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Effect of 2′-Deoxycoformycin Infusion on S-Adenosylhomocysteine Hydrolase and the Amount of S-Adenosylhomocysteine and Related Compounds in Tissues of Mice[1]

Svein Helland[2] and Per Magne Ueland

Department of Pharmacology, University of Bergen, School of Medicine, MFH-bygget [S. H., P. M. U.], and the Department of Dermatology, N-5016 Haukeland Sykehus [S. H], Bergen, Norway

ABSTRACT

Mice were given constant infusions of the adenosine deaminase inhibitor, 2′-deoxycoformycin, by i.p. implantation of microosmotic pumps, delivering the compound at a rate of 0.16 mg hr^{-1} kg^{-1}. In accordance with published data, we observed that adenosine deaminase in most tissues was nearly completely inhibited. In addition, the S-adenosylhomocysteine hydrolase activity decreased slowly and showed a half-life in liver of about 4 hr. The rate and extent of the inactivation were highest in spleen. The amounts of adenosine, 2′-deoxyadenosine, S-adenosylhomocysteine, and S-adenosylmethionine were determined in treated animals and control animals. The tissue levels of adenosine and, to a lesser degree, S-adenosylhomocysteine and S-adenosylmethionine were critically dependent on the procedure used for processing the tissues. Lowest concentrations were observed when the organs were frozen *in situ* by liquid nitrogen. Treatment with 2′-deoxycoformycin induced no or a moderate increase in tissue content of adenosine and S-adenosylhomocysteine, whereas the amount of 2′-deoxyadenosine increased markedly, especially in spleen and thymus. 2′-Deoxycoformycin treatment caused an increase in adenosine and 2′-deoxyadenosine, but not S-adenosylhomocysteine, in serum of mice.

INTRODUCTION

The antibiotic dCF[3] is a tight-binding inhibitor of isolated adenosine deaminase and also inhibits this enzyme *in vivo* (2, 10). dCF possesses lymphocytopenic and immunosuppressive activities which have been attributed to accumulation of dATP, an inhibitor of ribonucleoside diphosphate reductase. The lymphocytotoxic properties of dCF have stimulated clinical trials with dCF as an antileukemic agent (2, 10, 23, 25, 27, 33, 37). The potential usefulness of dCF as a chemotherapeutic agent is also indicated by the finding that dCF inhibits deamination of several adenosine analogues and thereby potentiates the biological effects of these agents both *in vitro* and *in vivo* (2, 10).

Our interest in dCF was stimulated by the finding that this agent potentiates the effect of ara-A on AdoHcy metabolism both in isolated cells and in whole animals (12, 13). ara-A is an irreversible inactivator of isolated AdoHcy hydrolase (16), the enzyme responsible for the metabolic degradation of the endogenous transmethylase inhibitor, AdoHcy (31). Several mechanisms may contribute to the enhancement of ara-A effects by dCF, all of which seem to be related to adenosine deaminase inhibition (13). We have recently reported that reactivation of the intracellular ara-A-AdoHcy hydrolase complex is partly blocked by dCF (12, 13).

The present investigation was carried out to provide further knowledge on the effect of dCF on AdoHcy hydrolase and AdoHcy metabolism when dCF was administered alone. This project was also stimulated by recent reports on low AdoHcy hydrolase activity in lymphoblasts from patients receiving dCF treatment (15) and in erythrocytes from patients afflicted with severe combined immunodeficiency disease associated with lack of adenosine deaminase (17).

MATERIALS AND METHODS

Chemicals. Sources of most chemicals have been given in previous publications (12, 13); 2′-deoxyadenosine was purchased from Sigma Chemical Co., St. Louis, Mo., and dCF was a gift from either the Developmental Therapeutics Program, Chemotherapy, National Cancer Institute, Bethesda, Md., or Parke-Davis Research Laboratories, Ann Arbor, Mich.

Treatment of Animals. dCF was dissolved in 0.9% sodium chloride solution, and the solution was sterilized by filtration through Millex single-use filters (Millipore, Bedford, Mass.). Alzet miniosmotic pumps (Model 2001; Alza, Palo Alto, Calif.) were filled with the solution under sterile conditions, as described by the manufacturer. The filled pumps were implanted i.p. into mice. For short-term experiments (less than 24 hr), the prefilled pumps were placed in 0.9% NaCl solution, for 4 hr at room temperature, before the implantation.

