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Research paper

Organ distribution of cisplatin after intraperitoneal administration of cisplatin-loaded microspheres

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Abstract

The aim of this study was to clarify the organ distribution of cisplatin (CDDP) after intraperitoneal (i.p.) administration of cisplatin-loaded microspheres (CDDP-MS). The distribution of CDDP to normal organs lying in the peritoneal cavity after i.p. administration of CDDP-MS was assessed by comparing with subcutaneous administration to non-cancerous mice. The organ distribution of CDDP after i.p. administration of CDDP-MS shows that CDDP released from microspheres was distributed to the organs lying in the peritoneal cavity and in the retroperitoneum. These are mainly from the systemic circulation, but are not directly from the organ surface. The distribution of CDDP to tumors was evaluated in sarcoma180 tumor-bearing mice by comparing with a bolus injection. The CDDP-MS delivered CDDP to tumors more effectively than did bolus injection. The distribution of CDDP-MS in the peritoneal cavity was in accord with the tumor distribution. This concordance and sustained exposure of CDDP to the tumors might play a critical role in enhancing the CDDP accumulation in tumors. It is concluded that CDDP-MS have a distinct regional pharmacokinetic advantage for peritoneal carcinomatosis, and that i.p. administration of CDDP-MS is an effective treatment for peritoneal carcinomatosis.

Keywords: Cisplatin; Microspheres; Organ distribution; Intraperitoneal administration; Tumor; Kidney

1. Introduction

Peritoneal carcinomatosis is a serious concern in therapy for abdominal tumors such as hepatic or gastric tumors. For the treatment of peritoneal carcinomatosis, intraperitoneal (i.p.) administration of anticancer drugs was introduced with the intention of achieving tumoricidal drug levels locally while minimizing systemic side effects [1,2]. Cisplatin (*cis*-dichlorodiammine-platinum[II]: CDDP), which is clinically effective against a variety of cancers, has been administered intraperitoneally in order to achieve a greater concentration in the compartment containing tumors, while reducing the patient's systemic exposure to the drug [3,4]. However, the CDDP-based i.p. chemotherapy in a clinical trial was restricted to a small tumor (<1–2 cm) [5] and a small number of tumor cells because penetration of CDDP into the tumor was not sufficient to kill the tumor cells located in the center of larger tumors [6,7]. To penetrate into a tumor deeply, CDDP must be in contact with a tumor for a longer period of time or at a considerably higher concentration. However, high concentrations of CDDP in the peritoneal cavity lead to a high plasma concentration, which results in systemic side effects. Therefore, to overcome the restricted anti-tumor activity, recent studies have focused on new chemotherapeutic approaches using biodegradable microspheres, which allow for a long-term, sustained release of CDDP (CDDP-MS) [8–11].

In order to develop CDDP-MS into a clinical agent, a series of preclinical studies have to be conducted. In our study using human tumor xenografts, the administration of CDDP-MS resulted in a continuous high CDDP concentration in ascites, and induced a sustained tumor growth inhibition along with a prolonged survival time (unpublished data). The therapeutic efficacy of CDDP-MS could be intimately related to the organ distribution of CDDP. For this reason, especially, a pharmacokinetic study is quite important in order to obtain a 'proof of concept' to justify an improved formulation. Nephrotoxicity is known to be the dose-limiting factor for CDDP chemotherapy. Moreover, a large amount of the drug administered intraperitoneally is

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known to be absorbed into systemic circulation through the portal vein [12]. Accordingly, the aim of this study is to clarify the distribution characteristics of CDDP in kidneys and liver. In this study, we compare the organ distribution characteristics of i.p. administration with subcutaneous administration of CDDP-MS to evaluate the distribution route of CDDP to these organs. The therapeutic efficacy of CDDP-MS is intimately related to the distribution of CDDP to tumors. Therefore, the other aim of this study is to clarify the tumor-distribution characteristics of CDDP after i.p. administration of CDDP-MS, compared with a bolus injection of CDDP solution. In order to assess the distribution of the total amount of CDDP, the total platinum (total Pt, which corresponds to the total concentration of protein-bound CDDP plus CDDP) concentrations in various organs were evaluated.

