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EFFECT OF 5'-DEOXY-5'-S-ISOBUTYL-ADENOSINE (SIBA) ON MOUSE MAMMARY TUMOUR CELLS AND ON THE EXPRESSION OF MOUSE MAMMARY TUMOUR VIRUS

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SUMMARY

5'-deoxy-5'-S-isobutyl-adenosine (SIBA), an analogue of S-adenosyl-homocysteine, inhibits the growth of mouse mammary cells transformed by mouse mammary tumour virus (MMTV) and the synthesis of MMTV particles in confluent cells. This compound modifies also the activity of reverse transcriptase (RNA dependent DNA polymerase) associated with MMTV particles. The stimulation of MMTV synthesis by dexamethasone is suppressed with low concentrations of SIBA.

5'-deoxy-5'-S- isobutyl-adenosine(SIBA), a synthetic analogue of S-adenosyl homocysteine, inhibits the oncogenic transformation of chick embryo fibroblasts by Rous Sarcoma virus (1) and of mouse fibroblasts by murine sarcoma virus (Chermann, Yoshikura, unpublished results). SIBA also blocks the plaque formation by polyoma virus in mouse embryo fibroblasts (2).

Mice also carry B type endogenous viruses directly involved in tumour formation. Thus, mouse mammary tumour virus induces the development of mammary carcinoma in several strains of mice (3) (4). Proliferating epithelioid cells were cloned from these tumours and studied in vitro (5) . These MMTV-transformed cells in cell culture secrete MMTV particules into the medium. In addition, both the synthesis of MMTV in cell culture (6) and the development of mammary carcinoma have been shown to be strongly stimulated by glucocorticoids. Therefore the MMTV -transformed cells were used to test the effect of SIBA on mammary carcinoma cells, on the expression of MMTV

SIBA : 5'-deoxy-5'-S-isobutyl-adenosine

MMTV : Mouse Mammary Tumour Virus

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particles and on the inducibility of MMTV by dexamethasone, an analogue of glucocorticoids.

MATERIALS AND METHOD

SIBA was obtained from Sefochem, Emek Hayarden (Israel).

The GR cell line was derived from a spontaneous mammary tumour in the GR strain of mouse (5). GR cells were cultivated in Dulbecco's modified MEM supplemented with 10 % horse serum (5). MMTV particles purified from the milk of RIII mice were kindly supplied by Meloy Labs, Rockville Maryland (Office of Program Resources and Logistics, National Cancer Institute).

Reverse transcriptase (RNA-dependent DNA-polymerase) activity of MMTV was measured by the incorporation of (^{3}H) dTTP into acid-insoluble material (7). Medium was harvested from confluent cultures and clarified at 8000 rpm for 10 min. Virus was then pelleted at 35 000 rpm for 45 min at 4°C in an SW 56 Spinco rotor. Virus pellets were resuspended in 0.1 ml of incubation mixture and incubated at 37°C for 1 hour. The reaction mixture contained 50 mM Tris buffer 0.2 % (v/v) nonidet P40 (nonionic detergent), 4mM $MgCl_2$, 2 % (v/v) β-mercaptoethanol, and 1.5 uM (^{3}H) dTTP (49 Ci/mM Amersham, Buckinghamshire, England). Samples precipitated with 10 % trichloroacetic acid were filtered on nitrocellulose filters, dried and counted in a scintillation counter.

RESULTS AND DISCUSSION

We have examined the effect of SIBA on growing cells cloned from mammary tumour in the GR mouse strain. These cells, after becoming confluent, form cell "domes" in culture and stop dividing (3).

