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Pharmacokinetic behaviour of cisplatin in peritoneal fluid after intraperitoneal administration of cisplatin-loaded microspheres

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Abstract

The objective of this study was to establish a pharmacokinetic model for the estimation of unchanged *cis*-dichlorodiammine-platinum (II) (CDDP) concentration in peritoneal fluid after intraperitoneal administration of cisplatin-loaded microspheres (CDDP-MS) and to elucidate the accuracy of this model by comparisons between actual and simulated values after intraperitoneal administration of CDDP-MS. We developed a method enabling the precise and quick assessment of the drug concentration in the peritoneal cavity. The pharmacokinetic parameters obtained after intravenous bolus injection at a dose of 2 mg kg^{-1} were total body clearance (1026 mL h^{-1} kg^{-1}), elimination rate constant (3.24 h^{-1}) and distribution volume of systemic circulation (316.7 mL kg^{-1}). After an intraperitoneal bolus injection at a dose of 5 mg kg^{-1}, the absorption rate constant from the peritoneal cavity (3.64 h^{-1}) and the distribution volume of the peritoneal cavity (13.5 mL kg^{-1}) were determined. The protein-binding rate constant in ascites was 0.58 h^{-1}. Using these pharmacokinetic parameters, we established a pharmacokinetic model consisting of two compartments. Administration of CDDP-MS at a dose of 10 mg kg^{-1}, which released CDDP over 7 days in-vitro, yielded sustained concentrations of unchanged CDDP (1–2 mg mL^{-1}) in the peritoneal cavity that persisted for 7 days, and that were predictable by applying the in-vitro dissolution profile to the pharmacokinetic model. The findings obtained from this study are useful for understanding the basic pharmacokinetic characteristics of unchanged CDDP in the peritoneal cavity and may also be important in the development of optimized CDDP-MS formulations.

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Introduction

Free tumour cells are often observed in the peritoneal cavity of patients with ovarian cancer and gastric cancer and they may cause the accumulation of ascites (Gilly et al 1999). The uncontrollable accumulation of ascites in the peritoneal cavity produces various intolerable symptoms such as abdominal pain and distension. Therefore, an effective treatment to prevent the accumulation of ascites is very desirable in order to improve the quality of life of patients. Cisplatin (*cis*-dichlorodiammine-platinum (II); CDDP) is one of the most frequently used anticancer agents and it is often administered intraperitoneally as an aqueous solution to control ascites (Furukawa et al 1993; Alexander & Fraker 1996). The intraperitoneal administration of CDDP aqueous solution achieves high concentrations in the peritoneal cavity (Casper et al 1983; Lopez et al 1985), however

plasma concentrations are also high, causing serious side-effects such as nephrotoxicity. The anti-tumour effect of CDDP against tumour cell lines is known to be dependent on its concentration–time product (Kurihara et al 1995, 1996). Furthermore, Nagai & Ogata (1997) have reported that the maximum blood urea nitrogen (BUN) level, as an indicator of renal failure, was related to the area under the curve (AUC) calculated by the plasma concentration of unchanged CDDP greater than 0.9 mg mL^{-1}, a relationship described by the sigmoid E_{max} model. Therefore, it is very important for intraperitoneal chemotherapy using CDDP to maximize the unchanged CDDP concentration–time product in the peritoneal cavity and minimize the peak plasma concentration. To establish the ideal formulation, recent work has focused on new chemotherapeutic approaches with biodegradable microspheres, which allow long-term, sustained release of CDDP (Wada et al 1991; Matsumoto et al 1997).

Our ultimate aim is to determine the optimal CDDP-loaded microspheres (CDDP-MS) formulation for clinical consideration. The therapeutic efficacy of the CDDP-MS may be intimately related to their release characteristic in-vivo and subsequent fate in the peritoneal cavity. Therefore, to develop the optimal microsphere formulation, it is necessary to understand the pharmacokinetic behaviour of CDDP in peritoneal fluid after intraperitoneal administration. However, there have been very few reports on the fate of CDDP after intraperitoneal administration of sustained-release formulations. One reason for this is probably because there is no suitable analytical method for the precise and quick assessment of the drug concentration in peritoneal fluid, the amount of which is quite small in the healthy condition. Although the dialysistic technique has often been used (Zakaria & Rippe 1995; Zakaria et al 1996), this would not be feasible for our purpose, because the catheter has to be inserted in the peritoneal cavity during the experimental period, thus putting a lot of stress on the animal. Also, quick assessment is mostly difficult with this method.

