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DESIGN AND TESTING OF A NEW CISPLATIN FORM USING A BASE MATERIAL BY COMBINING POLY-D,L-LACTIC ACID AND POLYETHYLENE GLYCOL ACID AGAINST PERITONEAL METASTASIS

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Microspheres containing cisplatin (CDDP) embedded in poly-d,l-lactic acid (PLA) and polyethylene glycol acid (CDDP-PPMS) were developed to improve treatment of malignant effusions. *In vitro* studies demonstrated that CDDP was released continuously for more than 4 weeks from CDDP-PPMS without initial burst. CDDP-PPMS was compared with CDDP aqueous solution (CDDP-SOL) by i.p. administration in rats for 1) tissue distribution, 2) toxicity and 3) therapeutic effects against Yoshida sarcoma. We found that the CDDP concentration in the omentum was maintained at a higher level than in the CDDP-SOL group, while the particles of CDDP-PPMS were observed in the stomata of the omentum by electron microscopy. Concentrations of CDDP in the lung, liver, kidney and blood were lower in the CDDP-PPMS group than in the CDDP-SOL group. All rats given CDDP-PPMS containing ≤28 mg/kg were alive, whereas in the CDDP-SOL group, all rats given ≥16 mg/kg died from side effects. The LD_{50} of CDDP-PPMS and CDDP-SOL were 32.8 and 14.8 mg/kg, respectively. The survival of rats with peritoneal metastasis was better in the CDDP-PPMS group than in the CDDP-SOL group. *Int. J. Cancer* 76:709–712, 1998.
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Despite recent advances in treatment, carcinomatous peritonitis in gastrointestinal cancers accounts for a significant number of cancer deaths. Examination of i.p. free cancer cells has been used to estimate the prognosis of cancer patients (Jaehne *et al.,* 1989; Boku *et al.,* 1990; Leach *et al.,* 1995). Water-soluble anticancer agents are one of the most common treatment modes for patients with peritoneal metastasis. When administered locally, water-soluble anticancer agents rapidly pass into circulating blood and selective distribution of the agents to the target organ is rarely achieved. Increasing interest has therefore been focused on targeting chemotherapy using drug delivery systems (Hagiwara *et al.,* 1992; Yan *et al.,* 1993; Natsugoe *et al.,* 1995; Vaage *et al.,* 1995).

The purpose of locoregional chemotherapy is to selectively supply a high concentration of anticancer agents to the organs bearing a tumor and to maintain locally high concentrations of the anticancer agents as long as possible. Poly-d,l-lactic acid (PLA) is one of the most desirable base materials for drug delivery. It is widely distributed in animals, microorganisms and plants. PLA can be degraded either enzymatically or non-enzymatically by dissolution of ester bonds (Fukuzaki *et al.,* 1989). PLA is a biodegradable and biocompatible material which is used for surgical sutures (Postema and Pennings, 1989), osteoplastic materials (Zimmerman *et al.,* 1987) and carriers for drug delivery (Asano *et al.,* 1989; Hagiwara *et al.,* 1993*a;* Chang *et al.,* 1996; Natsugoe *et al.,* 1997).

Cisplatin (CDDP) is one of the most effective anti-cancer agents. However, this drug has some serious side effects, including renal dysfunction, bone marrow suppression, nausea, emesis and anorexia. CDDP microspheres decrease the incidence of side effects and enhance the therapeutic effects compared with CDDP aqueous solution (Hagiwara *et al.,* 1993*a;* Sasakura *et al.,* 1992; Kumagai *et al.,* 1996). Microspheres containing CDDP have been shown to be superior to CDDP solution for both treatment effect and reduction of side effects (Hagiwara *et al.,* 1993*a;* Kumagai *et al.,* 1996; Ohta *et al.,* 1993). However, the initial burst of CDDP microspheres remains a problem. If microspheres are prophylactically administered just after surgery, side effects from the initial burst of drug may adversely affect patients who are recovering from surgical stress.

We have attempted to formulate a new CDDP form which reduces the initial burst release of CDDP from microspheres by changing the molecular weight (m.w.), the type of polyester, the diameter of the microsphere and the m.w. distribution in order to further reduce side effects. We devised a base material by combining PLA and polyethylene glycol acid (PEG). In this study, we investigated the basic properties of CDDP-PPMS, their toxicity and their efficacy against transplanted tumor in rats.