2. Materials and methods

2.1. Chemicals

CDDP was obtained from Haraeus GmbH Produktbereich Chemie (Hanau, Germany). Poly(DL-lactic acid), (PLA) was obtained from Mitsui Chemicals (Tokyo, Japan). Poly(vinyl alcohol) (EG-40) was purchased from Nippon Synthetic Chemical Co., Ltd (Osaka, Japan). Sodium carboxymethyl cellulose was purchased from Nichirin Chemical (Hyogo, Japan). Tween 80 was obtained from Sigma Chemical, (Missouri, USA). All other chemicals were of reagent grade.

2.2. Preparation of CDDP-MS

CDDP-MS was prepared according to the solvent evaporation method [9–11]. In brief, 1.1 g of PLA (Mw = 9000) and 0.8 g of PLA (Mw = 22,000) were dissolved in 4.5 g of methylene chloride (Katayama Chemical, Osaka, Japan), and then 100 mg of pulverized CDDP crystals were dispersed therein to develop an oil phase. This oil phase was then emulsified in 8 ml of the water phase containing poly(vinyl alcohol) at a concentration of 0.5% using a high-shear homogenizer (Polytron®, Kinematica Ag Littau, Switzerland). The resultant emulsion was poured into 1000 ml of water to extract the solvent. The solidified microspheres were washed with an adequate amount of water and filtered. The obtained microspheres were freeze-dried. The content of CDDP in microspheres was determined by the method reported elsewhere [10]. The particle size distribution of CDDP-MS was analyzed by SALD-1100 particle size analyzer (Shimadzu, Kyoto, Japan).

2.3. Pharmacokinetic study in non-cancerous mice

Animal experiments were carried out in accordance with the ethical guidelines established by the Animal Experimental Ethical Committee of Tanabe Seiyaku Co. Ltd. A predetermined amount of CDDP was dissolved in saline, and CDDP-MS were suspended in saline containing 0.5% sodium carboxymethyl cellulose and 0.1% Tween 80. CDDP dissolved in saline was administered i.p. at a dose of 20 mg/20 ml/kg to 40 male *ddy* mice (7 weeks of age, SLC, Shizuoka, Japan). CDDP-MS was administered by i.p. or subcutaneous injection at a dose of 20 mg/20 ml/kg to 32 mice. At 10 min, 0.5, 1, 1.5, 2 h and 1, 3, 7, 14, 21 days after CDDP bolus administration and at 3, 7, 10, 14, 17, 22, 27, 34 days after CDDP-MS administration, whole blood, liver, kidneys and lung were collected to determine the total Pt concentration. Whole blood was centrifuged at 12,000 rpm for 5 min to obtain plasma samples. All samples were stored in a refrigerator at −80°C before analysis.

2.4. Determination of total Pt concentration

The total Pt concentrations in plasma, kidneys, liver and lung were determined by flameless atomic absorption spectroscopy. In brief, 0.1 g of the organ samples were added to 1.0 ml of acidic solution (nitric acid:perchloric acid:sulfuric acid = 24:24:1) and heated at 200°C for 2 h. The Pt was then extracted in methyl isobutyl-ketone (Katayama Chemical, Osaka, Japan) after chelating with ammonium-pyroridine-carbamate (Wako Pure Chemical, Osaka, Japan). After washing the methyl isobutyl-ketone layer with 0.1 N hydrochloric acid, the organic phase was evaporated to dryness under nitrogen gas flow. This residue was dissolved in methyl isobutyl-ketone again immediately before assay. The Pt concentrations were determined using a model Z8200 atomic absorption spectrometer (Hitachi, Tokyo, Japan). A seven-stage heating program was used, consisting of drying at 50–140°C for 40 s, drying at 140°C for 10 s, ashing at 600–800°C for 5 s, ashing at 1000°C for 25 s, atomizing at 2700°C for 10 s, and conditioning at 2800°C for 5 s. Nitrogen gas was used as the inert carrier gas. The average value of duplicate or triplicate determinations was used for calculation of total Pt concentration.

