INHIBITION OF MITOGEN INDUCED BLASTOGENESIS BY 5'-DEOXY-5'-S-ISO-BUTYL ADENOSINE

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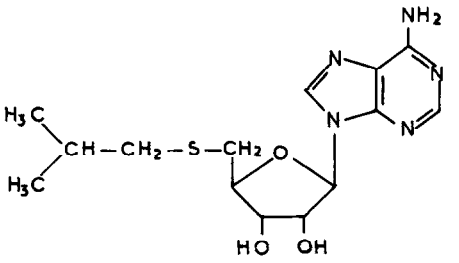
SUMMARY. The mitogen induced blastogenesis of human and rabbit lymphocytes is strongly inhibited by 100 and 300 µM 5'-deoxy-5'-S-isobutyl-adenosine (SIBA) (I), a synthetic analogue of S-adenosyl-homocysteine (SAH) which had previously been shown to prevent oncogenic transformation of chicken fibroblasts by Rous Sarcoma virus (15). Addition of 300 µM SIBA even 1 to 2 days after stimulation by Con A or a Water soluble mitogen from Nocardia (NWSM), inhibited strongly the incorporation of [^{3}H] thymidine. Lymphocytes recovered their ability to be stimulated by T or B mitogens after elimination of the inhibitor from the cultures.

INTRODUCTION

Methylation reactions mediated by various transmethylases seem to play an important role in blast transformation (1-3). Inhibition of blastogenesis is thus conceivable by compounds interfering with transmethylases, such as synthetic analogues of S-adenosyl-homocysteine (SAH) (4-14). - Recently, Robert-Géro et al. (15) have shown that one of these, 5'-deoxy-5'-S-isobutyl-adenosine (SIBA) (I) prevents in mM concentration the oncogenic transformation of chick embryo fibroblasts infected with Rous sarcoma virus. This compound inhibits also the virus production by transformed cells. SAH is inactive under the same conditions. SIBA and SAH have only cytostatic effects on normal fibroblasts.

Abbreviations :

ALS	: Anti-human lymphocyte serum	PPD	: Tuberculin
Antib 4	: antiallotype serum	SAH	: S-adenosyl-homocysteine
Con A	: Concanavalin A	SAM	: S-adenosyl-methionine
NWSM	: Nocardia water soluble mitogen	SIBA	: 5'-deoxy-5'-S-isobutyl-adenosine (I)
PG	: Peptidoglycan of E.coli	TCA	: trichloroacetic acid
PHA	: Phytohemagglutinine-P		



I.

We now report, that - as expected -, the same synthetic analogue of SAH, SIBA does indeed interfere with blastogenesis of human and rabbit lymphocytes and prevents the action of various mitogens tested.

MATERIAL AND METHODS

Separation of lymphocytes. For human peripheral blood lymphocytes the method followed was essentially that of Böyum (16). The lymphocytes of rabbit spleen were prepared as described previously (17).

Cultures of lymphocytes. 10^6 human lymphocytes were cultured for 5 days in 1 ml Eagle's minimum essential medium (MEM) (Grand Island Biological Company. Grand Island, N.Y.) supplemented with essential amino acids and 15 % pool of AB homologous serum which had previously been inactivated for 30 min. at 56°C. - The rabbit (Bouscot, Garches, France) spleen lymphocytes (2.5 x 10^6 cells) were cultured for 3 days in 1 ml Eagle's medium (Pasteur Institute) supplemented with 10 % of autologous serum which had previously been inactivated as described above.

The cultures were performed in an incubator model 1H-100 (Gallenkamp) under a continuous flow of a mixture of 5 % CO_2 and 95 % air.

The following commercial reagents were used : Concanavalin A (3 x crystallized, Miles Yeda Ltd, Israel), Phytohemagglutinine-P (Difco), [^{3}H]-thymidine 1 Ci/mM (CEA, Saclay), SIBA (Sefochem, Fine Chemicals Ltd, Emek-Hayarden, Israel), Horse Ig G antihuman lymphocyte and tuberculin were from Pasteur Institute, Paris. Nocardia Water Soluble mitogen (NSWM) was prepared according to Ciorbaru et al. (18) and peptidoglycan from E.coli as described by Martin et al. (19). Rabbit anti b4 antiallotype serum in rabbits with phenotype $b4^- b5^+ b6^+$ was prepared according to Oudin (20). The blast transformation of lymphocytes was determined by the scintillation method. 18 hours before harvest, the cells were incubated with 1 µCi thymidine for 10^6 cells. The processing of [^{3}H] thymidine treated cultures has been described previously (21).

