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Enhanced Antitumor Efficacy of a Combination of CPT-11, a New Derivative of Camptothecin, and Cisplatin against Human Lung Tumor Xenografts

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The objective of this study was to evaluate the antitumor efficacy of combined use of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11) and cisplatin (CDDP). The antitumor activities of CPT-11, CDDP and their combination against 3 human lung tumor xenografts were estimated using congenitally athymic BALB/c (*nu/nu*) mice. The doses were 47 mg/kg for CPT-11 and 6 mg/kg for CDDP on days 1, 5 and 9. In combination therapy, half of the single dosage of each agent was used. The doses were administered intraperitoneally. The antitumor activity and toxicity were evaluated in terms of the tumor volume and body weight change of mice, respectively. The combination therapy resulted in a statistically significant tumor regression compared to the use of only CPT-11 or CDDP in two tumor xenografts out of three. The toxicity of the combination therapy was no higher than that of CPT-11 or CDDP alone. These results suggest that the antitumor activity of the combination of CPT-11 and CDDP is superior to that of CPT-11 or CDDP alone.

Key words: CPT-11 — Cisplatin — Combination chemotherapy — Nude mouse — Lung cancer

7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11) is a new derivative of camptothecin, which has an unprecedented antitumor activity against a broad spectrum of experimental tumor models.[1,2] Phase I and II studies of this agent in patients with lung cancer have yielded excellent results[3–5]; the overall response rates of previously untreated non-small cell lung cancer and small cell lung cancer were 31.9% and 50%, respectively. These results suggest that CPT-11 could become one of the key drugs in lung cancer chemotherapy. Since the combined use of anticancer agents has generally been more effective than the use of a single agent, most current chemotherapy regimens have favored the use of combinations with the aim of increasing the response rate and the frequency of long-term survival. Therefore one important issue in treatment with CPT-11 is to determine which agent is most useful in combination.

First, we examined the *in vitro* synergistic effect of 7-ethyl-10-hydroxycamptothecin (SN-38), the major active metabolite of CPT-11,[6] in combination with some commonly used lung cancer chemotherapeutic agents, cisplatin (CDDP), etoposide, doxorubicin, vindesine and mitomycin C, against 5 human lung cancer cell lines.[7] Among these 5 agents, CDDP and etoposide frequently exhibited synergistic cytotoxicity with SN-38.

In this paper we deal with the *in vivo* antitumor activity of the combination of CPT-11 and CDDP against 3 human lung tumor xenografts serially implanted in nude mice.

MATERIALS AND METHODS

Animals and tumors All experiments were performed using male athymic nude mice of BALB/c background, 6 to 8 weeks of age, and weighing 22–25 g. The mice were purchased from Japan SLC Inc. (Shizuoka) and were kept in laminar air-flow rooms at a constant temperature (22–26°C) and humidity (30–50%). Food and bedding were sterilized and mice were given tap water *ad libitum* in sterilized bottles. Two lung tumor lines, Mqnul and Msnul, originally established in our institution, were maintained in the nude mice. The Mqnul tumor line had been established from surgical material of a 51-year-old male patient in December, 1986. This tumor was diagnosed as poorly differentiated squamous cell carcinoma. The patient had not previously been treated with any form of chemotherapy or radiation therapy. The Msnul tumor line had been established from biopsy material from a skin metastasis that had developed in a 66-year-old male. This tumor was diagnosed as small cell carcinoma. The patient had received 4 courses of the combination chemotherapy of cisplatin (80 mg/m^2, day 1) and etoposide (100 mg/m^2, days 1, 2, 3) with concurrent thoracic radiation therapy. The disease did not respond

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Table I. Characteristics of Human Lung Tumor Lines Xenografted in Athymic Nude Mice

Tumor line	Mqnu1	Msnu1	LX1
Origin	Lung	Skin metastasis	Lung
Histology	Squamous cell carcinoma	Small cell carcinoma	Mixed small cell/ large cell carcinoma
No. of passages	19–22	4–5	—
Doubling time (days)	9.4	7.3	7.9

to the chemo-radiotherapy. Skin metastasis developed, and he died of respiratory failure due to radiation pneumonitis 5 months after the initiation of the treatment. The LX1 tumor line was developed by B. Giovanella at the Stehlin Foundation for Cancer Research. This tumor line was used in a drug-screening tumor panel in the Division of Cancer Treatment, National Cancer Institute, USA.[8] The characteristics of the three tumor lines are summarized in Table I.

