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Cisplatin stimulates the expression from the human immunodeficiency virus long terminal repeat sequences in human fibroblasts

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A recombinant plasmid carrying the long terminal repeat (LTR) of the human immunodeficiency virus 1 (HIV-1) linked to the reporter chloramphenicol acetyl transferase (CAT) gene was stably introduced into human fibroblasts. The transfectant cells expressed CAT activity from the HIV LTR. The response to anti-neoplastic drugs, i.e. cisplatin, a platin derivative, and hexadecylphosphocholine, was studied. It was found that at 5×10^{-6} M concentrations cisplatin stimulates by 2.2-fold the expression of CAT from the HIV LTR. Our results extend our observations on the effect of cisplatin on HIV LTR in rodent fibroblast cells and suggest caution against therapy including cisplatin in the treatment of AIDS patients.

Key words: Cisplatin, HIV, AIDS.

Introduction

The discovery in 1969[1] that cisplatin has antitumor activity has led to the use of this compound in the chemotherapy of several types of human tumors (for a review see ref. 2). Although the site of action of cisplatin is not certain it is thought that DNA is the critical target.[3]

The human immunodeficiency virus (HIV) is the causative agent of the acquired immune deficiency syndrome (AIDS) (for a review see ref. 4). AIDS patients are at increased risk from a variety of cancers, including Kaposi's sarcoma, non-Hodgkin's lymphoma, squamous cell carcinoma, testicular cancers, malignant melanoma, primary hepatocellular carcinoma and Hodgkin's disease.[4]

The HIV-1 LTR contains a variety of regulatory sequences (for a review see ref. 5). We have previously shown that cisplatin transcriptionally activates the HIV LTR sequences in rat fibroblasts.[6] In the present study we have examined the effect of cisplatin on the HIV LTR in human fibroblasts and found that cisplatin also stimulates transcriptional activation significantly in this system.

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Materials and methods

Recombinant plasmids and cell lines

Plasmid pBHIV1 was derived from plasmid pBC12/HIV/CAT[7] by inserting a 1.9 kb *Bam*H1 fragment carrying the *aph* gene into the single *Bam*H1 site. Plasmid pBC12/HIV/CAT carries a 728 bp *XhoI-Hind*III DNA fragment containing the HIV LTR sequences linked to the CAT reporter gene.

The MRCSV40TGR human fibroblasts were obtained after immortalization of MRC cells with SV40 and are resistant to 6-thioguanine. SVTGHIV1-1 cells were obtained after transfection with plasmid pBHIV1 and selection in the presence of geneticin.

CAT assays

Cells were grown exponentially in Ham's medium containing 10% fetal calf serum and assayed for CAT activity as previously described.[8]

Assay for cell proliferation

The rapid colorimetric assay for cell proliferation of Mossmann[9] was used as previously described.[6]

