Jpn. J. Cancer Res. 85, 1050–1056, October 1994

In vitro and *in vivo* Effects of Cisplatin and Etoposide in Combination on Small Cell Lung Cancer Cell Lines

Hideki Kondo,[1,2] Fumihiko Kanzawa,[1] Kazuto Nishio,[1] Shiro Saito[2] and Nagahiro Saijo[1,3]

[1]*Pharmacology Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104 and* [2]*First Department of Internal Medicine, School of Medicine, University of Tokushima, 2-50-1 Kuramoto-cho, Tokushima 770*

The effects of cisplatin (CDDP) and etoposide (ETP) in combination were evaluated *in vitro* and *in vivo* using small cell lung cancer cell lines. The combination effects *in vitro* were investigated using isobologram analysis. Used together, CDDP and ETP showed a synergistic effect against cell growth on only 1 cell line (SBC-3), additive effects on 6 (SBC-2, SBC-5, Lu130, Lu134AH, Lu135T and H69) and an antagonistic effect on 1 (SBC-1). In the *in vivo* experiment, nude mice were inoculated with SBC-1, SBC-3 and SBC-5 cells. Two or 5 mg/kg CDDP and 10 or 30 mg/kg ETP were administered intraperitoneally alone and simultaneously in combination to nude mice. The *in vivo* effects of the combination were determined by comparing the observed growth ratio in mice treated with the combination with the expected value of this ratio calculated based on the assumption that the effects of the drugs were simply additive. According to this definition, synergistic effects were observed against all 3 tumors. Thus, the *in vivo* and *in vitro* effects differed. The toxicity of the combination therapy, which was analyzed by estimating the body weight change of mice, was no higher than that of CDDP or ETP alone. These results suggest that the excellent clinical effects of CDDP and ETP combination therapy may be attributable not to drug interaction at the cellular level but to the feasibility of combined use of them at full doses without overlapping side effects.

Key words: Cisplatin — Etoposide — Combination chemotherapy — Nude mouse — Lung cancer

Combination therapy with cisplatin (CDDP) and etoposide (ETP) plays a central role in the chemotherapy of lung cancer, malignant lymphoma and testicular cancer.[1–4] This combination has been used as a standard therapy for small cell lung cancer (SCLC) since the mid-1980s, due to its high anti-tumor activity and few side effects and, at present, no other combination is superior to it. The response rates with CDDP and ETP alone are only 10–15% and 40–60%, respectively, in patients with SCLC. However, the response rate with a combination of these two drugs is 85–100%, especially in patients with untreated SCLC, which suggests they have synergistic effects. The reason for the good results with such combination therapy still remains unclear. We have evaluated the *in vitro* and *in vivo* anti-tumor effects of CDDP and ETP in combination using SCLC cell lines and the possible synergistic mechanism involved is discussed.

MATERIALS AND METHODS

Tumor cell lines Eight SCLC cell lines (SBC-1, SBC-2, SBC-3, SBC-5, Lu130, Lu134AH, Lu135T and H69) were used; SBC-1, -2, -3 and -5 were established at Okayama University (Okayama), Lu130, Lu134AH and Lu135T at the National Cancer Center Research Institute (Tokyo) and H69 at the National Cancer Institute (USA). The cells were propagated in RPMI-1640 medium (Nikken Biomedical Laboratories, Kyoto) supplemented with 10% fetal bovine serum (FBS, Gibco), penicillin (100 units/ml) and streptomycin (100 μg/ml) in an atmosphere of 5% CO_2 in a highly humidified incubator at 37°C. The cell numbers were counted using a microcell counter (CC-108, TOA, Tokyo).

Animals Male, 6-week-old athymic nude mice (BALB/c-nu/nu) purchased from Charles River Japan Inc. (Yokohama) were used in this experiment. They were maintained under aseptic conditions with filtered air and sterilized food, water, bedding and cages.

