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# Differential interaction of cisplatin with the HIV-1 long terminal repeat in a resistant ovarian carcinoma cell line

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**We constructed a recombinant plasmid, pBHIV1, carrying the long terminal repeat (LTR) sequences of 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 a human ovarian cancer cell line A2780 and in a cisplatin resistant variant 2780CP, and obtained stable geneticin resistant A27HIV1-1 and 27CPHIV1-1 cells, respectively. Both transfectant cells express CAT activity from the HIV LTR promoter. The response to the anti-neoplastic drug cisplatin was studied on the LTR regulated CAT activity in both cell lines. It was found that cisplatin at $2.5 \times 10^{-5}$ M concentration stimulates the expression of CAT by 26-fold from the HIV LTR in A27HIV1-1, but requires a concentration of $5 \times 10^{-5}$ M to enhance expression by 4.1-fold in the cisplatin resistant 27CPHIV1-1 cells. Carboplatin, over a range of concentrations ($1 \times 10^{-6}$ to $1 \times 10^{-4}$ M), does not stimulate expression of CAT from the HIV-1 LTR in either of the transfected cells.**

*Key words:* Carboplatin, cisplatin, HIV-1.

## Introduction

Cisplatin [*cis*-diamminedichloroplatinum (II)] has become established as an antitumor agent of major clinical importance since its introduction in the early 1970s,[1] and is particularly active against gynecological malignancies such as ovarian carcinoma.[2] The antitumor activity of cisplatin appears to be mediated by the modification of DNA bases by forming multiple types of interstrand and intrastrand adducts.[3,4] However, the severe toxicity (particularly nephrotoxicity) or circumvention of cisplatin resistance in tumors has led to the development of second generation analogs.[5,6] Today, despite numerous synthetic chemistry initiatives, only one additional platinum complex, carboplatin [paraplatin, *cis*-diammine-1,1-cyclobutane dicarboxylatoplatinum (II)] has general acceptance.

The therapeutic effectiveness of carboplatin and cisplatin is similar even though the dose-limiting toxicities of these drugs are different.[7]

The molecular pharmacology of these agents is complex; however, their cytotoxicity has been correlated with the formation of mono- and bihydroxylated platinum intermediates which then bind to the $N^7$ residue of guanine, causing intra- and interstrand DNA cross-links.[3,4,8,9] The relatively low aquation rate of carboplatin as compared with cisplatin also explains why relatively high concentrations of this drug are required to obtain detectable adduct levels in cells in culture; hence, the different cytotoxicity of these two agents.

The HIV-1 long terminal repeat (LTR) flanks the proviral DNA and contains functional units and signals that govern transcriptional initiation and termination. 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, hexamethylene bisacetamide and mitomycin C), induce transcriptional activation through HIV-1 LTR.[13–20]

In a previous study we have found that anticancer drugs, i.e. cisplatin, doxorubicin, and hexamethylene bisacetamide as opposed to carboplatin, stimulate the expression of the reporter chloramphenicol acetyl transferase (CAT) gene from the HIV-1 LTR in rat and human fibroblasts, respectively.[15–19] In the present study we have investigated the effects of platinum coordination complexes on HIV-1 LTR-driven expression of the

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CAT gene in platinum sensitive and resistant human ovarian cancer cell lines.

## Materials and methods

### Recombinant plasmids and cell lines

Plasmid pBHIV1 carrying a 728 bp *Xho*I-*Hin*dIII 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*HI site of plasmid pBC12/HIV/CAT.[21]

A2780 is an ovarian carcinoma cell line derived from an untreated patient.[22] This cell line was made resistant to cisplatin by stepwise exposure with the drug.[23] These cell lines were used as recipients to obtain the A27HIV1-1 and 27CPHIV1-1 stable, geneticin resistant transfectants with plasmid pBHIV1.[15] DNA transfections were carried out using the calcium phosphate technique[24] as modified previously.[24]

