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In vivo screening models of cisplatin-resistant human lung cancer cell lines using SCID mice

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Abstract In vivo screening models of a cisplatin (CDDP)-resistant human small-cell lung cancer cell (SCLC) line, H69/CDDP, and a non-small-cell lung cancer cell (NSCLC) line, PC-14/CDDP, were evaluated. The transplantability of the tumor xenografts to SCID mice was more than 90%. Tumor xenografts of H69/CDDP and PC-14/CDDP showed CDDP resistance during in vivo treatment. The novel anti-cancer agent 254-S showed only a partial effect on the growth of H69/CDDP and PC-14/CDDP while ormaplatin showed no cross resistance to CDDP. The in vivo results correlated well with the results of the in vitro MTT assay. In this in vivo sensitivity test, H69/CDDP and PC-14/CDDP were more sensitive to ormaplatin than its parental cell lines. In vivo sensitivity testing using SCID mice bearing transplanted CDDP-resistant tumors was shown to be useful for evaluating the effects of new anti-cancer drugs, especially those that might overcome CDDP resistance.

Key words Cisplatin resistance · SCID mice
In vivo screening

Abbreviations SCLC, small-cell lung cancer; NSCLC, non-small-cell lung cancer; SCID, severe combined immunodeficiency; CDDP, cisplatin; PBS, phosphate-buffered saline; FBS, fetal bovine serum

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Introduction

Cisplatin (CDDP) is one of the most effective anti-cancer agents available at present [1]. However, acquired resistance to CDDP is common, and some tumors are naturally resistant to this agent. Both types of resistance are major obstacles to the successful treatment of cancer. The mechanisms of CDDP resistance have been studied in several types of CDDP-resistant cancer cell lines and have been demonstrated to be multifactorial. Elevation of cellular GSH [2, 3], metallothioneins [4, 5], increased DNA repair ability [6, 7] and reduced intracellular accumulation of CDDP [8–11] have been reported as mechanisms of CDDP resistance.

Recently, many anti-cancer drugs have been developed with the aid of in vitro screening systems. Some of these drugs show high cytotoxicity against CDDP-resistant cancer cell lines in vitro [11]. We have already reported that some new compounds and some sensitizing drugs have overcome CDDP resistance in CDDP-resistant human lung cancer cell lines established in our laboratory [12, 13]. However, we could not estimate their effectiveness in vivo, because there is no appropriate in vivo screening system for anti-cancer drugs against CDDP-resistant human lung cancers. In the present study, we established an in vivo screening system for CDDP-resistant human lung cancer cell lines in SCID mice, and here we discuss its usefulness for the in vivo evaluation of treatment modalities designed to overcome CDDP resistance.

Materials and methods

Chemicals

CDDP was donated by Bristol-Myers Squibb (Tokyo, Japan). Cis-diammine(glycolato)-platinum(II) (NSC 375101, 254-S) and tetrachloro(D,L-*trans*)-1,2-diaminocyclohexaneplatinum (IV) (ormaplatin) were provided by Shionogi Pharmaceutical Co. (Osaka, Japan) and Upjohn Pharmaceuticals (Tokyo, Japan), respectively. Thiazolyl blue

tetrazolium bromide (MTT) was purchased from Sigma (St. Louis, Mo.).

Cell lines

A human SCLC cell line (H69) established at the National Cancer Institute (Bethesda, Md.) was kindly provided by Dr. Y. Shimosato (National Cancer Center Central Hospital, Tokyo, Japan). A human NSCLC cell line (PC-14) derived from an untreated patient with pulmonary adenocarcinoma was kindly provided by Prof. Y. Hayata, Tokyo Medical College. Their CDDP-resistant sublines, H69/CDDP and PC-14/CDDP, were established in our laboratory by stepwise exposure to increasing concentrations of the drug [5, 11]. The cells were maintained as described elsewhere [14]. All CDDP-resistant cells were cultured in CDDP-free medium for at least 4 weeks before being used for experiments.

Sensitivity of the cell lines in vitro

The in vitro drug sensitivities of the cell lines to anti-cancer agents were determined by the tetrazolium dye assay (MTT assay) as described elsewhere [11]: 180 μl of cell suspension was incubated with anti-cancer drugs for 3 days in 96-well microtiter plates. After incubation, 20 μl 5 mg/ml MTT in PBS was added to each well and incubated at 37° C for a further 4 h. The medium was aspirated as completely as possible without disturbing the formazan crystals and cells on the plastic surfaces. Dimethylsulfoxide (200 μl) was added to each well, the plates were agitated on a plate shaker for 5 min to solubilize the formazan crystals, and the absorbance was measured at 562 and 630 nm using Delta-soft ELISA analysis (BioMetallics, Princeton, N.J.) for a Macintosh computer (Apple computer, Cupertino, Calif.) interfaced to a Bio-Tek Microplate Reader (EL-340, BioMetallics). Wells containing only the medium and MTT were used as controls. Each experiment was performed using six replicate wells for each drug concentration, and two independent experiments were carried out. The IC_{50} was defined as the drug concentration required to produce a 50% reduction of the optical density in each test and was calculated as (mean absorbance in 6 wells containing drugs-absorbance in 6 control wells)/(mean absorbance in 6 drug-free wells-absorbance in 6 control wells) × 100. The relative resistance ratio was defined as IC_{50} of the resistant subline/IC_{50} of the parental cell line.

