ANTIPROLIFERATIVE ACTION OF 5'-DEOXY-5'-S-ISOBUTYLADENOSINE IN VITRO AND IN VIVO ON NORMAL AND NEOPLASTIC MOUSE CELLS

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5'-Deoxy-5'-S-isobutyladenosine (SIBA) is a synthetic analog of S-adenosylhomocysteine, a natural inhibitor of DNA, RNA, and protein transmethylases. SIBA strongly and irreversibly inhibits virus transformation of cells in culture in vitro and reversibly inhibits DNA, RNA, and protein synthesis in normal fibroblasts [8] and blast transformation of normal human and animal lymphocytes [3], and has no inhibitory action on hematopoiesis or proliferation of intact hematopoietic stem cells (CFU_S) in mice [1]. In this connection it is very important to know whether SIBA possesses antiproliferative properties in vivo against tumor cells and normal hematopoietic cells.

The aim of this investigation was to study the antiproliferative properties of SIBA in vitro and in vivo in experiments on mice with transplantable lymphoma NK/Ly.

EXPERIMENTAL METHOD

SIBA was synthetized at the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, by Lederer's method [5].

Experiments in vivo were carried out on noninbred female mice with lymphoma NK/Ly, weighing 20–22 g, on the 5th–7th day after intraperitoneal transplantation of $9 \cdot 10^6$ NK/Ly ascites cells. Cells of the ascites tumor and bone marrow were studied in mice with lymphoma NK/Ly, and bone marrow cells in intact mice. SIBA in a concentration of 5 mg/ml physiological saline, was dissolved on a water bath at 85–95°C immediately before being injected intraperitoneally in a single does of 250 mg/kg body weight. DNA synthesis in ascites tumor cells and bone marrow cells was studied by measuring incorporation of [^{3}H]thymidine, injected intraperitoneally in a dose of 10 μCi per mouse (specific activity 23 mCi/mmole) 1 h after injection of SIBA. Mice receiving [^{3}H]thymidine alone served as the control.

To study reparative DNA synthesis replication was inhibited with hydroxyurea (HU) (from Serva, West Germany), which was injected intraperitoneally into the animals in a single dose of 200 mg/kg 30 min before injection of [^{3}H]thymidine. The animals were killed 1 h later and ascites tumor cells and bone marrow cells were removed. The effect of SIBA on reparative DNA synthesis was studied 30 min after injection of HU. The cells after removal were washed 3 times to remove the label in 50 ml of physiological saline, centrifuged at 1200 rpm for 10 min to obtain the cell residue, and distributed at the rate of $5 \cdot 10^6$ cells in a volume of 0.2–0.3 ml, to which 2 ml of cold 5% TCA was added for 30 min. Radioactivity of the samples was determined on a Mark 2 liquid scintillation counter (from Nuclear Chicago, USA).

In experiments in vitro ascites tumor cells from NK/Ly mice were washed once with medium No. 199, then transferred to incubation medium No. 199 containing 20% bovine serum and penicillin (100 U/ml medium). [^{3}H]Thymidine in a dose of 2 μCi was added to 2.5-ml samples containing $2 \cdot 10^6$ cells/ml after incubation with 300 μM SIBA for 1, 24, and 48 h. Incubation continued for 2 h, after which the cells were first washed 3 times with 0.9% NaCl, and then treated with 5% TCA to determine radioactivity in the tumor cells.

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TABLE 1. Effect of SIBA on DNA Synthesis in NK/Ly Ascites Tumor Cells in Vitro

Incubation time, h	Incorporation of [^{3}H]thymidine into DNA, cpm (M ± m)		Percent of inhibition
	untreated	treated with SIBA	
3	60 017±1299,7	11 017±971,0	81,7
24	16 451±859,5	838±254,1	94,9
48	1441,0±396,1	382±47,9	73,47

Legend. Each sample 2.5 ml in volume contained $5 \cdot 10^6$ cells and 300 μM SIBA.

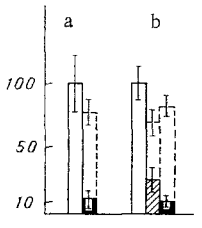

Fig. 1

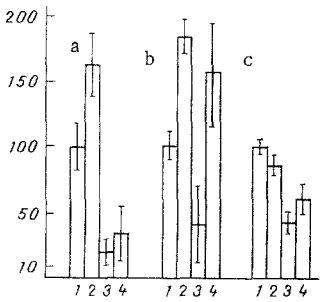

Fig. 2

Fig. 1. Effect of SIBA on incorporation of [^{3}H]thymidine into DNA of NK/Ly ascites tumor cells and bone marrow of intact mice in vitro. Ordinate, incorporation of [^{3}H] thymidine into DNA (in percent of control): a) ascites tumor cells incubated for 2 h in the presence of SIBA (500 μM to $5 \cdot 10^6$ cells in a volume of 2.5 ml); SIBA was removed by rinsing the cells and incubation continued for 2 h with [^{3}H]thymidine (2 μCi/$5 \cdot 10^6$ cells); b) bone marrow cells from intact mice (the same conditions). Unshaded columns – control; obliquely shaded – SIBA, 300 μM; black columns – SIBA, 500 μM; broken line indicates after rinsing cells.