PREPARATION AND PROPERTIES OF CDDP-PPMS

Cisplatin (CDDP, Nippon Kayaku, Tokyo, Japan) incorporated into PLA/PEG microspheres was prepared by a solvent evaporation method in an oil-in-oil emulsion system. After CDDP and PLA/PEG had been dissolved in acetonitrile solution as a dispersed phase, silicon oil was added and the mixture was stirred at 100 rpm in a 45°C bath for 24 hr. One hundred milligrams of microspheres was suspended in 20 ml of Tris buffer solution adjusted to pH 7.4. The solution was placed in a shaker bath with a shaking rate of 2.5/sec. Medium was periodically changed. The amount of CDDP released into the Tris buffer solution was measured by atomic absorption spectroscopy (Hitachi, Tokyo, Japan). The lower limit of the CDDP concentration was 0.05 µg/ml.

Some *in vitro* experiments were performed to determine the microsphere m.w., diameter and PLA/PEG ratio. Release profiles of CDDP from CDDP-PPMS were examined with different weight-averaged m.w. of PLAs: 5,000, 10,000, 20,000, 241,900 and 266,200. The release rate was reduced in proportion to the increase in weight-averaged m.w. of PLA (Fig. 1). Therefore, the 266,200 m.w. PLA was selected because it released CDDP at the slowest rate. Release profiles were also examined by changing the diameter of the microsphere (223, 109 and 68 µm); the larger the diameter, the slower the release (Fig. 2). Microspheres of 223 µm were most suitable. Release profiles of CDDP from PLA/PEG microspheres were finally examined at various rates of PEG: 0% wt, 10% wt, 15% wt, 20% wt and 30% wt using the 2,010 weight-averaged m.w. of PEG. Release profiles were controlled according to the proportion of PEG content (Fig. 3). No initial burst was found in microspheres containing 10% wt PEG.

Accordingly, we used the CDDP-PPMS with a 266,200 weight-averaged m.w. of PLA, 223 µm in diameter and containing 10% wt

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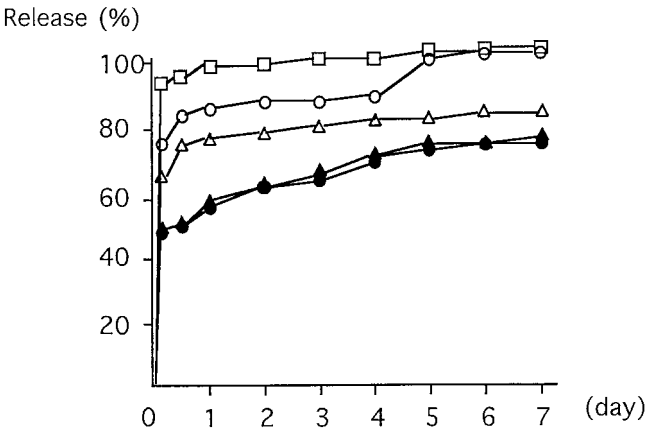

FIGURE 1 – Release rate of CDDP from CDDP-PLA at different m.w.: (○) 5,000, (△) 10,000, (□) 20,000, (▲) 241,900 and (●) 266,200.

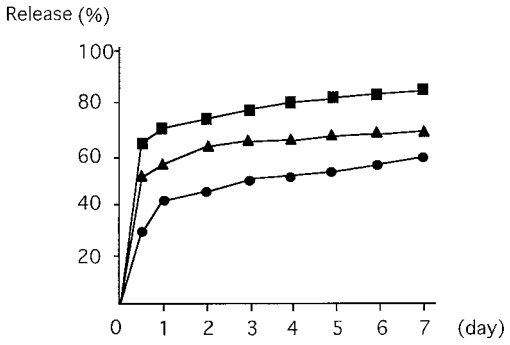

FIGURE 2 – Release rate of CDDP from CDDP-PLA at different diameters of PLA: (●) 223 µm, (▲) 109 µm and (■) 68 µm.

PEG. The release rates of CDDP from this CDDP-PPMS were 14% after 1 day, 25% after 5 days, 33% after 7 days, 50% after 14 days, 66% after 21 days and 85% after 30 days (Fig. 4).