### Treatment of cells and CAT assays

Cells were plated at $1.5 \times 10^6$ per 75 $cm^2$ flask in RPMI 1640 medium containing 10% fetal calf serum (FCS) at 37°C. At 24 h later the medium was replaced with RPMI 1640 containing 0.5% FCS and left for another 24 h at 37°C. Then the medium was changed with RPMI 1640 containing 5% FCS and the various concentrations of cisplatin and carboplatin, respectively. Cells were harvested 24 h later and tested for CAT activity as previously described.[26] In brief, the protein content was estimated using the BioRad protein assay; 100 $\mu$g of protein extract was mixed with 10 $\mu$l of 4 M acetyl-CoA (Sigma, St Louis, MO, USA) and 2 $\mu$l of [$^{14}$C]-chloramphenicol (57 mCi/mmol) (Amersham, Buckinghamshire, UK) were added. The mixture was incubated at 37°C for 90 min and 0.6 ml of ethyl acetate was then used to extract the chloramphenicol. The organic layer was dried and taken up in 30 $\mu$l of ethyl acetate, spotted on silica gel thin-layer plates and run with chloroform: methanol (95:5). After autoradiography, spots were cut out and counted.

### Assay for cell proliferation

Mosmann's rapid colorimetric assay for cell proliferation was used.[27] 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 $\mu$l per 100 $\mu$l medium) and plates were incubated at 37°C for each time interval. Then 110 $\mu$l 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 a wavelength of 540 nm. Plates were read within 1 h of adding the acid–isopropanol solution.

## Results

### Cisplatin enhances transcription from the HIV LTR sequences

The recipient A2780 and 2780CP human ovarian carcinoma cells and their derivative A27HIV1-1 and 27CPHIV1-1 transfectant cells were treated with cisplatin at concentrations ranging from $1 \times 10^{-6}$ to $2.5 \times 10^{-4}$ M. Representative CAT assays are shown in Figure 1(a) and the corresponding histograms are shown in Figure 1(b) for the sensitive and resistant cells.

At the optimal cisplatin concentration of $2.5 \times 10^{-5}$ M a 26-fold increase in CAT activity was observed for the A27HIV1-1 sensitive to cisplatin transfectant cell line. On the other hand, a 4.1-fold increase was observed for the 27CPHIV1-1 resistant cisplatin transfectant cell line after treatment with the optimal cisplatin concentrations in Figures 2(a) and 3(a) and the histograms in Figures 2(b) and 3(b), a time course revealed that 24 h exposure to cisplatin gave rise to maximal activation.

### Carboplatin does not stimulate transcription from the HIV-1 LTR sequences

The recipient A2780 and 2780CP human ovarian carcinoma cells and their derivative A27HIV1-1 and 27CPHIV1-1 transfectant cells were treated with carboplatin at concentrations ranging from $1 \times 10^{-6}$ to $1 \times 10^{-3}$ M. A representative CAT assay is shown in Figure 4(a) and the corresponding histogram is shown in Figure 4(b). At the optimal carboplatin concentration of $1 \times 10^{-3}$ M a 3.5-fold increase in CAT activity was observed in cisplatin