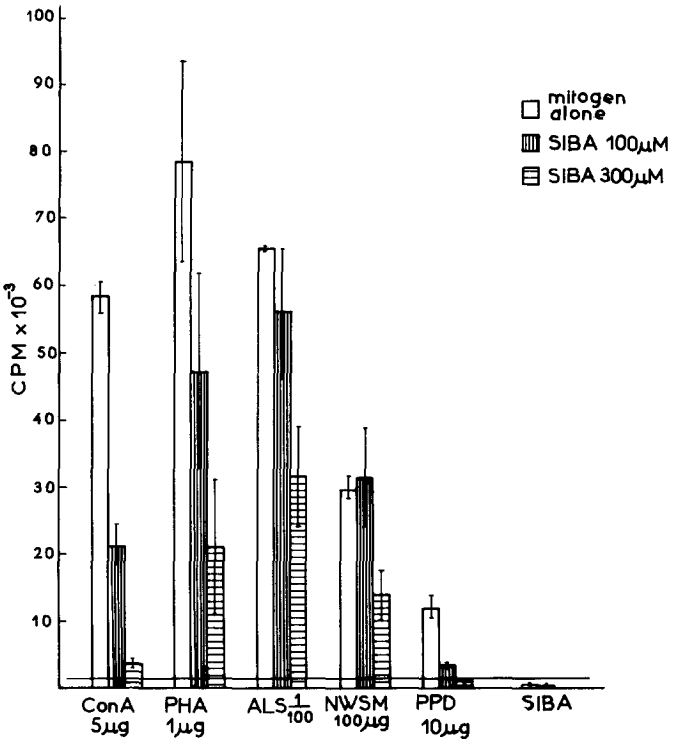


Fig. 1 : Effect of 100 µM and 300 µM SIBA on mitogen induced blast response of human peripheral blood lymphocytes. Mitogens are added simultaneously with the inhibitor. Eighteen hours before harvest, the cells were incubated with 1 µCi ^{3}H thymidine for 10^{6} cells. Incorporation was measured in the TCA insoluble fraction.

RESULTS

Effect of SIBA on mitogenic response of lymphocytes

In these experiments the inhibitor and the mitogen were added simultaneously at the beginning of the culture. As shown in Fig.1, 100 µM and 300 µM SIBA inhibit the action of Con A and of PHA (known to stimulate both B and T cells) (22-24) on human lymphocytes by 64 % and 94 % and by 40 % and 73 % respectively. - The effects of ALS, a T cell mitogen (Borchier, personal communication) and that of NWSM, a B cell mitogen (unpublished data) were inhibited to a lesser extent and only by 300 µM SIBA (52 % and 54 % respectively). - PPD stimulates specifically the T derived lymphocytes of subjects previously sensitized by mycobacteria (23,24). The blast

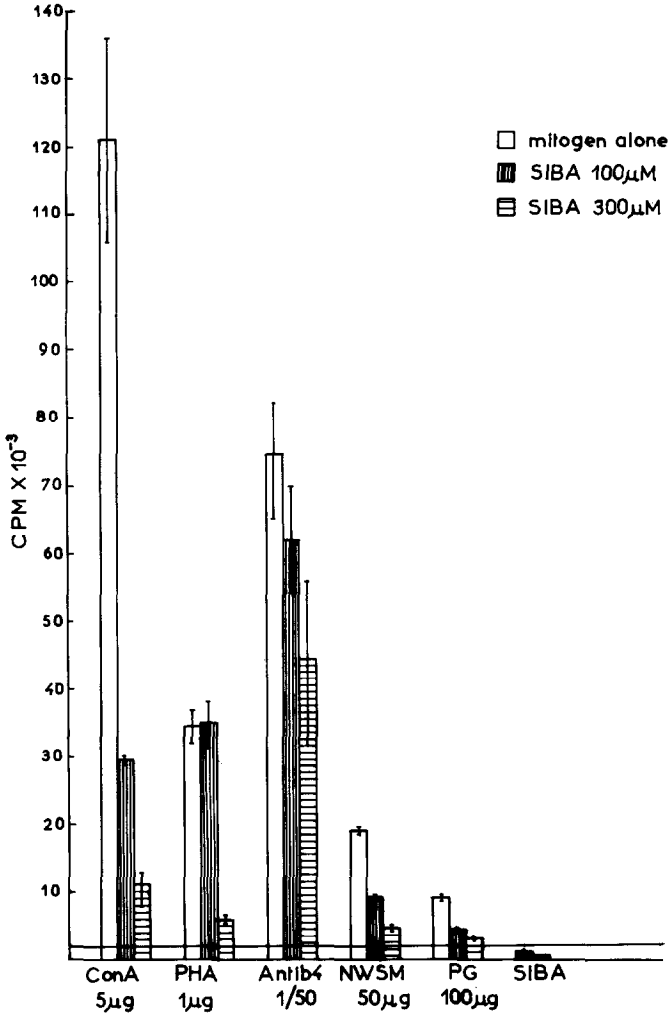


Fig. 2 : Effect of 100 µM and 300 µM SIBA on mitogenic response of rabbit lymphocytes. Mitogens were added simultaneously with the inhibitor ; [^{3}H] thymidine incorporation and the processing of treated cultures were the same as in Fig. 1.

formation by this mitogen was strongly inhibited : to 71 % by 100 µM and to 92 % by 300 µM SIBA. - As shown in the same figure, the inhibitor alone affects to a certain degree the incorporation of thymidine by non stimulated cells. However the cell viability was not affected, as estimated by the eosin exclusion test.

