

Inactivation of S-Adenosylhomocysteine Hydrolase during Adenine Arabinoside Therapy

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ABSTRACT To assess for possible inhibition of cellular transmethylation during adenine arabinoside (ara-A) therapy, S-adenosylhomocysteine hydrolase activity was analyzed in 10 patients with chronic hepatitis B virus infection. In six patients receiving ara-A, enzyme activity was suppressed to 0–2% of control erythrocyte enzyme activity. This decrease in enzyme activity was evident within 4 h of starting the drug infusion and continued for 7 d after cessation of therapy. S-adenosylhomocysteine hydrolase activity of peripheral mononuclear cells was also measured in two patients receiving ara-A. Suppression to as low as 3.5% of pretreatment levels was found; however, marked fluctuations with partial return of enzyme activity during therapy was also observed in mononuclear cells.

Inhibition of an enzyme involved in transmethylation reactions was observed in patients during ara-A therapy. This could contribute to the side effects and antiviral properties of ara-A.

INTRODUCTION

Adenine arabinoside (ara-A,¹ vidarabine, 9- β -D-ara-binofuranosyladenine) is a clinically effective compound with a relatively wide spectrum of antiviral activity. Although the basis for the antiviral action of ara-A remains unclear, preferential inhibition of a virus-specific DNA polymerase has been the most widely accepted explanation (1–4). However, more recent evidence has revealed, in fact, no preferential incorporation into viral DNA (5). The pharmacologic properties of ara-A therapy in the host may also contribute to toxic manifestations that occur in as many as 44% of certain groups of patients (6).

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¹ *Abbreviations used in this paper:* ara-A, adenine arabinoside; ara-H_x, ara-hypoxanthine; Ado-Hcy, S-adenosylhomocysteine.

Recent studies indicate that ara-A may interfere with biological methylation by inactivating S-adenosylhomocysteine (Ado-Hcy) hydrolase in vitro (7). Because other methylation inhibitors possess antiviral activity (8–12), and methylation reactions are widely distributed in intermediary metabolism (13), it is possible that this property could contribute to the antiviral action and systemic toxicity of ara-A. To clarify whether Ado-Hcy hydrolase inactivation occurs during ara-A therapy, and the kinetics of this reaction in vivo, we have examined the alterations of Ado-Hcy hydrolase during low-dose therapy of chronic hepatitis B virus infection.

METHODS

10 patients with chronic hepatitis B virus infection were selected for therapy with antiviral agents as described (14). Six patients received ara-A alone or in combination with human leukocyte interferon. Four patients received no ara-A during this period of the study and were used as controls. Ara-A was generously supplied by Parke-Davis & Co. (Detroit, Mich.). Treatment included 10 daily 12-h, i.v., infusions of 5.0 mg/kg per d of ara-A up to 450 mg/liter in warmed 5% dextrose in water.

Blood was collected in EDTA or heparin and kept at 4°C. Erythrocytes were then separated by centrifugation at low speed and washed twice with phosphate-buffered saline. Mononuclear cell preparations were collected by Ficoll-Hypaque separation of fresh, heparinized blood. The cells were stored at –70°C and coded by random number assignment before assay. Ado-Hcy hydrolase and purine nucleoside phosphorylase were measured by radiochemical methods (15, 16). Protein was assayed according to the method of Lowry et al. (17).

Statistical calculations were carried out using the paired or unpaired Student's *t* test.

RESULTS

Six patients undergoing eight separate treatment courses with ara-A were studied for enzyme changes

before, during, and after therapy. Pretreatment bloods from these patients, as well as blood from untreated patients, were used for controls. Ado-Hcy hydrolase activity in washed erythrocytes in controls was compared with enzyme activity at the end of a 12-h infusion of ara-A. These data are shown in Fig. 1. All 17 control samples fell within the previously established normal range of activity for this enzyme (3.9–9.8 nmol/h per mg). A decrease in enzyme activity was seen in all patients receiving ara-A. 37 samples from treated patients were analyzed with a mean of 0.2 ± 0.2 (SD) nmol/h per mg ($P < 0.001$).

Erythrocyte purine nucleoside phosphorylase was assayed on 17 of the 37 samples as a form of internal control. Activity of this enzyme in control bloods and postinfusion bloods was within the normal range (1,014.6 to 3,318.2 nmol/h per mg) with a mean of $1,861.5 \pm 253.5$ (SD) nmol/h per mg and $2,164.6 \pm 583.7$ (SD) nmol/h per mg, respectively (data not shown).

