposed to ara-A, but the activity was only 2 to 3% of the enzyme activity in nontreated cells (7). The fact that AdoHcy hydrolase is not completely inactivated may be explained by protection of the enzyme against inactivation by AdoHcy or other metabolites (7). The possibility that a continuous reactivation of AdoHcy hydrolase in cells exposed to ara-A may contribute to the residual AdoHcy hydrolase activity should also be considered.

dCF greatly enhances the accumulation of AdoHcy in tissues induced by ara-A (see "Results" and Charts 2 and 4), whereas the response of cells in suspension seems less dependent on dCF (6, 7). This observation should be related to the finding that dCF reduces the relatively high residual AdoHcy hydrolase activity in whole tissues (see "Results").

The metabolic response of mice treated with ara-A plus dCF varies from one organ to another (Chart 4). The accumulation of AdoHcy is particularly pronounced in liver and kidney (Chart 4). This could be explained by high activity of one or more methyltransferase(s), which are relatively insensitive to the inhibitory effect of AdoHcy. The differential response of various tissues to ara-A plus dCF should possibly be related to effects or side effects (2, 4, 12–14) of these agents.

AdoHcy is released into the extracellular medium from isolated cells accumulating AdoHcy in response to ara-A treatment (6). If AdoHcy egress from cells operates in whole animals, one would expect that the concentration of AdoHcy in serum increases in animals treated with ara-A plus dCF. This is certainly the case (Chart 5). Serum level of AdoHcy may be a useful parameter related to the metabolic response of tissues to treatment with ara-A.

In conclusion, ara-A is an inactivator of AdoHcy hydrolase *in vivo* in mice, and the inactive ara-A/AdoHcy hydrolase complex is slowly reactivated. dCF greatly enhances the ara-A-induced accumulation of AdoHcy in several tissues of mice. Several mechanisms may contribute to this effect observed with dCF. The possible mechanisms include inhibition of reactivation of the enzyme, lowering residual AdoHcy hydrolase activity, and inhibition of AdoHcy hydrolysis catalyzed by residual AdoHcy hydrolase. All these processes may be secondary to increased cellular content of ara-A or adenosine or other purines degraded by adenosine deaminase. The results presented in this paper add to data published previously (2, 4, 7), suggesting the usefulness of a combination therapy with ara-A plus dCF.

ACKNOWLEDGMENT

The authors are grateful to Eli Tepstad for preparing the tissue extracts.

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