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EFFECT OF 5'-METHYLTHIOADENOSINE ON INDUCTION OF MURINE ERYTHROLEUKEMIA CELL DIFFERENTIATION

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The polyamines putrescine, spermidine and spermine have been implicated in the regulation of proliferation and differentiation. The present study has monitored the effects of 5'-methylthioadenosine, the metabolic product of spermidine and spermine synthesis, on the appearance of a differentiated murine erythroleukemia cell phenotype. The results demonstrate that increasing concentrations of 5'-methylthioadenosine (1×10^{-6} to 5×10^{-4} M) progressively inhibit murine erythroleukemia cell heme synthesis and hemoglobin production. The results also demonstrate that this inhibition of differentiation is not related to depletion of intracellular spermidine or cytostasis. Since 5'-methylthioadenosine is also a known inhibitor of DNA methylation, this naturally occurring nucleoside may be an intermediate involved in both murine erythroleukemia cell proliferation and differentiation.

The polyamines putrescine, spermidine and spermine have been implicated in the regulation of cellular proliferation (1, 2) and differentiation (3-6). However, the involvement of polyamines in the interrelationship between proliferation and induction of differentiation has remained unclear. We have previously demonstrated that spermidine is required for MEL cell growth and that there is a highly significant relationship between intracellular spermidine levels and cellular proliferation (7). Furthermore, we have demonstrated that induction of MEL differentiation requires intracellular spermidine for both heme production and synthesis of α- and β- globin mRNA (8). These findings would suggest that spermidine is required for both proliferation and differentiation, although a requirement of cell division for expression of the mature phenotype could indirectly reflect the involvement of this polyamine in differentiation.

Abbreviations used are: 5'-methylthioadenosine: MTA; dimethyl sulfoxide: DMSO; hexamethylene bisacetamide: HMBA; butyric acid: BA; S-adenosylmethionine: AdoMet; S-adenosylhomocysteine: AdoHcy.

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MTA is formed during the synthesis of spermidine and spermine (9). The feed-back inhibition of spermidine synthase and spermine synthase by MTA is competitive with respect to decarboxylated AdoMet (9). MTA is also an inhibitor of AdoHcy hydrolase (10) and post-synthetic methylation of DNA (11). Thus, MTA affects pathways involved in both proliferation and expression of specific genes.

The present study has monitored the effects of MTA on proliferation and differentiation of MEL cells. The results demonstrate that MTA inhibits induction of MEL differentiation in a concentration-dependent manner. Further, the inhibition of MEL differentiation by MTA is not associated with spermidine depletion or cytostasis. These findings suggest that MTA formed during the synthesis of polyamines may regulate expression of a differentiated phenotype.

MATERIALS AND METHODS

CELL CULTURE

The murine erythroleukemia cell line PC4 provided by Dr. D. Housman, Massachusetts Institute of Technology, Boston, MA, was maintained in α medium (Gibco, Grand Island, NY) containing 10% fetal bovine serum (Gibco), 100 units streptomycin/ml, 100 μg penicillin/ml and 2mM L-glutamine at 37°C in a 5% CO_2 atmosphere. Cell density was maintained between 2 x 10^4 and 5 x 10^5 cells/ml to ensure continuous logarithmic growth. The MEL cells were grown in suspension culture in the presence of varying concentrations of MTA (Sigma Chemical Co., St. Louis, MO) and 1.25% DMSO (Fisher Scientific, Boston, MA). Cultures were scored for benzidine positive cells by the wet benzidine method (12). Heme concentration was determined fluorometrically (13). Viability was monitored by trypan blue exclusion.

POLYAMINE DETERMINATION

MEL cells (5 x 10^6) were harvested and washed twice with phosphate buffered saline. Polyamines were extracted in 4% sulfosalicylic acid and separated on a Beckman 121 MB amino acid analyzer (7, 14).

RESULTS

We have monitored the effects of varying concentrations of MTA (1 x 10^{-6} to 5 x 10^{-4}M) on proliferation of MEL cells. MTA had no detectable effect on MEL proliferation up to a concentration of 1 x 10^{-4}M. Figure 1 illustrates the effects of 5 x 10^{-4}M MTA which resulted in a partial slowing of MEL growth. Similar MEL growth curves were obtained with MTA alone or in combination with 1.25% DMSO (Figure 1).