MATERIAL AND METHODS

Distribution of CDDP after i.p. administration of CDDP-PPMS in vivo

Male Donryu strain rats, 6 weeks old and weighing 180–200 g, were obtained from Kyushu Animal Laboratory (Kagoshima, Japan) and were kept on a 12 hr light/12 hr dark schedule in a temperature- and humidity-controlled clean room (22°C, 66%) in the Institute for Laboratory Animal Research, Kagoshima University School of Medicine. A total of 128 rats were divided into 2 groups: CDDP-PPMS (n = 64) and CDDP aqueous solution (CDDP-SOL) (n = 64). In the CDDP-PPMS group, CDDP-PPMS containing 5 mg/kg of CDDP dissolved in 1.0 ml aqueous solution was administered i.p. In the CDDP-SOL group, 1.0 ml of CDDP-SOL containing 5 mg/kg of CDDP was administered similarly. On days 1, 3, 5, 7, 14, 21 and 30 after drug was administered to 8 rats in each group, the great omentum, lungs, liver, kidneys and blood were removed for measurement of CDDP concentrations. Each sample was weighed and then carbonized at 200°C in a Hot Block Bath (Iwaki) after 1 ml of nitrous acid had been added. During carbonization, a few drops of 60% HClO were added to evaporate NO_2 ($HClO_4$-HNO_3 method) (Kojima and Kiyozumi, 1974). CDDP concentrations were measured by atomic spectroscopy as described above. A portion of resected omentum was examined by scanning electron microscopy.

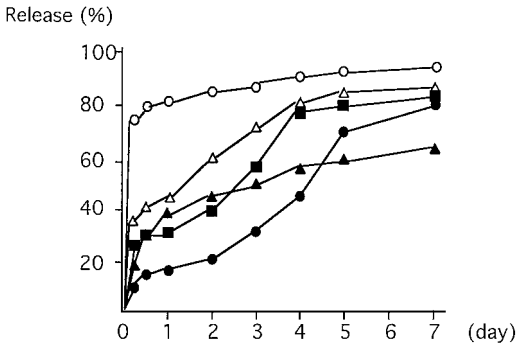

FIGURE 3 – Release rate of CDDP from CDDP-PPMS at different PEG acid contents: (△) 0% wt, (●) 10% wt, (■) 15% wt, (▲) 20% wt and (○) 30% wt.

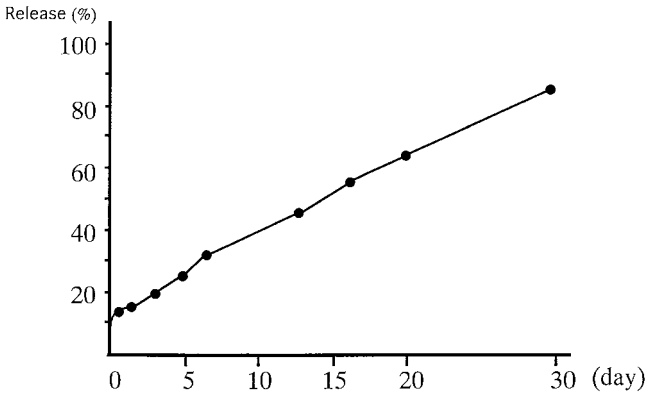

FIGURE 4 – Release profile of CDDP from CDDP-PPMS with 266,200 weight-averaged m.w., 223 µm in diameter and containing 10% wt PEG.

Animal toxicity

A total of 108 rats were divided into 12 groups according to CDDP amount: 8, 10, 12, 16, 24, 28, 32 and 40 mg/kg in the CDDP-PPMS group and 8, 10, 12 and 16 mg/kg in the CDDP-SOL group. After drug had been administered i.p., rats were checked daily for 14 days. Rats that died before 14 days were autopsied as were the remaining rats that were sacrificed on day 14.

Anti-tumor efficacy

A total of 54 male Donryu rats were prepared for this experiment. A rat Yoshida sarcoma cell line was used as an experimental tumor (Kyowa Hakko Kogyo, Shizuoka, Japan). Tumor was passaged weekly as ascites cells by i.p. transplantation into young adult Donryu rats. In preliminary experiments, we confirmed that 6×10^6 Yoshida sarcoma cells established peritoneal metastases 6–12 hr after tumor inoculation (Tobai, 1981). Therefore, 24 hr after Yoshida sarcoma cell inoculation, CDDP-PPMS or CDDP-SOL was administered i.p. Treatment protocols were as follows:

A group (n = 9): CDDP-PPMS containing 16 mg/kg of CDDP
B group (n = 9): CDDP-PPMS containing 12 mg/kg of CDDP
C group (n = 9): CDDP-PPMS containing 8 mg/kg of CDDP
D group (n = 9): CDDP-SOL containing 8 mg/kg of CDDP
E group (n = 9): microspheres without CDDP
F group (n = 9): no treatment

All rats were checked daily and monitored for 60 days.