Animals

Female C.B-17/IcrCrl-SCID Jcl mice (5 weeks old) were purchased from Nihon Clea (Tokyo, Japan) and maintained at the National Cancer Center Research Institute under standard conditions according to the Institutional Guidelines.

In vivo screening systems

All animals were given injections of 10^7 H69, H69/CDDP, PC-14 or PC-14/CDDP cells s.c. The tumor volumes increased to 500–1,000 mm^3 on days 15–20 in H69 and H69/CDDP cells, and on days 12–14 in PC-14 and PC-14/CDDP cells. In four different groups receiving H69, H69/CDDP, PC-14 or PC-14/CDDP, the mice were divided into four groups (5 mice/group), in which the average tumor volumes were almost equal. Anti-cancer drugs in several doses were injected i.p. on days 0, 4 and 8. On days 0, 4, 8, 12, 16 and 20, the diameters of the tumors were measured and tumor volumes were determined from the formula $V = ab^2/2$, (where a is the longest diameter and b is the shortest diameter of the tumor). The tumor growth rate was calculated from the formula:

$$\text{Tumor growth rate} = \frac{\text{Tumor volume on day X}}{\text{Tumor volume on day 0}} \times 100$$

Day X denotes the day on which the effects of the drugs were estimated and day 0 denotes the first day of treatment.

Calculation of therapeutic ratio

On day 20 (12 days after the last injection of drugs), the tumor sizes were measured and the growth inhibition rate and the therapeutic ratio were calculated from the formulas:

$$\text{Growth inhibition rate} = \frac{\text{Tumor growth rate of a treated mouse on day 20}}{\text{Average of the tumor growth rate of control mice on day 20}} \times 100$$

$$\text{Therapeutic ratio} = 100 - \text{growth inhibition rate}$$

Statistical analysis

The statistical significance of differences between test groups was analyzed using Student's two-tailed t-test.

Results

CDDP sensitivity in vitro

Table 1 shows the IC_{50} values for CDDP in H69, H69/CDDP, PC-14 and PC-14/CDDP cells during continuous drug exposure in vitro. The IC_{50} values of the parental cell lines, H69 and PC-14, against CDDP were 0.29 μg/ml and 0.60 μg/ml, respectively. The IC_{50} values of their CDDP-resistant sublines, H69/CDDP and PC-14/CDDP, were 1.04 μg/ml and 5.30 μg/ml, and the relative resistance ratios of H69/CDDP and PC-14/CDDP were 3.59 and 8.83, respectively.

CDDP sensitivity in in vivo screening system

Figure 1 and Table 2 show the growth inhibitory effect of CDDP during in vivo treatment. CDDP treatment suppressed tumor growth in the H69 and PC-14 cell lines (Fig. 1a, b), but not in their resistant sublines (Fig. 1c, d). Therapeutic ratios of CDDP (4 mg/kg) for H69, H69/CDDP, PC-14 and PC-14/CDDP were 77±17%, 5±3%, 41±11%, and 2±2%, respectively. The CDDP treatment suppressed tumor growth only during administration of CDDP.

Table 1 IC_{50} values for H69, H69/CDDP, PC-14 and PC-14/CDDP cell lines against several anti-cancer drugs. Drug sensitivity was determined by MTT assay as described in "Materials and methods." Each value is the average of 6 replicate wells for each drug concentration. Wells containing only RPMI-FBS and MTT were used as controls. The IC_{50} was defined as the drug concentration required to produce a 50% reduction of the optical density in each test

	IC_{50} value (relative resistance ratio)			
	H69	H69/CDDP	PC-14	PC-14/CDDP
CDDP (μg/ml)	0.29	1.04 (3.59)	0.60	5.3 (8.83)
254-S (μM)	30.5	63.7 (2.09)	9.01	54.7 (6.07)
Ormaplatin (μM)	11.1	9.5 (0.86)	0.74	0.49 (0.66)