Drugs CPT-11 was kindly supplied by Dai-ichi Pharmaceutical Co., Ltd. (Tokyo). CDDP was purchased from Nippon Kayaku Co., Ltd. (Tokyo).

Treatment and evaluation of antitumor activity The tumors were cut into fragments with a diameter of 2–3 mm and inoculated into the subcutaneous tissue of the lateral dorsum of mice. The tumor growth was followed by measuring the volume of the tumor twice a week with a sliding caliper. The tumor volume (V) was calculated by using the formula $V=(a^2\times b)/2$, where b is the largest diameter and a is the diameter perpendicular to b. When the tumor volume reached 70–150 mm^3, the mice were allocated according to tumor volume into different treatment groups and a control group, leading to similar mean tumor volumes in the groups, each of which consisted of 5 mice. Drugs were administered intraperitoneally to the treatment groups. The doses were 47 mg/kg for CPT-11 and 6 mg/kg for CDDP on days 1, 5 and 9. In combination therapy half of a single dose of each drug (23.5 mg/kg for CPT-11, 3 mg/kg for CDDP) was administered simultaneously on the same days. Drug doses and the treatment schedule were chosen based on a separate pilot study in which it was demonstrated that the three treatment arms produced almost equal toxicity.

To evaluate the antitumor activity, relative tumor volume (RV) was calculated as $RV\ (\%)=V_n/V_1\times 100$, where V_n is the tumor volume at day n and V_1 is the tumor volume at the initiation of the treatment (day 1). The antitumor activity of the treatments was also evaluated in terms of inhibition rate (IR), which was calculated as $IR\ (\%)=(1-Vtmn/Vcmn)\times 100$ where Vtmn is the mean tumor volume of the treated group at day n and Vcmn is the mean tumor volume of the control group at day n. The toxicity of the treatment was assessed in terms of change in the relative body weight during the experiments. It was calculated as $(W_n/W_1)\times 100$ where W_n is the body weight at day n and W_1 is the body weight at the initiation of the treatment (day 1).

The differences of RV at each day among the treatment groups were statistically analyzed by using Student's t test. All P values reported are two-tailed.

RESULTS

The growth curves of the three lung tumor xenografts treated with CPT-11, CDDP, and the combination therapy are shown in Figs. 1 to 3. All treatment arms resulted in significant tumor regression compared to the control group. In the treatment of the small cell carcinoma xenografts, Msnul and LX1, statistically significantly superior antitumor activities were observed in the combination therapy compared to single-drug treatment (Figs. 1 and 2). In mice bearing Msnul treated with CPT-11,

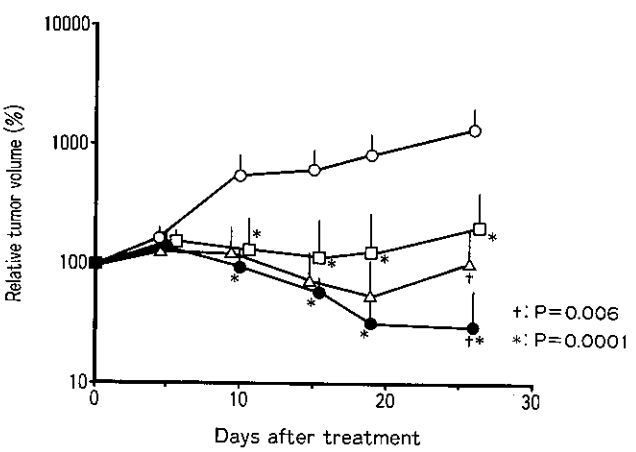

Fig. 1. Effects of CPT-11, CDDP, and their combination on the growth of human small-cell lung tumor xenograft, Msnul, in nude mice. The mice were divided into groups of 5 animals. Each agent was administered intraperitoneally on days 1, 5 and 9. The doses of CPT-11 alone and CDDP alone were 47 mg/kg/day and 6 mg/kg/day, respectively. The doses in combination were 23.5 mg/kg/day for CPT-11 and 3 mg/kg/day for CDDP. The values of the relative tumor volume are given as the mean+SD. ○; control, △; CPT-11, □; CDDP, ●; combination (CPT-11+CDDP).