Statistical analysis

The statistical significance of differences was examined using the Student's *t*-test. $p < 0.05$ was considered statistically significant.

TABLE I – CDDP CONCENTRATIONS IN THE OMENTUM, KIDNEYS, LIVER AND BLOOD

Organ	Group	Time after drug administration (days)				
		1	7	14	21	30
Omentum	CDDP-PPMS	12.5 ± 3.0 *	5.4 ± 4.0 *	3.9 ± 1.5 *	1.7 ± 1.3 *	0.4 ± 0.2
	CDDP-SOL	2.1 ± 1.2	0.9 ± 0.4	0.6 ± 0.3	0.4 ± 0.2	0.1 ± 0.1
Kidney	CDDP-PPMS	1.4 ± 0.3 *	1.4 ± 0.3 **	1.1 ± 0.2 **	1.1 ± 0.1	—
	CDDP-SOL	6.9 ± 3.1	4.8 ± 0.2	3.7 ± 2.7	1.0 ± 0.8	—
Liver	CDDP-PPMS	1.4 ± 0.2 **	0.2 ± 0.1 **	0.2 ± 0	0.2 ± 0.1	—
	CDDP-SOL	2.4 ± 1.0	1.5 ± 0.4	1.3 ± 0.2	0.5 ± 0.2	—
Blood	CDDP-PPMS	0.06 ± 0.05	—	—	—	—
	CDDP-SOL	0.33 ± 0.2	0.08 ± 0.05	—	—	—

*$p < 0.01$.–**$p < 0.05$.

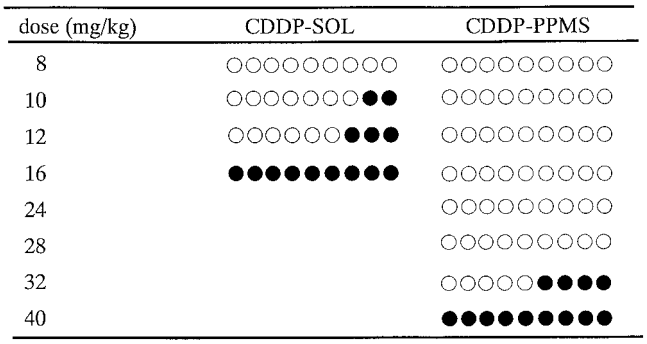

FIGURE 5 – Acute toxicity of CDDP-PPMS and CDDP-SOL: (○) alive and (●) dead.

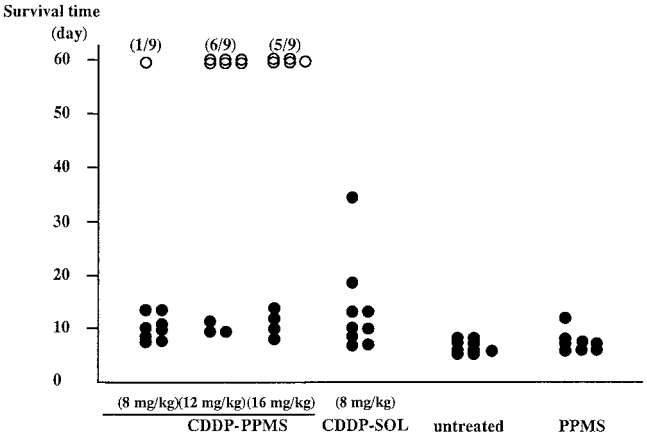

FIGURE 6 – Sixty-day survival with various treatments: (○) alive and (●) dead.

RESULTS

Drug distribution

The time course of changes in the CDDP concentration in the omentum was compared between CDDP-PPMS and CDDP-SOL (Table I). CDDP was maintained at a high level and was 4–6-fold higher than in the CDDP-SOL group during an observation period of 30 days. Even on day 30 after drug administration, the CDDP concentration after CDDP-PPMS remained >0.4 μg/g. Concentrations of CDDP in the lungs, liver, kidneys and blood are shown in Table I. In the CDDP-PPMS group, CDDP concentrations in these organs were lower than the concentrations in the CDDP-SOL group. Particles of CDDP-PPMS were observed at the stomata of the omentum by scanning electron microscopy.