Two procedures were used for isolation of organs: (*a*) The animals were put to death by decapitation, and the liver, kidney, spleen, lung, heart, brain, jejunum, and thymus were immediately removed and placed in liquid nitrogen. Liver was placed in liquid nitrogen within 15 sec; the brain, within 25 sec; and the other organs, within 1 min. The intestine (jejunum) was placed on ice and washed by a slow stream of 0.9% NaCl solution to remove residual intestinal content. (*b*) The mice were anesthetized with ether, and the abdominal cavity was opened to expose the liver and kidney. Skin and tissue were removed from the skull bone covering the brain. The animals were then allowed to breathe fresh air until just before they recovered and were then submerged in liquid nitrogen. The organs which could be identified and isolated while still frozen were liver, kidney, brain, lung, and heart. The organs were kept at −80° until use.

Preparation of Tissue Extracts. One part of each organ was homogenized at 0° in a buffer suitable for preservation of AdoHcy hydrolase activity [80 mM potassium phosphate buffer, pH 7.0, containing 2 mM dithiothreitol, 20% glycerol, 0.5% Triton X-100, and 3 mM DL-homocysteine (11)], and the other part (used for determination of purines) was homogenized in perchloric acid. Part of the perchloric acid extract (used

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[2] To whom requests for reprints should be addressed.

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

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for determination of adenosine and 2′-deoxyadenosine) was immediately neutralized to pH 7. Details have been given previously (13).

Assay for AdoHcy Hydrolase. This was carried out by a radiochemical method described elsewhere (11). The assay mixture used for the determination of the synthetic reaction contained 300 μM [8-^{14}C]adenosine and 3 mM DL-homocysteine.

Assay for Adenosine Deaminase. This was performed according to the method of Kalckar (21), recording the change in absorbance at 265 nm resulting from the conversion of adenosine to inosine, using a Kontron Spectrophotometer, Model Uvicon 810. The assay buffer was 0.1 M potassium phosphate, pH 7.5, containing 150 μM adenosine. For measurement of adenosine deaminase activity in brain extracts, a radiochemical method was used, based on separation of [8-^{14}C]adenosine (150 μM) from radioactive inosine and oxypurines by thin-layer chromatography (8).

Determination of AdoMet, AdoHcy, Adenosine, and 2′-Deoxyadenosine in Tissues. AdoMet, AdoHcy, and adenosine were determined by high-pressure liquid chromatography using cation exchange or reversed-phase columns, as described previously (12, 13). 2′-Deoxyadenosine was determined by high-speed reversed-phase chromatography, using the same chromatographic system as developed for measurement of adenosine (12). 2′-Deoxyadenosine (retention time, 5.67 min) eluted from the column immediately after adenosine. The chromatographic peaks corresponding to 2′-deoxyadenosine (and adenosine) were absent in extract treated with adenosine deaminase.

RESULTS

Adenosine Deaminase Activity. The adenosine deaminase activity in various tissues of mice was almost maximally inhibited after 4 hr of treatment with dCF. The residual enzyme activity varied from one tissue to another, and was high (about 50%) in jejunum, intermediary in brain, and low (1 to 2%) in the other tissues investigated (liver, kidney, lung, spleen, and thymus). These data (Table 1) confirm those obtained by Tedde *et al.* (29) in determining the effect of dCF infusion on mouse adenosine deaminase.

AdoHcy Hydrolase Activity. The AdoHcy hydrolase activity decreased slowly in most tissues of mice receiving continuous infusion with dCF. In liver, kidney, heart, spleen, lung, and thymus, the enzyme activity decreased at a rate of about 0.2 hr^{-1}, and reached a plateau corresponding to 5 to 20% of the enzyme activity in tissues of nontreated mice. The rate and extent of inactivation were relatively high in the spleen. In this organ, the residual AdoHcy hydrolase activity was about 5%. Only a moderate inactivation (residual activity, 30 to 40%) was observed in brain and jejunum (Chart 1).

Table 1

Adenosine deaminase activity in various tissues of mice given infusions of dCF

The adenosine deaminase activity in various tissues of untreated mice is compared with the activity in tissues exposed to dCF for 24 hr.

