Exposure of human ovarian carcinoma to cisplatin transiently sensitizes the tumor cells for liposome-mediated gene transfer

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ABSTRACT  Human ovarian carcinoma cells (line 2008) grown as subcutaneous solid tumor in the severe combined immunodeficient mouse can be transfected by directly injecting a plasmid DNA–liposome complex into the tumor (in situ lipofection). The level of reporter gene expression in the tumor cells was significantly elevated if the animal received a single i.p. injection of cisplatin 1 week before the lipofection. Sensitization of the tumor for lipofection peaked 1 week after cisplatin injection and declined thereafter. Cells exposed to low concentration of cisplatin in vitro for four to five doubling times also showed elevated sensitivity for lipofection in vitro. Cisplatin was the only anticancer drug tested that exhibited this activity. These results suggest a sequential combinational gene therapy protocol with cisplatin for the ovarian carcinoma.

Direct gene transfer to the tumor cells by intratumor injection of a plasmid DNA–liposome complex has been reported in animal models (1, 2) and in clinical trials (3). Expression of a major histocompatibility complex protein (class I) allolantigen in the tumor cells has resulted in the induction of tumorspecific cytotoxic T lymphocyte response in the host, leading to the inhibition of tumor growth and prolongation of the host survival (2). Direct injection of a DNA–liposome complex into the cutaneous melanoma lesions has also resulted in the allolantigen expression in the tumor cells and partial regression of the tumor in the treated individuals (3, 4). Thus, efficient transgene expression in the tumor by direct gene transfer can bring therapeutic benefit to the host.

We accidentally found that tumor cells resistant to cis-diaminedichloroplatinum(II) (cisplatin), an anticancer drug, were more transflectable with a cationic liposome–DNA complex than the parent cells (5). Andrews et al. (6) reported that ovarian cancer cells readily developed low-level cisplatin resistance upon cisplatin injection into the tumor-bearing animals. In looking for means to enhance the tumor cell transfection, we have tested whether injection of cisplatin into the host can enhance the sensitivity of the tumor to the subsequent injection of a DNA–liposome complex (lipofection). This hypothesis is tested with human ovarian carcinoma cells grown in the severe combined immunedeficient (SCID) mouse.

MATERIALS AND METHODS

Preparation of Plasmid DNA. We have used the bacterial chloramphenicol acetyltransferase (CAT) gene as a reporter to measure transgene expression level (7). pUCCMVCAT (5.1 kb) is a pUC18-based plasmid that contains the full-length CAT cDNA downstream from the immediate early promoter of the human cytomegalovirus element (generously provided by H. Farhood and N. Serbina, University of Pittsburgh, Pittsburgh). The plasmid was purified according to Sambrook et al. (8).

Animals and Solid Tumor Inoculation. Five- to 6-week-old female SCID mice (C.B. 17/IcrCrl-scid BR, Charles River Breeding Laboratories) were used in all experiments. Animal care was in accordance with the institutional guidelines. Solid tumors were established from the 2008 human ovarian carcinoma cell lines (generously provided by Paul A. Andrews, Georgetown University, Washington, DC). The 2008 cells were seeded in 150-mm tissue culture plates and grown to confluency in RPMI 1640 medium (GIBCO/3RL) supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, penicillin (100 unit/ml), and streptomycin (100 μg/ml). Cells were harvested by treatment with trypsin/EDTA (GIBCO/BRL), washed with complete medium, and resuspended in sterile PBS at 2 × 10^6 cells per ml. From this suspension, 0.1 ml was injected s.c. to the flanks and back of SCID mice by using a 25-gauge needle.

Treatment of SCID Mice with Cisplatin. When the tumors were about 8 mm in diameter, a single dose of cisplatin (5 mg/kg) dissolved in PBS was injected i.p. into mice. One week later, mice were injected with DNA–liposome complexes.