2.5. Pharmacokinetic study in tumor-bearing mice

Sarcoma180 (S180) tumor cells, purchased from Dainippon Pharmaceutical (Osaka, Japan), were maintained in RPMI 1640 medium (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) containing 10% fetal bovine serum. The tumor cells were collected and diluted into a single cell suspension containing 5×10^6 cells/ml. The male *ddy* mice (7 weeks of age, SLC, Shizuoka, Japan) received i.p. inoculations of 1×10^6 cells/mouse on day 0, and the drugs were administered by i.p. injection on day 5. To compare the organ distribution at equivalent doses for systemic toxicity, the maximum tolerated doses (MTD) of CDDP saline solution and CDDP-MS were determined in a pilot study. The MTD of CDDP saline solution and CDDP-MS at male *ddy* mice were 14 and 35 mg/20 ml/kg, respectively. The organ distribution was, therefore, evaluated at these doses. At 0.5 h, 1, 3, 7, 10, 14 days after CDDP bolus administration and at 1, 3, 7, 10, 14, 17, 21, 27 days after CDDP-MS

administration, the plasma, tumors and kidneys were collected periodically, and total Pt concentrations in these organs were assayed as described above.

2.6. Pharmacokinetic analysis

Pharmacokinetic parameters were determined from the total Pt concentration versus time data in each organ. The maximum organ concentration (C_{max}) of total Pt was read directly from concentration versus time data. The area under the curve of concentration versus time from zero to the time of last measurable concentration point (AUC_{0-t}) was determined according to the trapezoidal rule. The C_{max} and AUC_{0-t} were calculated using the mean value of each point.

2.7. Distribution characteristics of CDDP-MS and tumors in the peritoneal cavity

Sixteen male *ddy* mice (7-week-old, Japan SLC, Shizuoka, Japan) were anesthetized with diethyl ether. The mice received a midline laparotomy incision (approximately 0.5 cm) and S180 tumor cells were delivered through this incision (1×10^6 cells/mouse). This procedure was chosen because peritoneal metastasis often occurs after a surgical operation in the clinic. The fascia and skin were closed with a surgical adhesive (aron alpha A, Sankyo Co., Ltd, Tokyo, Japan) and with sutures made of silk (0.20–0.27 mm, Natume Co., Ltd, Tokyo, Japan). Five days after tumor cell inoculation, CDDP-MS were given at a dose of 35 mg/20 ml/kg intraperitoneally. Two days following administration, all mice were sacrificed. The CDDP-MS and tumors were carefully removed from the surface of the organs, and their weights were measured. The amount (%) distributed to each organ was calculated as a percentage of the total weight of recovered CDDP-MS and tumors.

2.8. Statistical analyses

All statistical analyses were accomplished using the Statistical Analysis System software (SAS Institute Inc., Cary, NC, USA). Statistical comparisons were performed with Student's *t*-test. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of CDDP-MS

The CDDP content and mean diameter of the CDDP-MS were $4.5 \pm 0.2\%$ and 47 μm, respectively. Fig. 1 shows the particle size distribution of CDDP-MS. Particles having diameters less than 10 μm made up about 2% of the total.

3.2. Pharmacokinetic study in non-cancerous mice

Fig. 2 shows the total Pt concentration–time profiles for plasma, kidneys, liver and lung after i.p. bolus injection of CDDP and i.p. or subcutaneous administration of CDDP-MS. Following bolus injection, reflecting a high plasma concentration, total Pt concentration in kidneys and liver reached up to 40 μg/g organ, and thereafter they declined biphasically. The total Pt concentration in the lung was lower than in other organs. After i.p. or subcutaneous administration of CDDP-MS, total Pt concentrations in kidneys and liver were maintained at around 2–6 μg/g organ for 35 days, and there was no sudden increment, as in the bolus method. Rather, there was a sustained plasma concentration of CDDP. The lung concentration was around 1 μg/g organ, which was lower than the concentration measured in liver and kidneys as well as that measured after bolus injection.