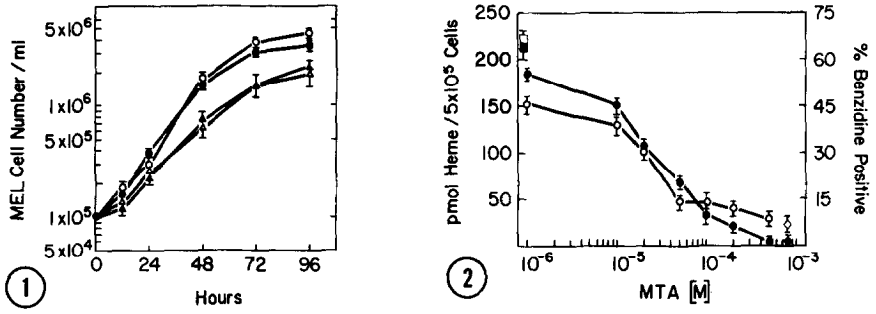

Figure 1: Effects of MTA and DMSO on MEL Cell Growth. MEL cells in logarithmic growth phase were treated with MTA (5 x 10^{-4}M) and/or DMSO (1.25%). Each point in this respresentative experiment is the mean ± standard deviation of 3 determinations. Viability was over 95% in each group. Control MEL cells (●), MTA-treated cells (▲), DMSO-treated cells (○); and MTA/DMSO-treated cells (△).

Figure 2: Effects of MTA and DMSO on MEL Heme Production and Percent Benzidine Positive Cells. MEL cells in logarithmic growth phase were treated with MTA and DMSO (1.25%). Cells were monitored for heme production (open symbols) and percent benzidine positive cells (closed symbols) at 96 hrs. Results of this representative experiment are expressed as the mean ± standard deviation of three separate determinations. Control MEL cells (◇,◆), DMSO-treated cells (□,■) and MTA/DMSO-treated cells (○,●). Mean MEL cell counts at 96 hrs were: Control, 3.7 x 10^6; 10^{-6}M MTA/DMSO, 4.5 x 10^6; 1 x 10^{-5}M MTA/DMSO, 3.8 x 10^6; 2 x 10^{-5}M MTA/DMSO, 4.4 x 10^6; 5 x 10^{-5}M MTA/DMSO, 4.3 x 10^6; 1 x 10^{-4}M MTA/DMSO, 3.9 x 10^6; 2 x 10^{-4}M MTA/DMSO, 2.9 x 10^6; and 5 x 10^{-4}M MTA/DMSO, 1.3 x 10^6.

The effect of varying concentrations of MTA (1 x 10^{-6} to 5 x 10^{-4}M) on the synthesis of heme and hemoglobin is illustrated in Figure 2. MTA treatment inhibited synthesis of both heme and the appearance of benzidine positive cells. Thus, 10^{-6}M MTA partially inhibited heme synthesis and increasing concentrations of MTA progressively blocked heme production. Similar results were obtained when monitoring for the appearance of hemoglobin by benzidine staining. Over 60% of the MEL cells were benzidine positive after 96 hrs of exposure to DMSO, while treatment with MTA progressively inhibited hemoglobin production. Benzidine positive cells were undetectable following exposure of MEL cells to DMSO and 5 x 10^{-4}M MTA (Figure 2).

Our previous findings demonstrating that depletion of intracellular spermidine inhibits DMSO induction of MEL differentiation (7) prompted the measurement of intracellular polyamines following MTA treatment. The results obtained with 5 x 10^{-4}M MTA are illustrated in Figure 3.

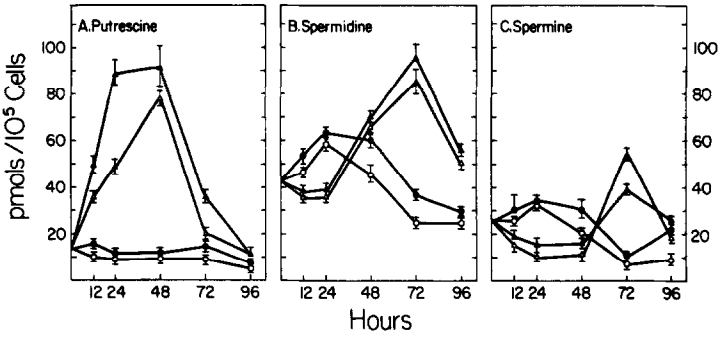

Figure 3: Effect of MTA on MEL Intracellular Polyamines. MEL cells in logarithmic growth phase were treated with MTA (5×10^{-4}M) and/or DMSO (1.25%) for 96 hrs. Control MEL cells (●), MTA-treated cells (▲), DMSO-treated cells (○), and MTA/DMSO-treated cells (△) were monitored for intracellular putrescine (A), spermidine (B) and spermine (C) at the indicated times. Each point represents the mean ± standard deviation of four separate determinations.

Intracellular putrescine, spermidine and spermine levels in cells treated with DMSO alone were comparable to levels obtained in untreated MEL cells. In contrast, MTA treatment resulted in marked increases in intracellular putrescine (Figure 3A) that reached maximal levels by 48 hrs. These increases in intracellular putrescine were accompanied by subsequent rises in intracellular spermidine (48, 72 hrs) and spermine (72 hrs). Similar results were obtained with 5×10^{-4}M MTA in combination with DMSO. Further, the addition of exogenous putrescine (5×10^{-5}M), spermidine (5×10^{-6}M) or spermine (5×10^{-6}M) failed to abrogate the inhibition of MEL differentiation by MTA.