MTT assay In order to measure the anti-tumor activity of the CDDP and ETP combination against the 8 cell lines *in vitro*, a chemosensitivity test was performed using the MTT assay, as described by Mosmann.[5] Exponentially growing cells were harvested by centrifugation and single-cell suspensions were obtained by mechanical disaggregation. These suspensions were diluted to the required seeding concentrations with RPMI-FBS, plated (100 μl per well) in 96-well microculture plates (Falcon), and incubated at 37°C in a 5% CO_2 incubator for 24 h. Drug solutions were added as follows. Aliquots of RPMI-FBS (100 μl) were added to the control wells, aliquots of 50 μl RPMI-FBS + 50 μl CDDP or ETP of the required concentration were added to single-drug wells and aliquots of 50 μl each of CDDP and ETP were added simultaneously to drug-combination wells. The

[3] To whom correspondence should be addressed.

duration of cell exposure to the drugs was determined according to the time required for 5-fold cell growth of each culture. After drug treatment, 20 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT: Sigma Chemical Co., USA) at a concentration of 5 mg/ml in phosphate-buffered saline was added to each well and the plates were incubated for 4 h at 37°C. The plates were centrifuged for 20 min at 2,000 rpm, the media were removed by decanting and blotting, and 200 μl of dimethyl sulfoxide (DMSO: Wako Pure Chemical Industries, Ltd., Osaka) was added to each well. The plates were agitated on an orbital shaker for 5 min to dissolve the formazan grains and the absorbance (A) of the contents of each well was measured at 560 and 630 nm using a scanning microplate spectrophotometer (Flow Laboratories Japan Inc., Tokyo). Cytotoxicity was defined as the cell survival fraction, which was determined by using the following formula: survival fraction (%) = [(A of treated cells − A of medium) / (A of control cells − A of medium)] × 100. Dose-response curves for CDDP and ETP alone and in combination were obtained by semilogarithmic plotting. The IC_{50} value was defined as the drug concentration needed to reduce the absorbance by 50% and was determined graphically from the dose-response curve. This experiment was performed in duplicate at least 3 times for each cell line.

Analysis of the effects of the combination *in vitro* The *in vitro* effects of the CDDP and ETP combination were analyzed using the isobologram method.[6,7] The IC_{50} values of CDDP and ETP alone were determined from the dose-response curves in single-drug treatment of each cell line, obtained by using the MTT assay, and their 95% confidence limits were calculated. From the dose-response curve for the combination, the IC_{50} of ETP at each CDDP concentration and that of CDDP at each ETP concentration (i.e., the concentration of each drug showing a 50% cytotoxic effect when used in combination with the other) were determined. The data were graphed by plotting the IC_{50} with 95% confidence limits for CDDP on the x axis and that for ETP on the y axis and the IC_{50} and the upper and lower 95% confidence limit values for the two drugs were each connected with diagonal lines (Fig. 1). The extent of drug interaction was determined by plotting the concentration of each drug that showed 50% cytotoxic effect on this graph. When this point fell in the area enclosed by the lines connecting the upper and lower 95% confidence limits of both drugs (shaded region) the effect of combined use was defined as additive, when it fell below the line connecting the lower 95% confidence limits the effect was defined as synergistic, and when it was above the line connecting the upper 95% confidence limits the effect was considered to be antagonistic.

***In vivo* evaluation of therapeutic effects** Exponentially growing tumor cells (10^7) were inoculated subcutaneously into the backs of nude mice. When each tumor had grown to a diameter of 2 cm, it was removed aseptically and minced into fragments in physiological saline in Petri dishes. A 50 μl aliquot was inoculated subcutaneously into the backs of nude mice using an 18G needle. Two weeks later, the drugs, dissolved in physiological saline, were injected intraperitoneally.

