Vol. 86, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1979 Pages 340-349

INDUCTION OF ERYTHROID DIFFERENTIATION IN FRIEND MURINE ERYTHROLEUKEMIC CELLS BY INORGANIC SELENIUM COMPOUNDS

by Paul S. Ebert
Laboratory of Molecular Virology
National Cancer Institute, N.I.H.
Bethesda, Maryland 20014

George I. Malinin
Physics Department, Georgetown University
Washington, D. C. 20057

Received December 8,1978

SUMMARY

Inorganic selenium compounds are shown to be inducers of hemoglobin synthesis in malignant murine erythroleukemia (MEL) cells. SeO_2 can induce hemoglobin synthesis at 1/20 the concentration of butyric acid and 1/5000 the concentration of dimethylsulfoxide (DMSO), two potent inducers of erythroid differentiation in MEL cells. SeO_2 and H_2SeO_3 showed an equivalent capacity to stimulate hemoglobin synthesis in three different MEL cell lines. The incorporation of ^3H-glycine into hemoglobin was demonstrated in lysates of SeO_2-induced MEL cells.

INTRODUCTION

Erythroid differentiation in Friend murine erythroleukemic (MEL) cells can be triggered by structurally and functionally diverse types of organic compounds including cyroprotective agents (1), polymethylene bisacetamides (2), anti-tumor antibiotics (3), certain fatty acids (4), and reagents primarily affecting DNA synthesis (3,5,6). Although the precise nature of the initiating signal triggering hemoglobin synthesis in these cells is unknown, various investigations suggest the site of initiation to be at the cell membrane (7,8), which may ultimately affect DNA transcription (9) and/or translation (10), or by modifying the expression of malignancy to allow the previously blocked differentiation to proceed to completion (1,3). We now report the first example of an inorganic inducer of erythroid differentiation in malignant MEL cells, SeO_2.

MATERIALS AND METHODS

Details of incubation techniques and hemoglobin assay were described previously (3). Briefly, MEL cells were diluted to a concentration of 10^5/ml

0006-291X/79/020340-10$01.00/0

and the inducers dissolved in 0.1 M Tris(pH 7.4)-0.9% NaCl were added 4 hours later. Cells were continuously cultured in the presence of the inducer for a total of 4-5 days. Cells were then counted in a hemacytometer and duplicate aliquots of $2x10^6$ cells were analyzed for hemoglobin content (3) by the modified benzidine assay of Crosby and Furth (11). Solutions of SeO_2 yielded no reaction in the benzidine assay.

The 6-thioguanine-resistant diploid clone 585 derived from MEL clone 745 (12) and the tetraploid MEL line TEL (13) were obtained from Dr. A. Deisseroth of the National Cancer Institute. The T3C12 line derived independently from a tumored DBA/2 mouse (6,14) was obtained from Dr. Yoji Ikawa.

RESULTS AND DISCUSSION

The effect of SeO_2 on the stimulation of erythroid differentiation in MEL cell line 745 is presented in Table 1. The response of hemoglobin production to varying doses of SeO_2 was compared to the response induced by optimal levels of the potent inducer DMSO. Doses of SeO_2 sufficient to induce optimal hemoglobin production depressed the growth rate of cells to 30% of controls. Maximal hemoglobin synthesis was obtained with 50-100 μM SeO_2. At doses up to 1 mM cell counts only doubled in 5 days. Under incubation conditions optimal for DMSO- and butyrate-mediated hemoglobin production, SeO_2 in this experiment showed the same inducing potential as DMSO, but considerably less than butyrate.

The time course of appearance of intracellular hemoglobin following exposure to SeO_2 and DMSO is presented in Figure 1. SeO_2, though inhibitory to cell growth, stimulated hemoglobin production greater than that of the control by the second day of incubation. Hemoglobin production continued to increase until the third day, but did not substantially increase later as did DMSO-mediated hemoglobin synthesis.