Table 1 summarizes the C_{max} and AUC_{0-t} of total Pt concentration in each organ after i.p. bolus injection of CDDP or after i.p. or subcutaneous administration of CDDP-MS. The C_{max} values in all organs after CDDP-MS administration were considerably lower than after bolus injection. In kidneys, the C_{max} and $AUC_{0-34\ days}$ after i.p. administration of CDDP-MS were 4.0 μg/g organ and 77.3 μg × day/g organ, respectively. These parameters were almost equal to values after subcutaneous administration of CDDP-MS (C_{max}; 5.2 μg/g organ, $AUC_{0-34\ days}$; 90.7 μg × day/g organ). A similar result was observed in liver and lung. The ratios of AUC_{0-t} in each organ to that in plasma (AUC_{organ}/AUC_{plasma}), which are characteristic of organ distribution from the systemic circulation, were similar according to measurements made using three experimental methods.

3.3. Pharmacokinetic study in tumor-bearing mice

Fig. 3 shows the total Pt concentration–time profiles of plasma, tumors and kidneys after i.p. bolus injection of CDDP or i.p. administration of CDDP-MS to S180 tumor-

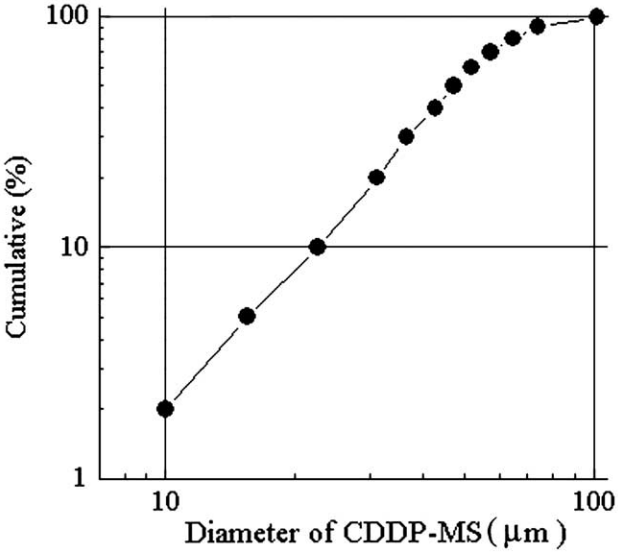

Fig. 1. Particle size distribution of CDDP-MS.

