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Mitomycin C stimulates the expression of human immunodeficiency virus long terminal repeat sequences in rat and human fibroblasts

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Abstract—We have employed a recombinant plasmid, pBHIV1, carrying the long terminal repeat (LTR) sequences 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 into 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 the antineoplastic drug mitomycin C was studied on the LTR regulated CAT activity in both cell lines. It was found that mitomycin C at 10 μg/mL concentration stimulates the expression of CAT from the HIV LTR 77-fold in rat RFBHIV1-1 and 3.1-fold in human SVTGHIV1-1 cells.

Mitomycin is a naturally occurring anticancer agent, isolated from *Streptomyces caespitosus* which has been shown to be active against a broad spectrum of animal [1] and human [2] solid tumors.

Studies show that the molecular mechanism of action of mitomycin C is intrinsically related to its ability to bind covalently to DNA both in a monofunctional and bifunctional manner, resulting in the latter case in stable cross-links between the complementary strands of the genetic material [3].

Human immunodeficiency virus type 1 (HIV-1*) is a cytopathic retrovirus and the primary etiological agent of the acquired immune deficiency syndrome (AIDS) and related disorders [4, 5]. The syndrome is associated with a range of malignancies including Kaposi's sarcoma, non-Hodgkin's lymphoma, squamous cell carcinoma, testicular cancers, malignant melanoma, primary hepatocellular carcinoma and Hodgkin's disease [6].

The HIV-1 is a highly regulated retrovirus. The HIV-1 long terminal repeat (LTR) has a complex structure comprised of protein binding sites which control the reactivation of a latent virus leading to further cycles of infection. A variety of events, such as infection of the host cells with other viruses and stimulation by some mitogens, cytokines, positive regulatory factors (e.g. NF-κB) induced by cell activation, gene products such as *tat*, protein *ras* p21 and chemotherapeutic drugs (cisplatin, doxorubicin and hexamethylene bisacetamide) induce transcriptional activation through HIV-1 LTR [7–14].

In a previous study we have found that cisplatin, doxorubicin and hexamethylene bisacetamide as opposed to carboplatin transcriptionally activate the HIV LTR sequences in rat and human fibroblasts. In the present study we have investigated the effects of mitomycin C on the HIV-1 LTR-driven expression of the chloramphenicol acetyl transferase (CAT) gene. We found that mitomycin C 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 constructed by inserting a 1.9 kb *Bam*HI fragment carrying the *aph* gene into the single *Bam*H1 site of plasmid pBC12/HIV/CAT [15].

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 [9]. DNA transfections were carried out using the calcium phosphate technique [16] as modified [17].

Treatment of cells and CAT assays. Cells were plated at $1.5 \times 10^6/75\ cm^2$ flask in Ham's SF12 medium containing 10% fetal calf serum (FCS) at 37°. Twenty four hours later the medium was replaced with Ham's SF12 containing 0.5% FCS and left for another 24 hr at 37°. Then the medium was changed to Ham's SF12 containing 5% FCS and the various concentrations of mitomycin C (from the Sigma Chemical Co., St Louis, MO, U.S.A.). Cells were harvested 24 hr later and tested for CAT activity as described previously [18].

Assay for cell proliferation. The rapid colorimetric assay for cell proliferation of Mosmann was used [19]. A stock solution of MTT [3-(4,5-dimethylthiazol-2,5-diphenyl)-tetrazolium bromide] (from Sigma) in phosphate-buffered saline (PBS) (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° for each time interval. One hundred and ten microliters of 0.04 N 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 MicroELIZA reader and optical density was recorded at the wavelength of 540 nm. Plates were read within 1 hr of adding the acid-isopropanol solution.

Results

Mitomycin C enhances transcription from the HIV LTR sequences. The recipient human MRCSV40TGR and its derivative SVTGHIV1-1 transfectant cells were treated with mitomycin C at concentrations ranging from 0.1 to 20 μg/mL. A representative CAT-assay is shown in Fig. 1a and the corresponding histogram in Fig. 1b. At the optimal mitomycin C concentration of 10 μg/mL a 3.1-fold increase in CAT activity was observed for the SVTGHIV1-1 transfectant cell line. Similar results were also obtained with the rat RFBHIV1-1 cells treated with mitomycin C. As shown in the autoradiogram of Fig. 2a and the histogram of Fig. 2b, a time course revealed that 24 hr exposure to mitomycin C gave rise to maximal activation (77-fold).

