From: PURINE AND PYRIMIDINE METABOLISM IN MAN V
Edited by W.L. Nyhan, L.F. Thompson and R.W.E. Watts
(Plenum Publishing Corporation, 1986)

DETERMINATION OF S-ADENOSYLHOMOCYSTEINE IN TISSUES FOLLOWING PHARMACOLOGICAL INHIBITION OF S-ADENOSYLHOMOCYSTEINE CATABOLISM

S. Helland* and P.M. Ueland

*Department of Dermatology and Department of Pharmacology
University of Bergen, 5000 Bergen, Norway

INTRODUCTION

S-Adenosylhomocysteine (AdoHcy) hydrolase (EC 3.3.1.1.), the enzyme responsible for the metabolic degradation of the endogenous transmethylase inhibitor, AdoHcy, is a target enzyme for various adenosine analogues. Some of these compounds have oncostatic and antiviral properties. Adenosine analogues, including 9-ß-D-arabino - furanosyladenine (ara-A), function as inactivators or inhibitors of AdoHcy hydrolase, and these analogues induce a massive accumulation of intracellular AdoHcy. Other adenosine analogues serve as substrates for AdoHcy hydrolase, and are metabolized to their corresponding nucleosidylhomocysteine (1).

The potential role of AdoHcy hydrolase as a chemotherapeutic target enzyme has demanded the development of methods for determination of AdoHcy in tissues. Hoffman et al. reported that the amount of AdoHcy in rat liver increased rapidly following death of the animal (2). We have recently confirmed and extended this observation by showing that the amount of AdoHcy in various tissues of mice under physiological conditions was less than 30% when the organs were frozen in vivo using liquid nitrogen, compared with values obtained with organs rapidly removed and frozen after the animal was put to death (3).

The increase in AdoHcy after death may be related to production of AdoHcy from methyl-transfer reactions still operating, and failure to metabolize the resultant AdoHcy because of accumulation of adenosine and/or homocysteine (2). Alternatively, the postmortal increase in AdoHcy may be related to reversal of the AdoHcy hydrolase reaction induced by accumulation of adenosine during tissue anoxia (4-6). However, we observed only minimal change in the amount of free homocysteine in tissues of mice following death of the animal (7). This observation led us to investigate whether postmortal increase in AdoHcy may contribute to the massive accumulation of AdoHcy in tissues of animals treated with inhibitors of AdoHcy catabolism (1). The present report focuses on the AdoHcy content in various tissues of mice treated with the drug combination ara-A plus 2'-deoxycoformycin (dCF), which nearly totally inactivates AdoHcy hydrolase (8,9).

METHODS

Mice were given repetitive i.p. injections with the drug combination ara-A plus dCF. The first injection was 50 mg ara-A and 0.16 mg dCF per kg, followed by ara-A (25 mg/kg) plus dCF (0.16 mg/kg) each hour for 8 hours. The drugs were dissolved in 0.9% sodium chloride. The animals were put to death 1 hour after the (last) injection.

Two procedures were used for isolation of organs:

1) The animals were put to death by decapitation, and the liver, kidney, brain, heart, lung and spleen were immediately removed and placed in liquid nitrogen. The liver was frozen within 15 seconds, the brain within 25 seconds, and the other organs within 30 seconds.

2) The animals were anesthesized with ether, and the abdominal cavity was opened to expose the liver, kidney and spleen. The skin and tissue were removed from the skull bone. The animals were then allowed to breath in air containing no ether, and were then submerged in liquid nitrogen just before they recovered. The organs were isolated while still frozen, and kept at -80°C until analysis.

Frozen tissue was homogenized (1:4, w/v) in perchloric acid, which was neutralized as described previously (8,9). AdoHcy was determined by high performance liquid chromatography on a Partisil 10 SCX column or a 3um ODS Hypersil column, as described (9).