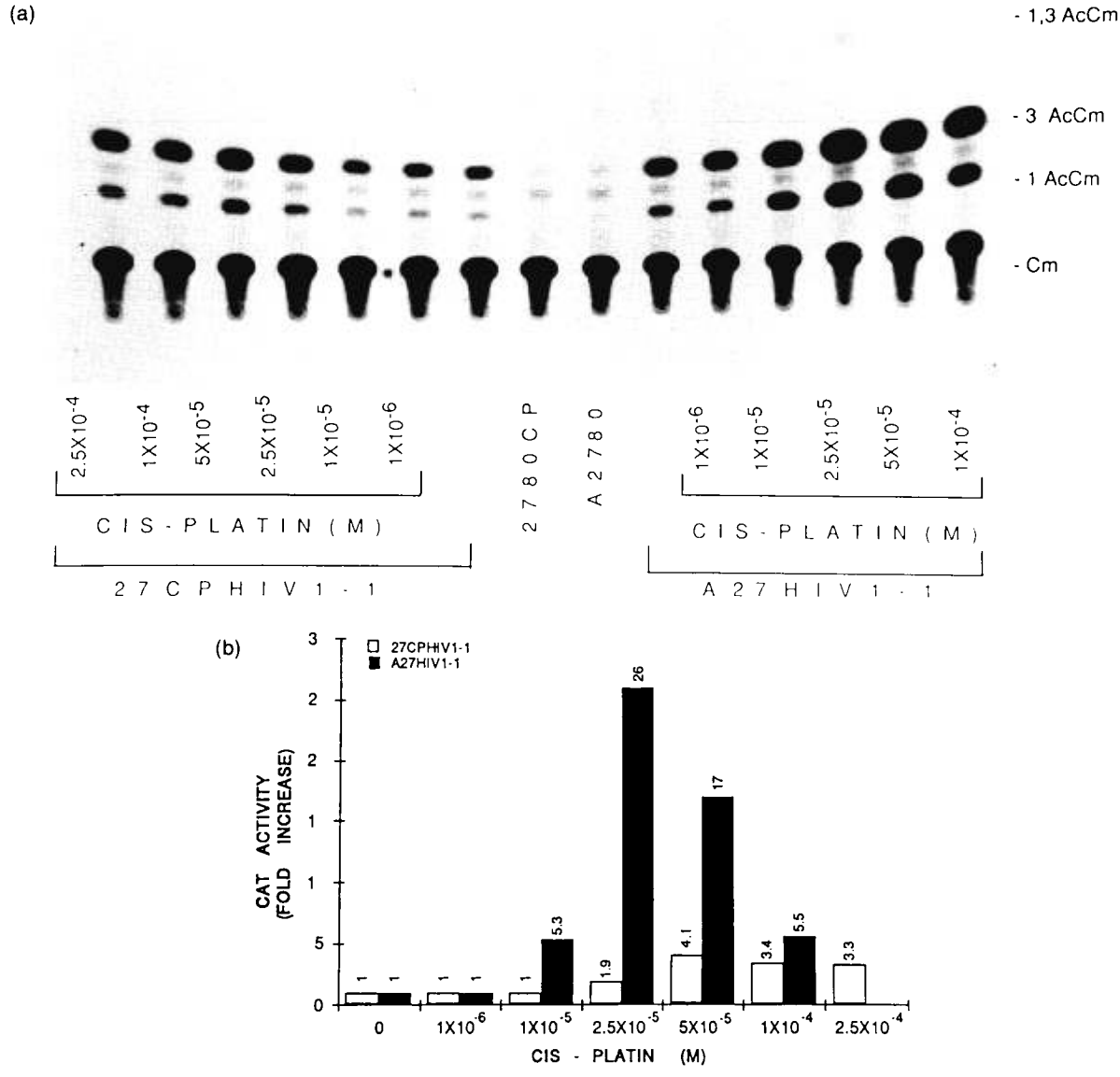


**Figure 1.** Induction of CAT activity in 27CPHIV1-1 and A27HIV1-1 cells by cisplatin. (a) Chromatogram for representative CAT assays with extracts from recipient 2780CP and A2780 and transfectant 27CPHIV1-1 and A27HIV1-1 cells with and without treatment with cisplatin. (b) Histogram of recorded CAT activities. Cells were tested for CAT activity as described in Materials and methods. Relative values of CAT activity in 27CPHIV1-1 and A27HIV1-1 cells were 1.2 and 2.1 pmol acetylated chloramphenicol/$\mu$g protein/h incubation, respectively. The average from three experiments is given. Standard deviation was less than 6% of the average values.

sensitive A27HIV1-1 cells, while at the same concentration of carboplatin, no corresponding stimulation was obtained in resistant cells.

## Cisplatin and carboplatin toxicity

The cytotoxic effect of cisplatin and carboplatin on A27HIV1-1 and 27CPHIV1-1 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 $1 \times 10^{-6}$ to $1 \times 10^{-3}$ M) (Figures 5 and 6). 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 cisplatin was most effective in stimulating the HIV LTR, it was strongly inhibitory for cell proliferation.

