Jpn. J. Cancer Res. 81, 425–430, April 1990

Advantages in Combination Chemotherapy Using Cisplatin and Its Analogues for Human Testicular Tumor Xenografts

Shuichi Hida, Kenichiro Okada and Osamu Yoshida[1]

Department of Urology, Faculty of Medicine, Kyoto University, 54 Shogoin-Kawahara-machi, Sakyo-ku, Kyoto 606

The antitumor effects and toxicities of combination chemotherapies using cisplatin (CDDP) and its analogues were compared with those of single drug therapies. Congenitally athymic nude BALB/c (*nu/nu*) mice were used to estimate antitumor activities of these compounds against human testicular tumor (Ht-14) xenografts and hetero-BALB/c (*nu/+*) mice were used to evaluate the toxic effects of the drugs. Combination therapy with half dosages of CDDP and carboplatin (JM8) (CDDP: 2, JM8: 20 mg/kg/day for 5 days), or of CDDP and (glycolato-O,O′)-diammineplatinum (II) (254S) (CDDP: 2, 254S: 4 mg/kg/day for 5 days), resulted in significant tumor regression. The combination of CDDP and JM8 had the highest therapeutic efficacy while the CDDP and 254S combination had a lower antitumor potency. In addition, the toxicities of the combination therapies were lower than what was produced by the highest dosage of CDDP (4 mg/kg/day for 5 days). These results demonstrated that the antitumor activities of these combination chemotherapies were equal or superior to the activity of CDDP or an analogue alone, and that the toxicities produced by these combinations were more manageable than those produced by single drug therapies.

Key words: Cisplatin — Cisplatinum analogue — Combination chemotherapy

Dramatic improvements have been made in the treatment of patients with disseminated testicular tumors by modern chemotherapy protocols, which are based on the dose-dependent antitumor activity of cisplatin (CDDP).[1,2] Unfortunately, the excellent antitumor activity of CDDP is accompanied with strong toxic effects. Recently, cisplatinum analogues with a high antitumor activity and lower toxicity have been clinically evaluated for the treatment of human cancers. Among them, carboplatin (JM8) and (glycolato-O,O′)-diammineplatinum(II) (254S) appear to have excellent antitumor activities and the toxic effects of these analogues are different from those of cisplatin.[3–8] Myelosuppression is the dose-limiting toxic effect of these analogues, while emesis and nephrotoxicity pose no problems. The use of JM8 or 254S in the first-line treatment of testicular tumors in lieu of CDDP, however, is not recommended because of the inferior antitumor activities of the analogues.[9] Considering that the antitumor activity is dose-dependent and that the toxicities do not overlap, it appears possible that the use of a combination of CDDP plus one of its analogues may improve the antitumor activity by increasing the permissible dose of platinum compounds while reducing the toxicity, when compared to the administration of high doses of CDDP or an analogue alone. Trump *et al.*[7] conducted a phase 1 study on the combination of JM8 and CDDP and indicated that the combination therapy was advantageous. It was, therefore, thought valuable to estimate the antitumor activities and toxicities of combination chemotherapies involving CDDP and its analogues *in vivo* and to compare these values with those found for single-drug therapy using a human testicular tumor xenograft. From these results, the possible roles that the combination chemotherapy might play in the treatment of testicular cancers could be assessed.

MATERIALS AND METHODS

Animals and tumor All experiments were performed using 6-week-old female BALB/c mice weighing 18–22 g, purchased from CLEA Japan Inc., Osaka. Antitumor activity was evaluated using congenitally athymic nude BALB/c (*nu/nu*) mice and the toxicity of chemotherapy was estimated using hetero-BALB/c (*nu/+*) nude mice. The mice were fed *ad libitum* with standard laboratory food from CLEA Japan Inc. and kept under pathogen-free conditions.

The transplantable testicular tumor xenograft (HT-14) was originally established in our laboratory and was maintained subcutaneously in this study.[10] The tumor was obtained from an orchiectomy sample with anaplastic seminoma. The patient tested negatively for tumor markers. The tumor take rate was nearly 100% and the median latency period, which was defined as time required for the tumor xenograft to reach the treatment

[1] To whom correspondence and reprint requests should be addressed.

size (over 100 mm^3) from the time of implantation, was 8 days. After transplantation in nude mice, the mean tumor doubling time was 2.5 days. The tumor was maintained using serial transplantation in nude mice. Prior to therapy, the mice with tumors were randomly distributed into different treatment groups. Each group consisted of 6 mice.