Mitomycin C toxicity. The cytotoxic effect of mitomycin C 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 hr) over a range of mitomycin C drug concentrations (from 0.01 to 20 μg/mL) (Fig. 3a and b). The same initial number of cells was used

* Abbreviations: HIV-1, human immunodeficiency virus 1; AIDS, acquired immune deficiency syndrome; LTR, long terminal repeat; CAT, chloramphenicol acetyl transferase; FCS, fetal calf serum.

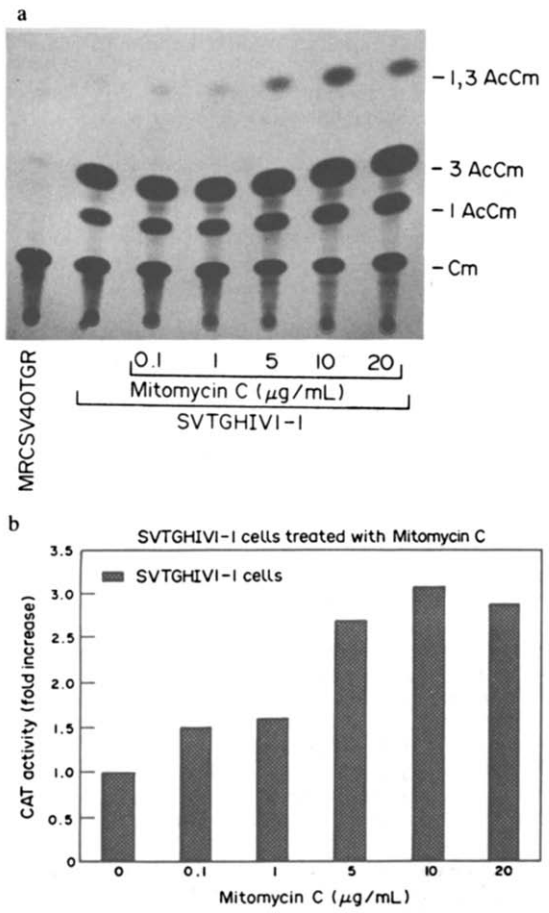

Fig. 1. Induction of CAT activity in SVTGHIV-1 cells treated with mitomycin C. (a) Chromatogram for representative CAT assays with extracts from recipient MRCSV40TGR and transfectant SVTGHIV1-1 cells treated with mitomycin C or left untreated. MRCSV40TGR = immortalized with SV40 human fibroblasts; Cm = [^{14}C]chloramphenicol; AcCm = acetylated [^{14}C]chloramphenicol. (b) Histogram of induction of CAT activity by mitomycin C as measured by a two-phase scintillation counting technique. Relative value of CAT activity in SVTGHIV1-1 was 23 pmol acetylated chloramphenicol/μg protein per hour incubation. The average from three experiments is given. Standard deviation was less than 6% of the average values.

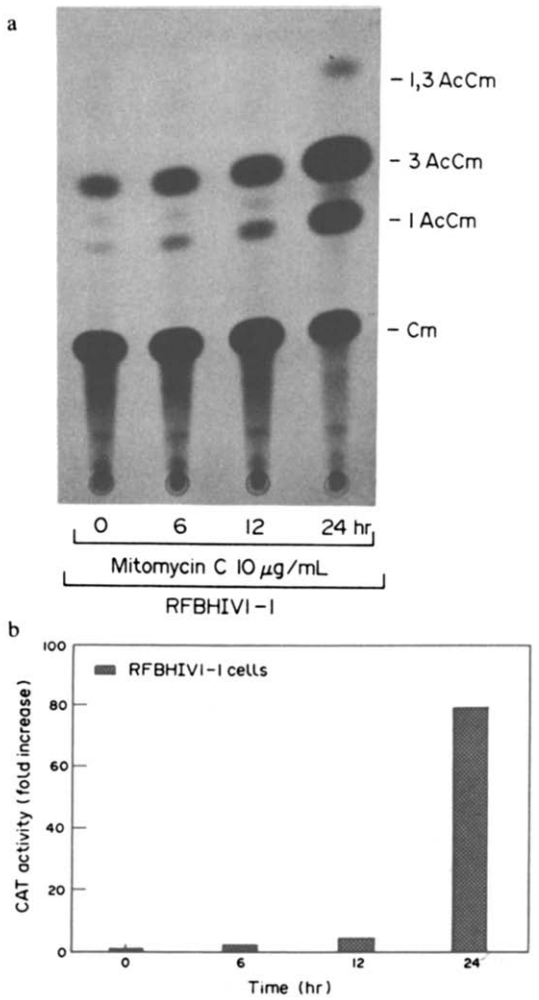

Fig. 2. Induction of CAT activity in RFBHIV1-1 cells by mitomycin C at various times post-treatment. (a) Chromatogram from representative CAT assays with extracts from RFBHIV1-1 cells treated with 10 μg/mL mitomycin C for 0, 6, 12 or 24 hr. Cm = [^{14}C]-chloramphenicol; AcCm = acetylated [^{14}C]chloramphenicol. (b) Representative histogram of induction of CAT activity by mitomycin C. Relative value of CAT activity in RFBHIV1-1 was 0.8 pmol acetylated chloramphenicol/μg protein per hour incubation. The average from three experiments is given. Standard deviation was less than 5% of the average values.