In Table 2 SeO_2 and H_2SeO_2 were compared with DMSO in their ability to stimulate hemoglobin production in three different MEL cell lines. SeO_2 and H_2SeO_3 were approximately equivalent in their capacity to stimulate induction of hemoglobin synthesis in all three lines. In two experiments the TEL line

TABLE 1. Comparison of DMSO and butyrate with doses of SeO_2 as inducers of hemoglobin synthesis in 745 MEL cells

Inducer	Inducer Concentration (mM)	Number of tests	Final Cell count ($\times 10^6$ cells)	Hemoglobin Concentration (μg Hb/10^6 cells)
None	---	3	3.6	0.15
DMSO	280	2	5.4	0.82
Butyrate	1.0	2	3.4	1.43
SeO_2	0.01	2	2.6	0.19
SeO_2	0.025	1	0.9	0.34
SeO_2	0.05	2	0.6	0.76
SeO_2	0.1	3	0.3	0.88
SeO_2	0.5	2	0.2	0.50
SeO_2	1.0	2	0.2	0.53

showed a superior response to both inducers compared to the other lines. Slight differences of inducibility observed with each Se compound could not be duplicated from experiment to experiment.

The red tint in SeO_2-induced MEL cells is visually distinguishable from that of cells induced by butyrate or DMSO. This color could not be readily extracted by repeated freeze-thawing of water suspensions of induced cells as determined by absorbance measurements of extracts at 415 nm. Since certain mammalian tissues can reduce selenium compounds to the red elemental form (15), it is necessary to exclude the possibility that the redness was due to the deposit of intracellular Se^o. For this reason we attempted to show the incorporation of a heme precursor into hemoglobin during incubation of MEL cells with SeO_2. Accordingly, the synthesis of hemoglobin in MEL cells (TEL) incubated with 0.05 mM SeO_2 and ^3H-glycine was demonstrated when the soluble fraction of sonicated induced cells was chromatographed on a DEAE-cellulose