Treatment and evaluation of antitumor activity Solid tumors produced on the backs of mice were measured every other day with a vernier caliper. For each tumor, the 3 perpendicular principal diameters were recorded, and the estimated tumor volume was expressed as $V=\pi/6\times(\text{length})\times(\text{width})\times(\text{height})$. When the tumor volume exceeded 100 mm^3, drugs were administered intraperitoneally. Experimental doses were chosen based on a separate pilot study. The doses were 2 mg/kg and 4 mg/kg for CDDP, 20 mg/kg and 40 mg/kg for JM8, and 4 mg/kg and 8 mg/kg for 254S. In these experiments, tumor-bearing mice were treated with drugs using a 5-day schedule according to a published clinical procedure.[2-4] Six mice served as the untreated control group.

To quantitate the antitumor activity, 3 parameters were used, as follows. (a) Relative tumor volume (%); $(V_n/V_0)\times100$, where V_n is the tumor volume at day n and V_0 is the volume at the initiation of treatment (day 0). Changes in the mean relative tumor volumes for each treatment group were used to construct a growth curve. (b) Maximum tumor load reduction (%); $(1-V_{min}/V_0)\times100$, where V_{min} is the minimum recorded tumor volume. (c) Maximum inhibition rate (IR%); $(1-T/C)\times100$, where T/C represents the ratio of the mean tumor volume of the treated group to that of control group at each measurement.

Toxic effects The toxic effects of the drugs were compared using hetero-BALB/c (*nu*/+) mice. The same doses and schedules as in the antitumor activity test were used. The drugs were given daily to each treatment group starting from day 0 to day 4. Each group consisted of 18 mice. The body weight of each mouse was recorded every other day from day 0 to day 14. Six mice in each group were killed and their blood was collected at random on day 6, day 10, and day 14. Blood cell counts and plasma analyses were done.

To evaluate side effects, 3 types of parameters were used, as follows. (a) Relative body weight (%); $(W_n/W_0)\times100$, where W_n is the body weight on day n and W_0 is the body weight at the initiation of treatment (day 0). Thus, changes in the mean relative body weight for each treatment group were used to construct a curve. (b) Maximum ratio of body weight reduction (the body weight at the initiation of treatment divided by the lowest recorded body weight). (c) Maximum toxicity ratio (the control blood cell count of each animal divided by the minimum count, and the maximum value of each biochemical parameter divided by the respective control value).

RESULTS

Studies with a single drug (CDDP, JM8 or 254S) A dosage of 20 mg/kg of JM8 or 4 mg/kg of 254S was less active than a dosage of 2 mg/kg of CDDP against the human testicular xenograft used in our study. A dosage of 2 mg/kg of CDDP showed a relatively good response with a maximum inhibition rate (IR) of 72%. On the other hand, although a dosage of 20 mg/kg of JM8 or 4 mg/kg of 254S had a positive activity against tumors, the maximum IR values obtained were only 63% and 49%, respectively (Fig. 1).

The maximum tolerated dosages of CDDP (4 mg/kg), JM8 (40 mg/kg) and 254S (8 mg/kg), individually, induced rapid tumor load reductions. The reductions became apparent from day 4 (Fig. 1). The maximum tumor load reduction rates were $96.3\pm3.6\%$ ($\pm$SD) with a 4 mg/kg dose of CDDP, $81.8\pm10.6\%$ with a 40 mg/kg dose of JM8 and $87.5\pm10.0\%$ with an 8 mg/kg dose of 254S. The maximum reduction induced by CDDP differed significantly ($P<0.01$) from those induced by JM8 and 254S.

Studies with combination chemotherapy (CDDP+JM8 or CDDP+254S) Combination therapy with CDDP (2 mg/kg) and JM8 (20 mg/kg) produced a marked reduction of tumor volume and the maximum tumor load reduction rate was $99.6\pm0.1\%$. A superior antitumor activity was observed compared to single drug treatment with 4 mg/kg of CDDP or 40 mg/kg of JM8. Combination therapy with CDDP and JM8 caused the complete disappearance of visible tumors, which rarely recurred. Tumor regression was also observed in the mice given combination therapy with CDDP (2 mg/kg) and 254S (4 mg/kg) and the maximum tumor load reduction rate was $86.0\pm8.7\%$. No difference was found between the mice treated with this combination therapy and those treated with 8 mg/kg of 254S alone. The antitumor efficacy of the CDDP-254S combination therapy was significantly ($P<0.01$) less than that achieved with 4 mg/kg of CDDP alone (Fig. 2).