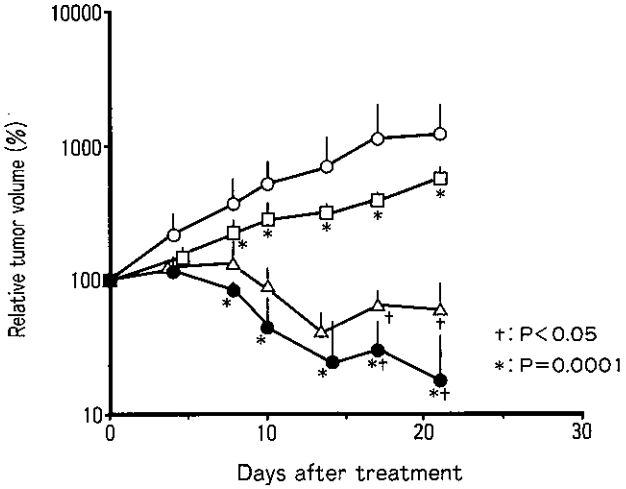

Fig. 2. Effects of CPT-11, CDDP, and their combination on the growth of human small-cell lung tumor xenograft, LX1, in nude mice. The mice were divided into groups of 5 animals. Each agent was administered intraperitoneally on days 1, 5 and 9. The doses of CPT-11 alone and CDDP alone were 47 mg/kg/day and 6 mg/kg/day, respectively. The doses in combination were 23.5 mg/kg/day for CPT-11 and 3 mg/kg/day for CDDP. The values of the relative tumor volume are given as the mean+SD. ○; control, △; CPT-11, □; CDDP, ●; combination (CPT-11+CDDP).

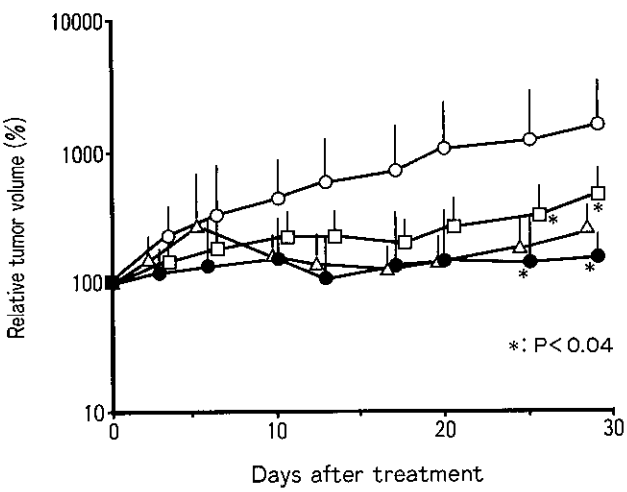

Fig. 3. Effects of CPT-11, CDDP, and their combination on the growth of human squamous cell lung tumor xenograft, Mqnul, in nude mice. The mice were divided into groups of 5 animals. Each agent was administered intraperitoneally on days 1, 5 and 9. The doses of CPT-11 alone and CDDP alone were 47 mg/kg/day and 6 mg/kg/day, respectively. The doses in combination were 23.5 mg/kg/day for CPT-11 and 3 mg/kg/day for CDDP. The values of the relative tumor volume are given as the mean+SD. ○; control, △; CPT-11, □; CDDP, ●; combination (CPT-11+CDDP).

