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Hexamethylene bisacetamide stimulates the expression of human immunodeficiency virus long terminal repeat sequences in rat and human fibroblasts

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We have employed a recombinant plasmid, pBHIV1, carrying the long terminal repeat (LTR) sequence of the human immunodeficiency virus-1 (HIV-1) linked to the reporter chloramphenicol acetyl transferase (CAT) gene and to the aminoglycoside phosphotransferase (*aph*) gene as a selectable marker. We have introduced pBHIV1 in rat 208F and human MRCSV40TGR fibroblasts and obtained stable geneticin resistant RFBHIV1-1 and SVTGHIV1-1 cells, respectively. Both transfectant cells express CAT activity from the HIV LTR promoter. The response to anti-neoplastic drug hexamethylene bisacetamide (HMBA) was studied on the LTR regulated CAT activity in both cell lines. It was found that HMBA at 5mM concentration stimulates the expression of CAT from the HIV LTR in rat and human cells by 28- and 1.9-fold, respectively.

Key words: Hexamethylene bisacetamide, HIV-1.

Introduction

A variety of agents, i.e. polar compounds,[1] vitamin D,[2] retinoic acid,[3] growth factors,[4] tumor promoters[5,6] and inhibitors of DNA or RNA synthesis,[7] can induce various transformed cell lines to express more differentiated characteristics.

The hybrid polar/apolar compound N,N'-hexamethylene-bisacetamide (HMBA) induces transformed cells to differentiate, accompanied by suppression of oncogenicity.[1] Clinical trials have shown that HMBA can cause positive therapeutic responses in some cancer patients, but clinical efficacy may be limited, in part, by dose-related toxicity.[8]

The murine erythroleukemia cells (MELC) have been proved a useful model to study inducer-mediated differentiation of transformed cells. HMBA-induced MELC differentiation to the erythroid phenotype[6] is a multistep process. The mechanism of action of HMBA is not known. There is evidence that protein kinase C (PKC) is involved in the pathway of inducer-mediated differentiation.[9] Also, in previous studies, a series of changes in the expression of genes has been reported—including α_1- and β-globin genes, the rRNA genes, and the proto-oncogenes c-*myb*, c-*myc*, c-*fos* and *p53*.[1]

Human immunodeficiency virus type-1 (HIV-1) has been clearly implicated as the primary cause of acquired immune deficiency syndrome (AIDS).[10,11] These viruses infect and destroy the T_4 lymphocytes, establish chronic infection,[12] and are associated with diseases including Kaposi's sarcoma, non-Hodgkin's lymphoma, squamous cell carcinoma, testicular cancers, malignant melanoma, primary hepatocellular carcinoma and Hodgkin's disease.[13]

The HIV-1 long-terminal repeat (LTR) has a complex structure comprised of protein binding sites which control the reactivation of latent virus leading to further cycles of infection. Stimuli which are known to affect HIV LTR activity include several mitogens, i.e. phytohemaglutin,[14] phorbol esters,[15] mitogenic lectins,[16] tumor necrosis factor-α, interleukin-1,[17] and gene products including the HIV *trans*-activator *tat*,[18] heterologous *trans*-activators from other viruses (e.g. HSV, CMV),[19] positive regulatory factors (e.g. NF-$\varkappa$B) induced by cell activation[20] and the oncogene *ras*.[21]

In a previous study we have found that cisplatin and doxorubicin stimulate the expression of the reporter chloramphenicol acetyl transferase (CAT) gene from the HIV-1 LTR in rat and human

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fibroblasts, respectively.[22,24] In the present study we have investigated the effects of HMBA on the HIV-1 LTR-driven expression of the CAT gene. We found that HMBA stimulates transcriptional activation in transfectant rat RFBHIV1-1 and human SVTGHIV1-1 cell lines.

Materials and methods

Recombinant plasmids and cell lines

Plasmid pBHIV1 carrying a 728 bp *Xho*I–*Hind*III DNA fragment containing the HIV-1 LTR sequences was costructed by inserting a 1.9 kb *Bam*HI fragment carrying the aminoglycoside phosphotransferase (*aph*) gene into the single *Bam*HI site of plasmid pBC12/HIV/CAT.[25]

The spontaneously immortalized rat 208F and the SV40 immortalized human MRCSV40TGR fibroblasts were used as recipients to obtain the RFBHIV1-1 and SVTGHIV-1 stable geneticin resistant transfectants with plasmid pBHIV1.[22] DNA transfections were carried out using the calcium phosphate technique[26] as modified elsewhere.[27]

CAT assays

Cells were grown exponentially in Ham's SF12 medium containing 10% fetal calf serum and assayed for CAT activity as previously described.[28]

Assay for cell proliferation

The rapid colorimetric assay for cell proliferation developed by Mosmann was used.[29] A stock solution of MTT, 3-(4,5-dimethylthiazol-2,5-diphenyl tetrazolium bromide (from Sigma) in phosphate buffered saline (5 mg/ml, filter sterilized), was prepared. This was added to each well (10 μl per 100 μl medium) and plates were incubated at 37°C for each time interval. Then 110 μl of 0.04N HCl in isopropanol was added to each well and after thorough mixing (to dissolve the dark blue crystals) was left for a few minutes at room temperature. Then the plates were placed on a Titertek Flow MicroELISA reader and the optical density was recorded at a wavelength of 540 nm. Plates were read within 1 h of adding the acid–isopropanol solution.