Tissue	Adenosine deaminase activity (μmol/min/g, wet wt)	
	Tissues of untreated mice	Tissues exposed to dCF for 24 hr
Liver	1.6 ± 0.3[a]	0.02 ± 0.01
Kidney	1.8 ± 0.4	0.03 ± 0.02
Heart	0.32 ± 0.10	ND[b]
Lung	1.5 ± 0.3	0.01 ± 0.01
Brain	0.16 ± 0.03	0.01 ± 0.008
Jejunum	29 ± 9	14 ± 6
Spleen	5.2 ± 1.2	0.04 ± 0.02
Thymus	10 ± 3.2	0.12 ± 0.06

[a] Mean ± S.E. of 4 to 8 observations.
[b] ND, not detectable.

The inhibition of AdoHcy hydrolase activity induced by dCF could not be reversed by subjecting tissue extracts to gel filtration on Sephadex G-25 columns (to remove low molecular weight compounds from the enzyme) (Table 2). These data suggest that dCF treatment irreversibly inactivates AdoHcy hydrolase in several tissues of mice.

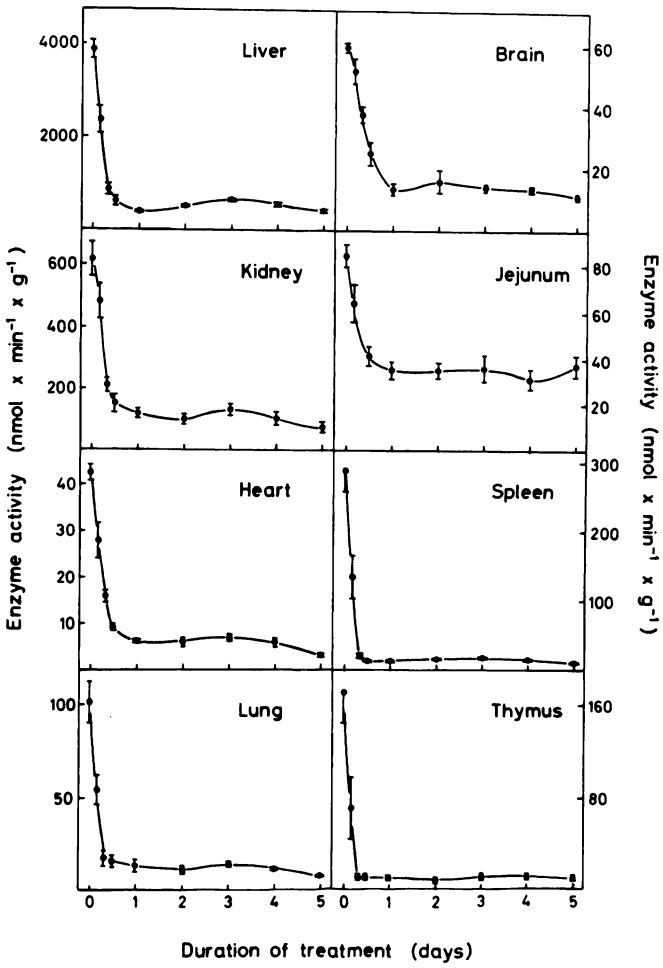

Chart 1. Inactivation of AdoHcy hydrolase and residual enzyme activity in various tissues of mice receiving constant infusion of dCF. *Values*, mean ± S.E. of 4 to 11 determinations.

Table 2

Effect of gel filtration of tissue extracts on residual AdoHcy hydrolase activity remaining after dCF infusion

Mice were treated with dCF for 4 days, and the tissues were processed as described under "Materials and Methods." Each extract was divided in 2 portions, one of which (100 μl) was subjected to gel filtration on Sephadex G-25 column (0.5 x 10 cm). The enzyme activity determined in these extracts was compared with the enzyme activity in the extracts not subjected to gel filtration. The activity is given as percentage of the activity in tissue extracts of nontreated animals.

Tissue	AdoHcy hydrolase activity (% of control)	
	After gel filtration	No gel filtration
Liver	11.0 ± 1.4[a]	13.8 ± 1.6
Kidney	23.3 ± 1.6	17.7 ± 3.0
Heart	20.4 ± 4.7	13.9 ± 2.4
Lung	12.3 ± 0.9	10.8 ± 0.8
Brain	26.5 ± 1.7	21.1 ± 1.3
Jejunum	35.9 ± 4.1	36.5 ± 5.4
Spleen	5.9 ± 0.8	4.8 ± 0.3
Thymus	10.9 ± 4.6	6.7 ± 1.1

[a] Mean ± S.E. of 4 determinations.

