LITERATURE CITED

1. L. V. Akhabadze, Ontogenez, 5, No. 2, 163 (1974).
2. G. B. Bol'shakova, Byull. Éksp. Biol. Med., No. 2, 234 (1980).
3. P. P. Rumyantsev and I. L. Sokolovskaya, in: Investigation of Cell Cycles and Nucleic Acid Metabolism during Cell Differentiation [in Russian], Moscow–Leningrad (1964), p. 71.
4. P. P. Rumyantsev and V. O. Mirakyan, Tsitologiya, No. 10, 1276 (1968).
5. P. P. Rumyantsev, Tsitologiya, No. 2, 132 (1978).
6. V. F. Sidorova, Postnatal Growth and Regeneration of the Internal Organs in Vertebrates [in Russian], Moscow (1969).
7. V. F. Sidorova and G. B. Bol'shakova, Byull. Éksp. Biol. Med., No. 4, 475 (1980).
8. O. Klinge, Arch. Path. Anat. Abt. B, Zellpath., 6, 208 (1970).
9. O. Klinge, Beitr. Pathol., 155, 79 (1975).

EFFECT OF 5'-DEOXY-5'-S-ISOBUTYLADENOSINE ON HEMATOPOIETIC STEM CELLS

S. B. Stepanova and N. A. Fedorov

UDC 612.419.014.46

KEY WORDS: hematopoietic stem cell; proliferation; cytostatic effect.

The compound 5'-deoxy-5'-S-isobutyladenosine (SIBA), a synthetic analog of S-adenosylhomocysteine, inhibits cell transformation induced by DNA- and RNA-containing viruses and viral replication [6, 10, 11], as well as methylation of tRNA and proteins [8, 14].

Raies et al. [10] and Robert-Gero et al. [11] observed a marked decrease in transport of labeled precursors when they studied the effect of SIBA on synthesis of nucleic acids and intracellular proteins. Pierre and Robert-Gero [9] showed that SIBA depresses transport of sugars and nucleotides equally in normal and virus-infected fibroblasts.

The antimitogenic action of SIBA on blast-transformed human and animal lymphocytes has been demonstrated by several investigations [2, 3].

According to Raies et al. [10], SIBA penetrates easily into cells. Carteni et al. [4] and Lawrence et al. [7] demonstrated the rapid enzymic degradation of SIBA to the less active 5'-deoxy-5'-S-isobutylinosine in eukaryotes.

Since SIBA has a powerful oncostatic action and possesses low toxicity toward normal cells, it shows prospects of being an effective antitumor preparation. Accordingly, in the investigation described below, the effect of SIBA was studied on normal hematopoiesis and, in particular, on proliferative activity of hematopoietic stem cells (CFUs).

EXPERIMENTAL METHOD

SIBA was generously provided by the French biochemists Lederer and Robert-Gero (Institut de Chimie des Substances Naturelles, France).

Experiments were carried out on female (CBA × C57BL)F_1 mice weighing 20–22 g.

In experiments in vitro bone marrow cells were flushed out of the femora of intact mice with medium TC 199 with the addition of 20 mM HEPES and antibiotics at 4°C, and then washed out once with medium. The cell suspension (4×10^6 nucleated cells in 1 ml) intended for transplantation was incubated with SIBA (3.1×

Laboratory of Biochemistry of Leukemia, Central Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 604-606, May, 1981. Original article submitted June 25, 1980.

TABLE 1. Effect of SIBA on Intact CFUs and CFUs Stimulated by Cyclic AMP in Vitro

Conditions	Concentration, M	Incubation time, h	Number of CFU_S on 9th day (M ± m)	Increase in number of CFUs, %	Decrease in CFU_S, %	P
Control (without treatment)	—	3	16,5±0,6 (9)	—	—	—
SIBA	$3,1 \cdot 10^{-4}$	3	16,5±1,4 (10)	—	—	—
SIBA	$3,1 \cdot 10^{-4}$	1	19,5±0,9 (10)	15,3	—	—
Cyclic AMP	10^{-8}	3	17,8±1,3 (10)	—	—	—
Cyclic AMP + SIBA	$10^{-8} + 3,1 \cdot 10^{-4}$	3 and 3	10,7±1,4 (10)	—	40,0	<0,01
Cyclic AMP + SIBA	$10^{-8} + 3,1 \cdot 10^{-4}$	3 and 1	19,5±1,2 (10)	—	—	—

Legend. Here and in Table 2, number of spleens shown in parentheses.

TABLE 2. Action of SIBA in vivo on CFU_S

Conditions	Dose of SIBA	Incubation time, h	Number of CFUs on 9th day (M ± m)	Increase in number of CFUs, %	P
Control (without treatment)	—	—	14,9±1,2 (9)	—	—
SIBA	100 mg/kg	3	17,7±1,2 (11)	15,8	<0,05
SIBA	0.1 mg/ml cell suspension	—	19,4±1,7 (8)	28,3	<0,05

10^{-4} M) for 1 and 3 h and with 3',5'-AMP (10^{-8} M) for 3 h at 37°C. SIBA also was added to the experimental samples with 3',5'-AMP. At the end of incubation, 4×10^4 living nucleated cells were injected intravenously into lethally irradiated mice. The mice were irradiated with ^{137}Cs γ-rays (dose rate 21 rads/min) in a dose of 1300 rads. The number of CFU_S was determined by the method of Till and McCulloch [13]. The mitogenic effect of cyclic AMP was determined from the decrease in the number of splenic colonies after transplantation of bone marrow cells incubated with SIBA for 3 h.