Toxicities of single drug therapies with CDDP and its analogues and combination therapies The rate of body weight reduction increased in proportion to increasing dosage of each drug. The weights of the mice treated with CDDP (4 mg/kg) did not recover while those of the mice treated with JM8 (40 mg/kg) or 254S (8 mg/kg) returned to their pretreatment levels by day 12 (Fig. 1). Weight losses were also observed immediately after the start of a combination therapy, but the losses were less severe than those of mice treated with 4 mg/kg of

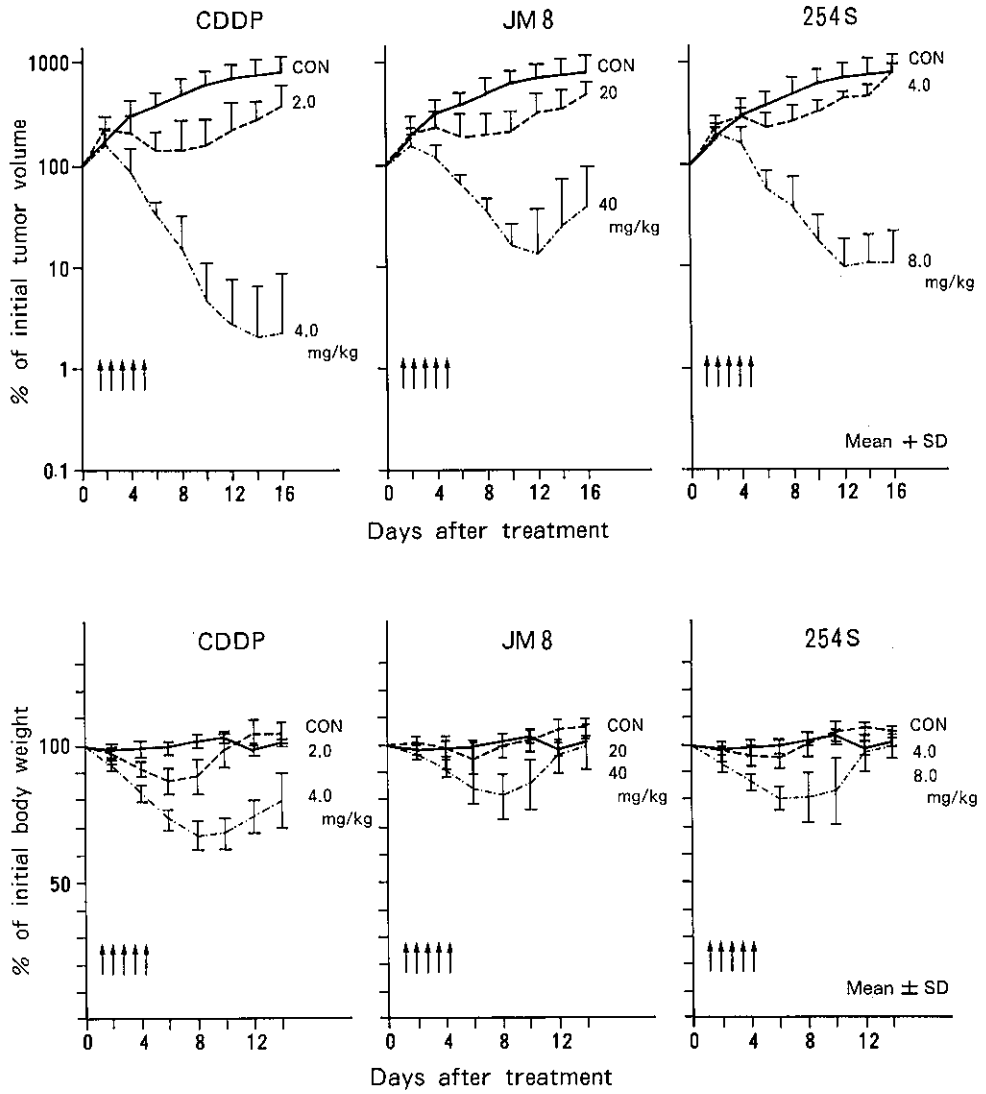

Fig. 1. Antitumor activities of CDDP, JM8, and 254S toward human testicular tumor xenografts (HT-14) in nude mice. Values for the relative tumor volume are given as the mean+SD. One group of 6 mice, implanted with tumors, was not treated (CON: control). Values for the relative body weight of hetero-nude mice are given as the mean±SD. Doses are shown in the figure. Arrows indicate administrations of drugs.

