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Short communication

Modulation of cytosine arabinoside-induced proliferation inhibition by exogenous adenosylmethionine

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Summary. The effect of cytosine arabinoside on adenosylmethionine synthesis in relation to its proliferation-inhibiting ability was investigated in HT/29 and SW 620 human colon-tumor cell lines. A significant decrease in adenosylmethionine synthetase (E. C.2.4.2.13) activity was found after 2.5 h incubation with the drug, suggesting that depletion of adenosylmethionine pools might occur. Both this possible loss of adenosylmethionine and the cytostatic effect of cytosine arabinoside could partly be reversed by the exogenous administration of the former drug. Our data show that the cytostatic effect of cytosine arabinoside may be due in part to a shortage of adenosylmethionine; this finding is important for the design of combination chemotherapy regimens.

Introduction

The major cytotoxic mechanism of the known antimetabolite cytosine arabinoside seems to be the inhibition of DNA synthesis via incorporation of the drug into DNA, resulting in the formation of an inadequate primer terminus for further chain elongation [6]. In addition, cytosine arabinoside exerts other effects on cell metabolism that are probably less singificant but may contribute to the antiproliferative activity of the drug, including inhibition of DNA polymerases [9, 15], inhibition of single- and double-strand DNA repair [16, 17] and hyper- or hypomethylation of DNA, depending on the concentration of the drug [1, 7, 18].

Considering the effect of cytosine arabinoside on DNA methylation, the drug presumably perturbs adenosylmethionine formation as well. The present study was undertaken to determine whether cytosine arabinoside actually influences adenosylmethionine synthesis and, if so, whether this influence has any impact on the cytostatic effect of the drug. In this paper we show that in the concentration range of 10^{-6}–10^{-4}M, cytosine arabinoside decreases the activity of adenosylmethionine synthetase (E. C.2.4.2.13), which leads in turn to a depletion of adenosylmethionine pools, whose loss can be readily reversed by exogenous administration of adenosylmethionine.

Materials and methods

RPMI 1640 tissue-culture medium was obtained from Gibco. *S*-Adenosyl-L-methionine (Sigma) was stored as a 1-mM stock solution in 0.9% NaCl at –20° C. The solutions of cytosine arabinoside were prepared immediately prior to their use. [^{3}H]-L-methionine (Sp. act., 17.24 GBq/mM) and [^{3}H]-*S*-adenosyl-L-methionine (sp. act., 555 GBq/mM) was supplied by Amersham.

HT/29 and SW 620 human colon-tumor cells were grown on RPMI 1640 medium supplemented with 10% fetal calf serum. After trypsinization, cells were seeded at an initial density of 1.5×10^5 cells/ml medium per well on a 24-well limbro-plate. Following adhesion, cells were continuously incubated in the presence of 50 μM adenosylmethionine. After 16 h, cells were treated with varying concentrations of cytosine arabinoside for 2.5 h. On the next day, viable cells were trypsinized and counted in a haemocytometer using the trypan-blue exclusion system. The reported values represent the means of three determinations.

The uptake of [^{3}H]-*S*-adenosyl-L-methionine over 60 min was quantified as follows. Following treatment with adenosylmethionine and/or cytosine arabinoside, the cells were incubated with 10 μM [^{3}H]-*S*-adenosylmethionine for 60 min and then washed two times with PBS containing 1 mM adenosylmethionine. The cells were solubilized in 0.5 ml 0.05 M NaOH-2% sodium dodecyl sulfate (SDS) and the radioactivity was determined using a dioxane-based scintillation cocktail.

A total of 10^7 cells were prepared for adenosylmethionine synthetase assay according to Cox and Goorha [3]. The assay mixture contained 0.1 M TRIS-HCl (pH 8), 25 mM $MgCl_2$, 150 mM KCl, 5 mM ditiothreitol (DTT), 10 mM adenosine triphosphate (ATP), and 25 μM [^{3}H]-L-methionine; the final volume of the reaction mixture was 100 μl. After cell incubation for 30 min at 37° C, 50 μl ice-cold 2 *N* $HClO_4$ containing 5 mM methionine was added. Following removal of the precipitate by centrifugation, the supernatant was applied onto P-81 phosphocellulose paper discs; the discs were washed five times in 0.1 M ammonium-formate (pH 3) and twice in acetone and the radioactivity was counted using a toluene-based scintillation cocktail. The results were expressed as counts per minute per 10^7 cells.