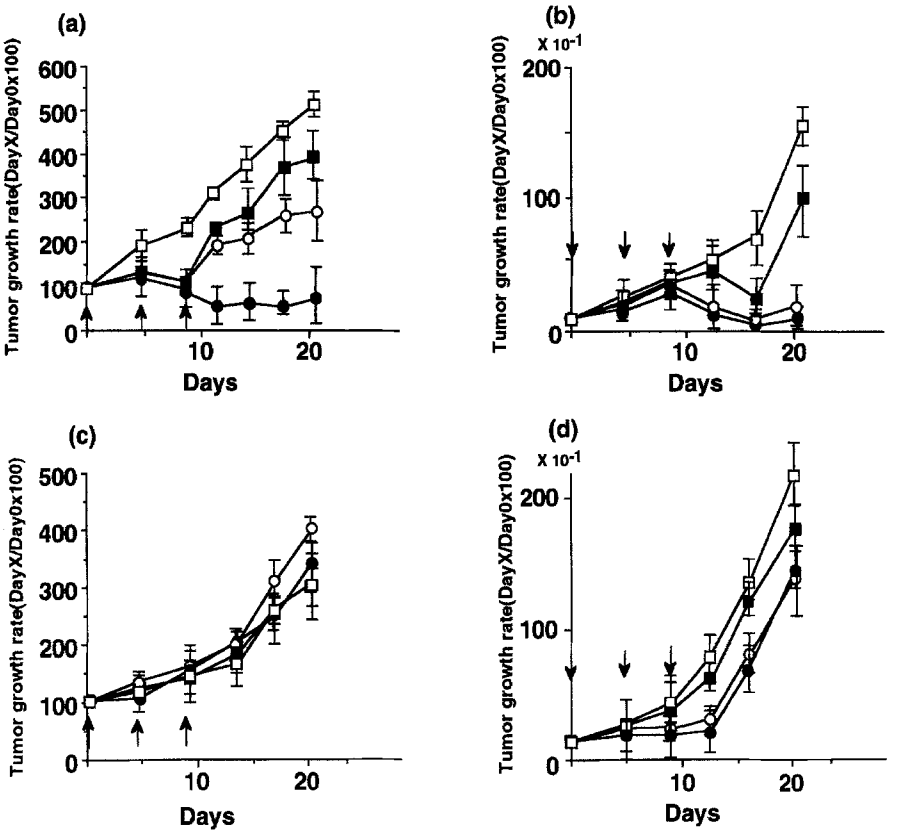

Fig. 1a–d Tumor growth rate after CDDP treatment in vivo. On day 0, the average tumor volumes were 500–1,000 mm^3 in all groups. Injection of CDDP i.p. was carried out on days 0, 4 and 8, and tumor volume was evaluated on days 0, 4, 8, 12, 16 and 20. Tumor growth rate was calculated as described in "Materials and methods." **a** H69, **b** PC-14, **c** H69/CDDP, **d** PC-14/CDDP. □; Control, ■; CDDP 1 mg/kg, ○; CDDP 2 mg/kg; ●; CDDP 4 mg/kg. Data are presented as means ± SD for 5 mice in each group

In vitro screening of CDDP derivatives

Table 1 shows the effects of CDDP derivatives, 254-S and ormaplatin on H69, PC-14 and their CDDP-resistant sublines in vitro. The IC_{50} values of the parental cell lines, H69 and PC-14, against 254-S were 30.5 μ*M* and 9.01 μ*M* respectively.

The IC_{50} values of 254-S against H69/CDDP and PC-14/CDDP were 63.7 and 54.7 and relative resistance ratios were 2.09 and 6.07. These data indicated that 254-S showed cross resistance to CDDP in vitro.

The IC_{50} values of ormaplatin against H69, PC-14, H69/CDDP and PC-14/CDDP were 11.1 μ*M*, 0.74 μ*M*, 9.5 μ*M* and 0.49 μ*M*, respectively. The relative resistance ratio of H69/CDDP was 0.86 and that of PC-14/CDDP was 0.66. There was no cross resistance between CDDP and ormaplatin in vitro.