To distinguish whether inactivation of Ado-Hcy hydrolase resulted from ara-A or its major metabolite, ara-hypoxanthine (ara- H_x), hemolysate from an untreated subject was analyzed for this enzyme activity after addition of these compounds to whole blood before separation of the erythrocytes. No inhibition was

observed after addition of ara- H_x (data not shown). Addition of ara-A in vitro led to a concentration-dependent decrease of enzyme activity. Significant suppression of activity occurred only at 100 μ g/ml, a level 50- to 100-fold greater than concentrations reached by this drug in vivo. This reduction of enzyme activity occurred in vivo because mixing the blood treated with ara-A in varying proportions with blood from a control subject gave the expected intermediate activity (data not shown).

The time-course of Ado-Hcy hydrolase inactivation was examined in cells from two patients (Fig. 2). Mean erythrocyte Ado-Hcy hydrolase activity was 8.0 nmol/h per mg before therapy. 4 h after the start of the drug infusion, mean enzyme activity had decreased to 0.6 nmol/h per mg or 8% of pretreatment levels. At the end of the first 12-h infusion, mean enzyme activity had decreased to 0.2 nmol/h per mg, or 2% of pretreatment values. A small rise in activity occurred each day during the infusion-free period with repeat suppression at the end of each subsequent infusion. After cessation of the 10-d course of therapy, enzyme levels slowly increased. Levels reached the normal range on day 9 post withdrawal of ara-A, but remained lower than pretreatment values for 30 d posttherapy. During the same period mononuclear cell Ado-Hcy hydrolase ranged from a pretreatment value of 244.0 nmol/h per mg to a low value of 5.2 nmol/h per mg (Fig. 3). However, rises and falls were seen in enzyme activity throughout the treatment period.

Circulating lymphocyte counts were measured in five patients analyzed for Ado-Hcy hydrolase activity. Fig. 4 displays these data. Pretreatment circulating lymphocytes were $1,988 \pm 823$ (SD) per mm^3 . These counts were decreased to 599 ± 296 (SD) per mm^3 or $34.4 \pm 23.9\%$ (SD) of pretreatment values after 7–10 d of therapy. This decrease was significant ($P < 0.01$ by paired t test) and was seen in all five patients studied.

DISCUSSION

A diminution of Ado-Hcy hydrolase occurs in erythrocytes and lymphocytes of patients receiving ara-A. The alteration in erythrocytes continues for many days after cessation of therapy, beyond the period of detectability of ara-A or ara- H_x , its major metabolite. In addition, this inactivation is the result of therapy with ara-A, rather than the effect of ara- H_x . Irreversible inactivation of Ado-Hcy hydrolase by ara-A (7) is the most likely explanation for our observations and a similar change in a single patient after completion of therapy (18).

Metabolic and biologic consequences of a block of Ado-Hcy hydrolase have been previously observed. In cell culture models, the intracellular accumulation of

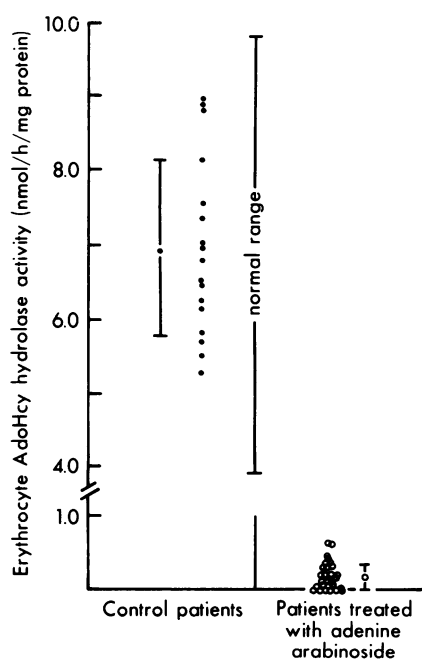


FIGURE 1 Effects of ara-A administration on erythrocyte Ado-Hcy hydrolase. Enzyme activity in washed erythrocytes in nanomoles per hour per milligram from 17 controls (left column ---●---) and 37 patients (right column ---○---) after 12 h infusion of drug (5.0 mg/kg per infusion). The established normal range, means, and standard deviations are also displayed.