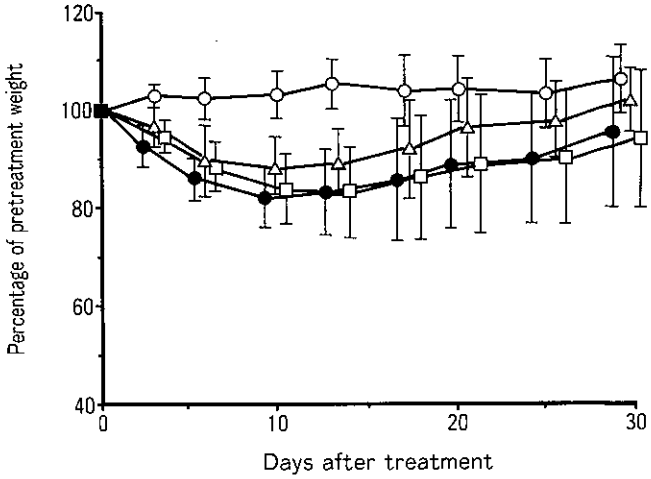

Fig. 4. Effects of the treatment on the body weight of mice carrying human lung tumor xenografts. The values for the relative body weight of mice are given as the mean±SD. ○; control, △; CPT-11, □; CDDP, ●; combination (CPT-11+CDDP).

CDDP and a combination of the two, the RV values on day 26 were 102.9%, 277.4% and 30.3%, respectively. The combination therapy produced a statistically significant reduction of the tumor volume compared to the single use of CPT-11 (P=0.006) or CDDP (P=0.0001). In mice bearing LX1, combination therapy also exhibited more potent antitumor activity than single-drug treatment on days 17 and 21; the RV values on day 21 for CPT-11, CDDP and the combination of the two were 59.6%, 557.1% and 17.6%, respectively, and the combination therapy produced a statistically significant reduction of the tumor volume compared to the single use of CPT-11 (P=0.041) or CDDP (P=0.0001). Therefore, the combined use of CPT-11 and CDDP appeared to show a synergistic effect against Msnul and LX1. The maximum inhibition rates in mice bearing Msnul and LX1 treated with the combination therapy were 98.0% and 97.1%, respectively. In the case of Mqnul, relative tumor volumes in animals treated with CPT-11, CDDP and a combination of the two on day 25 were 184.6%, 321.9% and 141.6%, respectively. The combination therapy was not significantly more effective than therapy using only CPT-11 (P=0.48) but was significantly more potent than therapy using only CDDP (P=0.04) (Fig. 3). The maximum inhibition rate in mice bearing Mqnul treated with combination therapy was 86.3%. The three treatment arms were obviously effective against Mqnul and their activities were almost equal.

With this dosage, all the mice in all the treatment groups survived during the experiments. Mice in the control group, moreover, gained weight during the exper-

iment (+6.2%). On the other hand, the mice in the three treatment groups significantly lost weight compared to the control group (P=0.0001). The maximum values of relative body weight loss in groups treated with CPT-11, CDDP and their combination were 11.8% (day 10), 16.5% (day 13) and 17.7% (day 10), respectively (Fig. 4). With this dosage, use of only CPT-11 appeared to be less toxic than use of cisplatin (10.8% vs. 16.5% on day 13 (P=0.142)) or use of a combination of the two (11.8% vs. 17.7% on day 10 (P=0.051)), though the differences were not statistically significant. Combined use was almost as toxic as single use of CDDP (17.7% vs. 16.0% on day 10 (P=0.59)).

A comparison of the antitumor activities of CPT-11 and CDDP alone at this dosage showed that CPT-11 was more active than CDDP in the treatment of Msnul (102.9% vs. 277.4% on day 26, P=0.01) and LX1 (59.6% vs. 557.1% on day 21, P=0.0001), and had almost the same activity as CDDP (184.6% vs. 321.9% on day 25, P=0.0867) in the treatment of Mqnul. In our pilot study involving treatment with half the dosage of these agents individually (23.5 mg/kg for CPT-11, 3 mg/kg for CDDP), CPT-11 proved to be statistically significantly more active against Mqnul than CDDP (data not shown). From the viewpoints of the antitumor activity (inhibition of tumor growth) and the toxicity (body weight loss) in the treatment of these 3 human lung tumors, the use of CPT-11 alone was more effective than the use of CDDP alone.