for each concentration. Toxicity was measured using Mosmann's colorimetric MTT assay. As seen in Fig. 3a and b, at the concentrations where the mitomycin C was most effective in stimulating the HIV LTR (1, 10 and 20 μg/mL) it was strongly inhibitory for cell proliferation.

Discussion

Recent studies indicate that the HIV-1 gene expression can be dramatically enhanced by certain heterologous viral and chemical agents, implicating these as potential reactivating agents of latent virus infection [7–14, 20]. The HIV-1 LTR plays an important role for viral behavior in the host cells as it carries *cis* or *trans*-acting sequences responding to cellular or viral gene products [7–14].

In a previous study we have examined the effect of cisplatin doxorubicin and hexamethylene bisacetamide on the transcriptional activation of the HIV-1 LTR employing transfectant cell lines of rat and human origin, expressing the reporter CAT gene from the HIV-1 LTR sequences. We have found that these antineoplastic drugs act as powerful inducers of CAT activity [9–13]. In the present study we found that mitomycin C causes a significant increase of 77- and 3.1-fold in transcriptional activity of the HIV-1 LTR regulatory sequences in RFBHIV1-1 and SVTGHIV1-1 transfectant cell lines, respectively.

The mechanism of action of mitomycin C is not known, but it is thought that DNA is the critical target. The antitumor antibiotic mitomycin C reacts exclusively with N2-positions of guanines in DNA. These reactions occur only upon reductive activation of mitomycin C [21]. A possible explanation for the effects of mitomycin C in RFBHIV1-1 and SVTGHIV1-1 cell lines is that its binding to *cis*-acting regulatory sequences of the HIV LTR inhibits the binding of negative regulatory proteins.

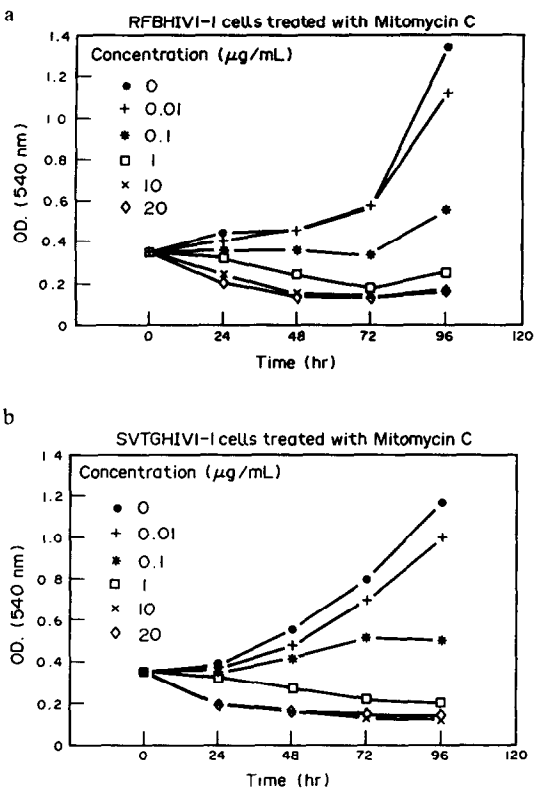

Fig. 3. Cell proliferation in response to mitomycin C at various times of exposure. Exponentially growing (a) RFBHIV1-1 or (b) SVTGHIV1-1 cells (4×10^{-3}) were plated in 96-well tissue culture clusters (Costar) in Ham's SF12 medium containing 10% FCS in the presence of the indicated concentrations of mitomycin C. At the indicated times cell proliferation was measured using Mosmann's rapid colorimetric assay.

It is clear from biochemical studies that mitomycin C can be activated by several pathways and that this drug has the potential to produce many different lesions in DNA [22–25]. The radicals which are produced by reduction of mitomycin C are probably the cause of cell death. However, the effect of mitomycin C on the transcription activation of the HIV-1 LTR could be mediated by activation of endogenous NF-κB through the production of superoxide radicals [26]. These findings may be significant in understanding the regulation of HIV by anti-tumor drugs, which might have potential clinical implications.

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