Evaluation of the Procedure Used for Isolation of Organs. The amounts of adenosine, 2′-deoxyadenosine, AdoHcy, and AdoMet in liver, kidney, brain, lung, and heart frozen *in situ* using liquid nitrogen were determined. The concentrations of these metabolites were compared with the corresponding values obtained when the organs were rapidly removed following decapitation. The comparison involved only organs which could be easily identified or isolated in a frozen state (Tables 3 to 5).

The method involving rapid freezing of the organs *in situ* gave adenosine concentrations in tissues far below the amounts observed when the organs were excised immediately after decapitation (Table 3). The former method also resulted in relatively low tissue level of AdoHcy and AdoMet (Tables 4 and 5). The amount of 2′-deoxyadenosine in some tissues (liver, kidney, and brain) was not related to the procedure used for isolation of the organs. In lung and heart frozen *in situ*, the 2′-deoxyadenosine content was somewhat lower than the amount in these organs frozen after death of the animal (Chart 2). (Data on heart frozen *in situ* should be interpreted with caution because of technical difficulties in removing intraventricular blood from frozen myocardium.)

AdoHcy hydrolase activity in tissues of treated mice and control animals was the same whether the organs were frozen *in situ* followed by immediate assay for enzyme activity, or the organs were removed after decapitation and stored for up to 7 days at −80° until assay (data not shown).

Tissue Content of Adenosine and 2′-Deoxyadenosine. dCF induced a marked increase in the amount of adenosine in some tissues when the organs were excised following decapitation, but no effect was observed in the brain (Table 3). In contrast, the concentration of adenosine in liver, kidney, heart, lung, and brain was essentially not increased following dCF treatment when the organs were frozen *in situ* (Table 3). This finding suggests that the elevation of adenosine concentration by dCF

Table 3

Concentration of adenosine in various tissues of mice given infusions of dCF for increasing periods of time

Mice were treated with dCF for 0 to 5 days. The tissues were either removed after decapitation or frozen *in situ* using liquid nitrogen. Details are given in the text.

	Adenosine in tissues during treatment (nmol/g, wet wt)				
Tissue	0 hr	8 hr	1 day	3 days	5 days
Removed after decapitation					
Liver	30 ± 10.2[a]	101.6 ± 11	217 ± 23.8	540.0 ± 85.8	441.2 ± 83.2
Kidney	145.9 ± 14.4	604.2 ± 101.2	741.7 ± 72.7	796.2 ± 90.0	373.5 ± 73.3
Heart	281.4 ± 54.2	472.2 ± 80.3	857.7 ± 201.3	766.4 ± 97.2	249.8 ± 45.7
Lung	21.7 ± 5.2	139.1 ± 30.2	136.9 ± 38.7	146.2 ± 17.9	107.1 ± 21.9
Brain	256.6 ± 43.3	257.9 ± 29.1	380.7 ± 52.4	432.5 ± 38.6	187.5 ± 35.6
Jejunum	19.9 ± 2.6	487.4 ± 119.0	816.5 ± 174.1	708.9 ± 97.7	868.7 ± 150.3
Spleen	11.5 ± 3.07	438.8 ± 103.2	536.6 ± 88.0	763.0 ± 116.0	944.3 ± 182
Thymus	23.8 ± 8.8	304.3 ± 41.2	198.8 ± 34.3	408.2 ± 48.0	602.1 ± 140.6
Frozen *in situ* using liquid nitrogen					
Liver	3.19 ± 0.90	3.79 ± 0.59	3.82 ± 1.02	9.63 ± 3.24	7.58 ± 1.9
Kidney	8.02 ± 2.0	4.29 ± 1.68	8.49 ± 2.87	9.24 ± 2.39	3.53 ± 0.38
Heart	27.7 ± 1.9	27.5 ± 3.9	34.8 ± 5.3	26.6 ± 5.6	11.7 ± 4.7
Lung	1.18 ± 0.2	1.08 ± 0.11	1.70 ± 0.43	1.01 ± 0.22	0.85 ± 0.26
Brain	0.71 ± 0.07	1.10 ± 0.15	0.77 ± 0.16	0.79 ± 0.16	0.86 ± 0.23

[a] Mean ± S.E. of 4 to 11 observations.