The SBC-1, SBC-3 and SBC-5 cell lines were used to generate tumors. In our preliminary experiment, CDDP or ETP alone was administered to nude mice (4 per group). One or 2 mice inoculated with each tumor died during the 3-week observation period when given 8 mg/kg CDDP and 60 mg/kg ETP, respectively. However, no mice given 5 mg/kg CDDP or 30 mg/kg ETP died. Therefore, these were considered to be the maximum acceptable doses (MAD) in nude mice. In the *in vivo* experiment, 2 and 5 mg/kg CDDP and 10 and 30 mg/kg ETP, dissolved in physiological saline, were administered intraperitoneally alone or simultaneously. There were 6 mice in each treatment group and 11–19 in the untreated control groups.

In order to evaluate the treatment effects, the estimated tumor volume before and 7, 14 and 21 days after drug administration was calculated from the tumor length (L, mm) and width (W, mm) using the following equation: $(L \times W^2)/2$ (mm^3). The mean tumor volume on each day for each treatment group was compared with that of the control group.

Animal body weights were measured immediately before and 2 weeks after treatment. The actual body weight was obtained by subtracting the mean tumor weight of each group from the mean whole body weight of its group. The percent change in body weights 2 weeks after treatment, relative to the pretreatment weights, was calculated.

Analysis of the *in vivo* effects of the combination The *in vivo* effects of these drugs in combination were evaluated as follows. The ratio of the mean tumor volume in each treatment group to that in the control group was calculated 7, 14 and 21 days after drug administration. Next, the expected tumor growth ratio with the combination therapy was calculated using the equation below and this was considered to be the expected tumor growth ratio if the effects of the two drugs were simply additive. An actual tumor growth ratio below this value in the combination therapy group indicated the drugs were synergistic and a ratio above it indicated their effects were antagonistic.

$$\text{Expected tumor growth ratio with combination therapy} = \frac{\text{mean tumor volume in CDDP group}}{\text{mean tumor volume in control group}} \times \frac{\text{that in ETP group}}{\text{that in control group}}$$

RESULTS

***In vitro* effects of CDDP and ETP combination therapy against 8 SCLC cell lines** Fig. 1 shows the isobologram analysis of the combined effects of CDDP and ETP on 3 SCLC cell lines (SBC-1, SBC-3 and SBC-5) with IC_{50} values of each drug obtained from MTT assay results. The use of these two drugs in combination had a synergistic effect on the cell growth of SBC-3, an additive effect on SBC-5 and an antagonistic effect on SBC-1. Similar analysis of the other 5 cell lines (SBC-2, Lu130, Lu134AH, Lu135T and H69) showed that this combination had additive effects on all these cell lines.[8] These results suggest that *in vitro* combination therapy can not necessarily demonstrate a synergistic effect of these two drugs against SCLC cell lines.

***In vivo* effects of CDDP and ETP combination therapy against human tumor xenografts** Nude mice were inoculated with SBC-1, SBC-3 and SBC-5 cells, against which different *in vitro* effects of the CDDP and ETP combination had been observed (antagonistic, synergistic and additive, respectively), and the effects of each drug