Fig. 2 shows the influence of SIBA on rabbit lymphocyte blast response. Con A and PHA are known to be T cell mitogens (25,26). Anti b4 antiallotype serum stimulates both T and B cells (27). NWSM and peptidoglycan stimulate selectively B cells (28,29). Strong inhi-

Table I : Effect of 300 µM SIBA on Con A and NWSM stimulated cells, when added after the mitogen

Time of addition* hrs	% inhibition			
	human cells		rabbit cells	
	Con A	NWSM	Con A	NWSM
3	-	-	98	84
6	-	-	98	73
12	-	-	97	66
24	88	37	93	66
48	83	24	90	58
72	75	47	81	0
96	56	63	-	-

* Delay between the addition of the mitogen and the inhibitor

bition was observed with both 100 and 300 µM SIBA when lymphocytes were stimulated by Con A, NWSM and PG whereas PHA treated cultures, were inhibited only with 300 µM SIBA (83 % inhibition). The effect of the compound on untreated cells was the same as in the case of human lymphocytes.

Inhibition of blastogenesis by 300 µM SIBA added after the mitogens

As shown in Table 1 Con A induced blast response was severely impaired even when SIBA was added 3 days after the lectin to the human or rabbit lymphocytes (75 % and 81 % inhibition respectively). NWSM induced blastogenesis of human lymphocytes was the most strongly inhibited when SIBA was added to the cells the third or fourth day of stimulation, while the action of the compound on NWSM stimulated rabbit cells decreased upon addition of the drug later than 48 hours after the mitogen.

Recovery of the mitogen response after preincubation with SIBA

Human rabbit or lymphocytes were treated for 24 hours with 300 µM SIBA, then washed three times to eliminate the inhibitor and

Table II : Recovery of mitogen response of rabbit and human lymphocytes after 24 hrs of preincubation with 300 µM SIBA

Preincubation with SIBA before mitogen	Mitogen added	Origin of lymphocytes	
		human	rabbit
		cpm*/culture	
-	-	4044±350	1724±276
-	Con A	34213±9806	52425±2423
-	NWSM	9945±1213	3216±1034
24h**	Con A	31669±6114	51534±2106
24h**	NWSM	12057±456	2676±135

* Average of triplicate cultures

** Cells washed free from SIBA after treatment.

incubated with either Con A or with NWSM under the same conditions as before. Thymidine incorporation was measured in the TCA insoluble fraction and compared to untreated cells. The lymphocytes recovered entirely their ability to respond to both mitogens after the elimination of the inhibitor (Table II).

DISCUSSION

Mitogen stimulated [^{3}H]-thymidine incorporation by human and rabbit lymphocytes is strongly inhibited by 100 and 300 µM SIBA (34, resp. 102 µg/ml). Studies of Riddick and Gallo (2) have shown that PHA stimulated human lymphocytes contain elevated levels of tRNA methylases which are qualitatively different from those formed in normal peripheral blood lymphocytes. It was also shown that this methylase activity was dependent on the synthesis of new RNA. However, it seems that tRNA methylase induction occurs late in, or after DNA synthesis and after morphologic transformation but prior to mitosis. An early protein synthesis at the level of translation after Con A stimulation of porcine lymphocytes was reported recently by Wettenhall et al. (31). - We showed in a former work (15) that SIBA inhibits protein, RNA and DNA synthesis in eucaryotic cells. Furthermore while SIBA is a poor competitive inhibitor of SAM in cell-free systems

it inhibits quite strongly the methylation in whole cells (A. Berneman, manuscript in preparation). Our recent investigations suggest that besides its action on macromolecular synthesis and nucleic acid methylation, SIBA acts at the cell membrane level by interfering with transport of certain molecules inside the cell (J. Enouf, unpublished results).

As quite the same extent of inhibition was obtained when 300 µM SIBA was added simultaneously, or 1 to 3 days after Con A stimulated human or rabbit lymphocytes one can speculate that either Con A and SIBA are not acting at the same sites on the cell surface (in this case binding of SIBA is masking the Con A binding sites) - or they are acting at the same sites with a greater affinity of SIBA to this sites.

The opposite seems to be true for NWSM : when lymphocytes are stimulated with this mitogen, the inhibition decreases strongly upon addition of SIBA after the mitogen (Table I). This suggest that NWSM has more affinity than SIBA for the same receptor sites, - or that binding of NSWM to the cell surface is hindering the "SIBA binding sites".

Lymphocytes which were cultured with SIBA and washed free of inhibitor did undergo blastogenesis upon addition of mitogen, indicating that SIBA is not lymphotoxic (Table II).

In conclusion, SIBA seems to be a potent inhibitor of mitogen induced blastogenesis in general and a valuable tool for the study of the specificity of cell surface binding sites of the various mitogens on lymphocytes and of the possible importance of transmethylation reactions in blastogenesis.

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