Table 4

Concentration of AdoHcy in various tissues of mice given infusions of dCF for increasing periods of time

Mice were treated with dCF for 0 to 5 days. The tissues were either removed after decapitation or frozen *in situ* using liquid nitrogen. Details are given in the text.

	AdoHcy in tissues during treatment (nmol/g wet wt)				
Tissue	0 hr	8 hr	1 day	3 days	5 days
Removed after decapitation					
Liver	25.5 ± 3.9[a]	24.0 ± 1.4	35.5 ± 4.4	31.3 ± 2.7	26.3 ± 3.3
Kidney	4.20 ± 0.80	5.62 ± 0.62	3.83 ± 0.42	4.02 ± 0.63	3.41 ± 0.90
Heart	0.41 ± 0.25	0.73 ± 0.17	1.25 ± 0.50	1.21 ± 0.43	0.72 ± 0.41
Lung	5.53 ± 0.86	6.81 ± 0.82	4.03 ± 0.44	4.00 ± 0.34	6.92 ± 1.19
Brain	0.76 ± 0.13	1.10 ± 0.27	0.59 ± 0.19	0.97 ± 0.26	0.89 ± 0.16
Jejunum	2.75 ± 0.39	3.52 ± 0.20	2.78 ± 0.50	3.49 ± 0.47	2.54 ± 0.31
Spleen	1.70 ± 0.40	3.70 ± 0.82	4.34 ± 0.46	2.63 ± 0.43	ND[b]
Thymus	1.19 ± 0.33	1.95 ± 0.70	2.40 ± 0.53	ND	ND
Frozen *in situ* using liquid nitrogen					
Liver	13.0 ± 4.6	6.73 ± 0.77	8.32 ± 0.54	6.69 ± 0.46	7.96 ± 0.67
Kidney	1.93 ± 0.63	1.51 ± 0.20	1.39 ± 0.25	2.13 ± 0.60	0.88 ± 0.08
Heart	0.31 ± 0.04	0.31 ± 0.03	0.51 ± 0.20	0.44 ± 0.02	0.29 ± 0.08
Lung	1.39 ± 0.19	2.07 ± 0.14	1.38 ± 0.07	2.2 ± 0.50	3.3 ± 0.32
Brain	0.32 ± 0.11	0.50 ± 0.09	0.77 ± 0.06	0.59 ± 0.20	0.89 ± 0.12

[a] Mean ± S.E. of 4 to 11 observations.
[b] ND, not determined.

Table 5

Concentration of AdoMet in various tissues of mice given infusions of dCF for increasing periods of time

Mice were treated with dCF for 0 to 5 days. The tissues were either removed after decapitation or frozen *in situ* using liquid nitrogen. Details are given in the text.

Tissue	AdoMet in tissues during treatment (nmol/g wet wt)				
	0 h	8 hr	1 day	3 days	5 days
Removed after decapitation					
Liver	112.8 ± 12.4[a]	106.2 ± 5.6	134.8 ± 10.9	111.3 ± 4.9	105.5 ± 7.9
Kidney	107.4 ± 5.5	90.6 ± 6.9	106.6 ± 7.2	92.1 ± 6.0	63.0 ± 4.6
Heart	58.5 ± 4.2	52.2 ± 3.9	53.1 ± 7.1	44.7 ± 1.6	40.9 ± 4.0
Lung	47.7 ± 3.6	50.1 ± 5.1	50.6 ± 7.1	34.2 ± 1.8	41.6 ± 6.3
Brain	35.8 ± 4.0	35.2 ± 3.2	45.0 ± 5.7	40.1 ± 2.4	33.2 ± 2.5
Jejunum	60.4 ± 9.7	45.5 ± 5.2	53.4 ± 4.2	59.5 ± 3.4	48.7 ± 8.3
Spleen	65.2 ± 8.1	45.8 ± 3.9	71.2 ± 11.0	58.6 ± 2.9	43.5 ± 9.3
Thymus	41.3 ± 11.5	49.3 ± 9.0	46.5 ± 12.0	27.6 ± 3.5	ND[b]
Frozen *in situ* using liquid nitrogen					
Liver	64.3 ± 9.8	52.1 ± 5.4	62.4 ± 3.3	51.7 ± 1.2	61.2 ± 3.6
Kidney	89.0 ± 6.4	69.0 ± 5.1	75.8 ± 5.2	53.2 ± 11.0	47.8 ± 1.2
Heart	20.6 ± 1.7	19.6 ± 3.0	15.21 ± 1.3	14.6 ± 1.0	16.1 ± 2.3
Lung	24.0 ± 3.0	27.3 ± 2.2	17.8 ± 3.7	20.2 ± 1.9	22.5 ± 1.6
Brain	24.0 ± 2.4	25.5 ± 4.3	21.7 ± 1.9	17.2 ± 2.8	23.2 ± 2.6

[a] Mean ± S.E. of 4 to 11 observations.
[b] ND, not determined.