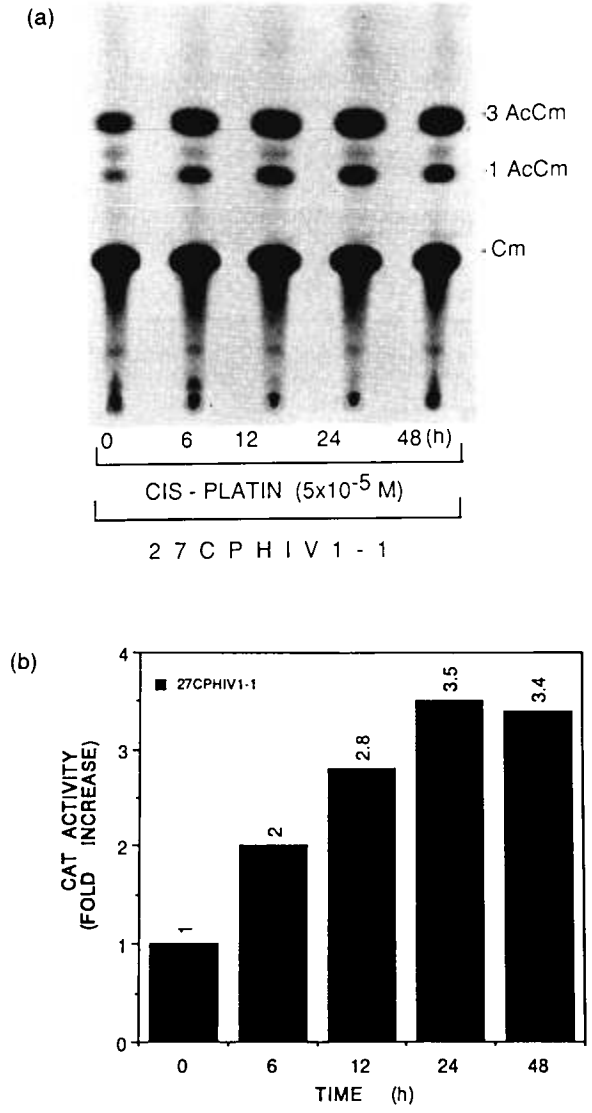


**Figure 2.** Induction of CAT activity in 27CPHIV1-1 cells by cisplatin at various times post-treatment. (a) Chromatogram of representative CAT assays with extracts from 27CPHIV1-1 cells treated with $5 \times 10^{-5}$ M cisplatin at various times. (b) CAT values were computed and are presented in histograms as described in Figure 1.

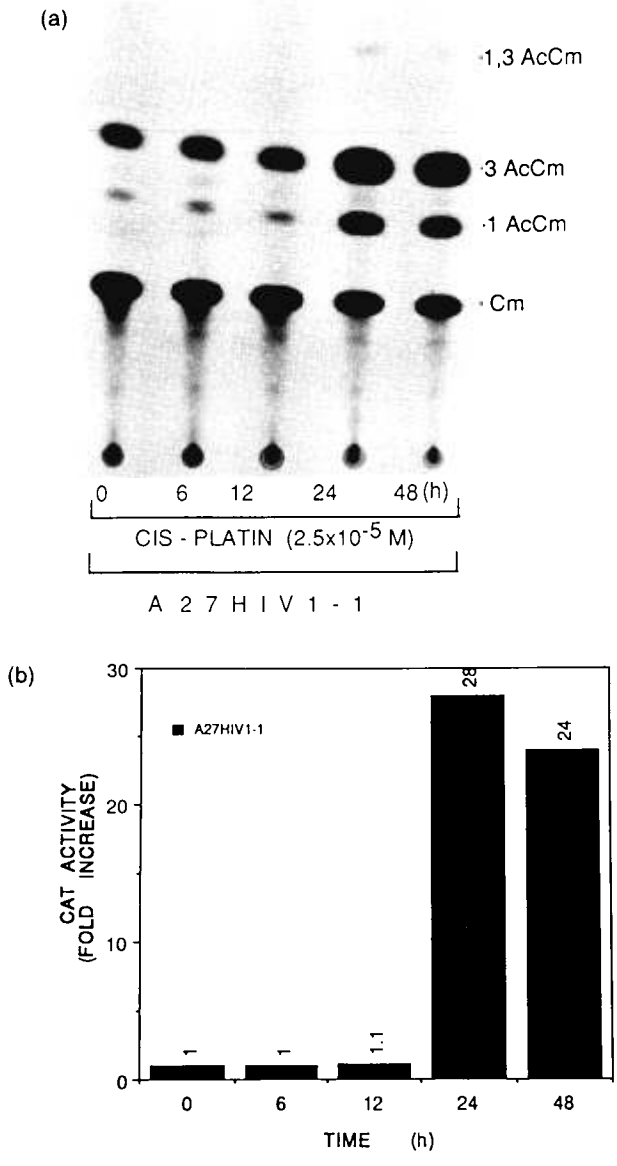


**Figure 3.** Induction of CAT activity in A27HIV1-1 cells by cisplatin at various times post-treatment. (a) Chromatogram from representative CAT assays with extracts from A27HIV1-1 cells treated with $2.5 \times 10^{-5}$ M cisplatin at various time intervals. (b) CAT values were computed and are presented in histograms as described in Figure 1.