CDDP. Statistically significant differences ($P<0.01$) were found between body weight reduction rates in the combination therapy groups and in the 4 mg/kg CDDP group, but there was no difference between the combination therapy groups and the 40 mg/kg JM8 or 8 mg/kg 254S group (Fig. 2).

Severe anemia was observed in the 40 mg/kg JM8 group ($P<0.01$) and the CDDP-JM8 combination therapy group ($P<0.01$). Thrombocytopenia was fairly severe in the 8 mg/kg 254S and 40 mg/kg JM8 groups ($P<0.01$). However, leukopenia was moderate in both groups. Strong hepatic toxicity was observed in the 4 mg/kg CDDP and 8 mg/kg 254S groups ($P<0.01$). Transient elevations of liver enzyme levels in the 4 mg/kg CDDP group appeared to be significant (at day 6, $P<0.001$). The levels of these enzymes in the 8 mg/kg 254S group increased progressively. CDDP and 254S seemed to be nephrotoxic, causing elevations in the levels of blood urine nitrogen (BUN) and/or creatinine (Cr) (Tables I and II).

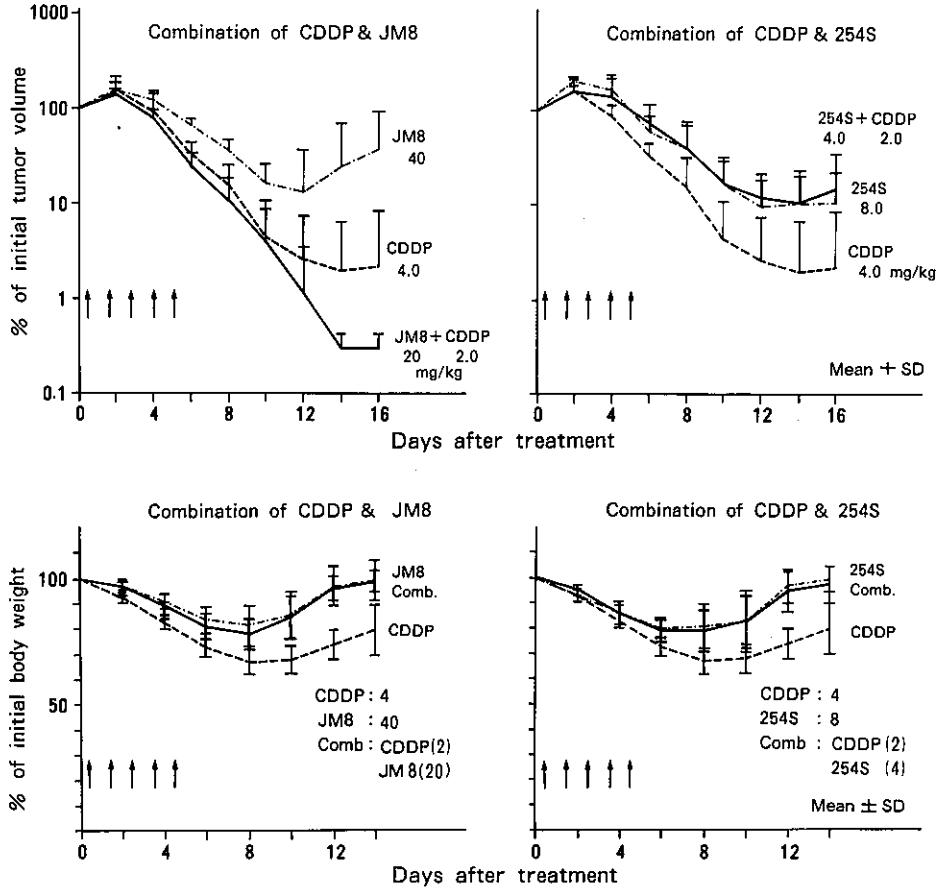

Fig. 2. Comparison of the effects of the highest dose of each drug with those of combination chemotherapies (Comb.) on the growth of human testicular tumor xenografts in nude mice. Values of the relative tumor volume are given as the mean+SD. The combination of CDDP and JM8 caused complete disappearance of the transplanted tumor. Values of the relative body weight are given as the mean±SD. Doses are shown in the figure. Arrows indicate administrations of drugs.