The first objective of the present study was to develop a method enabling the precise and quick assessment of the drug concentration in the peritoneal cavity. The ability to predict CDDP concentrations in the peritoneal cavity from the in-vitro dissolution profile would be very useful for the development of CDDP-MS formulations in the clinical situation. Therefore, the second objective of this study was to establish a pharmacokinetic model to predict unchanged CDDP in peritoneal fluid after intraperitoneal administration of CDDP-MS in rats. To determine the parameters for the pharmacokinetic model, in-vitro protein-binding and pharmacokinetic analysis after intravenous and intraperitoneal administration of CDDP aqueous solution were evaluated. The accuracy of this pharmacokinetic model was determined by comparisons between actual and simulated values after intraperitoneal administration of CDDP-MS. In general, the anti-tumour effect of CDDP is related to the unchanged CDDP concentration and therefore we evaluated the unchanged CDDP concentration in this study.

Materials and Methods

Chemicals

CDDP was obtained from Haraeus GmbH Produktbereich Chemie (Hanau, Germany). Poly(DL-lactic acid) (PLA; weight averaged MW 19000 kDa, determined by gel permeation chromatography using polystyrene as a standard; TOSOH, Tokyo, Japan) and poly(DL-lactic-co-glycolic acid) (PLGA; lactic acid/glycolic acid, 52:48, weight averaged MW 6700 kDa) were obtained from Mitsui chemicals (Tokyo, Japan). Poly(vinyl alcohol)(EG-40) was purchased from Nihon Synthetic Chemical Industries Ltd, (Tokyo, Japan). Phenol red was purchased from Nacalai Tesque (Kyoto, Japan). Sodium carboxymethylcellulose (TS-1) was purchased from Nichirin Chemical Kogyou (Hyogo, Japan). Tween 80 was obtained from Sigma Chemical Co. (St Louis, MO). All other chemicals were of reagent grade.

CDDP-MS

CDDP-MS were prepared according to the polymer-alloy method (Matsumoto et al 1997). Briefly, 1.9 g of a mixture of PLA and PLGA (55:40) was dissolved in 4.5 g methylene chloride, and then 100 mg pulverized CDDP crystals (mean diameter 1.46 μm; 99% range from 0.1 to 11 μm) was dispersed to make an oil phase. The 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 (Polytronâ, Kinematica Ag Littau, Switzerland). The resultant emulsion was poured into 1000 mL water to extract the solvent. The solidified microspheres were washed with an adequate amount of water and then filtered. The obtained microspheres were freeze-dried.

For the in-vitro release study of CDDP-MS, 10-mg samples of CDDP-MS were put into test tubes. The tubes were filled with 10 mL phosphate-buffered saline (PBS; 0.15 M, pH 7.4) as the test fluid. The tubes were incubated with stirring at 25 rpm in an air chamber

maintained at 37 ± 1°C. Each test tube was taken out at 1 h, and 1, 2, 3, 4, 5, 6 and 7 days. The eluate was assayed for the CDDP concentration by reversed-phase HPLC with an ODS 120A column (4.6 × 250 mm) at a constant temperature of 45°C. The mobile phase consisted of 6 mM 1-octanesulfonate, 6 mM tetra-*n*-butylammonium hydrogen sulfate and 20 mM potassium dihydrogen phosphate, which was adjusted to pH 5.0 with 1 M sodium hydroxide. The flow rate applied was 0.7 mL min^{-1}. The spectrophotometric detector was set at 301 nm. The detection limit was 100 ng mL^{-1} and the intra- and inter-day variations were less than 5%. The release profile was determined by the average of triplicate release studies.

Development of assay for unchanged CDDP concentration in peritoneal fluid

We aimed to develop a new method for the precise assessment of unchanged CDDP concentrations in peritoneal fluid. This method was designed on the basis of calculations using the unchanged CDDP and the phenol red concentrations in a phenol red-containing solution injected into the peritoneal cavity. Briefly, a constant amount of solution containing a known concentration of phenol red is initially injected into the peritoneal cavity. After mixing with peritoneal fluid by gently rubbing the abdomen, the injected solution is recovered from the peritoneal cavity as quickly as possible. To determine the peritoneal fluid volume, the phenol red concentration in the recovered solution is determined. The unchanged CDDP concentration in the recovered solution is determined, and thereafter the unchanged CDDP concentration in peritoneal fluid is corrected by the volume of peritoneal fluid.

In-vitro reliability study

All animal experiments were carried out in accordance with the ethical guidelines established by the Experimental Animal Care and Use Committee of Tanabe Seiyaku Co., Ltd. To validate the method, it is essential that the concentration of phenol red can be determined accurately without interference by its biological components, and that phenol red and its biological components do not interfere with the assay of unchanged CDDP concentration.