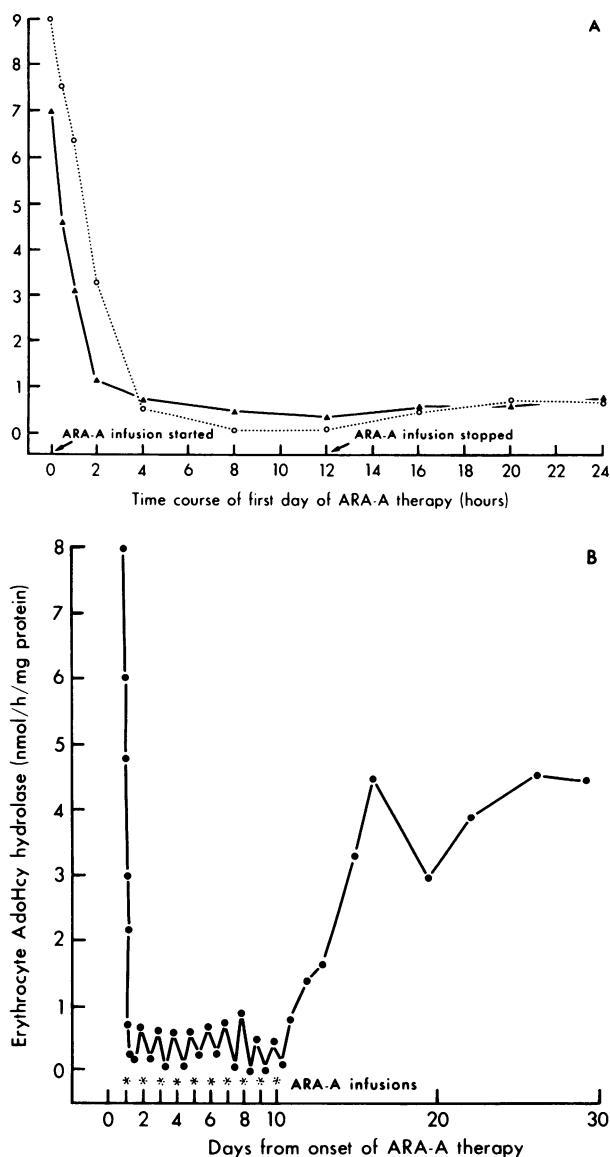


FIGURE 2 Time-course of suppression of erythrocyte Ado-Hcy hydrolase in two patients treated with ara-A for 10 d. (a) Enzyme activity in nanomoles per hour per milligram is plotted for each patient before and during the first 24 h of therapy. The actual times of drug infusion are shown (†). (b) Enzyme activity in nanomoles per hour per milligram (—●—) is plotted against time from the onset of therapy with ara-A. Each 12-h infusion with the drug is marked (*).

Ado-Hcy that follows enzyme inactivation inhibits cellular transmethylation reactions and leads to toxicity toward B lymphoblasts, T lymphoblasts, and macrophages (19–21). Severe immune dysfunction with lymphocytopenia and neurological abnormalities occurs in adenosine deaminase deficiency (22, 23), which is characterized by an almost complete absence of Ado-