Toxicity

When CDDP-PPMS containing 8, 12, 16, 24 and 28 mg/kg CDDP were administered, all rats were alive at 14 days. By contrast, CDDP-PPMS containing 32 and 40 mg/kg CDDP caused death in 44% (4/9) and 100% (9/9) of rats, respectively. Although all rats given CDDP-SOL containing 8 mg/kg CDDP were alive at 14 days, rats receiving CDDP-SOL at 10, 12 or 16 mg/kg CDDP exhibited serious side effects. The mortality rates for CDDP-SOL containing 10, 12 and 16 mg/kg CDDP were 22% (2/9), 33% (3/9) and 100% (9/9), respectively (Fig. 5). The LD_{50} values of CDDP-PPMS and CDDP-SOL were 32.8 and 14.8 mg/kg, respectively.

Thus, CDDP-PPMS groups consisting of 8, 12 and 16 mg/kg CDDP and a CDDP-SOL group of 8 mg/kg CDDP were prepared for the study of anti-tumor effects.

Anti-tumor efficacy of CDDP-PPMS

In the CDDP-PPMS group, peritoneal metastasis was absent in 11% (1/9), 67% (6/9) and 56% (5/9) of rats after treatment with 16, 12 and 8 mg/kg CDDP (A, B and C groups), respectively. All rats without peritoneal metastasis survived the entire 60 days.

Microscopic examination revealed no residual tumor in the omentum, peritoneum, lungs, liver, kidneys, gastrointestinal tract and lymph nodes in animals without macroscopic disease; these were deemed to exhibit a complete response. Microspheres were microscopically observed in the necrotic adipose tissues of the omentum. However, in a total of 15 rats that died within 60 days, autopsy findings revealed generalized metastases. Mean survival times were 8.0 ± 1.2, 43.0 ± 24.0, and 38.0 ± 24.6 days in the rats treated with 16, 12 and 8 mg/kg CDDP (A, B and C groups), respectively. In the CDDP-SOL group (D group), all rats died within 35 days with tumor metastases; mean survival was 11.5 ± 4.7 days. The survival time of rats treated with CDDP-PPMS containing 12 or 16 mg/kg CDDP was significantly better than in rats treated with CDDP-SOL containing 8 mg/kg CDDP. Rats given microspheres without CDDP (E group) and untreated rats (F group) all died within 12 days as a result of systemic tumor metastases (Fig. 6).

DISCUSSION

In vitro studies revealed that m.w. and diameter significantly affected the initial burst and release rate of CDDP from CDDP-PPMS, as did the PEG acid content. By controlling these variables, only 14% of total CDDP was released after 1 day and CDDP was slowly released from CDDP-PPMS for more than 30 days.

In experiments on CDDP distribution, the CDDP concentration of the omentum in the CDDP-PPMS group was higher than the CDDP-SOL group. Some reports have shown that cancer cells seeded i.p. specifically infiltrate the omentum in the early stages of peritoneal metastases (Kiyasu *et al.*, 1981; Lawrance *et al.*, 1991; Hagiwara *et al.*, 1993*b*). In this study, the presence of particles of CDDP-PPMS in the stomata of the omentum was confirmed using electron microscopy. This suggests that CDDP-PPMS slowly releases CDDP into the omentum by degradation of ester bonds, since the stomata of the omentum are one of the sites where CDDP-PPMS is selectively drained.

By contrast, the CDDP concentration in the blood, kidneys, liver and lungs was significantly lower in the CDDP-PPMS group than in the CDDP-SOL group, suggesting that local administration of CDDP-PPMS avoids major systemic side effects. Although renal dysfunction is one of the most common side effects caused by CDDP, administration of CDDP-PPMS might avoid renal toxicity, while allowing for a 3-fold higher CDDP dose to be administered by CDDP-PPMS.

In the CDDP-SOL group, rats given more than 10 mg/kg had serious side effects and rats given 8 mg/kg CDDP in both the CDDP-PPMS and the CDDP-SOL groups did not have improved survival duration. Only rats treated with CDDP-PPMS containing $\geq$12 mg/kg CDDP survived well. Thus, CDDP-PPMS provides a superior effect with high doses of CDDP and avoids significant toxicity.

In conclusion, our results demonstrate that CDDP-PPMS reduces the initial burst and delivers CDDP selectively to the omentum. Furthermore, CDDP-PPMS is superior to CDDP-SOL in both treatment efficacy and reduction of side effects. CDDP-PPMS may be a useful locoregional mode of chemotherapy; future studies showed investigate the clinical significance of this treatment modality.

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