To evaluate whether biological components in ascites interfere with the assay of phenol red, Yoshida sarcoma cells (1×10^7 cells) were inoculated intraperitoneally into 5-week-old male Donryu rats (Japan SLC, Shizuoka, Japan), and ascites removed at 5 days after the inoculation. Various amounts of ascites (0.25–9.0 mL) were put into test tubes. Then, 5 mL PBS containing phenol red at a concentration of 250 μg mL^{-1} was added to each tube. After mixing, the sample was diluted with 1 M sodium hydroxide and assayed for the phenol red concentration using a spectrophotometer (UV-2500PC, Shimadzu, Kyoto, Japan) at 560 nm. The volume of peritoneal fluid (V_{smp}) was calculated from the following equation:

$$V_{smp} = V_{std} \times (250 \times C_{smp}^{-1} - 1) \quad (1)$$

where C_{smp} is the phenol red concentration of the sample and V_{std} is the added volume of PBS containing 250 μg mL^{-1} phenol red (5 mL in this case).

The correlation between the actual amount of peritoneal fluid in the test tube and the determined values were calculated by the least-squares method. To validate the assay of the unchanged CDDP concentration, various concentrations of CDDP solution were added to the recovered solution from the peritoneal cavity after injection of PBS containing phenol red at 250 μg mL^{-1} to rats, and then assayed by post-column derivatization HPLC and atomic absorption using a spectrophotometer as described below.

In-vivo pharmacokinetics of unchanged CDDP

CDDP was dissolved in saline to give a 0.5 mg mL^{-1} CDDP solution and administered intravenously to 5-week-old male Donryu rats at the dose of 2 mg kg^{-1}. At a predetermined interval, a plasma sample was withdrawn from the jugular vein and the unchanged CDDP concentration in plasma was determined as described below.

CDDP was dissolved in saline to give a 0.5 mg mL^{-1} CDDP solution. CDDP-MS were suspended in saline containing 0.5% sodium carboxymethylcellulose and 0.1% Tween 80. The CDDP aqueous solution or the CDDP-MS suspension was administered intraperitoneally to 5-week-old male Donryu rats at the dose of 5 mg kg^{-1} and 10 mg kg^{-1}, respectively. The 20 rats receiving CDDP aqueous solution were divided into 5 groups (4 rats/group) and a group of animals was sacrificed at 10, 30, 60, 90 and 120 min after dosing. The 16 rats receiving CDDP-MS were divided into 4 groups (4 rats/group) and at 1, 3, 5 and 7 days after dosing, a group of animals was sacrificed. To collect the peritoneal sample, each rat was injected intraperitoneally with 5 mL PBS containing 250 mg mL^{-1} phenol red. After lightly rubbing the abdomen,

the peritoneal fluid was recovered as quickly as possible. A blood sample was collected from the vena cava.

The recovered peritoneal fluid and blood sample were centrifuged at 12000 rpm for 5 min at 4°C. To obtain an ultrafiltrated sample, a fraction (0.3 mL) of the supernatant was further centrifuged at 2000 *g* for 1 h at 4°C using an ultracentrifugator with a molecular weight cut-off of 30000 (Centifree-MPS3, Amicon Corp, MA). During the sample preparation, CDDP-MS was stable and further release of CDDP was not observed.

The peritoneal fluid sample obtained was diluted appropriately with 1 M sodium hydroxide, and then the phenol red concentration was analysed using a spectrophotometer at 560 nm. The peritoneal fluid volume was calculated from the absorbance of the sample according to equation 1 (V_{std} was 5 mL).

The unchanged CDDP concentrations in the ultrafiltrated sample of peritoneal fluid and plasma were determined by applying the post-column derivatization HPLC method described by Kizu et al (1995). The HPLC system consisted of a Shimadzu model LC-10A pump, a Sil-10AXL sample injector, a CTO-10A column oven for analysis column, a SPD-10A spectrophotometric detector operated at a wavelength of 290 nm, an Hitachi model L5020 column oven for post-column reaction and a Teflon post-column reaction coil (0.25 mm i.d., 5 m). A MCI gel CDR 10 column (100 mm × 4.6 mm i.d.,7 mm) (Mitsubishi Chemical, Tokyo, Japan) was used at 40°C. The mobile phase consisted of 50 mM sodium acetate buffer, adjusted to pH 4.8 with acetic acid. The post-column reagent solution consisted of 40 mM sodium bisulfite and 10 mM sodium acetate buffer, adjusted to pH 4.8 with acetic acid. The post-column reaction coil was maintained at 60°C. The unchanged CDDP concentration in peritoneal fluid (C_{peri}) was calculated from the following equation:

$$C_{peri} = C_{act} \times (1 + V_{std} \times V_{smp}^{-1}) \quad (2)$$

where C_{peri} and C_{act} are concentrations in the peritoneal fluid and ultrafiltrated sample, respectively; V_{std} is the volume of PBS containing 250 μg mL^{-1} phenol red (5 mL); and V_{smp} is the volume of peritoneal fluid calculated by equation 1.