In experiments in vivo SIBA was injected intraperitoneally into an intact mouse in a dose of 100 mg/kg. The bone marrow was removed 3 h later and a cell suspension prepared by the method described above. Irradiated recipients received an injection of 4×10^4 nucleated cells. In another series of experiments SIBA (0.1 mg/ml) was added to the cell suspension of intact bone marrow containing 10^4 nucleated cells/ml and was injected without incubation, in a dose of 4×10^4 nucleated cells, into the irradiated mice. The number of exogenous splenic colonies was counted on the 9th day. The change in the number of CFUs after treatment with SIBA was expressed in per cent.

EXPERIMENTAL RESULTS

The experiments showed (Table 1) that preincubation of bone marrow cells with SIBA for 3 h caused no change in the number of CFUs, but incubation under similar conditions for 1 h increased the number of CFUs by 15.3% compared with intact bone marrow. This suggests that SIBA acts not only on the donor's bone marrow cells, but also on T lymphocytes remaining in the recipients' spleens, suppressing mitogenic signals in response to transplantation of bone marrow.

To test this hypothesis the effect of SIBA was studied on the number of CFUs (Table 2) after simultaneous injection of SIBA with bone marrow cells and when the donors' hematopoietic cells were exposed to its action for 3 h after intraperitoneal injection. The increase in the number of splenic colonies (CFUs) in these experiments was by 28.3 and 15.8% respectively.

SIBA had an inhibitory effect on bone marrow cells stimulated by 3',5'-AMP, incubated with SIBA for 3 h and transplanted intravenously into lethally irradiated mice. No such effect was observed after incubation for 1 h with SIBA. The number of CFUs was reduced by 40% in the presence of SIBA compared with cells treated with 3'-5'-AMP alone (Table 1). The writers showed previously [1] that 3',5'-AMP stimulates proliferative activity of the CFUs by 60%. The increase in the sensitivity of the stem cells to the action of SIBA

probably took place as a result of emergence of the CFUs from a state of rest (G_0) under the influence of 3'-5'-AMP. Our results agree with those of Terrioux [12], who showed that SIBA acts on proliferating cells and not on cells blocked in the G_1 phase.

Evidently as a result of stimulation by 3'-5'-AMP there was an increase in activity of enzymes responsible for degradation of the products of S-adenosyl-methionine (SAM) metabolism, natural inhibitors of DNA synthesis and of methylation of RNA and proteins. The cytostatic effect of SIBA, according to Zappia, can be explained by competitive relations in vivo between 5'-methylthioadenosine (5'-MTA) and SIBA. Because of the great affinity of SIBA for these enzymes, a large quantity of 5'-MTA accumulates in the cell.

SIBA may perhaps exert its effect on CFUs at the cell membrane level. In agreement with observations by Hirata and Axelrod [5], S-adenosylhomocysteine (SAH) inhibits methylation of membrane phospholipids, and for that reason SIBA, as an analog of SAH, may act on this reaction to reduce the content of phosphatidyl-N-monomethylethanolamine (a methylation product of phosphatidylethanolamine). In the investigation cited it was shown that an increase in the content of this product in the membrane leads to a change in membrane fluidity; consequently, it can be tentatively suggested that SIBA, by changing membrane fluidity reduces the entry of certain molecules of sugar and nucleoside inside the cells [9].

It can be concluded from these data that SIBA does not inhibit the proliferative activity of intact stem cells but, on the contrary, increases the number of CFUs by 15-28% in vitro and in vivo. In the presence of SIBA the mitogenic effect of 3'-5'-AMP is inhibited, in the same way as proliferation of lymphocytes under the influence of various mitogens is inhibited.

LITERATURE CITED

1. S. B. Stepanova, N. A. Koreshkova, and N. A. Fedorov, Byull. Éksp. Biol. Med., No. 5, 590 (1980).
2. N. A. Fedorov, T. V. Borovkova, B. N. Borisov, et al., Dokl. Akad. Nauk SSSR, 244, 1266 (1979).
3. C. Bona, M. Robert-Gero, and E. Lederer, Biochem. Biophys. Res. Commun., 70, 622 (1976).
4. M. Carteni-Farrina, F. D. Radione, G. Ragosta, et al., FEBS Lett., 104, 266 (1979).
5. F. Hirata and J. Axelrod, Nature, 275, 219 (1978).
6. B. Jacquemont and J. Huppert, J. Virol., 22, 160 (1977).
7. F. Lawrence, M. Richou, M. Vebel, et al., Eur. J. Biochem., 87, 257 (1978).
8. A. Pierre, M. Richou, F. Lawrence, et al., Biochem. Biophys. Res. Commun., 76, 813 (1977).
9. A. Pierre and M. Robert-Gero, FEBS Lett., 101, 233 (1979).
10. A. Raies, F. Lawrence, M. Robert-Gero, et al., FEBS Lett., 72, 48 (1976).
11. M. Robert-Gero, F. Lawrence, G. Farrugia, et al., Biochem. Biophys. Res. Commun., 65, 1242 (1975).
12. C. Terrioux, M. Crepin, F. Gros, et al., Biochem. Biophys. Res. Commun., 83, 673 (1978).
13. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).
14. M. Vebel, M. Robert-Gero, M. Legraverend, et al., Nucleic Acids Res., 5, 2979 (1978).