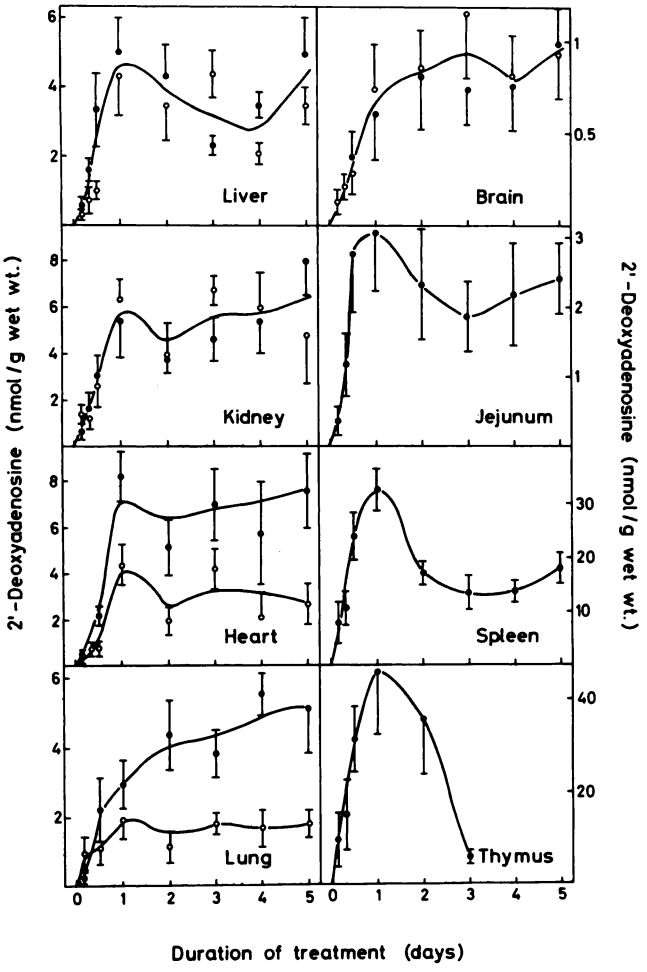

Chart 2. Concentration of 2′-deoxyadenosine in various tissues of mice receiving constant infusion with dCF. ●, amount of 2′-deoxyadenosine in tissues isolated immediately after the animal was put to death; ○, amount of 2′-deoxyadenosine in tissues frozen *in situ* using liquid nitrogen. Details are given in the text. *Values*, mean ± S.E. of 4 to 8 determinations.

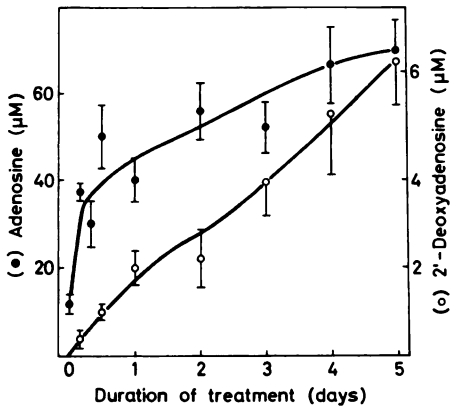

Chart 3. Concentrations of adenosine and 2′-deoxyadenosine in serum from mice receiving constant infusion of dCF. *Values*, mean ± S.E. of 4 to 8 determinations.

may be related to postmortem metabolic alterations.

Treatment of mice with dCF induced a marked increase in the concentration of 2′-deoxyadenosine in all tissues examined. Highest 2′-deoxyadenosine levels were observed in spleen and thymus (Chart 2).

Tissue Content of AdoHcy and AdoMet. The concentration of AdoHcy in most organs, except the spleen, did not increase following dCF treatment. In the spleen, only a slight increase was observed, but these data are limited to organs excised following decapitation (Table 4). Because of a marked atrophy of the thymus after 3 to 4 days of treatment, AdoHcy level in the thymus could not be obtained. dCF did not affect the amount of AdoMet in tissues (Table 5).