Pharmacokinetic analysis

The simple model shown in Figure 1 was used in this study. It consisted of two compartments: a peritoneal fluid compartment with a volume V_1 and a concentration C_1, and a systemic circulation compartment with a volume V_2 and a concentration C_2. In this scheme, after CDDP-MS administration, unchanged CDDP is assumed to be infused with a zero-order rate (k_0) into peritoneal fluid. In the case of bolus intraperitoneal administration, unchanged CDDP is assumed to be rapidly distributed into the peritoneal fluid. In the peritoneal fluid compartment, unchanged CDDP is assumed to be eliminated by two pathways: absorption into the systemic circulation through the serosal membrane with a first rate constant k_a, and metabolism to irreversibly protein-bound CDDP with a first rate constant k_{1b}. The unchanged CDDP in plasma is also assumed to be eliminated at a clearance rate of CL_{tot}, which contains the renal clearance and metabolism to irreversibly protein-bound CDDP in plasma. Unchanged CDDP is assumed to be distributed rapidly into apparent volumes V_1 and V_2. Our model is described by the following differential equations.

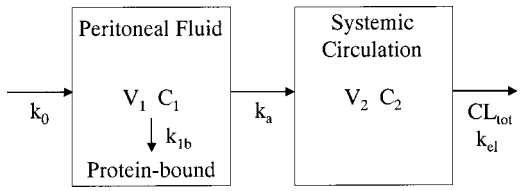

Figure 1 Pharmacokinetic model for unchanged CDDP disposition. V_1, volume of peritoneal fluid compartment; V_2, volume of systemic circulation compartment; C_1, unchanged CDDP concentration in peritoneal fluid; C_2, unchanged CDDP concentration in systemic circulation; k_0, zero-order rate to be infused into peritoneal fluid; k_a, absorption rate constant to the systemic circulation; k_{1b}, metabolism rate constant to irreversibly protein-bound CDDP in peritoneal fluid; k_{el}, elimination rate constant from systemic circulation; CL_{tot}, total clearance from systemic circulation.

$$dC_1\,dt^{-1} = (k_0 - k_a \times C_1 \times V_1 - k_{1b} \times V_1 \times C_1) \times V_1^{-1} \quad (3)$$

$$dC_2\,dt^{-1} = (k_a \times C_1 \times V_1 - CL_{tot} \times C_2) \times V_2^{-1} \quad (4)$$

To determine the protein-binding rate constant, ascites from Yoshida sarcoma tumour-bearing rats were used. The CDDP solution (10 μg mL^{-1} in saline) was added to 2.7-mL ascites samples to an initial concentration of 1 μg mL^{-1}, and the mixture was incubated at 37°C. Sequentially, samples were removed and protein-bound CDDP was removed by ultra-filtration (Centifree-MPS3, Amicon Corp, MA). The unchanged CDDP concentration in the filtrate was determined using the post-column derivatization HPLC method described above. To determine the protein-binding rate constant in ascites (k_{1b}), the first-order rate constant for protein binding of the parent drug was calculated by least

squares regression analysis on the log unchanged CDDP concentration–time profile.

The area under the curve of concentration–time data from time zero to the final sampling time (AUC_{0-t}) was determined according to the trapezoidal rule. The area under the curve of concentration–time data from time zero to infinity ($AUC_{0-\infty}$), k_a, V_1, CL_{tot}, and V_2 values were calculated from the following equations. The parameters in the peritoneal fluid compartment were calculated using the data of intraperitoneal bolus administration.

$$AUC_{i.p.0-\infty} = AUC_{i.p.0-t} + C_{t1} \times k_{app}^{-1} \quad (5)$$

$$V_1 = D_{i.p.} \times AUC_{i.p.0-\infty}^{-1} \times k_{app}^{-1} \quad (6)$$

$$k_a = k_{app} - k_{b1} \quad (7)$$

C_{t1} is the peritoneal fluid concentration at the final sampling time; k_{app} is the total rate constant of absorption of unchanged CDDP from peritoneal fluid to systemic circulation and protein binding, which was calculated by least squares regression analysis on log unchanged CDDP concentration in peritoneal fluid versus time data after intraperitoneal administration of CDDP aqueous solution; $D_{i.p.}$ is the administered dose of CDDP.

