***In Vitro* Characteristics and *in Vivo* Plasma Disposition of Cisplatin Conjugated with Oxidized and Dicarboxymethylated Dextrans**

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***In vitro* release behavior and cytotoxic activity, and *in vivo* plasma disposition of newly synthesized macromolecular derivatives of cisplatin (CDDP) were investigated and compared with CDDP. The derivatives included oxidized dextran conjugate of CDDP (OX-Dex/CDDP) and dicarboxymethylated dextran conjugate of CDDP (DCM-Dex/CDDP).**

***In vitro* release of platinum complex from dextran conjugated CDDP was determined by an equilibrium dialysis method. These dextran conjugates showed sustained release of the platinum complex. *In vitro* release half-life for DCM-Dex/CDDP was significantly longer (4.5 times) than that for OX-Dex/CDDP. *In vitro* cytotoxic activity of CDDP and dextran conjugated CDDP against colon 26, mouse colon cancer cell line, was measured using the MTT assay method. OX-Dex/CDDP showed a similar cytotoxic activity to CDDP. However, both cytotoxic activities were markedly decreased when preincubated with the medium containing serum. On the other hand, DCM-Dex/CDDP retained residual cytotoxic activity at a significantly higher level than OX-Dex/CDDP after preincubation with the medium containing serum, although it showed the lowest cytotoxic activity. This indicated longer maintenance of the *in vitro* antitumor activity of DCM-Dex/CDDP in serum compared with OX-Dex/CDDP. Plasma disposition of CDDP and dextran conjugated CDDP was determined by intravenous administration to rats. Although the total platinum plasma concentration–time profile for OX-Dex/CDDP was similar to that for CDDP, its markedly higher profile was achieved when DCM-Dex/CDDP was administered. The values of the total platinum AUC and MRT, where AUC is the area under the platinum concentration–time curve and MRT is the mean residence time, for DCM-Dex/CDDP were 11.2 times and 4.8 times significantly higher than with OX-Dex/CDDP in plasma, respectively. DCM-Dex/CDDP also showed a significantly lower total clearance compared with OX-Dex/CDDP. These results from the *in vivo* experiments revealed that retention of DCM-Dex/CDDP in blood circulation was much greater than that for OX-Dex/CDDP.**

DCM-Dex/CDDP thus has potential as a macromolecular derivative of CDDP for passive tumor targeting.

Key words cisplatin; dextran conjugate; macromolecular derivative; cytotoxic activity; plasma disposition; rat

In cancer chemotherapy it is important to control the biopharmaceutical behavior of antitumor drugs for effective treatment. Various approaches have been used to concentrate the cytotoxicity of the drugs on the tumor site and to minimize the burden on other tissues by modifying their biological and pharmacokinetic properties.¹⁾ In these approaches various drug carrier systems have been developed for controlled and site-specific delivery of drugs. Among them, conjugation of drugs to macromolecular carriers is one of the most promising systems.²⁾ Recently, macromolecular conjugates using the polymers poly-L-glutamic acid,³⁾ chitin,⁴⁾ and dextran^{5–10)} as drug carriers have been developed. These polymers possess some advantages as a drug carrier: high water solubility, the presence of multiple functional groups that are easily modified chemically, low immunogenicity and low toxicity. The macromolecularization of antitumor drugs is expected to overcome the problem of side-effects by improving the body's distribution of drugs and prolonging their activities.¹¹⁾

Cisplatin (*cis*-diamminedichloroplatinum (II); CDDP, Fig. 1), the most potent antitumor platinum complex, is widely used in the treatment of many solid cancers,¹²⁾ but accumulation of CDDP in kidney causes severe renal toxicity.¹³⁾ Moreover, CDDP is sparingly soluble, not only in water but also in lipid. Actually, the clinical application of CDDP has been limited. In our previous studies, we showed that sustained release of *cis*-dichloro-(cyclohexane-*trans*-1-1,2-diammine)-

platinum (II) (Dach-Pt), one of the antitumor platinum complexes, was achieved by conjugation with dextran derivatives, and revealed that the conjugation of Dach-Pt to oxidized and dicarboxymethylated dextrans led to a higher stability of Dach-Pt and a longer maintenance of its antitumor activity *in vitro*.^{14,15)} Takakura *et al.*¹⁶⁾ reported that the use of dextran as a drug carrier led to increase the longevity of therapeutic agents in blood circulation.