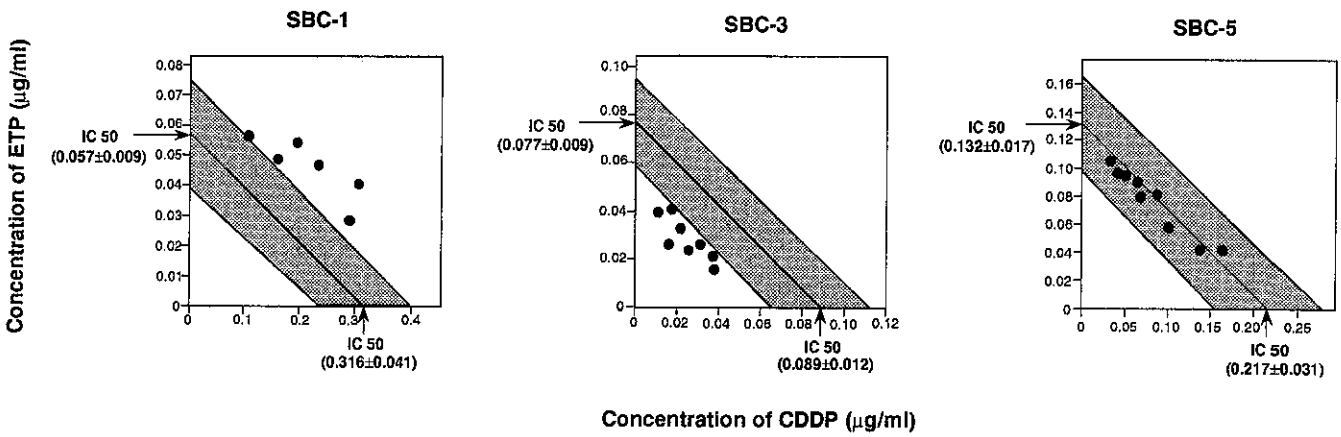

Fig. 1. Isobologram analysis of combined effects of CDDP and ETP on 3 SCLC cell lines. The IC_{50} points of CDDP and ETP are plotted on the x and y axes along with their 95% confidence limits, and the IC_{50} and the upper and lower 95% confidence limit values for the two drugs are each connected with diagonal lines. The degree of drug interaction is determined by plotting the concentration of each drug showing a 50% cytotoxic effect on this graph. When this point is situated in the area enclosed by the lines connecting the upper and lower 95% confidence limits of both drugs (shaded region), the effect of combined use is defined as additive. When it falls below the line connecting the lower 95% confidence limits, the effect is defined as synergistic and when it is above the line connecting the upper 95% confidence limits, the effect is defined as antagonistic.

Table I. Effect of CDDP, ETP or Their Combination Therapy on the Growth of SBC-1 Cells

Compound (mg/kg)	Mice (n)	Tumor volume (mm^3) Days after treatment 0	7	14	21
Controls	11	12.6±4.6	81.9±55.8	362.2±194.1	1047.6±550.4
CDDP (2)	6	13.0±8.7	75.3±66.6	271.8±250.4	892.5±844.8
CDDP (5)	6	13.7±11.4	67.0±67.0	247.3±274.5	875.3±956.9
ETP (10)	6	11.9±5.8	75.6±23.4	276.8±111.3	1127.4±483.8
ETP (30)	6	11.2±5.9	54.3±44.8	177.3±161.4	694.7±722.5
CDDP (2)+ETP (10)	6	12.5±13.4	29.2±36.2	152.4±229.1	621.3±757.7
CDDP (2)+ETP (30)	6	11.9±3.7	22.0±20.9*	109.4±120.3*	423.8±351.8*
CDDP (5)+ETP (10)	6	11.9±3.0	4.3±6.7***	7.2±9.1***	68.8±63.6***
CDDP (5)+ETP (30)	6	13.1±3.0	5.6±6.4***	10.7±8.7***	69.1±58.3***

Data represent mean values±SE.
* $P<0.05$, *** $P<0.001$ vs. control value.

Table II. Effect of CDDP, ETP or Their Combination Therapy on the Growth of SBC-3 Cells

Compound (mg/kg)	Mice (n)	Tumor volume (mm^3) Days after treatment 0	7	14	21
Controls	13	1154±367	3391±983	6451±1985	12505±4267
CDDP (2)	6	1233±332	3608±954	7142±2091	12975±2361
CDDP (5)	6	1080±84	2985±881	5966±1777	11563±4556
ETP (10)	6	1123±216	3775±1114	7123±2252	14239±4086
ETP (30)	6	1257±414	4067±1841	6557±2165	9120±3028
CDDP (2)+ETP (10)	5	1259±384	2823±785	5346±1139	9957±2227
CDDP (2)+ETP (30)	6	1156±416	1426±816**	3696±2583*	6060±4148*
CDDP (5)+ETP (10)	6	1102±383	1036±529***	3889±1623*	7994±3439*
CDDP (5)+ETP (30)	6	1213±429	335±264***	1385±1174***	3426±2984***

Data represent mean values±SE.
* $P<0.05$, ** $P<0.005$, *** $P<0.001$ vs. control value.