Preparation of Plasmid–Liposome Complexes and Injection to SCID Mice. Cationic liposomes were composed of 3β(N,N′,N′′-dimethylaminooethane)-carbamoylcholesterol (DC-chol) and dioleoyl phosphatidylethanolamine, 3:2 (mol/mol) (9). pUCCMVCAT (30 μg in 30 μl of 1× TE buffer (10 mM Tris-HCl, pH 8.0/1 mM EDTA) was diluted with 100 μl of 5% (wt/vol) dextrose and then mixed with 30 nmol of DC-chol–dioleoyl phosphatidylethanolamine liposomes in 15 μl of 20 mM Hepes (pH 7.8). After 10 min, this complex was injected directly into the tumor in three sites. The animals were sacrificed 2 days later for the examination of CAT gene expression.

Radiometric Assay of CAT Activity. The tumors excised from animals were homogenized in 40 mM Tris-HCl, pH 7.5/10 mM EDTA/150 mM NaCl. After homogenization, cells were lysed by three freeze–thaw cycles, and the lysate was heated at 65°C for 10 min and centrifuged at 16,000 × g for 10 min. The protein concentration of the extracts was measured with a Coomassie blue G250-based assay (Bio-Rad). The protein extract of each tumor (100 μg) was then assayed for the CAT activity using [14C]chloramphenicol as a substrate (10).

Lipofection in Vitro. Plasmid pUCCMVCAT in 1× TE buffer (0–2.0 μg) was mixed gently with 10 nmol of DC-chol–dioleoyl phosphatidylethanolamine liposomes at room temperature in 1 ml of serum-free RPMI 1640 medium, incubated for 10 min, and then added to cells. Cells at 30–40% confluency were washed once with serum-free RPMI 1640 medium.

The DNA–liposome complex was added to the cells and incubated at 37°C in 5% CO2/95% air for 6 h. Transfection medium was then removed and the washed cells were incubated in the growth medium containing 10% fetal bovine serum for 48 h before the CAT assay as described (11).

Abbreviations: SCID, severe combined immunodeficient; CAT, chloramphenicol acetyltransferase; DC-chol, 3β(N,N′,N′′-dimethylaminooethane)-carbamoylcholesterol.

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RESULTS

Fig. 1 shows an autoradiogram of a thin layer chromatography separation of the CAT assay mixture. Human ovarian tumors of animals without cisplatin injection were inefficiently transfectable with either DNA alone or with a DNA-liposome complex. There were variations in the degree of transfection between animals. For the animals receiving cisplatin injection, the level of CAT gene expression in the tumor transfected with DNA alone remained low and variable. However, tumors of cisplatin-injected animals became much more transfectable with the DNA-liposome complex in all animals and all tumors assayed. Other cationic liposomes such as Lipofectamine and Lipofectin could also be used for in situ lipofection, but their activities were not as strong as that of DC-chol. We optimized the dose of cisplatin that induced higher sensitivity in the tumor to lipofection and found that cisplatin at 5 mg/kg resulted in the highest and most reproducible effects. Animals injected with cisplatin at 10 mg/kg died of drug toxicity a few days after injection. At the single dose of 5 mg/kg, no antitumor effect was observed, as the tumor size did not reduce.

We next tested the time course of tumor sensitization. Mice were injected with a single dose of cisplatin (5 mg/kg). Lipofection was done at various times after the drug administration. Fig. 2 shows that there was not a significant enhancement in the efficiency of lipofection at day 3. However, the efficiency reached a peak at 1 week and declined thereafter. This result indicates that the tumor was only transiently sensitized for lipofection. Sensitization by cis-

![Fig. 1](image1.png)

**Fig. 1.** CAT reporter gene expression in 2008 tumors. Mice bearing tumors were i.p. injected with either PBS or cisplatin (5 mg/kg). One week later, pUCMVCAT DNA (30 \(\mu\)g) with or without liposomes (30nmol) was injected directly into the tumor. Animals were sacrificed 2 days later and the tumor protein extracts were assayed for CAT activity. Lanes 1 and 2, lanes 3 and 4, and lanes 5 and 6 are from duplicate animals, each bearing a single tumor; lanes 7 and 8 and lanes 9 and 10 are from duplicate animals, each bearing two tumors; lane 11 is standard *Escherichia coli* CAT (approximately 0.2 unit). Tumor from animals receiving no DNA showed no CAT activity (data not shown).