DISCUSSION

Phase I and II studies of CPT-11 disclosed that the dose limiting toxicities of this agent are myelosuppression and a gastro-intestinal effect, mainly diarrhea.[3-5,9] On the other hand, the major toxicities of CDDP are nephrotoxicity, nausea/vomiting, and ototoxicity. The spectrum of toxicity of CPT-11 does not overlap that of CDDP. CDDP is commonly used in lung cancer chemotherapy, and is considered to be one of the most active agents. Therefore, preclinical evaluation of the combined use of CPT-11 and CDDP is a very important issue. This is one of the reasons why CDDP was selected in the present study for the use in combination with CPT-11. Furthermore, we conducted an *in vitro* chemosensitivity test to evaluate the activity of combinations of SN-38, the major active metabolite of CPT-11, and other active agents using human lung cancer cell lines,[7] and we concluded that CDDP or etoposide was a better candidate for combined use with CPT-11 than were other agents. In the present study, we performed an *in vivo* preclinical evaluation of the efficacy of combined use of CPT-11 and CDDP using human lung tumor xenografts. Chemosensitivity to CPT-11, CDDP and a combination of the two depends on the tumor xenograft. In the treatment of two small cell lung carcinoma (SCLC) xenografts, Msnul and LX1, significant tumor regression was observed in the combination therapy compared to the single-drug treatment. The maximum IRs were 97.1 and 98.0%, respectively. However, in a squamous cell carcinoma xenograft, Mqnul, the combination therapy was not more effective than single-drug treatment. The maximum IR of the combination therapy was 86.1%. As we have been experienced in clinical studies, SCLC xenografts were more sensitive to this combination chemotherapy than the non-small cell lung carcinoma (NSCLC) xenograft. The activity of CPT-11 in a clinical phase II study in patients with SCLC was very high, even in previously treated patients, and CPT-11 alone showed the high response rate of 33.3%.[5]

At the dosage used in this experiment, which was determined on the basis of our pilot study, leading to equal toxicities in each group, CPT-11 alone proved to be more effective than CDDP alone, although the activities of CPT-11 toward the three tumor xenografts were different. The maximum IRs of CPT-11 to Msnul, LX1 and Mqnul were 91.1, 96.1 and 84.9%, respectively. In *in vitro* assay, synergism was obtained against NSCLC cell lines. However, in this study a higher effect of combination chemotherapy could not be obtained in a squamous cell carcinoma xenograft. We will have to examine other NSCLC xenografts to see whether or not a higher antitumor activity can be obtained in combination chemotherapy using CPT-11 and CDDP.

It is difficult to draw definite conclusions from our present study about whether combined use of CPT-11 and CDDP had a synergistic effect or not in lung tumor xenografts, because there is no established definition of synergism in preclinical evaluation. Many studies on combination treatment have expressed the combined effect in terms of enhancement,[10] augmentation[11] or advantage[12] instead of synergism. On the other hand, Fujita *et al.*[13] defined a synergistic effect to exist when the combination therapy was superior to each single drug therapy at double the dose. They compared the antitumor effects in terms of the inhibition rate. In our study, combination therapy in two SCLC xenografts produced higher inhibition rates compared to single use of CPT-11 or CDDP. So, in terms of this definition, combination therapy with CPT-11 and CDDP had a synergistic effect. But a final conclusion must await the results of a clinical study.

The mechanisms of cytotoxicity of CPT-11 and CDDP are different. This is another reason why we used CPT-11 and CDDP in combination chemotherapy. CPT-11 or camptothecin has been shown to block the rejoining step of the breakage-reunion reaction of DNA topoisomerase I by stabilizing the enzyme-DNA complex.[14] On the

other hand, CDDP has been shown to induce intrastrand DNA cross-links.[15] An *in vitro* study of the mechanism of the combined effect of CPT-11 and CDDP is under way. In the present study, only the combined effect of simultaneous administration of two drugs was evaluated. We still have to examine the effect of sequencing of the administration of the two drugs.

In conclusion, combination therapy using CPT-11 and CDDP proved to be superior to treatment using only CPT-11 or CDDP in two out of three human lung tumor xenografts. A clinical phase I study on the use of a combination of CPT-11 and CDDP should be conducted.