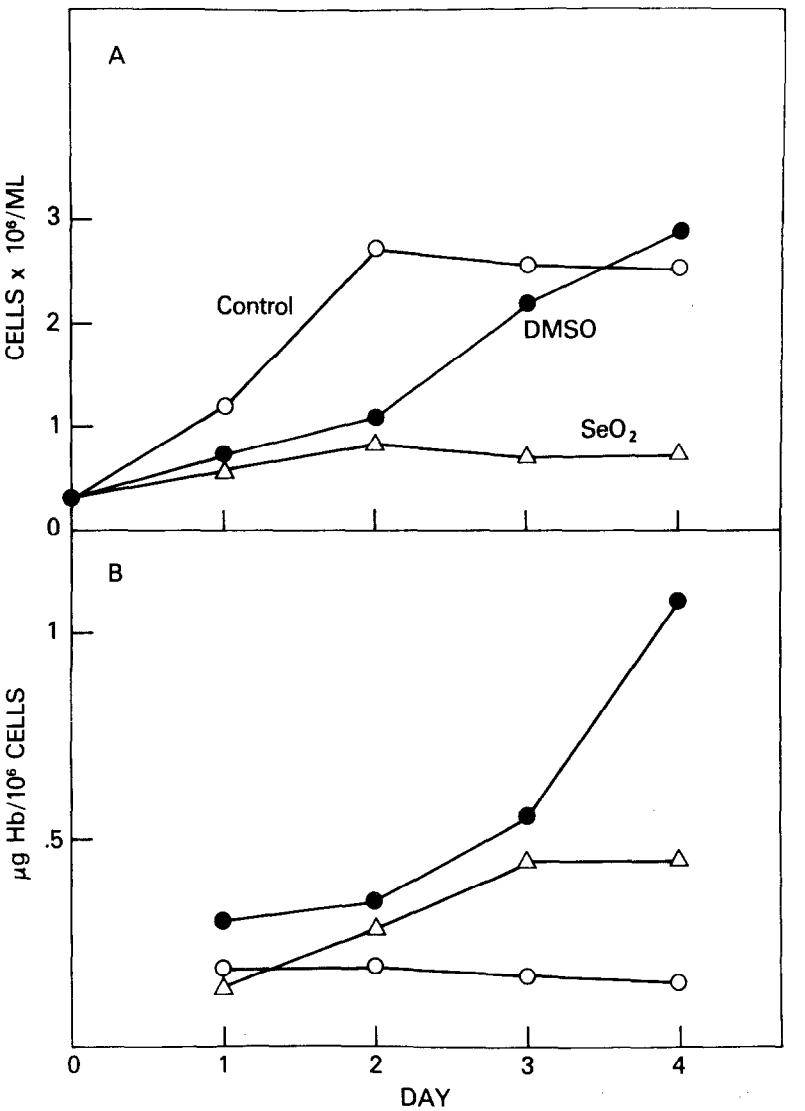


FIGURE 1. Time course of appearance of hemoglobin in 745 cells exposed to SeO_2 and DMSO. MEL cells were incubated in the presence of 280 mM DMSO or 0.05 mM SeO_2 for 4 days. Duplicate aliquots of MEL cells were analyzed for hemoglobin production at 24 hr intervals. Panel A shows the rate of cell growth for cells exposed to DMSO and SeO_2. Panel B shows the hemoglobin production in these cultures.

column (Figure 2). Two distinct peaks of ^3H-label co-chromatographed with carrier beef hemoglobin, while no labeled peaks were observed in extracts of non-induced cells.

TABLE 2. Effects of SeO_2 and H_2SeO_3 on hemoglobin production in 3 different MEL cell lines

Cell Line	Inducer	Number of Tests	Hemoglobin Production (μg Hb/10^6 cells)
585	--	1	0.11
585	DMSO, 280 mM	1	0.54
585	SeO_2, 0.05 mM	2	0.35 - 1.02
585	H_2SeO_3, 0.05 mM	2	0.35 - 0.82
TEL	--	1	0.30
TEL	DMSO, 280 mM	1	0.25
TEL	SeO_2, 0.05 mM	2	0.84 - 1.68
TEL	H_2SeO_3, 0.05 mM	2	0.91 - 1.45
T3C12	--	1	0.24
T3C12	DMSO, 280 mM	1	0.65
T3C12	SeO_2, 0.05 mM	2	0.55 - 0.89
T3C12	H_2SeO_3, 0.05 mM	2	0.70 - 1.19

A cytological examination of cells induced by DMSO and SeO_2 is shown in Figure 3 (A-D). Cells exposed to 0.05 mM SeO_2 generally appeared larger and frequently contained nuclei with vacuoles in comparison with DMSO-treated cells. Exposure to 0.1 mM SeO_2 produced greater toxicity as evidenced by cytolysis, shrinkage, and picnotic nuclei. The formation of intercellular bridges was a prominent feature as shown in Figure 3-D.

Selenium compounds may be affecting MEL cells by a number of different mechanisms. By virtue of their ability to inhibit various enzymes which require sulfhydryl groups for their function, Se compounds can inhibit a number of glycolytic enzymes as well as succinic dehydrogenase (oxidase) in the Krebs cycle (15). The inhibition of these enzymes most likely is responsible for the potent retardation of cell growth. Se can induce increased