The parameters in the plasma compartment were calculated using the data of intravenous bolus administration.

$$AUC_{i.v.0-\infty} = AUC_{i.v.0-t} + C_{t2}\, k_{el}^{-1} \quad (8)$$

$$CL_{tot} = D_{i.v.} \times AUC_{i.v.0-\infty}^{-1} \quad (9)$$

$$V_2 = CL_{tot} \times k_{el}^{-1} \quad (10)$$

C_{t2} is the plasma concentration at the final sampling time; k_{el} is the first-order rate constant for elimination from systemic circulation, calculated by least squares regression analysis on log unchanged CDDP concentration in plasma versus time data after intravenous administration of CDDP aqueous solution; $D_{i.v.}$ is the administered dose of CDDP.

Results and Discussion

In-vitro reliability study

For the animal studies, the new analytical method, enabling the volumetric assessment of peritoneal fluid and the drug concentration, was validated. To validate the method, an in-vitro reliability test was first performed. An excellent correlation ($r = 0.99$) was observed between the actual amount of peritoneal fluid in the test tube and the determined value (data not shown). This suggests that the volume of peritoneal fluid can be accurately determined without interference by biological components. Considering the in-vivo situation, the absorption of phenol red from the peritoneal cavity might result in the overestimation of the peritoneal volume. The osmotic pressure of the phenol red solution that was injected into rats was isotonic, and the recovery of phenol red solution was carried out within 10 s after injection. Because the amount of phenol red (MW 354) absorbed within 10 s was less than 3%, calculated using the clearance value of ofloxacin (MW 361) from the peritoneal cavity (48.48 mL h^{-1} kg^{-1}) (Sato et al 1988), overestimation owing to the absorption of phenol red from peritoneal fluid could be ignored. The influence of the phenol red solution recovered from the peritoneal cavity on the quantification of unchanged CDDP was also examined. The detection limit of this assay was 1.0 ng mL^{-1}. Furthermore, the intra- and inter-day variation was less than 5% (data not shown). These results demonstrate that the method was valid for the precise assessment of the amount of peritoneal fluid and unchanged CDDP concentration.

Determination of model parameters

Parameters in systemic circulation

The concentration–time profile of unchanged CDDP in plasma after intravenous administration of CDDP aqueous solution is shown in Figure 2. The unchanged CDDP was rapidly eliminated from plasma according to the first-order rate. The pharmacokinetic parameters

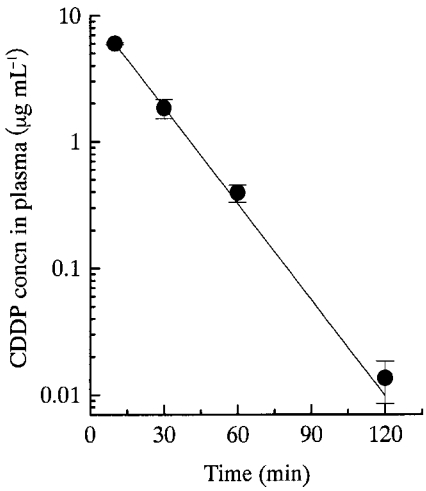

Figure 2 Unchanged CDDP concentration–time profile in plasma after intravenous administration of CDDP (2 mg kg^{-1}) aqueous solution to rats. Data represent mean ± s.d., n = 4.

Table 1 Pharmacokinetic parameters of unchanged CDDP in rats.

		IV[a] Plasma	IP[b] Peritoneal fluid
AUC_{0-2}	$\mu g \cdot h\ mL^{-1}$	1.92±0.90	87.60±3.74
$AUC_{0-\infty}$	$\mu g \cdot h\ mL^{-1}$	1.95±0.89	87.60±3.74
CL_{tot}	$(mL\ h)^{-1}\ kg^{-1}$	1026±50	–
k_a	$1\ h^{-1}$	–	3.64±0.11
k_{el}	$1\ h^{-1}$	3.24±0.44	–
V_1	$mL\ kg^{-1}$	–	13.5±0.58
V_2	$mL\ kg^{-1}$	316.7±27.6	–

[a]CDDP was administered at a dose of 2 mg kg^{-1}; [b]CDDP was administered at a dose of 5 mg kg^{-1}. Data represents the mean±s.d., n = 4.