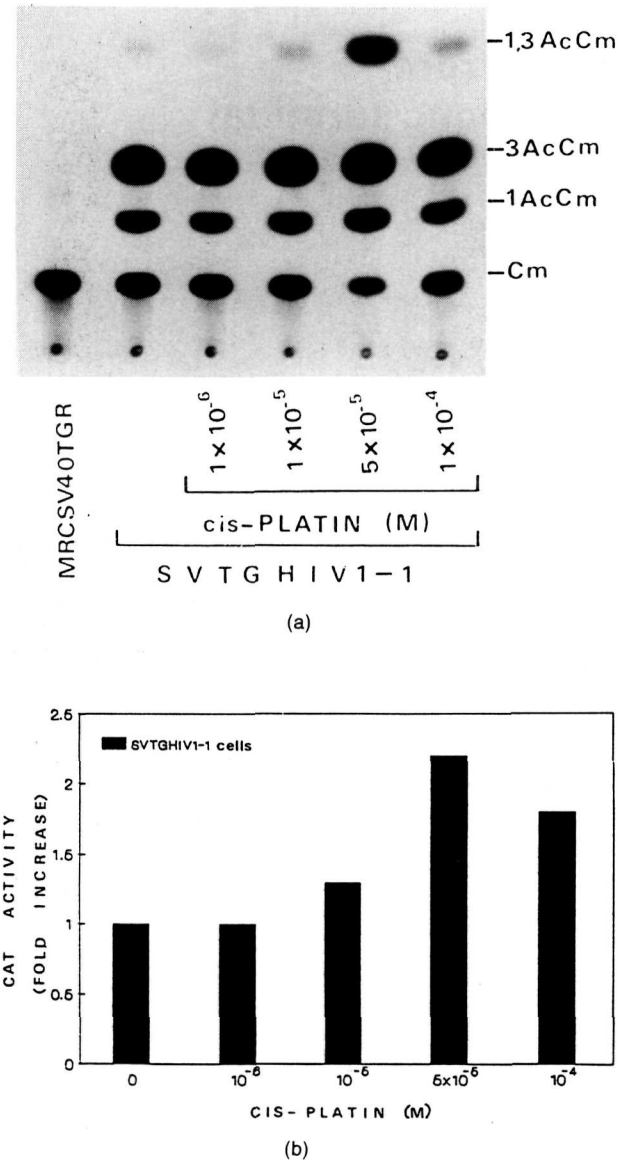


Figure 1. Induction of CAT activity by cisplatin. (a) Chromatogram of representative CAT assay with extracts from recipient MRCSV40TGR and transfectant SVTGHIV1-1 cells with and without treatment with cisplatin. (b) Histogram of recorded CAT activities. MRCSV40TGR and SVTGHIV1-1 cells were plated at 1.5×10^6 cells/75 cm^2 flask in Ham's SF12 medium containing 10% FCS at 37°C. 24 h later the medium was replaced with Ham's SF12 medium 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 the varying concentrations of cisplatin. Cells were harvested 24 h later and tested for CAT activity as described in Materials and methods. Relative values of CAT activity in SVTGHIV1-1 cells were 48 pmole acetylated chloramphenicol/μg protein per hour incubation. Average from three experiments is given. Standard deviation was less than 5% of the average values.

Results

Cisplatin enhances transcription from the HIV LTR sequences

The recipient MRCSV40TGR and the transfectant SVTGHIV1-1 cells were treated with cisplatin at various concentrations. A representative CAT assay is shown in Figure 1(a) and the corresponding histogram in Figure 1(b). Optimal stimulation was obtained at 5×10^{-5} M, where CAT activity increased 2.2-fold. As shown in Figure 2 a time course revealed that 24 h exposure to cisplatin gave rise to maximal activation.