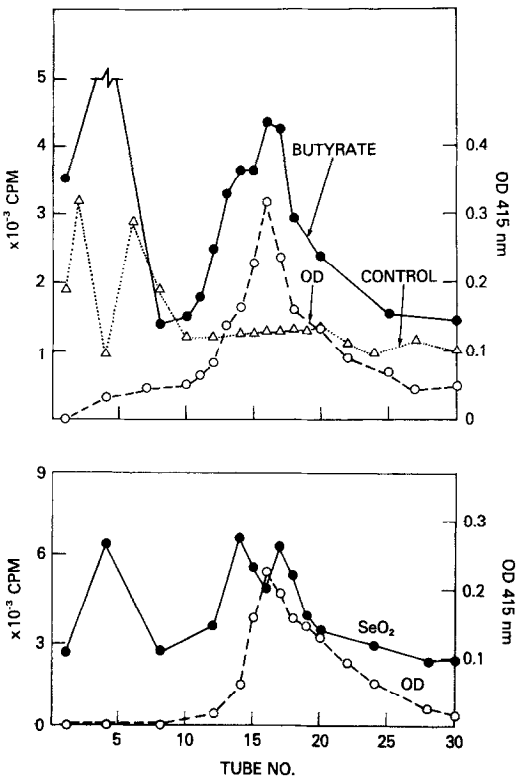


FIGURE 2. Chromatography of SeO_2-induced hemoglobin. TEL cells were incubated with no inducer, 0.05 mM SeO_2, or 1 mM butyrate for 5 days. On day 1 ^{3}H-glycine (15 Ci/mmole) was added to the incubation media at a final concentration of 5 μCi/ml and the cells were incubated 4 days longer. The cells were centrifuged, washed twice with Tris-NaCl and sonicated for 2 min in 0.2 M glycine-0.01% KCN (Developer A) (16). Following centrifugation, the soluble fractions were added to 1 × 11 cm columns of DEAE-cellulose (DE-52, Whatman), and following a wash with 5 ml of Developer A, the hemoglobin was eluted with a continuous gradient of NaCl from 0 to 0.2 M in Developer A. Fraction size was 2.5 ml.

glutathione peroxidase activity in neuroblastoma cells (17), as well as δ-aminolevulinic acid (ALA) synthetase and heme oxygenase of the heme biosynthetic and degradative pathways in normal liver (19). Apparently in MEL cells heme is synthesized and not quickly degraded as it is in liver tissue. However, since hemoglobin production does not increase markedly beyond the second day, unlike the DMSO-mediated response, some heme degradation must also be occurring. SeO_2 has been shown to induce chromosomal aberrations in cultured lymphocytes

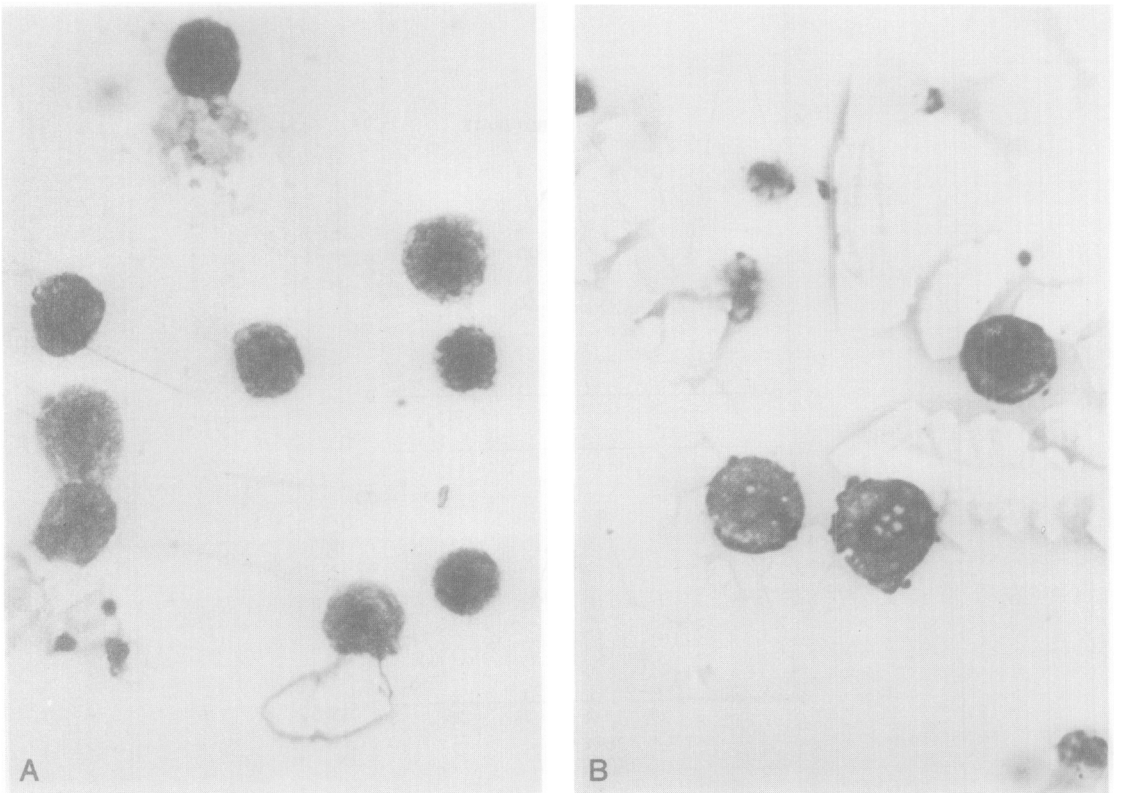
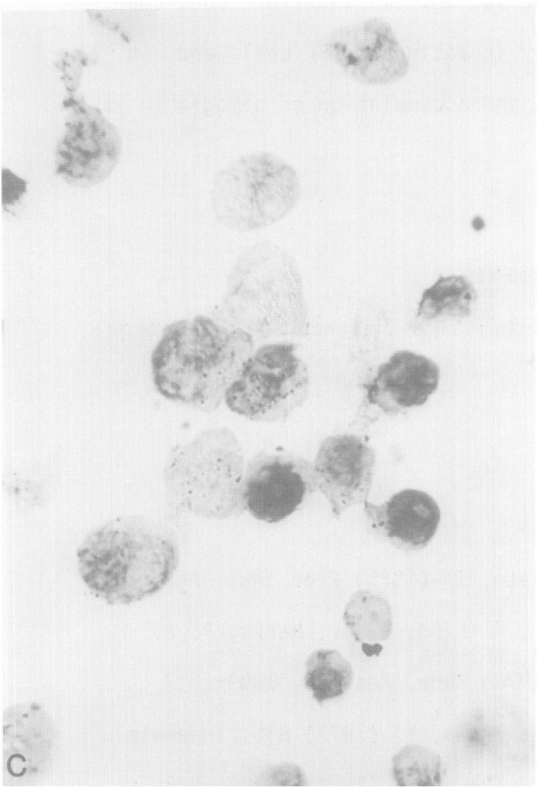