Table I. Minimum Body Weight Rates or Minimum Blood Cell Counts, and Maximum Blood Enzyme Levels

Drugs	Minimum	Minimum			Maximum				
	W_n/W_0 (×100%)	RBC (×10⁴)	WBC (×10²)	PLT (×10⁴/mm³)	GOT (KU)	GPT (KU)	LDH (WU)	BUN (mg/dl)	Cr (mg/dl)
Control	99±2 (2)	887±35 (10)	66±23 (10)	94±25 (10)	123±31 (10)	40±3 (10)	616±173 (10)	24±2 (14)	0.54±0.04 (14)
CDDP/2	87±5 (6)	837±50 (14)	42±11 (6)	71±44 (6)	400±111 (6)	35±10 (6)	2046±519 (6)	33±4 (10)	0.66±0.1 (10)
JM8/20	95±6 (6)	823±74 (14)	37±11 (6)	44±20 (14)	270±72 (6)	68±17 (10)	1254±250 (6)	30±6 (14)	0.73±0.08 (14)
254S/4	95±4 (4)	849±40 (10)	44±15 (6)	61±19 (10)	222±84 (6)	47±3 (14)	1303±407 (6)	37±4 (14)	0.82±0.28 (14)
CDDP/4	67±5 (8)	855±47 (14)	39±11 (6)	24±4 (10)	799±173 (6)	240±204 (6)	2648±141 (6)	45±8 (10)	0.82±0.28 (6)
JM8/40	81±8 (8)	658±104(14)	34±5 (14)	21±5 (14)	261±93 (6)	47±8 (10)	1102±529 (6)	32±4 (14)	0.71±0.23 (14)
254S/8	80±4 (6)	757±62 (14)	34±11 (10)	18±5 (14)	440±192 (10)	139±89 (10)	2032±606 (14)	41±4 (14)	1.03±0.18 (10)
CDDP/2 +254S/4	79±8 (8)	731±91 (14)	31±9 (14)	40±15 (10)	415±163 (6)	62±28 (14)	1815±821 (6)	38±4 (14)	0.75±0.25 (14)
CDDP/2 +JM8/20	78±6 (8)	710±41 (14)	32±10 (14)	25±12 (14)	322±54 (10)	100±54 (10)	1375±356 (10)	35±4 (10)	0.78±0.18 (10)

Drugs were injected ip into hetero-female BALB/c mice. Each group consisted of 18 mice. Values are mean±SD.
The numbers in parentheses show on what days the measurements were taken after the start of treatment.

Table II. Ratio of Body Weight Reduction and Toxicity Ratio of Organs

Drug/Dose	Maximum ratio of body weight reduction[a]	Average of maximum toxicity ratio[b]		
		Bone marrow	Liver	Kidney
CDDP/2	1.13	1.32	2.48	1.30
JM8/20	1.04	1.70	1.98	1.30
254S/4	1.04	1.36	1.70	1.53
CDDP/4	1.48	2.22	5.60	1.70
JM8/40	1.22	2.59	1.70	1.32
254S/8	1.24	2.78	3.45	1.81
CDDP/2 +254S/4	1.25	1.90	2.62	1.49
CDDP/2 +JM8/20	1.27	2.36	2.45	1.45

a) Maximum ratio of body weight reduction: the body weight at the initiation of treatment divided by the lowest recorded body weight.
b) Maximum toxicity ratio: the control blood cell counts divided by the minimum counts and the maximum values of blood enzymes divided by the respective control values.

Considering both the antitumor activity and side effects, the combination therapy of CDDP and JM8 appeared to have advantages over the other therapies tested.

DISCUSSION

In this study, we used a transplantable human testicular tumor xenograft (Ht-14) model[10] in BALB/c nude mice. It has been reported that the volume doubling times of testicular tumor xenografts range between 1.6 and 14 days.[11-16] A volume doubling time of 2.5 days in our study with Ht-14 was consistent with these estimates. The transplanted tumor had a high dose-dependent sensitivity to the platinum compounds, which indicated that this tumor model would be suitable for estimating the clinical antitumor activities of the drugs.