Results

HMBA enhancers transcription from the HIV LTR sequences

The recipient rat 208 F and human MRCSV40TGR and their derivative RFBHIV1-1 and SVTGHIV1-1 transfectant cell lines, respectively, were treated with HMBA at concentrations ranging from 1 to 50 mM. Representative CAT assays are shown in Figures 1(a) and 2(a) and the corresponding

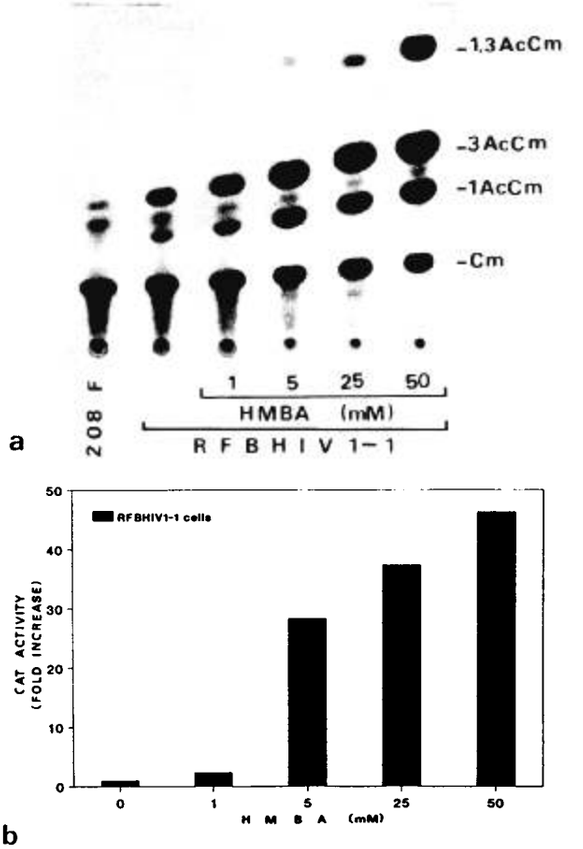

Figure 1. Induction of CAT activity in RFBHIV1-1 cells by HMBA. (a) Chromatogram for representative CAT assays with extracts from recipient 208 F and transfectant RFBHIV1-1 cells treated with and without HMBA. (b) Histogram of recorded CAT activities. 208 F and RFBHIV1-1 cells were plated at 1.5×10^6/75 cm^2 flask in Ham's SF12 containing 10% FCS at 37°C. After 24 h the medium was replaced with Ham's SF12 containing 0.5% FCS and left for another 24 h at 37°C. The medium was then changed with Ham's SF12 containing 5% FCS and varying concentrations of HMBA. Cells were harvested 24 h later and tested for CAT activity. Relative values of CAT activity in RFBHIV1-1 were 1.6 pmol acetylated chloramphenicol/μg protein/h incubation. The average from three experiments is given. Standard deviation was less than 3% of the average values.