In the present study, two dextran conjugates of CDDP, oxidized dextran conjugate of CDDP (OX-Dex/CDDP, Fig. 1) and dicarboxymethylated dextran conjugate of CDDP (DCM-Dex/CDDP, Fig. 1), were newly synthesized and their *in vitro* characteristics and *in vivo* plasma disposition were investigated and compared with CDDP.

MATERIALS AND METHODS

Materials Dextran with an average molecular weight of 60000 and platinum standard solution (1000 ppm) were purchased from Wako Pure Chemical Ind., Ltd., (Osaka, Japan). CDDP, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethylsulfoxide were obtained from Sigma Chemical Co., (St. Louis, U.S.A.). RPMI 1640 and fetal bovine serum were obtained from Gibco BRL (Grand Island, U.S.A.). All other chemicals were of special reagent grade.

Syntheses of Dextran Conjugates of CDDP Oxidized

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Fig. 1. Representative Chemical Structures of CDDP and the Dextran Conjugated CDDP

A, CDDP; B, OX-Dex/CDDP; C, DCM-Dex/CDDP.

and dicarboxymethylated dextrans were synthesized as reported previously.^{14,15} Syntheses of OX-Dex/CDDP and DCM-Dex/CDDP were carried out according to the method reported in the conjugation of Dach-Pt to oxidized dextran¹⁴ and dicarboxymethylated dextran¹⁵ with minor modifications, respectively. In brief, CDDP (50 mg) in 30 ml distilled water was mixed with aqueous silver nitrate solution (0.1 M, 0.22 ml) and stirred at 60 °C for 6 h. The precipitates of silver chloride were removed by filtration. The filtrate containing *cis*-diammineplatinum nitrate was passed through a column packed with an anion-exchange resin (Diaion SA-10A OH⁻ type) to convert it to *cis*-diamminedi-hydroxyplatinum. The solution obtained was added to oxidized dextran (226 mg) in distilled water and stirred at 60 °C for 24 h. The product was purified by gel-filtration chromatography using a Sephadex G-25 column. The high molecular weight fraction was collected and freeze-dried to yield 210 mg of OX-Dex/CDDP (76%) as a white powder. DCM-Dex/CDDP was synthesized using dicarboxymethylated dextran and *cis*-diamminedi-hydroxyplatinum by the same procedure as above. The apparent molecular weights of OX-Dex/CDDP and DCM-Dex/CDDP, measured by gel-permeation chromatography using a Shodex OHpack KB-803 column (Showa Denko K. K., Tokyo, Japan), were 30000. OX-Dex/CDDP and DCM-Dex/CDDP were estimated to contain CDDP to an almost equal extent of 22% and 9%, respectively, by determining platinum content using atomic absorption spectrophotometry.

In Vitro Release Experiments The release of platinum

complex from dextran conjugated CDDP was determined by the equilibrium dialysis method using 3 ml chambers. Phosphate buffer saline (PBS, pH 7.4) containing CDDP or its dextran conjugates at a concentration of 10 µg/ml equivalent CDDP (donor side) was dialyzed against the same volume of PBS (receptor side) using a cellulose tube (molecular weight cutoff value of 12000, Viskase Sales, Chicago, U.S.A.) maintained at 37 °C. At appropriate time intervals, aliquots of the receptor fluid were withdrawn. The amount of platinum in the samples was determined by atomic absorption spectrophotometry.