Table III. Effect of CDDP, ETP or Their Combination Therapy on the Growth of SBC-5 Cells

Compound (mg/kg)	Mice (n)	Tumor volume (mm^3) Days after treatment 0	7	14	21
Controls	19	185.3±64.4	701.8±229.4	1073.2±436.9	1451.3±615.6
CDDP (2)	6	207.1±47.1	744.1±254.3	1202.8±443.7	1583.0±729.3
CDDP (5)	6	202.1±50.3	641.7±208.0	932.5±398.9	1227.8±335.6
ETP (10)	6	202.7±55.1	662.4±220.1	1088.8±310.8	1335.8±409.2
ETP (30)	6	194.8±62.4	505.4±248.1	729.0±330.2	1011.8±427.1
CDDP (2)+ETP (10)	6	181.3±56.4	375.6±206.0*	516.8±226.3**	729.3±476.5**
CDDP (2)+ETP (30)	6	181.1±40.8	252.1±76.8***	264.6±149.8***	239.6±122.8***
CDDP (5)+ETP (10)	6	185.6±29.3	286.0±141.2***	325.4±177.5***	311.0±157.9***
CDDP (5)+ETP (30)	6	201.6±94.3	162.8±69.4***	171.3±78.7***	203.9±83.9***

Data represent mean values±SE.
* $P<0.05$, ** $P<0.005$, *** $P<0.001$ vs. control value.

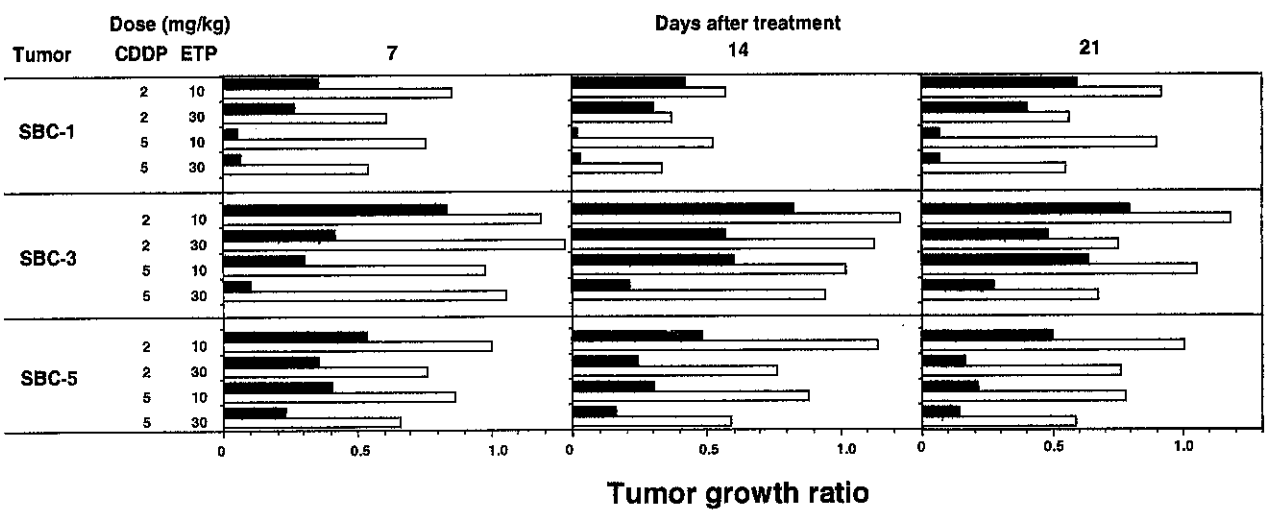

Fig. 2. Comparison of the observed and expected tumor growth ratio of SBC-1, SBC-3 and SBC-5 tumors in combination with CDDP and ETP. Closed bars represent the observed growth ratio in the presence of the combination of CDDP and ETP. Open bars represent the expected growth ratio in the case of simple additivity.