## Discussion

The phenomenon of drug resistance in genito-urinary malignancies such as ovarian carcinoma is of great clinical importance. The mechanism of tumor resistance to cisplatin is not yet clear but there is *in vitro* evidence suggesting that resistance is at least in part due to increased repair of cisplatin DNA adducts with DNA polymerase $\beta$.[29] It is presumed that adduct repair requires recognition of the adduct, its excision from the DNA double-helix, replacement of the excised bases and their relegation. In human leukemia and ovarian carcinoma cells, the initial accumulation of cisplatin (2–10 $\mu$M) during the first 10 min after the addition of the drug is similar between sensitive and resistant cells.[30] There are transport differences in human carcinoma cells that are sensitive and resistant to cisplatin at higher concentrations (50–100 $\mu$M) and at longer time points,[31] and intracellular glutathione

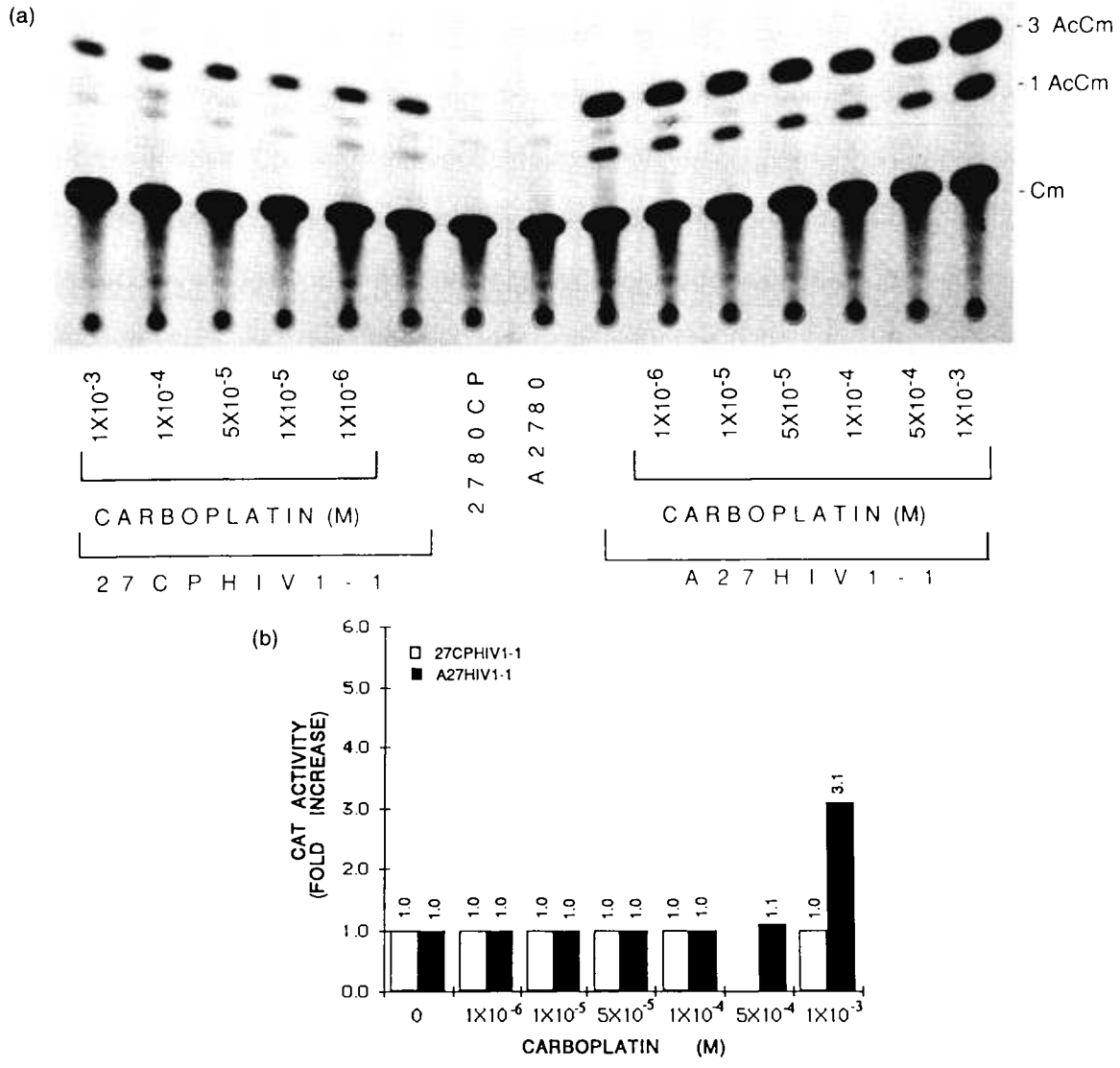


**Figure 4.** Induction of CAT activity in 27HIV1-1 and A27HIV1-1 cells by carboplatin. (a) Chromatogram from representative CAT assays with extracts from recipients 2780CP and A2780 and transfectants 27CPHIV1-1 and A27YHIV1-1 cells with and without treatment with carboplatin. (b) CAT values were computed as described in Figure 1 and are presented in histograms. Relative values of CAT activity in 27CPHIV1-1 and A27HIV1-1 were 1.2 and 2.1 pmol acetylated chloramphenicol/$\mu$g protein/h incubation, respectively. The length of exposure time to carboplatin was 24 h. Values are means from three experiments. Standard deviation was less than 4% of the average values.