In the study on the comparative activities of the drugs, the maximum tolerated dosages of CDDP, JM8, and 254S induced rapid tumor reductions. However, while CDDP showed a significant antitumor activity, the activities of JM8 and 254S were lower. These results are consistent with previous reports.[8, 9]

The present study on combination therapies provided evidence that the combination therapy of CDDP plus JM8, with its greater antitumor activity, offered distinct advantages over the other therapies tested and induced the tumor to disappear almost completely. The CDDP plus 254S combination regimen also induced rapid tumor regression but the reduction rate was less than expected, though it had been reported that the pharmacokinetic behavior of 254S was similar to that of JM8.[8] A possible reason for the difference in antitumor activities is the difference in the potentials for cross-resistance. Wiltshaw *et al.*[5] conducted a prospectively randomized trial comparing CDDP and JM8. They found a 15% to 20% cross-over response rate to JM8 after disease progression in a tumor being treated with CDDP and hypothesized that there was a lack of cross-resistance between the two drugs. However, studies concerning the cross-resistance between CDDP and 254S have not been reported. The combination of CDDP and JM8 appears to be the most useful when antitumor activities are considered.

We used hetero-BALB/c (*nu*/+) mice to examine the toxic effects of chemotherapy. The reason for this choice was that, with two closely related species of animal, it would be possible to correlate changes during the course of a therapeutic study and to adjust dosage levels according to the levels of toxicity encountered. There appear to be large discrepancies between the results using nude mice in the determination of antitumor activities and those using other animals in the determination of toxic effects of drugs used. The use of large athymic animals is not practical for both antitumor activity and toxic studies.

In nude mice models, change in body weight is usually used as an indicator of non-lethal toxic effects. The change in body weight alone, however, seems to be insufficient as an indicator of toxicity and the nature of the toxic effects cannot be discussed in detail. Unfortunately, no definitive method has been established to evaluate different types of toxicity. Therefore, to quantitate the toxic effects of antitumor agents, we ventured to employ a ratio system to evaluate each side effect. However, there may be many problems with this system. For example, (1) When a toxic substance is administered, the size of the change for each metabolic function may be different, and hence the ratios of test sample values to control values can be quite different depending upon the parameters chosen. For example, while the RBC count may be affected only slightly, the platelet count may be affected greatly upon the administration of a drug. Thus the use of the ratio system to define the toxic effects of drugs is probably an oversimplification of the actual situation. (2) Unfortunately, we do not know which parameters are most important to represent the toxic effects of the drugs in question. The ratio system was employed, however, to quantify the side effects of drugs as much as possible, within the current limits of our ability.

It became clear from the toxicity study that CDDP caused severe weight reduction and liver dysfunction, and that JM8 and 254S induced myelosuppression, as expected. But 254S caused greater-than-expected hepatic

and renal toxicities, whereas JM8 appeared to be better tolerated and more useful as far as toxicities are concerned. The CDDP-JM8 combination caused moderate toxicities characteristic of each of these factors, but caused greater than expected myelosuppression. Trump *et al.*[7] also reported that thrombocytopenia and leukopenia were the dose-limiting toxicity factors when using this combination.

Based on clinical results obtained through modern chemotherapy in patients with metastatic diseases, it has become well recognized that CDDP is the most important drug for use in chemotherapy and that the dose-response relationship for CDDP is very clear-cut in the treatment of testicular tumors with high sensitivities to platinum. At the same time, however, toxicities are also encountered in these regimens. The goal of chemotherapy is to cure the patient without inducing intolerable toxicity. In the further development of chemotherapy, a combination of CDDP plus one of its analogues should offer distinct advantages, which are enhanced antitumor activity and reduced toxicity. Using a combination, it will be possible to administer higher doses of drugs with a more manageable toxicity than can be obtained using a single drug.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the excellent technical assistance of Miss K. Ishida.

