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A NEW PHOSPHODIESTERASE INHIBITOR IN HUMAN LYMPHOCYTES: N-METHYL-ISATIN-β-THIOSEMICARBAZONE

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N-methyl-isatin-β-thiosemicarbazone (Me-IBT) is an inhibitor of the growth of pox viruses (1, 2) and is prophylactic for small pox, as well as therapeutic for eczema vaccinatum, a complication of small pox vaccination (3, 4). This drug is also effective in vitro against the infectivity of a wide variety of other virus groups (5, 6).

In recent reports (7, 8), it was shown that Me-IBT inhibited the RNA-dependent DNA polymerase of Rous sarcoma virus and inactivated the transforming ability of the virus. Also, experimental evidence was presented which suggested that the mode of action of Me-IBT was related to its ability to chelate metallic ions, particularly copper. The spectrum of activity of Me-IBT has been extended to include other polymerases, such as purified E. coli DNA and RNA polymerases (8). However, not all enzymes which utilize nucleic acids are inhibited since tRNA nucleotide transferase, DNA terminal transferase, pancreatic DNase and pancreatic RNase were not affected (8). The site of action of Me-IBT may be the nucleic acids since it has been shown that Cu^{++}-Me-IBT complexes bind to DNA and RNA (P. Mikelens and W. Levinson, unpublished observations).

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In view of the possible involvement of nucleic acids in the activity of Me-IBT, it was of interest to determine whether adenyl cyclase, which utilizes adenosine triphosphate as substrate, was inhibited. Our initial approach was to determine the amount of cAMP in human peripheral blood lymphocytes (HPBL) in the presence of the drug. In this report we present evidence that the cAMP level increases significantly following exposure to Me-IBT, apparently as a result of inhibition of the cAMP-specific phosphodiesterase.

MATERIALS AND METHODS

The HPBL were isolated from normal donors by density centrifugation (11) and were cultured at a concentration of 1×10^7/ml in Hanks BBS. At the end of each incubation period, the cultures were centrifuged and washed twice in ice-cold PBS. The cell pellets were then prepared using procedures outlined by Whitfield et al. (12). Assay of cAMP levels was performed using a cAMP binding protein prepared in this laboratory and assayed according to methods outlined by Gilman (13).

RESULTS AND DISCUSSION

The cyclic AMP content of HPBL incubated with Me-IBT, a Cu^{++}-Me-IBT complex and/or isoproterenol (a synthetic catecholamine which elevates cAMP levels in lymphocytes) is shown in Table 1. Me-IBT alone or Cu^{++}-Me-IBT complex augmented intracellular cAMP to levels similar to those found in cells stimulated by isoproterenol. It is of interest to note that DMSO appears to potentiate somewhat the effects of isoproterenol ($CuSO_4$ was found to have no effect by itself or with DMSO). Further in the presence of both isoproterenol and either Me-IBT or Cu^{++}-Me-IBT complex, cAMP levels are elevated to a much greater degree. The addition of Me-IBT or Cu^{++}-Me-IBT complex 20 min after the addition of isoproterenol also causes cAMP levels to rise when measured 20 min later.

TABLE 1. Effect of Me-IBT and isoproterenol on cAMP levels in HPBL.

	cAMP Levels picomoles / 10^6 HPBL									
Time (min)	Control	DMSO	Isoprot. 10^{-6} M	Iso + DMSO + $CuSO_4$	Me-IBT	Cu + Me-IBT	Iso + Me-IBT	Iso + Cu + Me-IBT	Iso + Me-IBT	Iso + Cu + Me-IBT
0 - 5	0.98[a]	1.06	3.33	6.94	3.26	4.10	17.06	17.40	--	--
10	--	1.06	3.83	5.82	2.67	3.07	22.23	14.96	--	--
20	--	0.92	2.98	4.95	4.42	2.43	17.91	16.95	--	--
40	--	0.66	1.89	3.68	3.58	2.34	17.57	--	14.02[b]	7.30[b]

The values reported here represent the mean of duplicate determinations at each point. The HPBL were isolated from normal donors by density centrifugation (11) and were cultured at a concentration of 1 x 10^7/ml in Hanks BSS. At the end of each incubation period, the cultures were centrifuged and washed twice in ice-cold PBS. The cell pellets were then prepared using a procedure outlined by Whitfield et al. (12). cAMP was assayed by the competition binding method of Gilman (13). The Me-IBT was dissolved in DMSO prior to addition; its final concentration was 200 µM in 10% DMSO. The concentration of $CuSO_4$ was 200 µM.