It was also of interest to determine whether similar results would be obtained with other inducers of MEL differentiation such as HMBA and BA. MEL cells were treated with 5×10^{-4}M MTA and either inducer. The results obtained are listed in Table 1. Induction of differentiation with HMBA was completely inhibited by MTA treatment. Similar results were obtained with BA.

DISCUSSION

Although the polyamines have been implicated in regulating both proliferation (1, 2) and differentiation (3-6), a direct role has been demonstrated only for spermidine in DNA replication (15). We have

Table 1. Effect of 5×10^{-4} M MTA on other inducers of MEL differentiation

Drug	% Benzidine Positive [1]
HMBA (4 mM)	82.2 ± 0.4
MTA/HMBA	9.0 ± 0.5
BA (1.75 mM)	31.3 ± 2.0
MTA/BA	12.1 ± 1.5

[1] Mean ± standard deviation of four determinations performed at 96 hr of exposure to HMBA and BA.

previously demonstrated that spermidine is required for both MEL proliferation and induction of differentiation by DMSO, HMBA and BA (7, 8). The requirement of spermidine for induction of MEL differentiation may have reflected a necessity for cell division in the expression of a differentiated phenotype. In this regard, cessation of MEL proliferation during the commitment period by total depletion of intracellular spermidine was required for inhibition of differentiation.. Other studies with erythroid precursor cells *in vivo*, however, suggest that the polyamines play a role only in proliferation and not differentiation (16).

MTA is formed from AdoMet and as a by-product in the synthesis of spermidine and spermine (9). MTA also regulates polyamine synthesis by directly inhibiting spermidine synthase and spermine synthase (9). In the present study, MTA treatment resulted in a rise in intracellular putrescine and a delay in progression to spermidine and spermine. In contrast to our previous work demonstrating a requirement of spermidine in induction of MEL differentiation, there was no significant depletion of intracellular spermidine following MTA treatment. Furthermore, MTA caused only a partial inhibition of cellular proliferation, while our previous study (7) demonstrated that spermidine depletion and cytostasis was necessary for complete inhibition of MEL differentiation. Thus, MTA is clearly acting by mechanisms other than spermidine depletion or complete inhibition of DNA replication.

MTA is also an inhibitor of AdoHcy hydrolase (10). Thus, MTA could serve as an intermediate between the polyamine pathway and a pathway involved in transmethylation. In this regard, MTA is an effective inhibitor of DNA methylation (11) and therefore may regulate specific gene expression and differentiation. Since MTA does not appear to be inhibiting MEL differentiation by spermidine depletion, it is possible MTA inhibits heme synthsis and hemoglobin production by interfering with transmethylation mechanisms. Whatever the mechanism, the present study suggests that MTA may play a role in the relationship between MEL proliferation and differentiation.

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REFERENCES

1. Heby, O. (1981) Differentiation 19:1-20.
2. Tabor, C.W. and Tabor, H. (1976) Ann. Rev. Biochem., 45:285-306.
3. Emmanuelsson, H. and Heby, O. (1978) Proc. Natl. Acad. Sci. 75:1039-1042.
4. Fozard, J.R., Part, M.L., and Prakash, N.J. et al. (1980) Science 208:505-508.
5. Takigawa, M., Ishida, H., Takano, T. and Suzuki, F. (1980) Proc. Natl. Acad. Sci. 77:1481-1485.
6. Verma, D.S., and Sunkara, P.S. (1982) Cancer Res. 42:3026-3049.
7. Sugiura, M., Shafman, T. and Kufe D. (1984) Cancer Res. 44:1440-1444.
8. Watanabe, T., Shafman, T. and Kufe, D.W. (in press) J. Cell. Phys.
9. Williams-Ashman, H.G., Seidenfeld, J. and Galletti, P. (1982) Biochem. Pharmacol. 31:277-288.
10. Ferro, A.J., Vandenbark, A.A. and MacDonald, M.R. (1981) Biochem. Biophys. Res. Comm. 100:523-531.
11. Woodcock, D.M., Adams, J.K., Allan, R.G. and Cooper, L.A. (1983) Nucl. Acids Res. 2:489-499.
12. Orkin, S., Swan, D. and Leder, P. (1975) Proc. Natl. Acad. Sci. 72:98-102.
13. Sassa, S. (1976) J. Expt. Med. 143:305-315.
14. Gehrke, C., Kuo, K. and Ellis, R. (1977) J. Chromatogr. 143:345-361.
15. Porter, C.W. (1983) Science 219:1083-1085.
16. Niskanen, E., Kallio, A., McAnn, P., Pou, G., Lyda, S. and Thornhill, A. (1983) Cancer Res. 43:1536-1540.