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S-adenosyl-L-homocysteine: a non-cytotoxic hypomethylating agent

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Abstract. The cytotoxic effect caused by the hypomethylating agent S-adenosyl-L-homocysteine (SAH) was compared with that of two drugs commonly used to induce DNA hypomethylation, 5-azacytidine and 5-aza-2′-deoxycytidine. Two in vitro cytotoxicity tests, the tetrazolium MTT assay and the intracellular lactate dehydrogenase (LDH) activity test, suggest that SAH induces hypomethylation without causing any cytotoxic effect. We propose the use of SAH as a non-cytotoxic agent which may be more suitable for inducing experimental DNA hypomethylation.
Key words. Cytotoxicity; S-adenosyl-L-homocysteine; hypomethylating agent; cytidine analogs.

The role of DNA methylation as a locking mechanism for gene expression in somatic cells is well established, but much remains to be done to improve our understanding of the dynamic changes in DNA methylation which occur during development[1]. It has also been suggested that modification of DNA-protein interactions induced by methylation of cytosines is related to changes in the organization of the eukaryotic chromatin[2]. Thus, the availability of hypomethylating agents is of great interest in cellular and molecular biology.

5-azacytidine (5-aza-CR) and 5-aza-2′-deoxycytidine (5-aza-CdR) are the drugs commonly used to induce DNA hypomethylation. Both analogs are incorporated into replicating DNA, and act by inhibiting enzymes that methylate cytosine residues in eukaryotic DNA[3]. However, these agents have been found to be highly cytotoxic for mammalian cells, as demonstrated by the low plating efficiency exhibited by cells in their presence[4,5]. Recently, Davidson et al.[6] have suggested a close correlation between the cytotoxicity caused by these agents and their inhibitory effect on DNA methylation.

A new hypomethylating agent, S-adenosyl-L-homocysteine (SAH), has recently been assayed. Barbés et al.[7] reported that SAH, at the same final concentration used by us, inhibits the DNA methyltransferase activity in cell free systems by about 45%, and comparable results have been obtained in vivo in our laboratory[8].

Nevertheless, to date there are no reports of the possible effects of this compound on cell viability. Basal cytotoxicity from a chemical is related in many cases to an assault on cell membrane integrity or on mitochondrial activity, since these are fundamental metabolic functions that are common to all cells. Therefore, we have used in this work two in vitro cytotoxicity tests in combination: the tetrazolium MTT test, which measures mitochondrial integrity, and the intracellular lactate dehydrogenase (LDH) activity assay, which detects alterations on cell membrane permeability[9]. We compare the cytotoxic effects induced by SAH with those produced by both 5-aza-CR and 5-aza-CdR after different length incubations and at concentrations that induce DNA hypomethylation[10].

Materials and methods

Human oral fibroblasts (primary cultures) were cultured in 12-well plates using Dulbecco's modified Eagle's medium (DMEM), supplemented with 5% foetal calf serum, and kept in a CO_2/O_2 cell incubator. The plates were incubated for 24 h to achieve about 60–70% confluency. Then, the medium was removed and the cells were cultured with unmodified medium (control) or supplemented with the agents under study, at concentrations inducing DNA hypomethylation: 8 μM 5-aza-CR (Serva), 0.2 μM 5-aza-CdR (Sigma)[10] and 150 μM SAH (Boehringer-Mannheim)[7,8]. Cells were incubated for 24, 48 and 72 h, and culture solutions were renewed each 24 h.

The leakage of intracellular LDH after exposure of cells to xenobiotics was measured essentially as described by Vassault[11]. The MTT test was carried out according to Edmonson et al.[12]. This method is based on the reduction of the soluble yellow MTT tetrazolium salt to a blue insoluble MTT formazan product by mitochondrial succinic dehydrogenase[13]. The quantification of viable cells after incubation with toxins was by colorimetric methods.

All the experiments were performed at least three times, using triplicate wells for each time and test agent.

Results and discussion

The cytotoxicity caused by the hypomethylating agent S-adenosyl-L-homocysteine, is compared here with that of cytidine analogs at concentrations that induce DNA hypomethylation, previously indicated by different au-

Comparative cytotoxicity of hypomethylating agents as determined by the tetrazolium MTT and the intracellular LDH tests.

Test agent	MTT assay[a]			LDH assay[b]		
	24 h	48 h	72 h	24 h	48 h	72 h
Control	100	100	100	152.3 ± 8.06 (100)	285.0 ± 11.00 (100)	157.5 ± 8.19 (100)
5-aza-CR	100	83.1	76.0	137.0 ± 7.64 (89.9)	267.3 ± 10.60 (93.7)	162.2 ± 8.32 (100)
5-aza-CdR	100	100	91.0	96.1 ± 6.40 (63.0)	141.5 ± 7.77 (49.6)	78.4 ± 5.78 (49.7)
SAH	100	100	92.3	115.2 ± 7.01 (75.7)	258.0 ± 10.40 (90.5)	148.3 ± 7.90 (94.1)

[a]Cell viability is expressed as percentage of that of control cultures.
[b]Enzymatic activities of LDH are expressed as units/litre ± SEM and compared with those of controls (percentage values in brackets).

thors[8,10]. Results obtained by two standard cytotoxicity tests are shown in the table. Both assays quantify viable cells after incubation with toxins, but each test has a different physiological endpoint[14].

Under our experimental conditions, the hypomethylating agent SAH is revealed as nontoxic by both assays. The concentration used to cause DNA hypomethylation does not reduce the respiratory activity of the cells as measured by the MTT test. Neither is there any effect on the cell membrane permeability, since values of intracellular LDH in treated cells are not significantly different from those observed in control cells ($p < 0.005$).

5-aza-CR seems to interfere with mitochondrial activity as revealed by the MTT data, but permeability of fibroblast cell membrane is not significantly altered, according to LDH results. Conversely, 5-aza-CdR was shown to be highly toxic by the intracellular LDH activity assay, but it appeared as non-cytotoxic by the MTT test. The present data indicate that in vitro cytotoxicity methods can give different results for the same chemical, which may also give some information about the biochemical mechanism of the compounds.

It is important to note that the cytotoxic effects caused by both drugs became significant after 48 h of treatment. This result is in line with the fact that the DNA of cells treated with these cytosine analogs attains maximal hypomethylation levels after 48 h[10].

On the other hand, both tests revealed that SAH has no cytotoxic effects after 48 or 72 h treatments. The innocuousness of SAH may be related to the mechanism by which it promotes hypomethylation. Thus, whereas cytidine analogs are directly incorporated into replicating DNA, SAH is a cellular metabolite. SAH is known to be a byproduct of transmethylation reactions and carries out a feedback control on such reactions Consequently, SAH acts as a potent inhibitor of DNA methyltransferase[15].

The absence of cytotoxicity exhibited by SAH makes it, in our opinion, the agent of choice to induce effective DNA hypomethylation.

Abbreviations: 5-aza-CR = 5-azacytidine; 5-aza-CdR = 5-aza-2′-deoxycytidine; DMEM = Dulbecco's modified Eagle's medium; LDH = Lactate dehydrogenase; SAH = S-adenosyl-L-homocysteine.

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