(Received November 4, 1989/Accepted February 3, 1990)

REFERENCES

1) Samson, M. K., Rivkin, S. E., Jones, S. E., Costanzi, J. J., LoBuglio, A. F., Stephens, R. L., Gehan, E. A. and Cummings, G. D. Dose-response and dose-survival advantage for high versus low-dose cisplatin combined with vinblastine and bleomycin in disseminated testicular cancer. A Southwest Oncology Group study. *Cancer,* **53,** 1029–1035 (1984).
2) Ozols, R. F., Ihde, D. C., Linehan, W. M., Jacob, J., Ostchega, Y. and Young, R. C. A randomized trial of standard chemotherapy vs a high-dose chemotherapy regimen in the treatment of poor prognosis nonseminomatous germ-cell tumors. *J. Clin. Oncol.,* **6,** 1031–1040 (1988).
3) Rozencweig, M., Nicaise, C., Beer, M., Crespeigne, N., Rijmenant, M. V., Lenaz, L. and Kenis, Y. Phase I study of carboplatin given on a five-day intravenous schedule. *J. Clin. Oncol.,* **10,** 621–626 (1983).
4) Van Echo, D. A., Egorin, M. J., Whitacre, M. Y., Olman, E. A. and Aisner, J. Phase I clinical and pharmacologic trial of carboplatin daily for 5 days. *Cancer Treat. Rep.,* **68,** 1103–1114 (1984).
5) Wiltshaw, E., Evans, B. and Harland, S. Phase III randomized trial cisplatin versus JM8 (carboplatin) in 112 ovarian cancer patients, stage III and IV. *Proc. Am. Soc. Clin. Oncol.,* **4,** 121 (1985).
6) Peckham, M. J., Horwich, A. and Hendry, W. F. Advanced seminoma: treatment with *cis*-platinum-based combination chemotherapy or carboplatin (JM8). *Br. J. Cancer,* **52,** 7–13 (1985).
7) Trump, D. L., Grem, J. L., Tutsh, K. D., Wilson, J. K. V., Simon, K. J., Alberti, D., Storer, B. and Tormey, D. C. Platinum analogue combination chemotherapy: cisplatin and carboplatin — A phase I trial with pharmacokinetic assessment of the effect of cisplatin administration on carboplatin excretion. *J. Clin. Oncol.,* **5,** 1281–1289 (1987).
8) Sasaki, Y., Tamura, T., Eguchi, K., Shinkai, T., Fujiwara, Y., Fukuda, M., Ohe, Y., Bungo, M., Horichi, N., Niimi, S., Minato, K., Nakagawa, K. and Saijo, N. Pharmacokinetics of (glycolato-O,O′)-diammine platinum(II), a new platinum derivative, in comparison with cisplatin and carboplatin. *Cancer Chemother. Pharmacol.,* **23,** 243–246 (1989).
9) Harstrick, A., Casper, J., Guba, R., Wilke, H., Poliwoda, H. and Schmoll, H. J. Comparison of the antitumor activity of cisplatin, carboplatin and iproplatin against established human testicular cancer cell lines *in vivo* and *in vitro. Cancer,* **63,** 1079–1083 (1989).
10) Okada, K. and Yoshida, O. Study on heterotransplantation of malignant urogenital tumors in nude mice: results of transplantation and the characteristics of the explants. *Acta Urol. Jpn.,* **30,** 1749–1767 (1984).
11) Selby, P. J., Heyderman, E., Gibbs, J. and Peckham, M. J. A human testicular teratoma serially transplanted in immune-deprived mice. *Br. J. Cancer,* **39,** 578–583 (1979).
12) Tveit, K. M., Fodstad, O., Brogger, A. and Olsnes, S. Human embryonal carcinoma grown in athymic mice and *in vitro. Cancer Res.,* **40,** 949–953 (1980).
13) Edler von Eyben, F., Trope, C., Ljungberg, O., Alm, P., Wennerberg, J. and Gulberg, B. Histologic pattern and growth in two human testis cancers before and after transplantation to nude mice. *Cancer,* **50,** 2845–2853 (1982).
14) Steel, G. G., Countenay, V. D. and Peckham, M. J. The response to chemotherapy of a variety of human tumour xenografts. *Br. J. Cancer,* **47,** 1–13 (1983).
15) Lapis, P., Kopper, L., Bodrogi, I., Sugar, J., Lapis, K. and Eckhardt, S. Characteristics and chemotherapeutic sensitivity of a human testicular cancer grown in artificially immunosuppressed mice. *Oncology,* **42,** 112–118 (1985).
16) Schlappack, O. K., Bush, C., Delic, J. I. and Steel, G. G. Growth and chemotherapy of a human germ-cell tumour line (GCT 27). *Eur. J. Cancer Clin. Oncol.,* **24,** 777–781 (1988).