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REFERENCES

1) Kunimoto, T., Nitta, K., Tanaka, T., Uehara, N., Baba, H., Takeuchi, M., Yokokura, T., Sawada, S., Miyasaka, T. and Mutai, M. Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. *Cancer Res.*, **47**, 5944–5947 (1987).
2) Matsuzaki, T., Yokokura, T., Mutai, M. and Tsuruo, T. Inhibition of spontaneous and experimental metastasis by a new derivative of camptothecin, CPT-11, in mice. *Cancer Chemother. Pharmacol.*, **21**, 308–312 (1988).
3) Negoro, S., Fukuoka, M., Masuda, N., Takada, M., Kusunoki, Y., Matsui, K., Takifuji, N., Kudoh, S., Niitani, H. and Taguchi, T. Phase I study of weekly intravenous infusions of CPT-11, a new derivative of camptothecin, in the treatment of advanced non-small-cell lung cancer. *J. Natl. Cancer Inst.*, **83**, 1164–1168 (1991).
4) Fukuoka, M., Niitani, H., Suzuki, A., Motomiya, M., Hasegawa, K., Nishiwaki, Y., Kuriyama, T., Ariyoshi, Y., Negoro, S., Masuda, N., Nakajima, S. and Taguchi, T. A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. *J. Clin. Oncol.*, **10**, 16–20 (1992).
5) Negoro, S., Fukuoka, M., Niitani, H. and Taguchi, T. Phase II study of CPT-11, new camptothecin derivative, in small cell lung cancer (SCLC). *Proc. Am. Soc. Clin. Oncol.*, **10**, 241 (1991).
6) Kaneda, N., Nagata, H., Furuta, T. and Yokokura, T. Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse. *Cancer Res.*, **50**, 1715–1720 (1990).
7) Takada, M., Fukuoka, M., Kudoh, S., Masuda, N., Nakagawa, K. and Kishimoto, S. Synergistic effects of CPT-11 and cisplatin or etoposide on human lung cancer cell lines and xenografts in nude mice. *Proc. Am. Assoc. Cancer Res.*, **33**, 226 (1992).
8) Goldin, A., Venditti, J. M., Macdonald, J. S., Muggid, F. M., Henney, J. E. and Devita, V.T., Jr. Current results of the screening program at the Division of Cancer Treatment, National Cancer Institute. *Eur. J. Cancer*, **17**, 129–142 (1981).
9) Ohno, R., Okada, K., Masaoka, T., Kuramoto, A., Arima, T., Yoshida, Y., Ariyoshi, H., Ichimaru, M., Sakai, Y., Oguro, M., Ito, Y., Morishima, Y., Yokomaku, S. and Ota, K. An early phase II study of CPT-11: a new derivative of camptothecin, for the treatment of leukemia and lymphoma. *J. Clin. Oncol.*, **8**, 1907–1912 (1990).
10) Ning, S. and Hahn, G. M. Combination therapy: lonidamine, hyperthermia, and chemotherapy against the RIF-1 tumor *in vivo*. *Cancer Res.*, **51**, 5910–5914 (1991).
11) Krosnick, J. A., Mulé, J. J., McIntosh, J. K. and Rosenberg, S. A. Augmentation of antitumor efficacy by the combination of recombinant tumor necrosis factor and chemotherapeutic agents *in vivo*. *Cancer Res.*, **49**, 3729–3733 (1989).
12) Hida, S., Okada, K. and Yoshida, O. Advantages in combination chemotherapy using cisplatin and its analogues for human testicular tumor xenografts. *Jpn. J. Cancer Res.*, **81**, 425–430 (1990).
13) Fujita, F., Fujita, M., Shimozuma, K. and Taguchi, T. Combination chemotherapy of human gastrointestinal and breast cancer xenografts in nude mice with 5′-deoxy-5-fluorouridine and mitomycin C. *J. Jpn. Soc. Cancer Ther.*, **21**, 1386–1396 (1986) (in Japanese).
14) Hsiang, Y. H., Hertzberg, R., Hecht, S. and Liu, L. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J. Biol. Chem.*, **260**, 14873–14878 (1985).
15) Lippard, S. J. Overview. *In* "Platinum Coordination Complexes in Cancer Chemotherapy," ed. M. P. Hacker, E. B. Douple and I. H. Krakoff, pp. 11–13 (1984). Martinus Nijhoff Publishing, Boston.