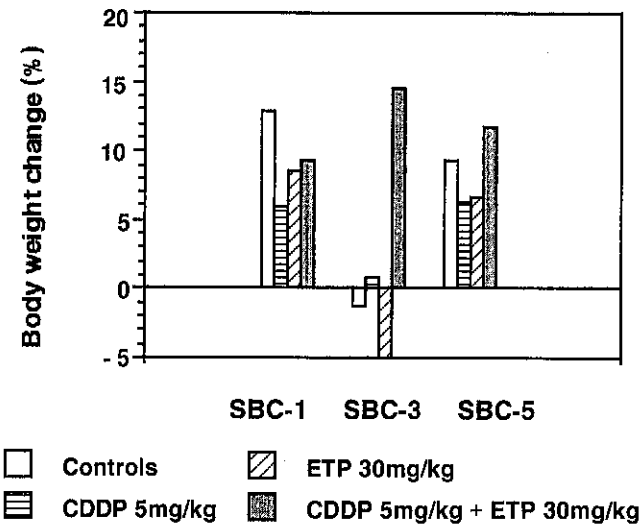

Fig. 3. Effects of the administration of CDDP and ETP alone or in combination on the body weight of mice inoculated with SBC-1, SBC-3 and SBC-5 cells.

alone and in combination *in vivo* were evaluated (Tables I, II and III). Neither CDDP nor ETP alone showed significant effects against any tumor. However, the combination inhibited tumor growth, and the inhibition was particularly marked with 2 mg/kg CDDP plus 30 mg/kg ETP, 5 mg/kg CDDP plus 10 mg/kg ETP and 5 mg/kg CDDP plus 30 mg/kg ETP.

The expected tumor growth ratio, based on the assumption that the effects of the two drugs were additive, was determined by calculating the ratio of tumor growth in the groups treated with each dose of CDDP and ETP alone to that of the control group and multiplying them together. The observed tumor growth ratios in the combination therapy groups were evaluated 7, 14 and 21 days after drug administration (Fig. 2).

In the mice inoculated with SBC-3 cells, against which the combination therapy showed synergistic effects *in vitro,* synergistic anti-tumor effects were observed 7, 14 and 21 days after drug administration. The CDDP and ETP combination also had synergistic effects *in vivo* against SBC-5 and SBC-1, which contrasted with the additive and antagonistic effects respectively observed *in vitro.* Thus, regardless of the *in vitro* effects of this combination, *in vivo* combination therapy with CDDP and ETP demonstrated synergistic effects against all three inoculated tumors. In particular, marked anti-tumor effects were observed in the groups treated with 5 mg/kg CDDP or 30 mg/kg ETP, which suggests that substantial synergistic effects may be observed with high-dose combination therapy.

With these doses, all the mice in the treatment groups survived during the experiments. Changes in body weight reflect the overall toxicity of the treatment. The body weight of mice treated with the combination of 5 mg/kg CDDP and 30 mg/kg ETP the (MADs for nude mice of each drug) was not reduced during 2 weeks after the treatment (Fig. 3). Indeed, the percent increase in the body weight was rather greater in this combination group than in the single drug treatment group of 5 mg/kg CDDP or 30 mg/kg ETP. These results indicate that the toxic effects of CDDP and ETP in combination are minimal and less than additive on mice inoculated with tumors.

DISCUSSION

The simultaneous *in vitro* CDDP and ETP combination had synergistic anti-tumor effects only on the SBC-3 cell line, while it showed additive or less effects on the other 7 lines. Despite the marked clinical efficacy of CDDP and ETP combination therapy in patients with SCLC, few workers have demonstrated synergistic effects of this combination *in vitro*. On the contrary, the hypothesis proposed by Tsai *et al.*[9] that this combination has no synergistic effect at the cellular level has been generally accepted. Our *in vitro* results support this hypothesis.

It is not appropriate to speculate on the mechanism responsible for the clinical effects of combination therapy on the basis of *in vitro* experimental results alone, as the mechanism whereby the drugs interact *in vivo* is unclear and experimental conditions *in vitro* differ markedly from those *in vivo*. In order to assess the clinical effects of combination therapy with two drugs, evaluation should be carried out using an *in vivo* system.