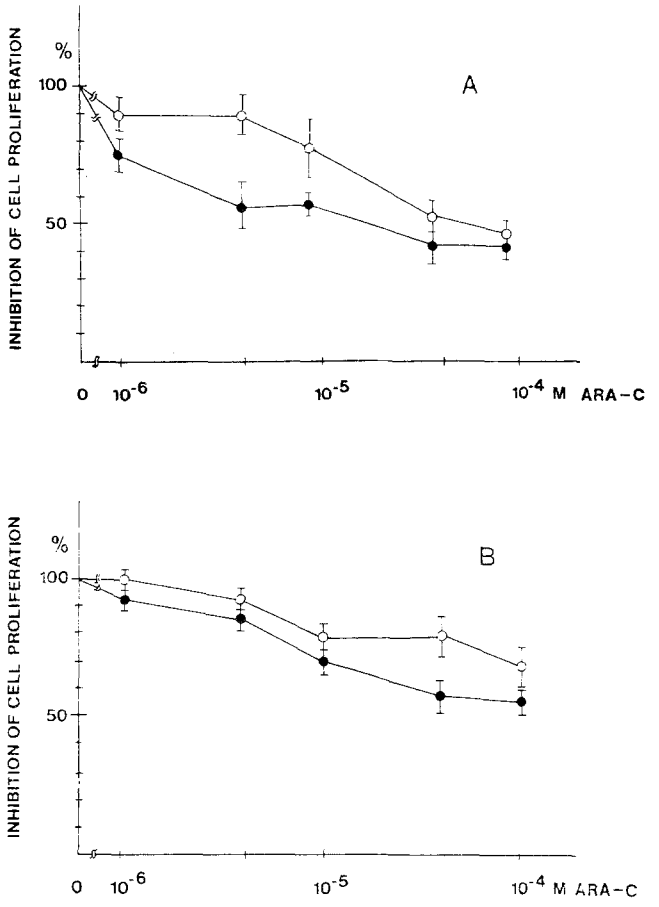

Fig. 1 A, B. Effects of 2.5 h incubation with cytosine arabinoside (●, *ARA-C*) and a combination of 16 h treatment with adenosylmethionine and 2.5 h incubation with Ara-C (○) on the proliferation of cell lines HT/29 (**A**) and SW 620 (**B**)

Table 1. Changes in adenosylmethionine synthetase activities and total adenosylmethionine pools after different treatments

Cell line	Treatment	Enzyme activity		Total SAM uptake	
		cpm/10^7 cells	% of control	cpm/10^5 cells	% of control
HT/29	Control	4,900	–	3,410	–
	1 μM Ara-C	3,900	80	4.530	133
	10 μM Ara-C	3,300	67	5,560	163
	50 μM Ara-C	1,420	29	5,860	172
	50 μM SAM	2,600	53	4,380	128
	1 μM Ara-C +50 μM SAM	3,310	68	4,100	120
	10 μM Ara-C +50 μM SAM	3,080	63	4,470	131
	50 μM Ara-C +50 μM SAM	900	18	5,150	150
	10% DMSO in vitro	3,430	70	–	–
SW 620	Control	5,130	–	4,320	–
	1 μM Ara-C	4,080	80	5,490	127
	10 μM Ara-C	2,680	52	6,180	143
	50 μM Ara-C	2,160	42	6,960	161
	50 μM SAM	2,260	50	5,310	123
	1 μM Ara-C +50 μM SAM	3,330	55	5,230	121
	10 μM Ara-C +50 μM SAM	3,540	69	5,620	130
	50 μM Ara-C +50 μM SAM	1,800	35	6,000	139
	10% DMSO in vitro	4,030	79	–	–

Ara C, Cytosine arabinoside; SAM, [^{3}H]-*S*-adenosyl-L-methionine; DMSO, dimethylsulfoxide

Results

As shown in Fig. 1, cytosine arabinoside induced a concentration-dependent inhibition of cell proliferation in HT/29 and SW 620 cell lines. The presence of adenosylmethionine in the tissue-culture medium reduced the influence of cytosine arabinoside on cell proliferation. Adenosylmethionine alone did not exert stimulatory or inhibitory effect on cell growth when used at a concentration of 1–100 μM (data not shown).