FIGURE 3 Cytology of TEL cells exposed to DMSO or SeO_2. A. DMSO-induced TEL cells showed nearly uniform size and large intensely basophilic nuclei and eosinophilic cytoplasm. All slides were stained with Wright-Giemsa and examined at ×1200 magnification. B. MEL cells incubated with 0.05 mM SeO_2 were larger than those incubated in DMSO. Markedly basophilic nuclei frequently contained a number of vacuoles.

(19) at concentrations above 65 μM. Recent investigations have shown an association of a decrease in the coefficient of sedimentation of Friend cell DNA with erythroid differentiation of MEL cells (20). SeO_2 can also function as a specific oxidant for the conversion of an α-methylene ketone to a 1,2-diketone (21). This possible reaction is analogous to the stimulation of mitogenic activity by periodate in lymphocytes (22). There is presently no experimental evidence to suggest that a similar triggering event may be occurring in MEL cells.



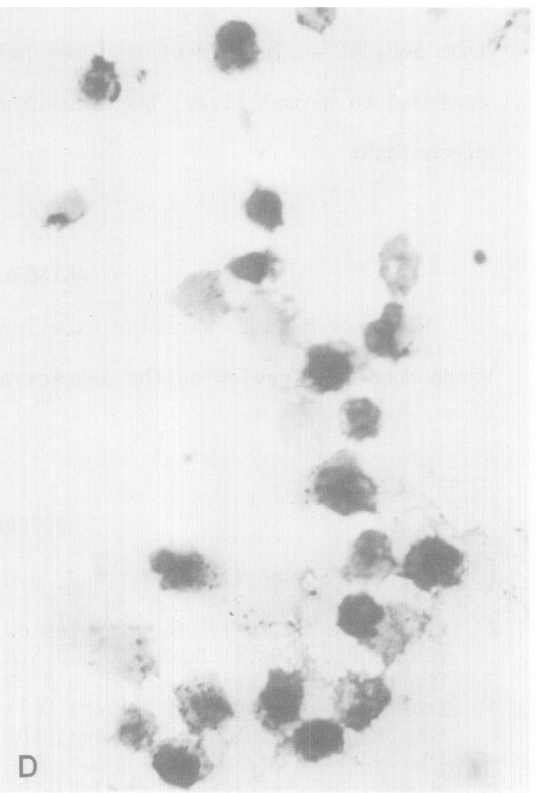


FIGURE 3 cont'd. C. MEL cells incubated for 5 days in the presence of 0.1 mM SeO_2 showed more pronounced evidence of cytoxicity as compared with Fig.3B. Cytotoxicity of SeO_2 was expressed by a decrease in a number of large cells containing basophilic nuclei, presence of intensely eosinophilic and vacuolated cells devoid of nuclei, and cell fusion. D. MEL cells incubated with 0.1 mM SeO_2 showing manifestations of cytotoxicity as evidenced by the presence of a large number of cells in various stages of cytolysis, cell shrinkage and picnotic nuclei. Cell clumping and intercellular bridges are observed frequently. Cytoplasmic granules also seen in Figure 1C are possibly depositions of elemental Se.