Therefore, we evaluated the *in vivo* anti-tumor effects of CDDP and ETP combination therapy in nude mice inoculated with SBC-1, SBC-3 and SBC-5 cells, against which this combination showed different effects *in vitro* (antagonistic, synergistic and additive, respectively). There is no generally accepted established method for the evaluation of the effects of combination therapy *in vivo* and the methods used in different studies vary. The definition of synergy we adopted was that the observed growth ratio in the combination therapy group was lower than the value expected if their effects were simply additive, which was calculated by multiplying the growth ratio of the CDDP-treated to the control group by that of the ETP-treated to the control group.[10] According to this definition, *in vivo* combination therapy with CDDP and ETP had synergistic effects not only against SBC-3, as it did *in vitro*, but also against SBC-5 and SBC-1, which differed from the additive and antagonistic effects respectively observed *in vitro*. The observed growth ratios were markedly lower than the expected simple additive

values in the SBC-1-inoculated groups treated with 5 mg/kg CDDP + 10 mg/kg ETP and 5 mg/kg CDDP + 30 mg/kg ETP, even though this combination showed antagonistic effects against SBC-1 cells *in vitro*. Thus, the *in vivo* and *in vitro* effects differed.

In the *in vitro* system, synergistic effects of the two drugs were observed in only 1 (SBC-3) of the 8 cell lines. Previously, we suggested that the biochemical mechanism responsible for the synergistic effects of these two drugs against SBC-3 was enhancement of the DNA topoisomerase II inhibitory activity of ETP by CDDP.[8] However, the fact that the *in vivo* and *in vitro* effects differed suggests that the *in vivo* synergistic effects of the combination therapy are caused not by biochemical modulation but by pharmacokinetic changes. Some workers have suggested that the beneficial effects of the combination therapy with CDDP and ETP are due to a decrease in ETP clearance as a result of rapid renal function deterioration following CDDP administration.[11, 12] Recently, Pflüger *et al.*[13] observed an increase in the AUC value and decreases in $t1/2\alpha$ and $t1/2\beta$ values, systemic clearance and the volume of distribution at the steady state (Vd_{ss}) of ETP in patients who received ETP after CDDP administration, and they suggested that inhibition of ETP clearance by CDDP was the primary cause of the *in vivo* synergistic effects of these drugs. The results of our study have confirmed that these two drugs have synergistic anti-tumor effects in nude mice inoculated with various tumor cells. These results support the hypothesis that changes in the pharmacokinetics of ETP as a result of CDDP administration are responsible for the potent anti-tumor effects of this combination therapy. It is important to establish whether such pharmacokinetic changes are observed or not in mice treated with these drugs in combination. Unfortunately, it is difficult to analyze the pharmacokinetics in combination therapy in mice for the following reasons; i) a large amount of sample is required to measure the plasma concentration of drugs, and ii) a large number of mice is necessary to obtain such an amount.

It appears that the feasibility of using the two drugs in combination at full doses without overlapping side effects is a major factor in determining the clinical success of the combination chemotherapy. In the present *in vivo* experiment, it is noteworthy that we could administer both CDDP and ETP at full doses (the MADs of each drug) to mice inoculated with SCLC cells without any death during the experiment. Furthermore, we observed less than additive toxic effects by estimating body weight changes of mice treated with CDDP and ETP both or alone. These results suggested that the excellent clinical effects of CDDP and ETP combination therapy were due to the feasibility of administering full doses of them both without overlap of their toxicities.

The evaluations of the *in vitro* and *in vivo* effects of a combination of CDDP and ETP in this study suggest that the less-than-additive effect on toxicity is involved in the marked clinical effects of this combination therapy. To our knowledge, no other experimental studies have been reported in which the *in vitro* and *in vivo* effects of CDDP and ETP against the same cell lines have both been evaluated.