Figure 1 shows the effect of various concentrations of SIBA on cells growing exponentially and on cells maintained confluent during 48 hours. Results clearly show that SIBA is toxic to growing cells but has no effect on cells blocked in the G1 phase of the cell cycle. In the presence of SIBA, cells do not stick to the culture dish, become vacuolized and are not stained by Giemsa. Thus a decrease of 50 % in alive cell number was observed with a concentration as low as 5 μg/ml (0.015 mM) of SIBA. In order to study this toxic effect, we have measured the cloning efficiency of these GR cells, after treatment with various concentrations of SIBA. Growing cells were dispersed into single cells and incubated for 150 min with SIBA. After plating in fresh medium cell clones develop only from untreated cells or cells treated with low concentrations of SIBA (Figure 2). Thus a brief treatment of GR cells with 10 μg/ml of SIBA (0.03 mM) almost completely destroys the cloning efficiency of these cells. These two types of experiment

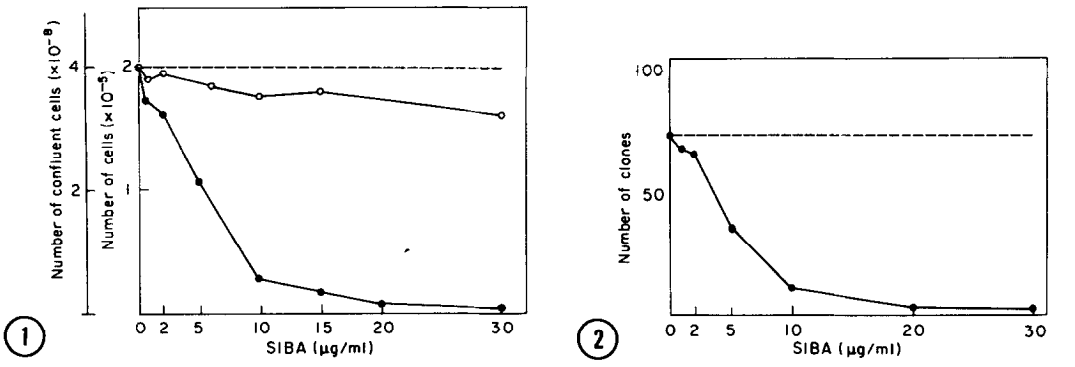
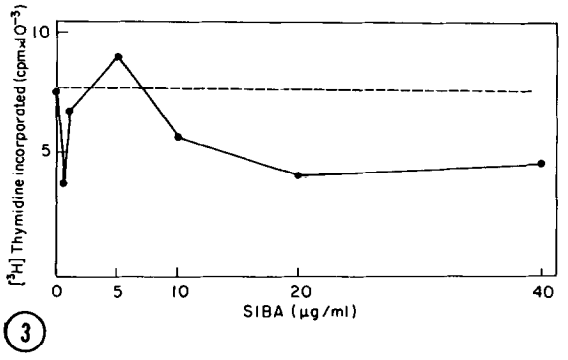


Figure 1 : Effect of SIBA on the growth of GR mammary cells : 10^5 cells per 10 cm diameter culture dish were treated for 22 hours with various concentrations of SIBA (——●——). Cells stained with Giemsa were counted in each dish. Non-growing confluent cells were treated for 22 hours with same concentrations of SIBA (——o——). In each experiment cells dissociated with trypsin (0.3%) and EDTA (2mM) were counted with hemocytometer after staining.

Figure 2 : Effect of SIBA on the cloning efficiency of GR mammary cells : Cells growing exponentially were dissociated with trypsin (0.3%) and EDTA (2 mM) and treated for 150 min with various concentrations of SIBA. After 3 days of culture clones were fixed with ethanol, stained with Giemsa and the number in each culture dish counted.

show that SIBA is strongly toxic for MMTV transformed cells. Since previous studies have shown a very low toxicity of SIBA on normal chick and mouse embryo fibroblasts (1) (2), the present work indicates a preferential destruction of virus-transformed cells.