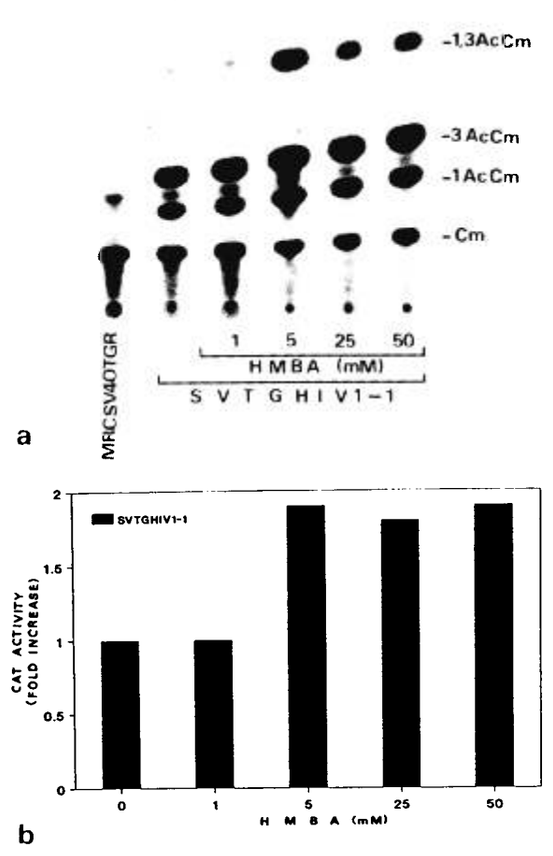

Figure 2. Induction of CAT activity in SVTGHIV1-1 cells by HMBA. (a) Chromatogram of representative CAT assays with extracts from recipients MRCSV40TGR and transfectants STVGHIV1-1 cells treated with and without HMBA. (b) Representative histogram of induction of CAT activity by HMBA. Relative values of CAT activity in SVTGHIV1-1 were 37 pmol acetylated chlorampenicol/μg protein/h.

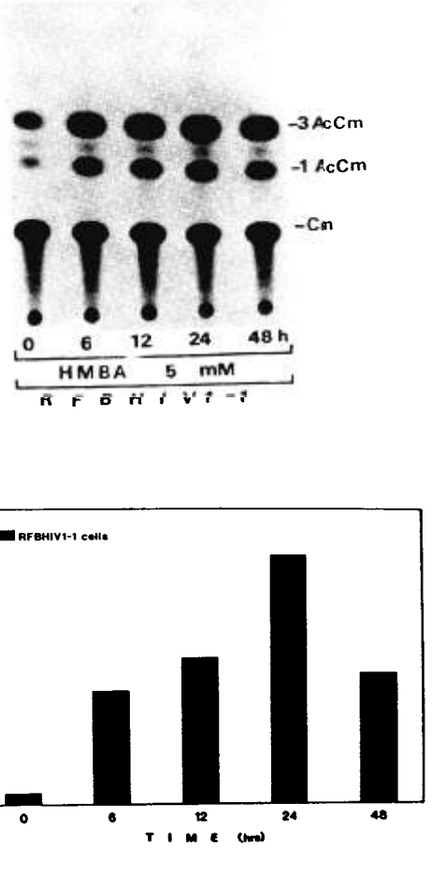

Figure 3. Induction of CAT activity in RFBHIV1-1 cells by HMBA at various times post-treatment. (a) Chromatogram from representative CAT assays with extracts from RFBHIV1-1 cells treated with 5 mM HMBA at various times. (b) CAT values were computed and are presented in histograms as described in Figure 1.

histograms in Figures 1(b) and 2(b) for the rat and human cells, respectively.

At the optimal HMBA concentration of 50 mM a 46-fold increase in CAT activity was observed for RFBHIV1-1 transfectant cell line. Similarly, a 1.9-fold increase was observed for the SVTGHIV1-1 transfectant cell line after treatment with the optimal HMBA concentration of 5 mM. As shown in the autoradiograms of Figures 3(a) and 4(a), and the histograms of Figures 3(b) and 4(b), a time course revealed that 24 h exposure to HMBA gave rise to maximal activation.

HMBA toxicity

The cytotoxic effect of HMBA on RFBHIV1-1 and SVTGHIV1-1 cells was measured by a rapid cell proliferation assay, for different time exposures (0, 24, 48, 72, 96 and 120 h) over a range of drug concentrations (from 0.1 to 50 mM) (Figure 5a and b). The same initial number of cells was used for each concentration. Toxicity was measured using Mosmann's colorimetric MTT assay. As seen in Figure 5, at the concentrations where the HMBA was most effective in stimulating the HIV LTR (5, 25 and 50 mM) it was strongly inhibitory for cell proliferation.

Discussion

Several regulatory elements have been defined on the HIV-1 LTR. The HIV-1 LTR plays an important role for viral behavior in the host cell