ACKNOWLEDGEMENTS

This research was supported in part by a Grant-in-Aid for the Comrehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare, by Grants-in-Aid for Scientific Research from the Ministry of Health and Welfare and the Ministry of Education, Science and Cultuer, Japan, and by funds from Bristol-Myers Squibb Co.

(Received March 28, 1994/Accepted July 21, 1994)

REFERENCES

1) Einhorn, L. H. Initial therapy with cisplatin plus VP-16 in small-cell lung cancer. *Semin. Oncol.*, **13** (Suppl. 3), 5–9 (1986).
2) Loehrer, P. J., Einhorn, L. H. and Greco, F. A. Cisplatin plus etoposide in small cell lung cancer. *Semin. Oncol.*, **15** (Suppl. 3), 2–8 (1988).
3) Von Heyden, H. W., Scherpe, A. and Nagel, G. A. *cis*-Dichlorodiammineplatinum (II) (*cis*-platinum) and etoposide for patients with refractory lymphomas. *Cancer Treat. Rev.*, **9** (Suppl. A), 45–52 (1987).
4) Hainsworth, J. D., Williams, S. D., Einhorn, L. H., Birch, R. and Greco, F. Successful treatment of resistant germinal neoplasms with VP-16 and cisplatin. *J. Clin. Oncol.*, **3**, 666–671 (1985).
5) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application of proliferation and cytotoxicity assays. *J. Immunol. Methods*, **65**, 55–63 (1983).
6) Church, M. W., Dintcheff, B. A. and Gessner, P. K. The interactive effects of alcohol and cocaine on maternal and fetal toxicity in the Long-Evans rat. *Neurotoxicol. Teratol.*, **10**, 355–361 (1988).
7) Cho, Y. L., Christensen, C., Saunders, D. E., Lawrence, W. D., Deppe, G., Malviya, V. K. and Malone, J. M. Combined effects of 1,25-dihydroxyvitamin D_3 and platinum drugs on the growth of MCF-7 cells. *Cancer Res.*, **51**, 2848–2853 (1991).
8) Kondo, H., Kanzawa, F. and Saijo, N. Analysis of *in vitro* combined effects of cisplatin and etoposide against small

cell lung cancer cell lines. *J. Jpn. Soc. Cancer Ther.*, **28**, 592–601 (1993) (in Japanese).
9) Tsai, C. M., Gazdar, A. F., Venzon, D. J., Steinberg, S. M., Dedrick, R. L., Mulshine, J. L. and Kramer, B. S. Lack of *in vitro* synergy between etoposide and *cis*-diamminedichloroplatinum (II). *Cancer Res.*, **49**, 2390–2397 (1989).
10) Ishikura, K., Fujioka, T., Hasegawa, M., Nomura, K., Okamoto, T., Tanji, S. and Kubo, T. *In vivo* antitumor effect of combination therapy with α/β-interferon and γ-interferon on a murine renal cell carcinoma. *J. Jpn. Soc. Cancer Ther.*, **27**, 2020–2028 (1992) (in Japanese).
11) Newell, D. R., Eeles, R. A., Gumbrell, L. A., Boxall, F. E., Horwich, A. and Calvert, A. H. Carboplatin and etoposide pharmacokinetics in patients with testicular teratoma. *Cancer Chemother. Pharmacol.*, **23**, 367–372 (1989).
12) D'Incalci, M., Rossi, C., Zucchetti, M., Urso, R., Cavalli, F., Mangioni, C., Willems, Y. and Sessa, C. Pharmacokinetics of etoposide in patients with abnormal renal and hepatic function. *Cancer Res.*, **46**, 2566–2571 (1986).
13) Pflüger, K. H., Hahn, M., Holz, J. B., Schmidt, L., Köhl, P., Fritsch, H. W., Jungclas, H. and Havemann, K. Pharmacokinetics of etoposide: correlation of pharmacokinetic parameters with clinical conditions. *Cancer Chemother. Pharmacol.*, **31**, 350–356 (1993).