Table 2 Therapeutic ratio of CDDP, 254-S and ormaplatin on H69, H69/CDDP, PC-14 and PC-14/CDDP cell lines in vivo. All mice were given injections of the cells s.c. After 15–20 days (H69, H69/CDDP cells) or 12–14 days (PC-14 and PC-14/CDDP cells), i.p. injections of anti-cancer drugs (1, 2, or 4 mg/kg) were started. Injections were repeated 3 and 6 days later. On day 20 (12 days after the last injection of drugs), the tumor sizes were measured and the therapeutic ratio was calculated as described in "Materials and methods." Data are presented as means ± SD for 5 mice in each group

		Therapeutic ratio (%)			
		H69	H69/CDDP	PC-14	PC-14/CDDP
Control		0	0	0	0
CDDP	1 mg/kg	19±8	0±3	8±6	1±3
	2 mg/kg	34±6	−2±4	37±9	3±6
	4 mg/kg	77±17	5±3	41±11	2±2
254-S	1 mg/kg	6±4	3±3	–	–
	2 mg/kg	39±11	14±5	–	–
	4 mg/kg	44±8	19±7	38±9	18±6
Ormaplatin	1 mg/kg	19±4	21±3	–	–
	2 mg/kg	34±7	41±7	–	–
	4 mg/kg	40±12	56±8	36±5	41±10

In vivo screening of CDDP derivatives

Table 2 shows the therapeutic ratio of CDDP, 254-S and ormaplatin for H69, H69/CDDP, PC-14 and PC-14/CDDP. Therapeutic ratios of CDDP (2 mg/kg), 254-S (2 mg/kg), and ormaplatin (2 mg/kg) for H69 were 34±6%, 39±11%, and 34±7%, respectively. Those for H69/CDDP were −2±4%, 14±5%, and 41±7%, respectively. The therapeutic ratios of CDDP (4 mg/kg), 254-S (4 mg/kg), and ormaplatin (4 mg/kg) for PC-14 were 41±11%, 38±9%, and 36±5%, respectively, and those for PC-14/CDDP were 2±2%, 18±6%, and 41±10%, respectively. These data indicate that there was cross resistance between 254-S and CDDP but not between ormaplatin and CDDP in vivo.

Discussion

Both acquired and natural resistance to CDDP are major obstacles to the successful treatment of cancer. We have reported on some new anti-cancer drugs that show high cytotoxicity against CDDP-resistant cancer cell lines in vitro [11–13]. However, we had not been able to estimate their effectiveness in vivo, because so far there has been no appropriate in vivo screening system for anti-cancer drugs against CDDP-resistant human lung cancers. In this report, we showed an in vivo screening system for CDDP-resistant human lung cancer cell lines in SCID mice.

A few reports of in vivo evaluation of anti-cancer drugs for CDDP-resistant human ovarian cancer cells using nude mice have been published [15–18]. We also tried to establish an in vivo treatment model for H69, PC-14 and their CDDP-resistant sublines in order to overcome CDDP resistance in nude mice, but the rate of tumor transplantability was less than 20%. As the transplantability of these cells to SCID mice is more than 90%, SCID mice could be a useful tool for in vivo evaluation of anti-cancer drugs for CDDP-resistant tumors or sensitizing drugs. The price of SCID mice is only 1.14 times that of nude mice. The screening system in SCID mice is more economical, as it yields more benefit than that in nude mice.

Previously, we reported that a CDDP derivative, ormaplatin, showed a lack of cross resistance to CDDP in vitro, even though other derivatives such as carboplatin, 254-S or *cis*-dichloro(ethylenediamine) platinum (*cis*-DEP) demonstrated cross resistance to CDDP [12]. As shown in Table 2, ormaplatin inhibited the tumor growth of H69/CDDP and PC-14/CDDP to the same extent as that of their parent lines, H69 and PC-14. 254-S showed partial cross resistance with H69/CDDP and PC-14/CDDP in vivo. The results of the in vivo experiment showed good agreement with those of in vitro treatment. Interestingly, the sensitivity of H69/CDDP and PC-14/CDDP to ormaplatin was significantly higher than that of H69 and PC-14, especially in vivo.

Several workers have studied the mechanisms of CDDP resistance and have carried out trials of methods to overcome them. Morikage et al. reported that amphotericin B increased the accumulation of CDDP in CDDP-resistant cell lines and modulated CDDP sensitivity in an in vitro system [14]. Many drugs have been shown to overcome CDDP resistance in vitro [19]. The evaluation of the effects of these drugs in vivo is extremely important for further investigation of their clinical applications.

One of the few disadvantages of using SCID mice was that we could not estimate the size of the tumors precisely because of their hair. This meant that we had to start treatment after the tumors became too large to be cured. We solved this problem by using a depilatory cream (e.g., those produced by Shiseido and Kanebo). After this treatment, we were able to measure the diameters of the tumors precisely, as in nude mice. The depilatory cream had no influence on the growth of the transplanted tumors.

In this report, we present in vivo treatment models for CDDP-resistant human lung cancer cell lines. We are now constructing in vivo treatment models for cancer cell lines resistant to vindesine [20], taxol [21], and etoposide [22].

Resistance to anti-cancer drugs is one of the most important problems in cancer chemotherapy. We believe that this in vivo treatment model provides a promising tool for the detection of new anti-cancer drugs effective against tumors that are resistant to currently available agents.

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