The schedule of treatment with adenosylmethionine and cytosine arabinoside turned out to be an important factor. We observed this antagonistic effect only when the addition of adenosylmethionine preceded treatment with cytosine arabinoside; the former substance did not modulate the cytostatic effect of cytosine arabinoside when it was added simultaneously with or after the latter. In our experiments on the uptake of adenosylmethionine by cells that had been treated with cytosine arabinoside, we recorded a 30%–70% increase in the total adenosylmethionine pool. Pretreatment with adenosylmethionine constrained this increase to 20%–50%. The data shown in Table 1 reveal that a 2.5-h treatment with cytosine arabinoside reduced the activity of adenosylmethionine synthetase in a concentration-dependent manner. The presence of adenosylmethionine in the medium caused a further decrease in the activity of this enzyme.

On the basis of the data shown in Table 1, we believe that the adenosyl-methionine synthetase present in these two cell lines is probably the kidney-type γ isozyme, which is significantly inhibited by 10% DMSO in vitro and is characteristically a tumor-type adenosylmethionine synthetase isozyme.

Discussion

Cytosine methylation patterns have been postulated to participate in the modulation of cellular differentiation [4, 22] and to play a role in base mismatch repair through alterations in the structure of chromatin [2, 8]. The existence of a relationship between DNA hypo- and hypermethylation and cytosinearabinoside induced differentiation has been supported by previous studies [5, 12]. We designed experiments to determine whether the cytostatic effect of cytosine arabinoside is dependent on changes in the DNA methylation pattern and, if so, whether such alterations represent a cause or a consequence of the effect of the drug. The treatment schedule for cytosine arabinoside was based on previous pharmacokinetic and clinical data [14, 20]. The highest level of cytosine arabinoside achievable in blood plasma was found to be 10^{-4} M for 2 h using a high-dose cytosine arabinoside regimen. For this reason, we applied the drug for 2.5 h in the concentration range of 10^{-6}–10^{-4} M.

We observed a pronounced decrease in adenosylmethionine synthetase activity immediately following treatment with cytosine arabinoside and, probably as a

consequence, an increase in the uptake of adenosylmethionine. On the other hand, the administration of adenosylmethionine prior to cytosine arabinoside could partly modulate the cytostatic effect of the drug. This finding suggested the possibility that cytosine arabinoside induces a depletion of adenosylmethionine pools. On the basis of these data, we could not determine whether this impact on the synthesis of adenosylmethionine was due to an immediate effect of cytosine arabinoside-ATP, the active metabolite of the drug, on adenosylmethionine synthetase.

The main pathways in the cell that are impaired by this shortage of adenosylmethionine have yet to be defined; however, a shift in the adenosylmethionine/adenosylhomocysteine ratio may be expected. Adenosylmethionine is a central intermediate in methionine metabolism, being a methyl donor in the methylation of nucleic acids [10], drugs and xenobiotics [24], an aminopropyl donor in polyamine synthesis [19] and an intermediate in the transsulfuration pathway leading the formation of to cysteine and cysteine derivatives. It also regulates certain enzymatic reactions, including its own synthesis [13].

Decreased adenosylmethionine synthetase activity might interfere with maintenance of the highly methylating environment that is necessary for DNA and RNA methylation. Consequently, our results fit well with those reported in previous studies [3, 7], showing that cytosine arabinoside used in the present concentration range causes DNA hypomethylation. Nevertheless, considering the influence of the drug on methionine metabolism, the formation of adenosylmethionine may play a more significant role in alterating the DNA methylation pattern than in changing the DNA-methyltransferase activity. Resistance to cytosine arabinoside might be developed not only via a decrease in cytidine kinase or an increase in the deoxycytidinetriphosphate (dCTP) pool or in cytidine deaminase, but via an increase in the adenosylmethionine pool as well. In a former study, mutant murine lymphoma cells exhibiting cross-resistance to various adenosine nucleoside and methionine analogues were isolated; this resistance was apparently derived from an elevated adenosylmethionine level [11]. We suggest that cytosine arabinoside belongs to the group of drugs that perturbate adenosylmethionine/adenosylhomocysteine metabolism.

Our results may have important implications for the design of combination chemotherapy regimens. Drugs that block methionine uptake, such as methotrexate [23], inhibit the formation of methionine from homocysteine, or cause a depletion of polyamine pools, e.g., difluoromethylornithine [21], might produce a synergistic effect if they are applied prior to cytosine arabinoside.

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