[a] Control culture received no treatment and indicates the basal level of activity of these cultures.

[b] These cultures received their drug additions 20 min after culture initiation and were assayed 40 min after culture initiation.

Since measurement of changes in cAMP levels could not differentiate between an effect on the synthetic enzyme adenylate cyclase or the degradative cAMP-specific phosphodiesterase (PDE), experiments were performed measuring the effect of Me-IBT or thiosemicarbazide, on PDE isolated from HPBL. Thiosemicarbazide was shown to be the portion of the molecule active against the RNA-dependent DNA polymerase of Rous sarcoma virus. The results in Fig. 1 show that Me-IBT may act by blocking phosphodiesterase activity. In this experiment increasing amounts of Me-IBT in either 2.5% DMSO or 5% DMSO lead to decreasing PDE activity. Thiosemicarbazide was ineffective at all doses except possibly 200 µM. This correlates with results obtained with HPBL (not shown) which indicates that thiosemicarbazide has only a very weak effect on cAMP levels in the presence or absence of isoproterenol.

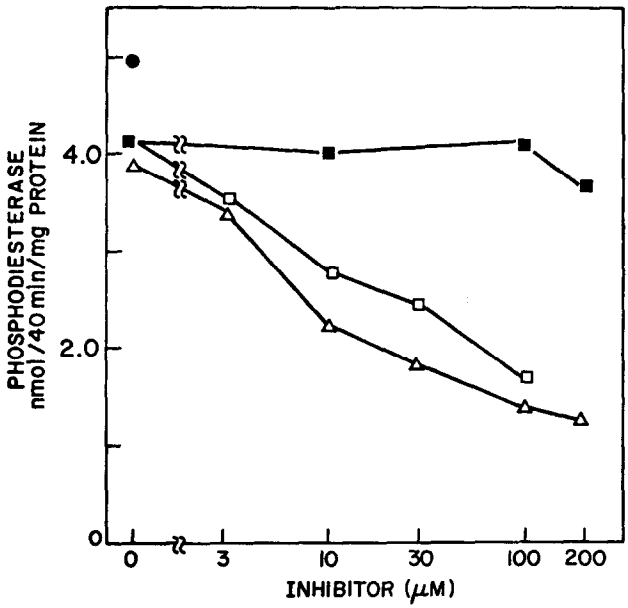

Fig. 1. Assay of phosphodiesterase activity was performed on HPBL according to the method of Beavo et al. (14). The points represent the average of duplicate determinations. Concentration of ^{3}H-cAMP was 1.0 μM (specific activity 2.4 Ci/mmole). The (-●-) represents the negative control; the (-■-) represents thiosemicarbazide in 2.5% DMSO; the (-□-) Me-IBT in 2.5% DMSO; the (-Δ-) Me-IBT in 5% DMSO.

A similar experiment performed on a lymphoid cell line derived from a mouse lymphoma revealed no phosphodiesterase inhibition. Me-IBT appears to be a specific cAMP phosphodiesterase inhibitor which is effective so far only in normal human peripheral blood lymphocytes, as tests of human lymphocytic cell lines and HeLa cells yielded negative results. The mechanism by which the Me-IBT exerts its effect on lymphocyte phosphodiesterase remains to be determined. That Me-IBT alters cAMP levels in lymphocytes may present potential difficulties in the use of this drug therapeutically. Changes in cAMP levels in lymphocytes have been shown to influence immune response both positively and negatively (9, 10). Thus, the possibility exists that use of Me-IBT might lead to a suppression of the host's natural defenses. In fact, that is the case. The response to sheep erythrocytes (sRBC) of mice exposed to both the drug and sRBC is less than half that of untreated controls (15). Whether such an effect

also occurs in response to viral antigens remains to be determined.

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