Fig. 2. Effect of SIBA on [^{3}H]thymidine incorporation into DNA of NK/Ly ascites tumor cells and bone marrow cells in vivo in mice. Ordinate, incorporation of [^{3}H]-thymidine into DNA (in percent of control); 1) control (untreated); 2) SIBA, 250 mg/kg; 3) HU, 200 mg/kg; 4) HU, 200 mg/kg + SIBA, 250 mg/kg (with 30-min intervals). a) NK/Ly ascites tumor cells; b) bone marrow cells from mice with NK/Ly lymphoma; c) bone marrow cells of intact mice.

In experiments with preliminary incubation for 2 h, $5 \cdot 10^6$ cells in a sample measuring 2.5 ml were incubated in the presence of SIBA in a final concentration of 300 and 500 μM. After incubation, ascites tumor cells and intact bone marrow cells were washed twice with medium No. 199 in a volume of 20 ml to remove SIBA, centrifuged for 5 min at 1200 rpm, and the cell residue was isolated and treated with fresh medium containing [^{3}H]thymidine (at the rate of 1 μCi to $2.5 \cdot 10^6$ cells). Incubation then continued for 2 h at 37° C. The cell suspension in a volume of 0.2 ml was then treated with 5% TCA and radioactivity was measured in acid-insoluble fractions.

EXPERIMENTAL RESULTS

Considerable differences were found in the antiproliferative activity of SIBA in vivo and in vitro on NK/Ly. The results of the study of the action of SIBA on NK/Ly ascites tumor cells are given in Table 1. SIBA in a dose of 300 μM inhibited incorporation of [^{3}H]thymidine into DNA of the test cells by more than 80% as early as

after 3 h of incubation, and after 24 h of incubation the percentage of inhibition exceeded 90, in agreement with data obtained in the writers' laboratory [2] on the effect of SIBA in the same dose in vitro on L5178 mouse leukemia cells. SIBA, as an analog of S-adenosylhomocysteine, undergoes rapid enzymic hydrolysis [6] and other conversions [7]. During short-term culture of tumor cells and of normal explantable cells (lymphocytes and bone marrow cells), however, the activity of these enzymes particularly in the extracellular medium is very low, and SIBA is able to exert its inhibitory action on various transmethylase reactions.

It was observed previously [8] that the effect of inhibition of nucleic acid and protein synthesis in normal and transformed cells can be partially or completely abolished after removal of SIBA from the incubation medium by rinsing the cells.

Restoration of [^{3}H]thymidine incorporation into DNA of NK/Ly ascites cells and bone marrow cells after rinsing to remove SIBA also was found in experiments in vitro.

Some increase in antiproliferative activity of SIBA took place as the result of an increase in its dose (Fig. 1). For instance, the percentage of incorporation of [^{3}H]thymidine for bone marrow cells was 15.7 and 8.9 compared with the control for doses of 300 and 500 μM, whereas after rinsing the cells to remove SIBA, incorporation of the label rose sharply to 69 and 82% respectively.

The results of the experiments in vivo are given in Fig. 2. After a single intraperitoneal injection of SIBA in a dose of 250 mg/kg incorporation of [^{3}H]thymidine into DNA of normal mouse bone marrow cells was unchanged, but incorporation of [^{3}H]thymidine into bone marrow DNA of mice with NK/Ly lymphoma and of the tumor cells themselves was significantly increased compared with the control. Consequently, instead of its strong antiproliferative action, SIBA in vivo either had no effect on proliferation, as in the case of bone marrow cells of normal mice, or it had the directly opposite action: It stimulated proliferation just as in the case of bone marrow cells of mice with NK/Ly lymphoma and of ascites tumor cells.

After consecutive injections of HU, an inhibitor of replicative synthesis, and SIBA in the same dose, with a 30 min interval, there was some decrease in the antiproliferative action of HU in the case of bone marrow cells from intact mice and ascites tumor cells, whereas in bone marrow cells of mice with NK/Ly lymphoma incorporation of [^{3}H]thymidine was stimulated up to 155% of the control.

Since SIBA in vivo stimulates proliferation of hematopoietic cells against the background of HU, this property of SIBA may prove useful for combination chemotherapy.

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