In Vitro Cytotoxic Activity Experiments Mouse colon cancer cell line, colon 26, obtained from The American Type Culture Collection (Rockville, U.S.A.), was used. Cytotoxic activity of CDDP and dextran conjugated CDDP against colon 26 was measured by the MTT assay method.¹⁷ In brief, the cell line was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO₂ and 95% air at 37 °C. Exponentially growing cells were plated in 96-well flat-bottomed microplates (Falcon 3072, Becton Dickinson Co., Lincoln Park, U.S.A.) at a density of 30000 cells (100 µl) per well and cultured in a medium containing drugs at various concentrations. After 48 h incubation, 10 µl of an MTT stock solution (5 mg/ml) was added to each well, followed by further incubation for 4 h. The supernatant was aspirated, and the resulting pigment was dissolved in dimethylsulfoxide (100 µl per well). Absorbance was measured at 570 nm with a microplate reader Model 3550 (Bio-Rad Laboratories, Hercules, U.S.A.). Growth inhibition was calculated from the absorbance of the reduced coloration.

The residual cytotoxic activity of CDDP and the dextran conjugated CDDP against colon 26 was also measured. CDDP and its dextran conjugates (100 µM equivalent CDDP) were preincubated with the medium containing 10% fetal bovine serum for appropriate time periods before measurement of the cytotoxic activity. The cytotoxic activity of drugs against colon 26 was measured in the same manner as above, and the residual cytotoxic activity (*T/C*%) was calculated as follows:

$$T/C \times 100 \quad (1)$$

where *T* and *C* are the cytotoxic activities of drugs with (treated) and without (control) preincubation, respectively.

In Vivo Experiments Male SPF Wistar rats (Ohtsubo Inc., Nagasaki, Japan), weighing 240–260 g, were housed in cages in an air-conditioned room, and had free access to a standard laboratory diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and water. The animals were anesthetized with urethane (1.5 g/kg as 300 mg/ml, i.p.), and anesthesia was continued throughout the experimental period. OX-Dex/CDDP (3.40 mg/ml) and DCM-Dex/CDDP (8.33 mg/ml) were prepared in PBS, corresponding to 0.75 mg/ml as CDDP. A volume of 4 ml/kg of the drug solution was intravenously administered. These doses correspond to 3 mg/kg as CDDP. Whole blood samples (400 µl) were collected at various times (5–360 min) and immediately centrifuged to separate the plasma. The plasma sample (150 µl) was divided into two and analyzed for total and free platinum concentrations, free platinum being determined after ultrafiltration of the plasma sample (Ultrafree C3-LGC, Nippon Millipore

Co., Ltd., Tokyo, Japan).

Platinum Assay The concentration of platinum in samples was determined by flameless atomic absorption spectrophotometry (Z-8000, Hitachi Ltd., Tokyo, Japan) according to the procedure described previously.¹⁸⁾ Standards (0.2—2.0 $\mu\text{g/ml}$) were prepared from platinum standard solution by diluting with 0.1 M HCl. The standard curve was linear for the entire range of standard samples. The limit of assay detection was 0.02 $\mu\text{g/ml}$.

Data Analysis Pharmacokinetic parameters were calculated using the MULTI program.¹⁹⁾ Platinum concentration data were fitted to the following biexponential equation:

$$C_t = A \cdot e^{-at} + B \cdot e^{-bt} \quad (2)$$

where C_t is the drug concentration at time t , and A , a , B and b are the biexponential equation constants. The area under the platinum concentration–time curve (AUC) from time 0 to time infinity was calculated by the linear trapezoidal rule. Noncompartmental analysis was performed to obtain the mean residence time (MRT) for platinum in plasma.

Statistical analysis was performed using Student's t -test.

RESULTS

In vitro release behavior of platinum complex from dextran conjugated CDDP, determined by the equilibrium dialysis method, is shown in Fig. 2. The sustained release of the platinum complex from the dextran conjugates was achieved *in vitro*. Both of them showed the monoexponential liberation of the complex. The observed *in vitro* release half-life was 9.9 h for OX-Dex/CDDP and 45.0 h for DCM-Dex/CDDP. DCM-Dex/CDDP was 4.5 times and significantly longer ($p < 0.01$) than OX-Dex/CDDP.