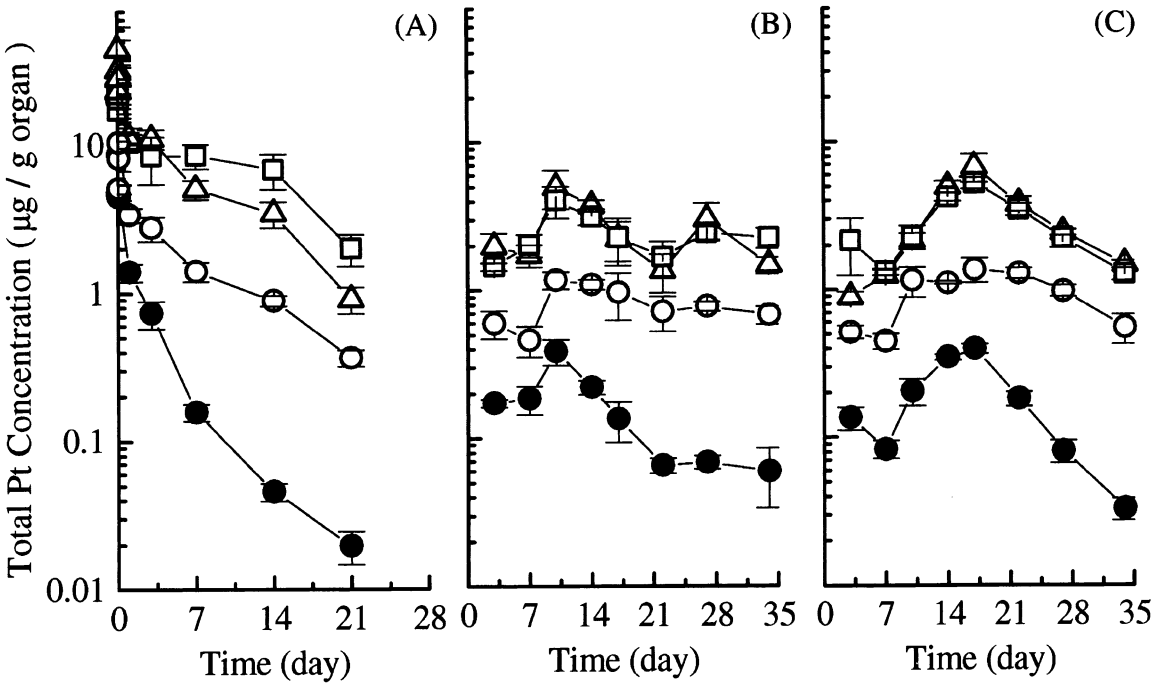

Fig. 2. The total Pt concentration–time profiles in plasma (●), kidneys (△), liver (□) and lung (○) after (A) i.p. bolus injection of CDDP, (B) i.p. administration of CDDP-MS and (C) subcutaneous administration of CDDP-MS to non-cancerous mice. All experiments were carried out at a dose of 20 mg/kg. Each point represents the mean ± SE from four mice.

bearing mice. Following bolus injection of CDDP, the total Pt concentration in tumors was initially 20 μg/ml, and thereafter it declined biphasically. The total Pt concentration in kidneys was the same as in tumors at all time points. After i.p. administration of CDDP-MS, the concentration of total Pt in tumors was approximately 100 μg/g organ, which was significantly higher than that of the kidney at all time points. This concentration was maintained for 28 days.

Table 2 summarizes the characteristics of organ distribution after i.p. bolus injection of CDDP and i.p. administration of CDDP-MS to S180 tumor-bearing mice. The $AUC_{0-14\ days}$ in kidneys and tumors after bolus injection were 72.3 and 36.8 μg/g organ, respectively. The $AUC_{0-27\ days}$ in these organs after CDDP-MS were 178 and 2566 μg/g organ, respectively. In kidneys, the AUC_{organ}/AUC_{plasma} values were no different between bolus and CDDP-MS administration, and also did not differ for non-cancerous mice. On the other hand, the AUC_{organ}/AUC_{plasma} value in tumors after CDDP-MS (270) was considerably higher than after bolus injection (8.2).

3.4. Distribution characteristics of CDDP-MS and tumors in peritoneal cavity

Table 3 summarizes the distribution characteristics of CDDP-MS and tumors in the peritoneal cavity. CDDP-MS was distributed to the greater omentum, the surgical repair site, and the scrotum with high frequency. The amounts distributed to the greater omentum and the surgical repair site were 28 and 30% of that found in these organs, which in turn contained 58% of total Pt. The tumors were located primarily in the greater omentum and at the surgical repair site.

Table 1
Pharmacokinetic parameters of total Pt after administration of CDDP saline solution or CDDP-MS at a dose of 20 mg/kg to non-cancerous mice

Formulation	Route	Plasma		Liver		Kidney		Lung	
		C_{max} [a]	AUC_{0-t} [b]	C_{max} [a]	AUC_{0-t} [b]	C_{max} [a]	AUC_{0-t} [b]	C_{max} [a]	AUC_{0-t} [b]
CDDP solution	i.p.	20.2	8.2	22.4	157.2 (19.2)	44.5	125.5 (15.3)	10.4	42.8 (5.2)
CDDP-MS	i.p.	0.38	4.9	5.0	82.6 (16.9)	4.0	77.3 (15.8)	1.2	26.6 (5.4)
CDDP-MS	s.c.	0.39	5.8	6.7	97.8 (16.9)	5.2	90.7 (15.6)	1.4	30.9 (5.3)

[a] The C_{max} was determiued using mean values of each point (μg/g organ).

[b] AUC_{0-t} is the AUC from time 0 through the last sampling point (μg × day/g organ). The AUC_{0-t} were calculated using mean values of each point. Values in parentheses are the ratios AUC_{0-t} in each organ to that in plasma (AUC_{organ}/AUC_{plasma}).