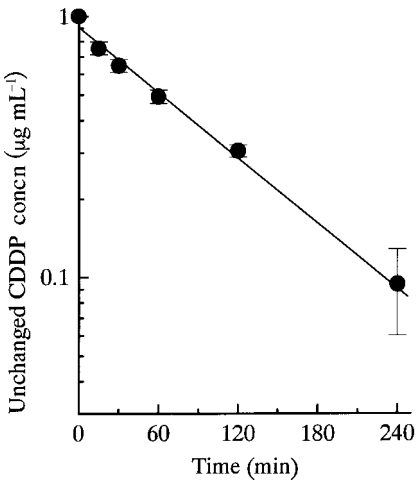

Figure 3 Concentration–time profile of unchanged CDDP after incubation with ascites. Data represent the mean±s.d. of four runs.

of unchanged CDDP in plasma calculated from intravenous administration are summarized in Table 1. The $AUC_{0-\infty}$, CL_{tot}, k_{el} and V_2 were 1.95 μg h mL^{-1}, 1026 mL h^{-1} kg^{-1}, 3.24 h^{-1} and 316.7 mL kg^{-1}, respectively. Among the pharmacokinetic parameters obtained, k_{el} and V_2 were quite similar to the reported values calculated from the intravenous administration data (King et al 1986; Litterst & Magin 1988). The CL_{tot} calculated from the plasma concentration of unchanged CDDP was considered the sum of renal clearance and protein-binding clearance. In rats with peritoneal carcinomatosis, we speculated that there is little change in systemic clearance compared with non-cancerous rats, because the distribution volume, except in the peritoneal cavity, and renal clearance were not changed in both animal models.

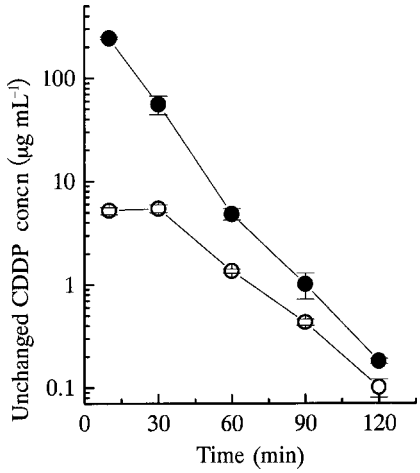

Figure 4 Unchanged CDDP concentration–time profile in peritoneal fluid (●) and in plasma (○) after intraperitoneal administration of CDDP (5 mg kg^{-1}) aqueous solution to rats. Data represent mean±s.d., n = 4.

Parameters in peritoneal fluid

Figure 3 shows the concentration–time profile of unchanged CDDP after incubation with ascites. The unchanged CDDP concentration was decreased at the first-order rate constant of 0.58±0.01 h^{-1}. The concentration–time profiles of unchanged CDDP in peritoneal fluid and plasma after intraperitoneal administration of CDDP aqueous solution are shown in Figure 4. These profiles indicated that unchanged CDDP was rapidly eliminated from the peritoneal cavity according to the first-order rate. In plasma, an absorption phase was found at an early stage; after 30 min, unchanged CDDP was eliminated, according to first-order rate. Although the unchanged CDDP concentrations in peritoneal fluid were much higher than those in plasma immediately after administration, they gradually decreased and reached levels similar to those in plasma within 2 h.

The pharmacokinetic parameters of unchanged CDDP in peritoneal fluid calculated from intraperitoneal administration are summarized in Table 1. $AUC_{0-\infty}$ of unchanged CDDP in peritoneal fluid was 87.60 μg h mL^{-1}. The k_a and V_1 values were 3.64 h^{-1} and 13.5 mL kg^{-1}, respectively. In general, elimination of unchanged CDDP was considered to occur through two pathways: absorption into systemic circulation and metabolism to protein-bound CDDP. The k_a was 6-fold larger than k_{1b}, suggesting that absorption is the major route of elimination of unchanged CDDP from peritoneal fluid.

Molecular weight and hydrophilicity were considered important factors influencing drug absorption from the

peritoneal cavity into systemic circulation (Myers & Collins 1983; Kuzuya et al 1997). CDDP is a hydrophilic compound with MW 300. The apparent first-order absorption rate constant of theophylline (MW 180) was reported to be 1.9 h^{-1} (Kuzuya et al 1997). Furthermore, it was reported that the clearance rates of norfloxacin (MW 319) and ofloxacin (MW 361) from the peritoneal cavity were 44.16 and 48.48 mL h^{-1} kg^{-1}, respectively (Sato et al 1988). Thus, the absorption rate constant (k_a = 3.64 h^{-1}) and clearance from peritoneal cavity (49.14 mL h^{-1} kg^{-1}, calculated from $k_a \times V_1$) of unchanged CDDP were approximately equal to that of xanthine derivatives, ofloxacin and norfloxacin, because these compounds have a similar molecular weight and hydrophilicity.