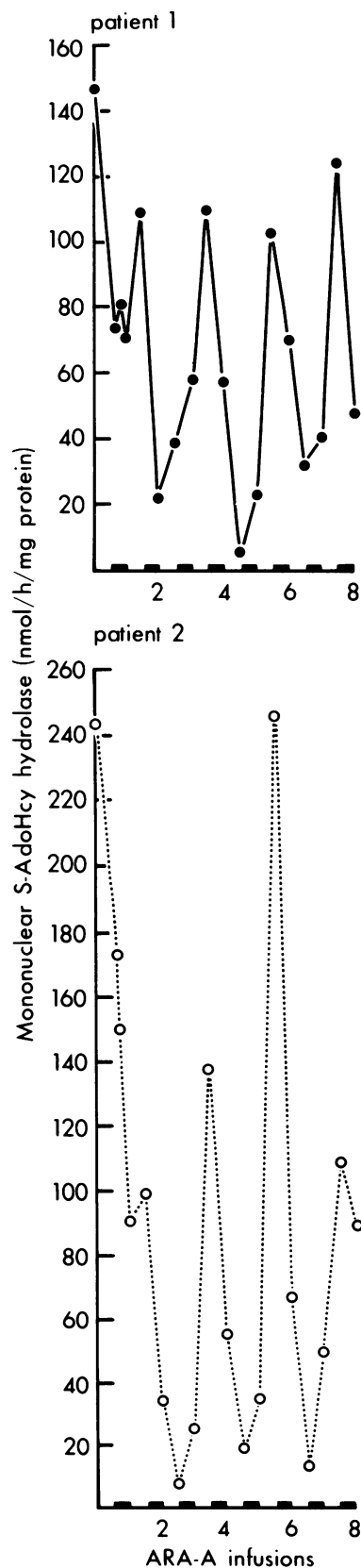


FIGURE 3 Time-course of suppression of mononuclear cell Ado-Hcy hydrolase in each of two patients treated with ara-A for 10 d. Enzyme activity in nanomoles per hour per milligram is plotted against time from the onset of therapy with adenine arabinoside. Each 12-h infusion with the drug is marked (■).

Hcy hydrolase in erythrocytes (15, 24). However, the exact contribution of the Ado-Hcy hydrolase deficiency to the disorders associated with adenosine deaminase deficiency remains unclear.

It is possible that inactivation of Ado-Hcy hydrolase could mediate certain toxic manifestations of ara-A therapy. Lymphocyte counts were diminished in 98% of all treatment courses, reaching a mean nadir of 48% of pretreatment lymphocyte counts by day 5 of therapy (6). We have also demonstrated similar findings in a subgroup of five patients studied both for enzyme activity and lymphocyte effects. Apparent lympholytic properties of ara-A (25) could be mediated by inactivation of Ado-Hcy hydrolase. While adenine arabinoside triphosphate accumulation has been previously associated with ara-A cytotoxicity in cultured T lymphoblasts (26), it is possible that adenine arabi-

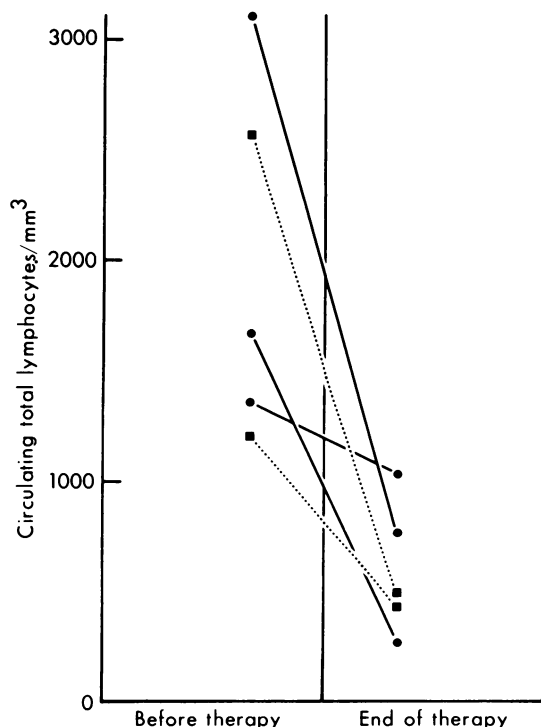


FIGURE 4 Circulating lymphocyte counts in five patients undergoing therapy with ara-A in whom erythrocyte Ado-Hcy hydrolase activity was measured. Pretreatment and posttreatment lymphocyte counts are compared in each patient. The patients whose mononuclear Ado-Hcy hydrolase activity were measured are marked separately (■).

noside triphosphate accumulation is not a factor in peripheral lymphocytes. The latter biochemically resemble B lymphoblasts (27), which accumulate little or no adenine arabinoside triphosphate (28) and which are 10-fold less sensitive to the toxic properties of ara-A than are cultured T lymphoblasts (26).

Ado-Hcy hydrolase inactivation during ara-A therapy may have clinical relevance. Lymphocytotoxicity of this compound could be mediated in part through this mechanism. Secondly, inhibition of transmethylation reactions could have significance as a mechanism of antiviral action for ara-A, especially because inhibition of transmethylation reactions has established antiviral properties (8–12). Whereas no specific data is presented here to implicate this pathway as the antiviral mechanism, the *in vivo* potential for transmethylation inhibition is provided by Ado-Hcy inactivation. Further investigation of the mechanisms of action and toxicity of ara-A should be undertaken.

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