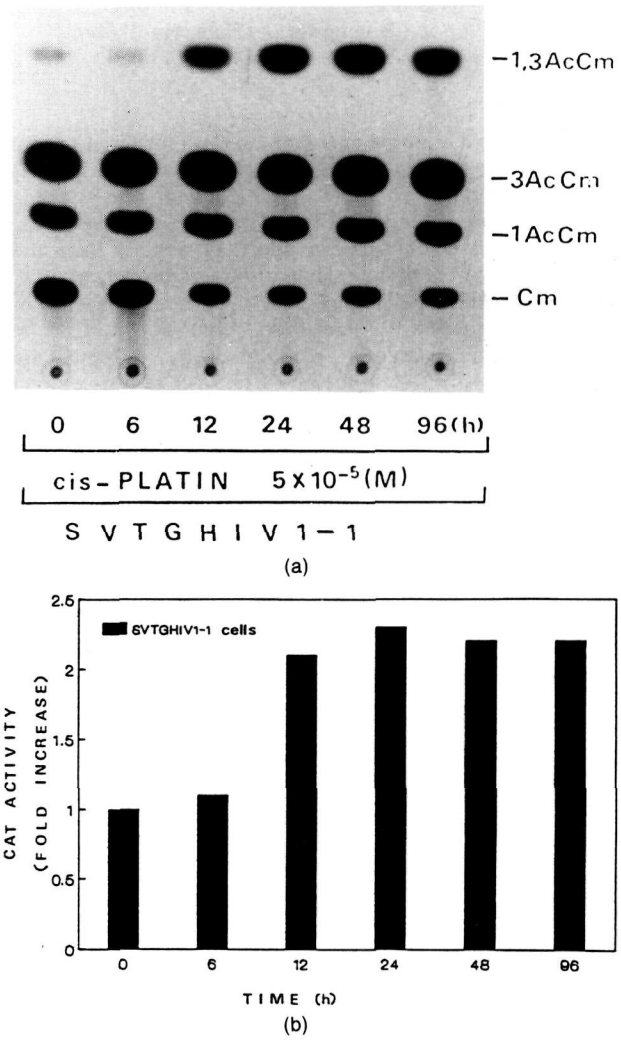
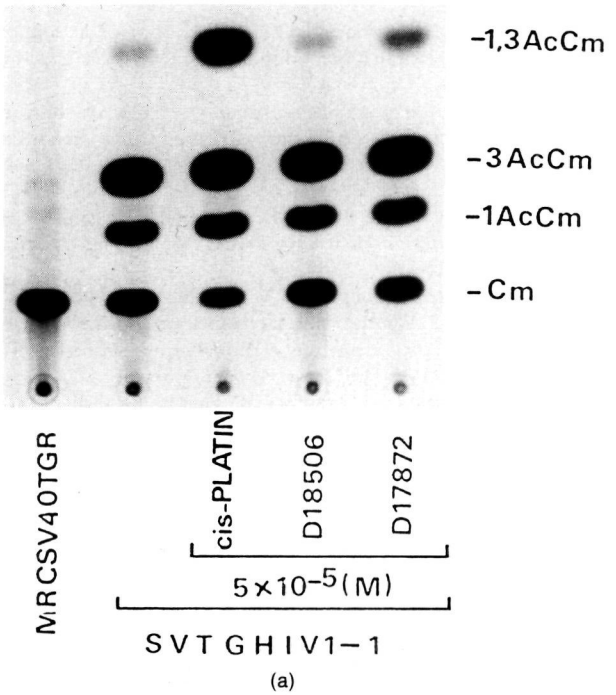
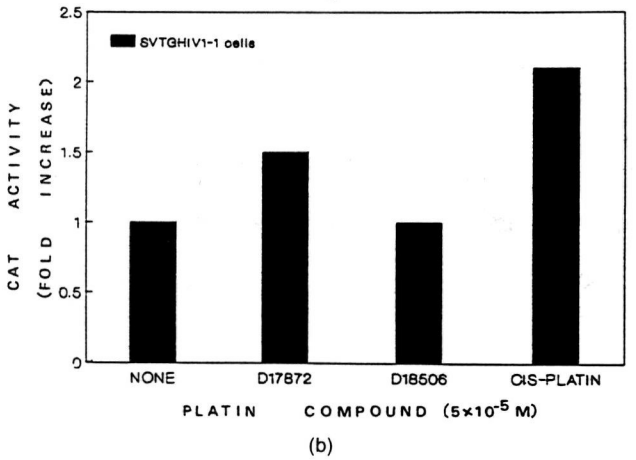


Figure 2. Induction of CAT activity in SVTGHIV1-1 cells by cisplatin at various times post-treatment. (a) Chromatogram for representative CAT assays with extracts from SVTGHIV1-1 cells treated with 5×10^{-5} M cisplatin at various times. (b) CAT values were computed and are presented in histograms as described in Figure 1.



(a)



(b)

Figure 3 Induction of CAT activity by cisplatin and D17872 and D18506. (a) Chromatograms for representative CAT assays with extracts from SVTGHIV1-1 cells treated with 5×10^{-5} M cisplatin and the antineoplastic compounds D18506 and D17872. (b) CAT values were computed and are presented in histograms as described in Figure 1.