Characteristics of CDDP-MS

The CDDP content of CDDP-MS was 4.5 ± 0.2 %. The mean size of CDDP-MS was approximately 42 μm and 95 % of microspheres were over the range of 20–100 μm. The morphology of CDDP-MS was a multi-reservoir type (Matsumoto et al 1997), in which the CDDP crystals were distributed in the inner small particles and not near the surface of microspheres. The CDDP-MS had a smooth surface with no pit-holes (data not shown). The in-vitro drug release characteristics of the CDDP-MS are shown in Figure 5. The amount of CDDP released within 1 h was less than 3 %, which means that this formulation gives essentially no "initial burst", a common problem observed in microsphere formulations (Itoi et al 1996). Some researchers have reported the preparation of microspheres using an oil-in-oil emulsion method, in which CDDP and polymer were dissolved in dimethylformamide and this oil phase emulsified in liquid paraffin, resulting in non-crystal CDDP in microspheres (Itoi et al 1996). These CDDP-MS showed a large initial burst because of the rapid dissolution of CDDP distributed near the surface of the microspheres. In contrast, in this study, CDDP crystals were imbedded in the interior of the microspheres and consequently the CDDP-MS showed essentially no initial burst.

The steady release of CDDP from CDDP-MS was observed over 7 days, during which approximately 99 % of the drug was released. The average steady-state release rate in-vitro was 0.69 % h^{-1} under the conditions used. Release of encapsulated molecules from microspheres typically occurs by two mechanisms: the release of the drug by diffusion through pores formed in the polymer matrix after hydration, and the release of the drug as the polymer hydrolyses and the microspheres degrade (Fung and Saltzman 1997). The steady release from CDDP-MS might be explained by well-regulated diffusion of CDDP and polymer degradation. However, further experiments are necessary to clarify the dissolution mechanism, particularly because the microspheres contained both PLGA and PLA.

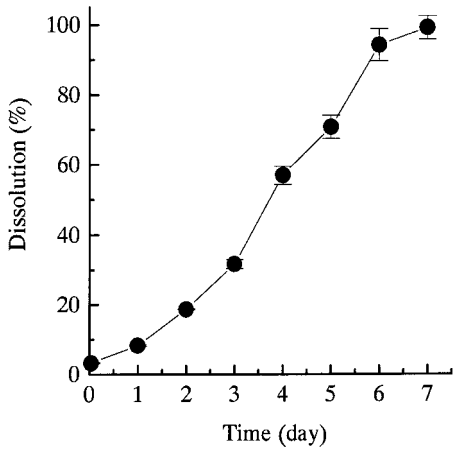

Figure 5 In-vitro CDDP release profile from CDDP-loaded microspheres. Data represent the mean ± s.d. of three runs.

In-vivo pharmacokinetics of unchanged CDDP after administration of CDDP-MS

The concentration profiles of unchanged CDDP in peritoneal fluid and plasma after intraperitoneal administration of CDDP-MS are shown in Figure 6. In peri-

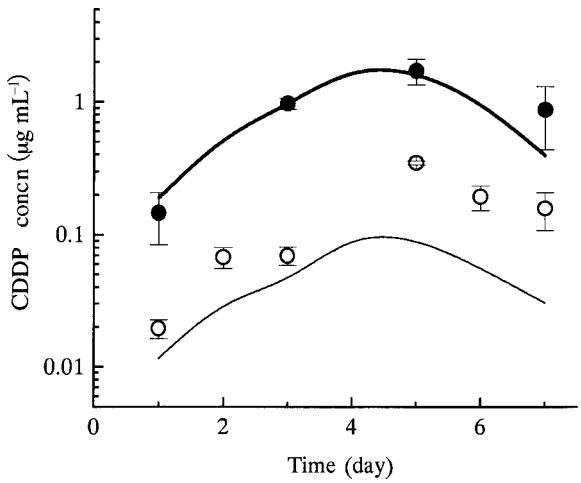

Figure 6 Unchanged CDDP concentration–time profile in peritoneal fluid (●) and in plasma (○) after intraperitoneal administration of CDDP-loaded microspheres (thick line: simulated unchanged CDDP concentration in peritoneal fluid; thin line: simulated unchanged CDDP concentration in plasma). Dose: 10 mg kg^{-1} as CDDP. Data represent mean ± s.d., n = 4.

toneal fluid, the unchanged CDDP concentration was gradually increased to approximately 1–2 μg mL^{-1} within 5 days, and then remained at the same level until 7 days. The unchanged CDDP concentration in plasma remained at a low level (0.02–0.2 μg mL^{-1}). Thus, our CDDP-MS continuously released unchanged CDDP into the peritoneal cavity in-vivo as well as in-vitro.