We have demonstrated the stimulation of erythroid differentiation of MEL cells by the inorganic compound SeO_2. Since Se has recently been shown to stimulate the synthesis of ALA synthetase in rat hepatic cells (18), stimulation of this rate-controlling enzyme, and other heme synthetic enzymes as well, must also occur in MEL cells. The difference between normal liver and these transformed cells appears to be that the heme synthesized in MEL cells

is not as rapidly degraded as in hepatic cells. It is therefore concluded that SeO_2 is an inducer of the heme pathway in malignant MEL cells and, in contrast to normal liver, the biosynthesis and accumulation of hemoglobin is predominant.

ACKNOWLEDGEMENTS

We thank Mrs. I. Wars for excellent technical assistance and Dr. George Vande Woude for reviewing the manuscript.

REFERENCES

1. Friend, C., Scher, W., Holland, J.G., Sato, T. (1971) Proc. Nat. Acad. Sci. U.S.A. 68, 378-382.
2. Reuben, R. C., Wife, R. L., Breslow, R., Rifkind, R. A., Marks, P. A. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 862-866.
3. Ebert, P. S., Wars, I., Buell, D. N. (1976) Canc. Res. 36, 1809-1813.
4. Leder, A. and Leder, P. (1975) Cell 5, 319-322.
5. Sugano, H., Furusawa, M., Kawaguchi, T., Ikawa, Y. (1973) Bibl. Haematol. 39, 943-954.
6. Sugano, H., Furusawa, M., Kawaguchi, T., Ikawa, Y. (1974) Recent Results in Canc. Res. 44, 30-44.
7. Bernstein, A., Hunt, D. M., Crichley, V., Mak, T. W. (1976) Cell 9, 375-381.
8. Tabuse, Y., Furusawa, M. Eisen, H., Shibata, K. (1977) Exp. Cell Res. 108, 41-45.
9. Ross, J., Gielen, J., Packman, S., Ikawa, Y., Leder, P. (1974) J. Mol. Biol. 87, 697-714.
10. Harrison, P. R., Gilmour, R. S., Affara, N. A., Conkie, D., Paul, J. (1974) Cell Differ. 3, 23-30.
11. Crosby, W. H. and Furth, F. W. (1956) Blood 11, 380-383.
12. Deisseroth, A., Burk, R., Picciano, D., Minna, J., Anderson, W. F., Nienhuis, A. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 1102-1106.
13. Deisseroth, A., Velez, R., Burk, R. D., Minna, J., Anderson, W. F., Nienhuis, A. (1976) Som. Cell Genet. 2, 373-384.
14. Ikawa, Y., Furusawa, M., Sugano, H. (1973) Bibl. Haematol. 39, 955-967.
15. Rosenfeld, I, Beath, O. A. (1964) In Selenium, pp. 338-360, Academic Press, New York.
16. Abraham, E. C., Reese, A., Stallings, M. and Huisman, T. H. J. (1976-77) Hemoglobin 1, 27-44.
17. Germain, G. S. and Arneson, R. M. (1977) Biochem. & Biophys. Res. Commun. 79, 119-123.
18. Maines, M. D. and Kappas, A. (1976) Proc. Nat. Acad. Sci. U.S.A. 73, 4428-4431.
19. Nakamuro, K., Yoshikawa, K., Sayato, Y., Kurata, H., Tonomura, M., Tonomura, A. (1976) Mut. Res. 40, 177-184.
20. Terada, M., Nudel, U., Fibach, E., Rifkind, R. A., Marks, P. A. (1978) Canc. Res. 38, 835-840.

21. Stewart, R. (1964) In Oxidation Mechanisms pp. 107, W.A. Benjamin, Inc., New York.
22. Parker, J. W., O'Brien, R. L., Steiner, J., Paolilli, P. (1973) Exp. Cell Res. 78, 279-286.