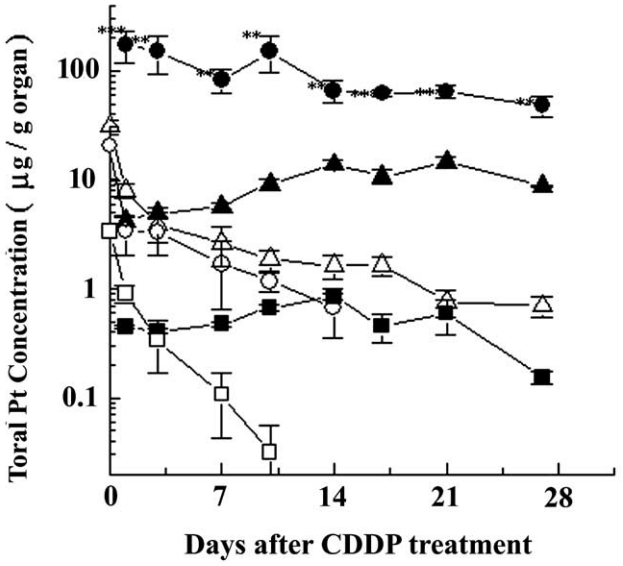

Fig. 3. The total Pt concentration–time profiles in plasma (□,■), tumors (○,●) and kidneys (△,▲) after i.p. bolus injection of CDDP or i.p. administration of CDDP-MS to S180 tumor-bearing mice. ○, △, □; CDDP bolus injection, ●, ▲, □; CDDP-MS. S180 tumor-cells were inoculated into mice on day 0, then CDDP saline solution and CDDP-MS were i.p. administered at doses of 14 and 35 mg/kg on day 5, respectively. Each point represents the mean ± SE from four mice. *** and ** designate $P < 0.001$ and $P < 0.01$, respectively, compared with total Pt concentration of kidneys after CDDP-MS administration.

Table 3
Distribution characteristics of CDDP-MS and tumors in peritoneal cavity after i.p. administration

Tissue	CDDP-MS[a]		Tumors[a]	
	Frequency	Amount (%)[b]	Frequency	Amount (%)[b]
Greater omentum	16/16	28	16/16	87
Surgical repair site	14/16	30	10/16	13
Liver surface	7/16	12	0/16	–
Kidney surface	1/16	3	0/16	–
Spleen surface	8/16	7	0/16	–
Scrotum	12/16	17	0/16	–
Abdominal wall	2/16	2	0/16	–
Cecum	2/16	2	0/16	–

[a] S180 tumor cells inoculated into mice on day 0, then CDDP-MS were i.p. administered at dose of 35 mg/kg on day 5. Two days following administration, all mice were sacrificed and assessed.
[b] Amount (%) was expressed as a percentage of the total weight of recovered CDDP-MS and tumors.

4. Discussion

The delivery of chemotherapeutic agents using polymeric microspheres has become a popular area of research because of the possibilities of achieving controlled release and of localizing the delivery of cytotoxic agents. Microspheres that remain in the peritoneal cavity and release CDDP for a long period, while limiting systemic exposure and toxicity, would represent an improvement over the current systemic formulation. However, microspheres with a diameter less than 8 μm [13] may disappear from the peritoneal cavity through the lymphatic capillaries [14,15]. The mean diameter of CDDP-MS used in this study was 47 μm and the number of microspheres less than 10 μm was about 2% of the total. Therefore, most of this CDDP-MS preparation might remain in the peritoneal cavity for a long period.

To characterize the organ distribution of CDDP after i.p. administration of CDDP-MS, the AUC_{organ}/AUC_{plasma} values after i.p. or subcutaneous administration of CDDP-MS are compared in Table 1. The AUC_{organ}/AUC_{plasma} value after subcutaneous administration characterizes the distribution of CDDP from the systemic circulation to each organ. The AUC_{organ}/AUC_{plasma} values of the lung, liver and kidneys after i.p. administration of CDDP-MS were very similar to that of subcutaneous administration, suggesting that CDDP released from microspheres in peritoneal cavity is mainly distributed to these organs from the systemic circulation, after being absorbed from the peritoneal cavity. This is probably due to the large surface of the peritoneal membrane, which consists of both visceral and parietal peritoneum. When CDDP released from CDDP-MS is exposed to this large membrane surface, rapid absorption may occur. On the other hand, there was a distribution of CDDP-MS to liver and kidney surfaces, as shown in Table 3. Nishida et al. [16] reported that low-molecular-weight drugs were absorbed from the liver surface. The liver and kidneys are covered by serous membranes consisting of squamous epithelial cells. There is a space between the serous membrane and parenchyma supported by connective tissue. In addition, liver and kidneys are covered by peritoneum. In