metabolism may contribute to platinum resistance. The polymerase chain reaction and other methods have demonstrated gene amplification and enhanced gene expression for DNA polymerase $\beta$, *fos*, H-*ras* and *myc* in carcinoma patients who have failed cisplatin.[30,32,33]

In a previous study we 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 anti-neoplastic drugs act as powerful inducers of CAT activity.[15–19] In the present study we have found that cisplatin causes a significant increase of 26- or 4.1-fold in transcriptional activity of the HIV-1 LTR regulatory sequences in sensitive and resistant human ovarian carcinoma, and transfectant cells A27HIV1-1 and 27CPHIV1-1 respectively (Figure 1a and b). In addition we have confirmed that the HIV-1 LTR promoter does not respond to carboplatin in either sensitive or

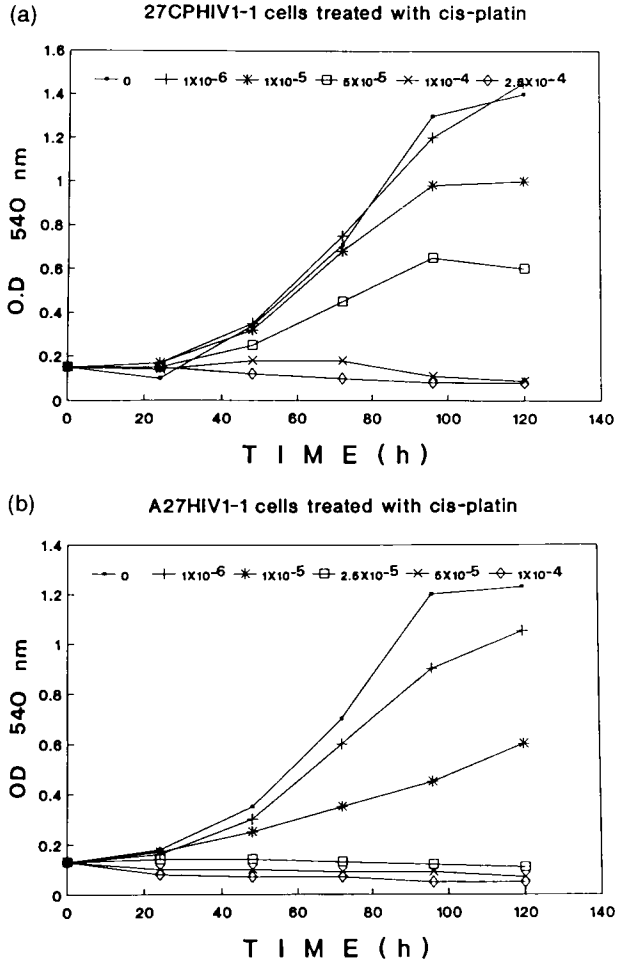


**Figure 5.** Cell proliferation in response to cisplatin after various exposure times. Exponentially growing (a) 27CPHIV1-1 and (b) A27HIV1-1 cells ($3 \times 10^{-3}$) were plated in 96-well tissue culture clusters (Costar) in RPMI 1640 medium containing 10% FCS in the presence of the indicated concentration of cisplatin. At the indicated times cell proliferation was measured using Mosmann's rapid colorimetric assay.