RESULTS AND DISCUSSION

The amount of AdoHcy in several tissues has recently been determined in our laboratory (3). In accordance with data published by others (2, 10, 11), we observed a rapid increase in AdoHcy (from 13 to 26 nmol/g in liver) in several tissues following death of the animal. This phenomenon could be avoided by freezing the organs in vivo using liquid nitrogen (3). If the same amount of AdoHcy accumulates after death in tissues of mice treated with ara-A plus dCF as that observed with non-treated mice, this postmortal phenomenon will be totally obscured under conditions of the high AdoHcy content induced by ara-A plus dCF (8,9).

In the present paper data are presented suggesting that a large fraction of AdoHcy in several tissues of mice treated for 8 hours with the drug combination stems from postmortal increase in AdoHcy. The amount of AdoHcy in the liver, and to a lesser degree in kidney, heart and lung was significantly lower when the organs were frozen in vivo compared with the amounts determined when the organs were isolated and frozen after the animal was put to death. In the liver of treated mice, the AdoHcy content increased from 150 nmol/g to 370 nmol/g within 15 seconds after death. In brain and spleen, the amount of AdoHcy was essentially the same whether the organs were frozen in vivo or isolated and frozen after decapitation (Table 1).

Four mice treated with ara-A plus dCF for 8 hours were anesthesized with ether and operated, as described in a preceding paragraph. The animals were killed by decapitation, and the organs removed and frozen immediately after death. The amount of AdoHcy in liver, kidney, heart, brain, lung and spleen of these animals was the same as the amount in these organs of treated mice not anesthesized and operated (data not shown). These data suggest that the low AdoHcy content in organs frozen in vivo (Table 1) is not induced by the anesthesia and/or the operative trauma.

Table 1

AdoHcy Content in Various Tissues of Mice Injected with ara-A plus dCF for 8 Hours

Mice were given i.p. injections of the drug combination ara-A plus dCF each hour for 8 hours, as described in the text.

Tissue	Concentration of AdoHcy (nmol/g wet wt)		
	Organs frozen after death[a]	Organs frozen in vivo[b]	p-values[c]
Liver	370±36	146±12	<0.0001
Kidney	644±53	374±42	<0.001
Heart	24.5±5.2	11.1±0.9	<0.01
Brain	29.0±1.8	24.5±1.7	<0.05
Lung	45.7±2.4	25.2±2.4	<0.001
Spleen	69.6±5.5	51.3±5.5	<0.05

[a] Mean + S.E.M. of 9 animals. The animals were killed by decapitation and the organs were immediately removed and frozen.

[b]Mean +S.E.M. of 12 animals. The exposed organs were frozen in vivo using liquid nitrogen, as described in the text.

[c]The p-values for the difference between AdoHcy content in organs frozen in vivo and frozen after death, were calculated using Student's t-test for two means.

Treatment of mice with ara-A plus dCF almost completely inactivates AdoHcy hydrolase in several tissues of mice (9). Therefore it seems unlikely that the postmortal increase in AdoHcy content in tissues of treated mice is related to further inhibition of AdoHcy catabolism by adenosine or to condensation of endogenous homocysteine with adenosine formed in response to postmortal tissue anoxia (4-6).

There was essentially no postmortal increase in AdoHcy in brain of mice injected with ara-A plus dCF (Table 1). Alternatively, the data obtained (Table 1) may be interpreted as failure to prevent postmortal increase of AdoHcy by freezing the brain in vivo. There are consistent reports on a rapid, massive accumulation of adenosine in brain during anoxia (4-6, 12), and the postmortal accumulation of this nucleoside can be avoided by freezing the brain in vivo through the intact skull bone (6, 12).

CONCLUSION

A large fraction (from 50 to 75%) of the AdoHcy content in liver, kidney, heart and lung of mice treated with ara-A plus dCF may rapidly accumulate following death of the animal. The postmortal increase in AdoHcy in brain and spleen was less pronounced. This source to erratic results can be avoided by freezing the organs in vivo, using liquid nitrogen. The mechanism(s) behind the postmortal elevation of AdoHcy content in tissues is unknown. Nevertheless, attention should be paid to this phenomenon when determining the metabolic response to compounds interfering with AdoHcy catabolism.

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