Adenosine, 2′-Deoxyadenosine, and AdoHcy in Serum. No 2′-deoxyadenosine and only trace amounts of AdoHcy were detected in serum of mice not exposed to dCF. Infusion with dCF induced a progressive increase in the amount of adenosine and 2′-deoxyadenosine in serum (Chart 3), whereas no increase in the AdoHcy concentration was observed (data not shown).

DISCUSSION

Inhibition of adenosine deaminase by dCF leads to accumulation of adenosine and 2′-deoxyadenosine in tissues (Table 3; Chart 2). Determination of the former metabolite is critically dependent on the procedure used for isolation of the organs (Table 3), and high levels may result from tissue anoxia following death of the animal. This problem has been addressed by others (3) and has been studied in detail in brain (26, 35, 36), but postmortem increase in adenosine concentrations in tissues is often overlooked (9, 34).

Postmortem increase in the amount of AdoHcy in tissues has been reported by Hoffman *et al.* (18) for rat liver. When the organs were frozen *in situ* by liquid nitrogen, we obtained values for AdoHcy in liver and other tissues (Table 4) below those reported previously by others (6, 9, 18, 28). Unexpectedly, this procedure also gave relatively low concentrations of AdoMet (Table 5).

AdoHcy hydrolase is inactivated in tissues of mice treated with dCF (Chart 1). This observation is in agreement with the finding of low level of AdoHcy hydrolase in erythrocytes from patients with adenosine deaminase deficiency (17, 22). dCF decreased the AdoHcy hydrolase activity in spleen, thymus, and liver of mice (14) and in human leukemic cells (15). AdoHcy hydrolase activity in cultured cells from patients afflicted with hereditary absence of adenosine deaminase was low in relation to the activity in cells from normal individuals (30). A mechanism for a secondary inactivation of AdoHcy hydrolase under conditions of impaired nucleoside catabolism has been suggested. Low adenosine deaminase activity leads to accumulation of both adenosine and 2′-deoxyadenosine (17), and the latter compound is a mediocre inactivator of AdoHcy hydrolase (16).

AdoHcy hydrolase was not completely inactivated in the tissues examined, even after prolonged treatment with dCF. The residual enzyme activity was remarkably stable, and only decreased slightly in some tissues. A similar observation has been made for AdoHcy hydrolase activity in several tissues of mice (13), in the liver of rats (7) given injections of ara-A, and in intact cells exposed to ara-A (11). The residual enzyme activity has been explained by protection of the intracellular enzyme by AdoHcy accumulating in response to ara-A (7, 11, 13).

A similar explanation can be offered for the residual AdoHcy hydrolase activity in tissues of mice exposed to dCF. Adenosine, accumulating in the presence of dCF (Table 3), protects AdoHcy hydrolase against inactivation by 2′-deoxyadenosine (16). However, the finding of essentially no adenosine accumulation when precautions were taken to avoid postmortem elevation of adenosine (Table 3) is not in favor of this explanation. The possibility exists that the residual AdoHcy hydrolase activity represents a balance between the inactivation process and mechanism(s) reactivating AdoHcy hydrolase (11, 13). This explanation is in accordance with the observation that the residual activity is low in tissues accumulating large amounts of 2′-deoxyadenosine in response to dCF (Charts 1 and 2).

During preparation of this manuscript, a paper by Kajander (20) appeared, showing that AdoHcy hydrolase was inactivated in extracts from the liver of rats given injections of the adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine. The inactivation occurred even at 0° and was inhibited by homocysteine. The author suggests that the inactivation induced by erythro-9-(2-hydroxy-3-nonyl)adenine was a postmortem phenomenon mediated by adenosine accumulating in the liver after death of the animal. Because of this, he recommends that the AdoHcy hydrolase inactivation under conditions of disturbances of purine metabolism should be reconsidered (20).

In accordance with the data of Kajander (20), we observed a marked inactivation of AdoHcy hydrolase in extract of rat hepatocytes, but the inactivation was prevented by extraction of the cells in phosphate buffer containing homocysteine and dithiothreitol (11). This procedure was used in the present work.

The following observations strongly favor the possibility of AdoHcy hydrolase inactivation under conditions of impaired adenosine catabolism (Ref. 22 and the present work) as being an *in vivo* phenomenon.