The AUC_{0-t} of unchanged CDDP in peritoneal fluid was 198 μg h mL^{-1}, which was equivalent to the $AUC_{0-\infty}$ for aqueous solution at same dose. The cytotoxic effect of CDDP against tumour cell lines is known to be dependent on its concentration–time product (Kurihara et al 1995, 1996). To compare the anti-tumour effects of CDDP-MS and CDDP bolus administration, further studies in tumour-bearing animals are necessary.

Prediction of unchanged CDDP concentration in peritoneal fluid and plasma from in-vitro release study

To demonstrate the usefulness of the in-vitro release study of CDDP-MS, we tried to estimate the unchanged CDDP concentration in peritoneal fluid after administration of CDDP-MS from the in-vitro dissolution profile (Figure 5). The unchanged CDDP concentration was estimated from differential equations 3 and 4 using pharmacokinetic parameters (Table 1). Figure 6 shows the comparisons between the concentration profiles determined from the in-vitro dissolution rate of CDDP (Figure 5) with the pharmacokinetic model (Figure 1) and also shows the actual concentration in peritoneal fluid and plasma after CDDP-MS administration. In the peritoneal cavity, the simulated values were almost identical to the actual concentration at any time point, suggesting that the in-vitro release study of CDDP-MS reflects the dissolution in-vivo. This result provides important information for the optimization of the microsphere formulations, because the effectiveness of CDDP for peritoneal carcinomatosis is intimately related to its concentration in the peritoneal cavity. However, in this study, we examined the pharmacokinetics of CDDP using non-cancerous rats. The tumour cells seeded intraperitoneally tend to infiltrate the milky spots, which are lymphoid tissue distributed mainly in the greater omentum (Tsujimoto et al 1995). Furthermore, it has been reported that drugs are absorbed from the peritoneal cavity to systemic circulation not only via the portal vein, but also through lymph drainage (Lukas et al 1981; Rasheid and Rippe 1995). Therefore, the growth of tumour cells at the omentum may cause a decrease in the absorption rate from ascites to systemic circulation through lymph drainage. Moreover, tumour growth and ascites accumulation lead to a larger distribution volume in the peritoneal cavity (V_1). Thus, in the clinical situation, these parameters are considered to vary according to the stage of carcinomatosis and the therapeutic efficacy of CDDP-MS. Further study is required to solve this problem, especially the relationship between unchanged CDDP concentration, ascites accumulation and absorption rate.

In plasma, the simulated values were approximately 3-fold lower than the actual values. Such a large difference is probably owing to the unique renal excretion of unchanged CDDP. In fact, it was recently demonstrated that the accumulation of CDDP in renal tubular cells could occur through re-absorption after renal tubular secretion (Hanada et al 1999). Furthermore, it was reported that the renal clearance of unchanged CDDP is concentration-dependent with saturation of renal tubular re-absorption (Reece et al 1987). CDDP-MS would result in lower plasma levels of unchanged CDDP and avoid saturation of renal tubular absorption and hence renal clearance would be expected to be lower. The elimination rate from systemic circulation used in this study was calculated from intravenous administration of CDDP aqueous solution. This suggests that the k_{el} might be defined as a saturable parameter. Further studies are required to determine the contribution of re-absorption of CDDP to renal clearance.

Conclusions

In this report, we demonstrated a novel method designed to determine the concentration of unchanged CDDP in the peritoneal cavity. A new pharmacokinetic model was established to predict the unchanged CDDP concentration in peritoneal fluid after intraperitoneal administration of CDDP-MS. After CDDP-MS administration, the in-vivo behaviour of unchanged CDDP in the peritoneal cavity was predictable from the in-vitro dissolution profile. Although the present study was conducted using non-cancerous rats, the physiological condition of which is somewhat different from that of tumour-bearing rats, the findings obtained are useful for understanding the basic pharmacokinetic characteristics of unchanged CDDP in the peritoneal cavity, and may also be important in the development of optimized CDDP-MS formulations.

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