Effect of other anti-neoplastic compounds on HIV LTR activation

Two other compounds with known antineoplastic properties—D17872, a benzylethylenediamine derivative of cisplatin,[10] and D18506, a hexadecylphosphocholine compound[11]—were employed at concentrations similar to that of cisplatin (5×10^{-5} M) to test their effect on HIV LTR activation. As shown in Figure 3, only the D17872 compound activated the HIV LTR at 1.7-fold, much below the activation caused by cisplatin.

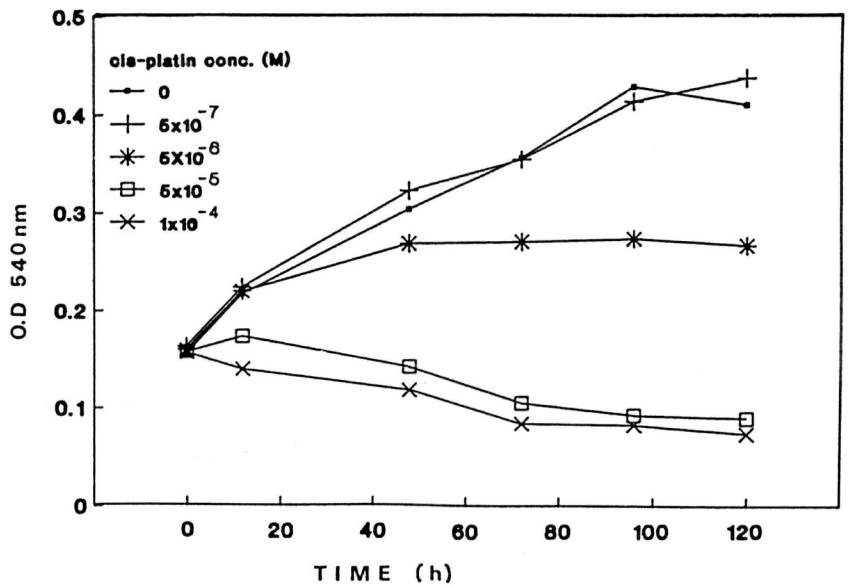


Figure 4. Cell proliferation in response to cisplatin at various times of exposure. 5×10^3 exponentially growing SVTGHIV1-1 cells were plated in 96-well tissue culture clusters (costar) in Ham's SF12 medium containing 10% FCS in the presence of the indicated concentration of cisplatin. At the indicated times cell proliferation was measured using Mossman's rapid colorimetric assay.[9]

Cisplatin toxicity

The effect of cisplatin on SVTGHIV1-1 cells was measured by a rapid cell proliferation assay. SVTGHIV1-1 cells were exposed for various times (0, 24, 48, 72, 96, 120 h) at a range of concentrations of the drug (from 5×10^{-7} M to 1×10^{-4} M (Figure 4).

The same initial number of cells was used for each concentration. Toxicity was measured, using Mosmann's colorimetric MTT assay. As seen in Figure 4, at the concentration where the cisplatin was most effective in stimulating the HIV LTR (5×10^{-5} M) it was strongly inhibitory for cell proliferation.

Discussion

The human immunodeficiency virus LTR contains several defined regulatory domains.[5] These can be *cis*-[12,13] or *trans*-acting sequences responding to cellular[14] or viral[15] gene products.

In a previous study we have found that cisplatin at 5×10^{-5} M concentrations stimulated by 22-fold the expression of the reporter CAT gene from the HIV LTR in rat fibroblasts.[6] In the present study we have found that in human fibroblasts cisplatin enhances the HIV LTR although at a much lower level (2.2-fold) (Figure 1). Again, it is of interest that the effect of cisplatin is observed when cell proliferation is strongly suppressed (Figure 4). Since cisplatin potentiates the activity of HIV LTR as shown before in rat,[6] and as shown here in human, fibroblasts we urged further caution when considering therapy including this compound in the treatment of AIDS patients carrying tumors.

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