Incubation of Rous Sarcoma-transformed chick embryo fibroblasts with 1 mM SIBA for 24 hours irreversibly inhibits protein and nucleic acid synthesis and the expression of this virus in transformed cells (1). We have analysed the effect of SIBA on the synthesis of MMTV particles under the same conditions. The number of particles secreted in the culture medium was estimated by measuring the reverse transcriptase activity (RNA dependent DNA polymerase) associated with these particles. Figure 3 shows the effect of various concentrations of SIBA on this activity. Inhibition of activity observed at very low concentrations of SIBA is completely reversed by higher concentrations of the compound, but appears again when the concentration is raised to 10 μg/ml (0.03 mM). The reversal of the inhibitory effect



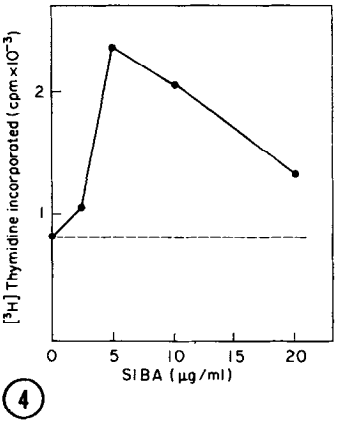


Figure 3 : Effect of SIBA on the production of MMTV particles by GR mammary cells : Confluent mammary cells were treated for 14 hours with various concentrations of SIBA. Culture supernatants were centrifuged as described in Materials and Method. Reverse transcriptase activity was measured on the virus pellet resuspended in incubation buffer as described in Materials and Method. After 1 hour of incubation at 37°C incorporation of (^{3}H)-thymidine triphosphate in DNA was measured by precipitation with 10% trichloroacetic acid.

Figure 4 : Effect of SIBA on the reverse transcriptase activity associated with MMTV particles : From about 10^5 MMTV particles purified from mouse milk reverse transcriptase activity was measured as described in Figure 3.

at the intermediate concentrations may be due to an effect on the reverse transcriptase itself. In order to test this possibility we measured the activity associated with MMTV purified from mouse milk in the presence of the same amounts of SIBA and found (Fig. 4) that SIBA stimulates reverse transcriptase activity in this concentration range. An analogous stimulation of the activity of the reverse transcriptase of AMV (Avian Myoblastosis Virus) by low concentrations of SIBA has been observed by A. Berneman, A. Pierré and M. Robert-Géro (unpublished results). These observations may explain the apparent reversal of inhibition observed in the previous experiment (Figure 3).

Since SIBA and some of its analogues are inhibitors of protein methylases and tRNA methylases (8) its oncostatic action on cells and viruses might be mediated via this effect. This compound could possibly interfere with the methylation of viral mRNA by inhibiting the viral or host methylase activities which have been described recently (9-17).

Glucocorticoid stimulation of the tRNA methyl-transferase has been

TABLE I : EFFECT OF SIBA ON THE INDUCIBILITY OF MMTV PARTICLES BY DEXAMETHASONE.

	Reverse Transcriptase Activity [a]		
	acid insoluble cpm / 30 min / 10^8 cell		
	- dexamethasone	+ dexamethasone	stimulation
- SIBA	600 ± 90	6800 ± 100	11-fold
+ SIBA (20 ug/ml)	460 ± 90	850 ± 80	2-fold

[a] 10^8 GR mammary cells were treated for 14 hours with SIBA in the presence or absence of dexamethasone (5×10^{-7} M). Particles released in the culture medium were concentrated by ultracentrifugation and reverse transcriptase activities were measured as describes in Materials and Method. Each value represents the average of three independent experiments.

reported during mouse mammary gland differentiation (18). Since dexamethasone stimulates the production of viral particles in GR mammary cells, we have tested the effect of SIBA on the MMTV production in the presence and absence of this hormone analogue.

Table 1 shows that the inducibility of MMTV particles by dexamethasone ($5. 10^{-7}$ M) is almost completely suppressed in the presence of 20 µg/ml (0. 06 mM) of SIBA. The two compounds, which enter very quickly into the cells presumably do not affect each others permeability. Furthermore, SIBA could decrease the secretion of viral particles at the membrane level or could interfere with the synthesis of viral proteins. Since the inducibility of MMTV particles by dexamethasone occurs directly at the transcription level (19, 20), its suppression by SIBA could be related to the transcription process. Further studies are in progress to analyse the mechanism of action of this compound.

SIBA has been patented by ANVAR (Agence Nationale pour la Valorisation de la Recherche), patent N° 7517972 (1975)

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