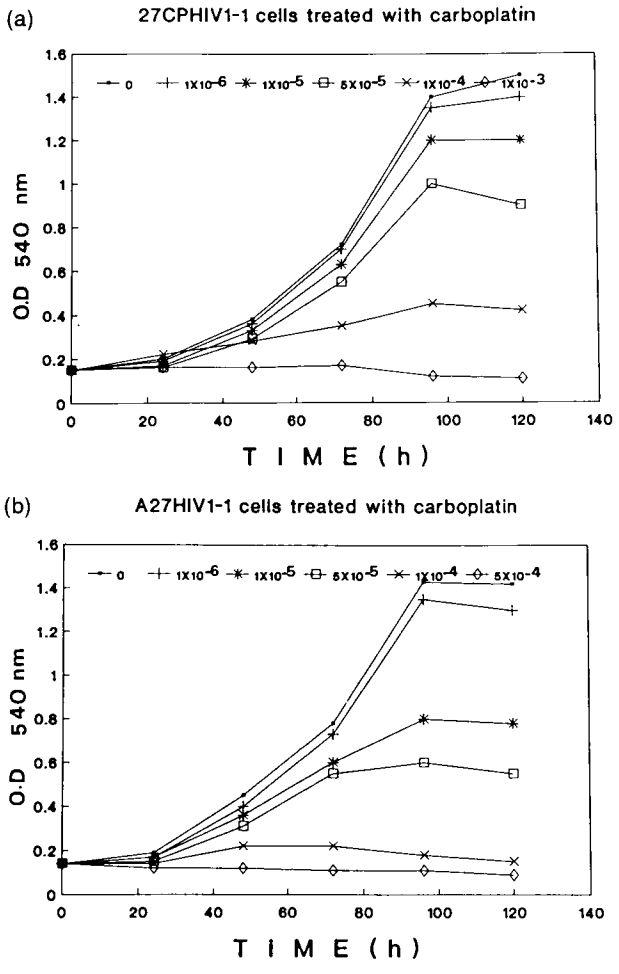


**Figure 6.** Cell proliferation in response to carboplatin after various times of exposure. At the indicated times cell proliferation of (a) 27CPHIV1-1 and (b) A27HIV1-1 was measured as described in Figure 5.

resistant human ovarian carcinoma transfectant cells (A27HIV1-1 and 27CPHIV1-1, respectively). Despite the fact that the active molecular principle is the same for cisplatin and carboplatin, there is a clear difference in their ability to interact with the HIV-1 LTR promoter. This could be explained partly by the differences in the kinetics of hydration of the two compounds, which has been shown to affect the rate of binding to DNA (slower for carboplatin).[6,8] A plausible explanation for the effects of cisplatin in sensitive cell lines is that it binds to *cis*-acting regulatory sequences of the HIV LTR and inhibits the binding of negative regulatory proteins. In contrast, in resistant 27CPHIV1-1 human ovarian carcinoma cells, the changes in expression due to the mechanisms involved in conferring relative resistance to cisplatin [e.g. DNA polymerase $\beta$ and oncogenes (*fos*/*ras*/*myc*)] increase the rate of repair of *cis*-platin–DNA damage. Therefore, the negative regulatory protein could gain access to the DNA regulatory elements and suppress CAT expression controlled by HIV-1 LTR sequences.

It is interesting to note that the relative cellular resistance to cisplatin correlates inversely with the ability of cisplatin to reduce transcription from HIV-1 LTR. It is possible that the biochemical mechanisms responsible for drug resistance (e.g. increased DNA repair) are associated with the mechanisms responsible for damping down the transcriptional inductive effect of cisplatin.

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