Table 2
Pharmacokinetic parameters of total Pt after i.p. administration of CDDP saline solution or CDDP-MS to S180 tumor-bearing mice

Formulation	Route	Dose (mg/kg)	AUC_{0-t}(μg × day/g organ)[a]		
			Plasma	Tumor	Kidney
CDDP solution[b]	i.p.	14	4.5	36.8 (8.2)	72.3 (16.1)
CDDP-MS[b]	i.p.	35	9.5	2566 (270)	178 (18.7)

[a] $AUC_{0-t}$is the AUC from time 0 through the last sampling point. The AUC_{0-t} were calculated using mean values of each point. Values in parentheses are the ratios of AUC_{0-t} in each organ to that in plasma (AUC_{organ}/AUC_{plasma}).
[b] S180 tumor cells inoculated into mice on day 0, then CDDP saline solution and CDDP-MS were i.p. administered at doses of 14 and 35 mg/kg on day 5, respectively.

order for CDDP to access the organ parenchyma, CDDP must penetrate the peritoneum and a connective tissue layer. It is suggested that peritoneum and connective tissue act as a physiologic barrier, restricting the distribution of CDDP to organ parenchyma from the organ surface.

The $AUC_{tissue}/AUC_{plasma}$ value after CDDP bolus injection was almost the same as that after CDDP-MS for all organs examined. A large amount of the drug administered intraperitoneally is known to be absorbed into systemic circulation through the portal vein [12]. Moreover, Yang et al. [17] reported that the extraction rate of CDDP from the liver was relatively high (approximately 60%) Therefore, there is a possibility that a slight change of the extraction rate at the liver, which might be caused by sustained exposure, alters the organ distribution of CDDP. However, the $AUC_{tissue}/AUC_{plasma}$ value of bolus injection was similar to that of the CDDP-MS after i.p. administration, suggesting that the distribution of CDDP to the liver was not affected by sustained release.

The CDDP-MS delivered CDDP to tumors more effectively than did bolus injection, as shown in Fig. 3 and Table 2. Tumor parenchyma disseminated in the peritoneum is separated from the peritoneal cavity only by a loose connective stroma [18]. Therefore, it is considered that the penetration of CDDP from a tumor surface is considerably faster than from a normal organ surface. It has been reported that penetration of doxorubicin [19] and paclitaxel [20] from tumor surfaces are time and concentration dependent. Likewise, CDDP must be in contact with a tumor for a long period of time or at a high concentration. Because CDDP is in contact with tumors for a much longer period of time after administration via MS, it is speculated that CDDP could penetrate into the tumor efficiently. The tumor cells metastasized and/or disseminated from a primary tumor to the peritoneal cavity tend to infiltrate the milky spots, which are lymphoid tissue distributed mainly in the greater omentum [21]. Furthermore, in humans surgical repair sites have also been shown to be preferentially seeded by tumor cells [22]. As shown in Table 3, our results supported these reports. CDDP-MS is also located in the greater omentum and surgical repair site frequently. Thus, distribution of CDDP-MS in the peritoneal cavity was in accord with the tumor distribution. It is considered that both CDDP-MS and tumor cells are particles with diameters in the range from 20 to 60 μm, and that they lead to similar distribution characteristics in the peritoneal cavity. This concordance might also play a critical role in enhancing the CDDP accumulation in tumors while lowering its concentration in kidneys.

5. Conclusions

The organ distribution of CDDP after i.p. administration of CDDP-MS shows: (1) CDDP released from microspheres was distributed to the organs lying in the peritoneal cavity and in the retroperitoneum. These are mainly from the systemic circulation, but not directly from organ surface; (2) CDDP-MS produced an effective accumulation of CDDP in tumors not only due to sustained exposure of CDDP to the tumors, but also to their location in the peritoneal cavity. It is concluded that CDDP-MS have a distinct regional pharmacokinetic advantage for peritoneal carcinomatosis, and that i.p. administration of CDDP-MS is an effective treatment for peritoneal carcinomatosis. This multimodality approach is feasible and pharmacokinetically advantageous enough to undergo further investigations.

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