AdoHcy hydrolase inactivation (Chart 1) correlated better with accumulation of 2′-deoxyadenosine (Chart 2) than postmortem increase in adenosine (Table 3).

One hr after i.p. injection of dCF into mice, there was a maximal adenosine deaminase inhibition and postmortem adenosine accumulation in various tissues, whereas essentially no AdoHcy hydrolase inactivation was observed.

The AdoHcy hydrolase activity in tissues of mice treated with dCF was not dependent on the procedure used for isolation of the organs (see "Materials and Methods"). The same degree of inactivation of the enzyme was observed whether the assay was carried out immediately after death of the animal or after some days of storage of the organs at −80°.

Kaminska and Fox (22) could not demonstrate factors inactivating AdoHcy hydrolase in hemolysates from patients with adenosine deaminase deficiency.

No or only a moderate increase in the amount of AdoHcy in tissues has been observed after dCF treatment (Table 4). Besides, the slight increase in the AdoHcy content in spleen should be interpreted with caution, because these data are limited to organs frozen after death of the animals (Table 4). Lack of a marked increase in the amount of AdoHcy (Table 4) under conditions of a profound inhibition of AdoHcy hydrolase (Ref. 13; Chart 1) suggests that the enzyme level exceeds the activity required to handle the amount of AdoHcy formed as a product of cellular transmethylation reactions. However, data presented in this paper do not rule out the possibility that dCF affects the turnover of AdoHcy or AdoMet.

Treatment of patients with dCF leads to increased concentrations of AdoHcy and reduction in RNA methylation in leukemic cells from these individuals (15). This observation appears to be in conflict with the finding of no inhibition of DNA or RNA methylation in mouse spleen lymphocytes incubated with dCF (5), and with the present data showing essentially no increase in AdoHcy level in tissues of mice treated with dCF (Table 4).

Some difficulties exist when trying to interpret and reconcile data of the present paper. Assuming that AdoHcy hydrolase is inactivated by 2′-deoxyadenosine (16, 17), it is remarkable that the rate of inactivation of the enzyme proceeds at about equal rates in liver and spleen (Chart 1) despite the fact that the rate and extent of accumulation of the inactivator are much higher in spleen than in liver (Chart 2). Accumulation of 2′-deoxyadenosine seems to correlate better with the degree of inactivation (residual activity) than the rate of the process. Furthermore, we are also puzzled by the high degree of inactivation required before AdoHcy accumulates (Ref. 13; Chart 1; Table 4) even in the liver,

where the turnover of AdoHcy is high (19). The possibility exists that some nucleosides and nucleoside analogues, which irreversibly inactivate AdoHcy hydrolase in cell-free systems (31), function as regulators of the intracellular enzyme (32). Some intracellular factor(s) may be critical for a dynamic, allosteric regulation of AdoHcy hydrolase. Allosteric regulation of the enzyme by nucleosides in the intact cell should be considered in light of the recent finding that AdoHcy hydrolase has 2 classes of adenosine-binding sites, and 2 of 4 sites participate in the catalytic cycle (1). These sites probably reside on 2 nonequivalent pairs of subunits (1, 4).

Previous and present data (13) show that ara-A and dCF, when given alone, only induce a moderate or no increase in AdoHcy content in tissues *in vivo*. In contrast, the combination treatment with dCF plus ara-A gives a massive build-up of AdoHcy. This enhancement of the ara-A effect by dCF has been explained by inhibition of ara-A degradation, inhibition of reactivation of AdoHcy hydrolase, and inhibition of residual enzyme activity by adenosine (13). The present data indicate that accumulation of 2′-deoxyadenosine may contribute to the increase in AdoHcy concentration observed when ara-A is given in combination with dCF. Newly reactivated AdoHcy hydrolase (12, 13) may be trapped by forming an inactive complex with 2′-deoxyadenosine.

The observation that dCF does not induce an elevation of AdoHcy content in tissues argues against the possibility that the biological and toxicological properties of dCF (2, 10, 24) are mediated by AdoHcy. However, the marked reduction of AdoHcy hydrolase activity observed under conditions of impaired purine catabolism (Chart 1; Refs. 15, 17, 22, 30) may have biological implications not readily apparent in light of the present knowledge of the role of AdoHcy hydrolase in cellular function.

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