![Fig. 2](image2.png)

**Fig. 2.** Time course of tumor activation by cisplatin (CP). SCID mice bearing 2008 tumors were i.p. injected with cisplatin (5 mg/kg). After various periods, the tumor was injected with PBS (○), 30 \(\mu\)g of pUCMVCAT plasmid DNA alone (△), or a complex containing 30 \(\mu\)g of DNA and 30 nmol of DC-chol liposomes (□). The tumor was excised 2 days later and processed for the CAT assay. Each data point is the mean ± SD from eight tumors.

![Fig. 3](image3.png)

**Fig. 3.** *In vitro* sensitization of 2008 ovarian carcinoma cells by cisplatin (CP). Cells were grown in 12-well plates in RPMI 1640 medium supplemented with 10% bovine fetal serum containing 0 (○), 1 (●), or 2 (▲) \(\mu\)M cisplatin for 6 weeks. Cells were then transfected with a complex of pUCMVCAT plasmid DNA (0.5–2 \(\mu\)g) and DC-chol liposomes (10 nmol). Transfected cells were grown for 2 days in complete growth medium before being harvested and processed for CAT assay (11). Each data point is the mean ± SD of triplicate cultures.
platin was not limited to the 2008 cells. Human lung carcinoma A549 cells and human squamous carcinoma SCC 25 (head and neck tumor) cells had both shown enhanced lipofection activity after cisplatin injection (data not shown). However, it is not clear whether these tumors followed the same transient kinetics as the 2008 cells.

Since there are many different cell types in a solid tumor, it is possible that the cells sensitized by cisplatin were not the tumor cells. We thus directly exposed 2008 cells grown in vitro to low doses of cisplatin for several weeks. The cells did not grow for about a week and then grew slowly. Fig. 3 shows that cells treated continuously with cisplatin for 6 weeks were more transfactable than the untreated cells. Cells treated with 2 μM cisplatin were about 2-fold more transfactable than those treated with 1 μM cisplatin. It was estimated that cells had grown for only four or five doubling times during this period. Since tumor cells could be directly sensitized in vitro, it is likely that they are also sensitized in vivo. However, other cell types in the tumor could also be sensitized.

We have tested a number of anticancer drugs to see whether the sensitization activity was unique to cisplatin. Fig. 4 shows that only cisplatin could significantly sensitize the tumor for in situ lipofection. Other anticancer drugs including methotrexate, etoposide, cytosine arabinoside, doxorubicin, and vincristine had no effect. Transplatin, a geometric isomer of cisplatin that has no anticancer activity, also showed no effect. Since carboplatin has similar antitumor activity as cisplatin (12–14), it is surprising that it showed no sensitization activity. Perhaps, higher doses of carboplatin are required for the activity.

We have also tested whether cisplatin injection nonspecifically increases the transfectivity of normal cells in the mouse. DNA alone or complexed with liposomes was injected intramuscularly into cisplatin-injected mice. Expression of the CAT gene in the muscles at the injection site was measured. Data in Fig. 5 show that muscles of the cisplatin-treated animals did not express higher CAT activity than the muscles from control un.injected animals. Thus, the enhanced sensitivity to lipofection seems to be limited to the tumor cells of the cisplatin-treated animals.

**DISCUSSION**

Cisplatin induces DNA damage by forming adducts with guanine (15, 16). Cells undergo a series of complicated genetic and biochemical changes including the induction of DNA repair (17–20), glutathione (21, 22), metallothionein...