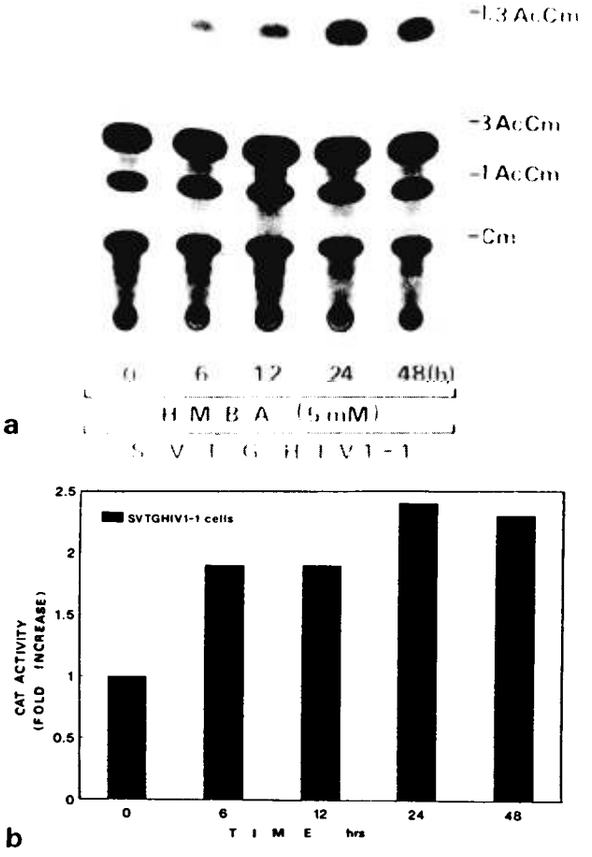

Figure 4. Induction of CAT activity in SVTGHIV1-1 cells by HMBA at various times post-treatment. (a) Chromatogram from representative CAT assays with extracts from SVTGHIV1-1 cells treated with 5 mM HMBA at various times. (b) CAT values were computed and are presented in histograms as described in Figure 2.

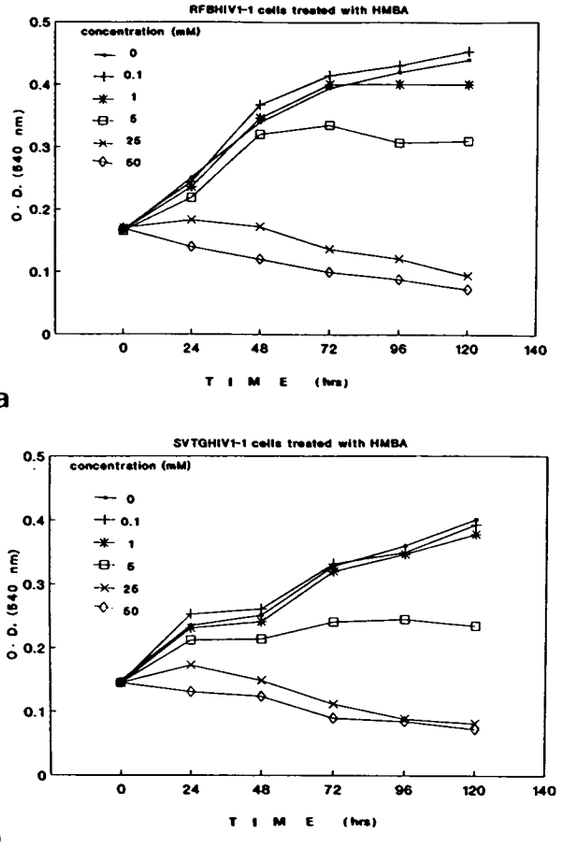

Figure 5. Cell proliferation in response to HMBA at various times of exposure. A total of 4×10^3 exponentially growing RFBHIV1-1 or SVTGHIV1-1 cells were plated in 96-well tissue culture clusters (Costar) in Ham's SF12 medium containing 10% FCS in the presence of the indicated concentration of HMBA. At the indicated times cell proliferation was measured using Mosmann's rapid colorimetric assay.

as it carries *cis*- or *trans*-acting sequences responding to cellular[21,30] or viral gene products.[18 20].

In a previous study we have examined the effect of cisplatin and doxorubicin on the transcriptional activation of the HIV-1 LTR employing transfectant cell lines or rat and human origin, expressing the reporter CAT gene from the HIV-1 LTR sequences. We have found that both anti-neoplastic drugs act as powerful inducers of CAT activity.[22-24] In the present study we have found that HMBA causes a significant increase of (46- or 1.9-fold) in transcriptional activity of the HIV-1 LTR regulatory sequences in RFBHIV1-1 and SVTGHIV1-1 transfectant cell lines, respectively. The site(s) of action of HMBA is not defined. Also, the mechanism by which the drug–target interaction leads to cell death has not been explained. Most of the anticancer drugs interact with proteins, RNA or with genomic DNA. Evidence from the studies of the action of the anti-neoplastic drugs has implicated DNA as the critical target for cytotoxicity.[31]

The mechanism of action of HMBA is not known, but there is evidence that this hybrid polar–apolar compound initiate a number of changes in cells. Among these changes are alterations in membrane ion permeability, in membrane fluidity, changes in concentration of the cyclic AMP, as well as in the expression of photo-oncogenes *c-myb, c-myc, c-fos* and *p53*.[1] These metabolic changes and the modulation of gene expression may contribute directly or indirectly in the regulation of the expression from the HIV-1 LTR. The activity of PKC is increased

in the MEL cells after treatment with HMBA.[9] In our system of stable transfectant cell lines of rat and human fibroblasts, PKC probably plays a key role for the *trans*-activation through the HIV-1 LTR. Experiments are in progress to test this possibility.

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