Figure 3 shows *in vitro* cytotoxic activity of CDDP and dextran conjugated CDDP against colon 26, measured using the MTT assay method. CDDP showed the highest cytotoxic activity, and that of OX-Dex/CDDP was similar. However, their cytotoxic activities were markedly decreased when they were preincubated with the medium containing 10% fetal bovine serum before measurement (Fig. 4). Although DCM-Dex/CDDP showed significantly lower cytotoxic activity than OX-Dex/CDDP (Fig. 3), it retained residual cytotoxic activity at a significantly higher level than OX-Dex/CDDP after 36 h preincubation with the medium containing 10% fetal bovine serum (Fig. 4).

The concentration–time profiles for total and free platinum in plasma after intravenous administration of CDDP and dextran conjugated CDDP are shown in Fig. 5. The total and the free platinum after administration of CDDP were represented as total and unbound CDDP, respectively. After administration of 2 dextran conjugated CDDP, however the total platinum was represented as both conjugated and released platinum complex, and the free platinum was represented as unbound platinum complex released from these dextran conjugates. All plasma-decay curves showed biexponential kinetics. Although the total platinum plasma concentration–time profile for OX-Dex/CDDP was similar to that for CDDP, its profile was markedly higher when DCM-Dex/CDDP was administered.

The profiles of the platinum concentrations in the plasma after administration of CDDP were analyzed pharmacokinet-

Fig. 2. *In Vitro* Release Behavior of Platinum Complex from Dextran Conjugated CDDP in a pH 7.4 Buffer at 37 °C

Symbols: (●) CDDP; (▲) OX-Dex/CDDP; (■) DCM-Dex/CDDP. Each point represents the mean $\pm$ S.D. of results from at least 4 experiments.

Fig. 3. *In Vitro* Cytotoxic Activity of CDDP and Dextran Conjugated CDDP against Colon 26

Symbols: (●) CDDP; (▲) OX-Dex/CDDP; (■) DCM-Dex/CDDP. Concentration of the dextran conjugated CDDP represents the equivalent concentration of CDDP included. Each point represents the mean $\pm$ S.D. of results from at least 4 experiments. * $p < 0.05$, compared with OX-Dex/CDDP.

Fig. 4. Effect of Preincubation with Serum on the *in Vitro* Drug Cytotoxic Activity against Colon 26

Symbols: (●) CDDP; (▲) OX-Dex/CDDP; (■) DCM-Dex/CDDP. Each point represents the mean $\pm$ S.D. of results from at least 4 experiments. * $p < 0.01$, compared with OX-Dex/CDDP.

Fig. 5. Concentration-Time Profiles for Total and Free Platinum in Plasma after Intravenous Administration of CDDP and Dextran Conjugated CDDP in Rats

Total platinum (●) and free platinum (○) after administration of CDDP (3 mg/kg) were represented as total and unbound CDDP, respectively. After administration of the dextran conjugated CDDP (3 mg/kg equivalent CDDP) total platinum (▲, OX-Dex/CDDP; ■, DCM-Dex/CDDP) was represented as both conjugated and released platinum complex, and free platinum (△, OX-Dex/CDDP; □, DCM-Dex/CDDP) was represented as unbound platinum complex released from the conjugates, respectively. Each point represents the mean $\pm$ S.D. of results from 4 to 5 experiments.

Table 1. Pharmacokinetic Parameters for Total and Free Platinum (as Total and Unbound CDDP) in Plasma after Intravenous Administration of CDDP (3 mg/kg) to Rats

Parameter	Total platinum	Free platinum
AUC (min $\cdot$ μ g/ml)	1145 $\pm$ 69	435 $\pm$ 86
MRT (min)	188 $\pm$ 14	86 $\pm$ 18
K_{12} (min ⁻¹)	0.025 $\pm$ 0.006	0.041 $\pm$ 0.004
K_{21} (min ⁻¹)	0.010 $\pm$ 0.004	0.010 $\pm$ 0.001
K_{el} (min ⁻¹)	0.014 $\pm$ 0.004	0.009 $\pm$ 0.005
Vd_c (ml/kg)	112 $\pm$ 17	—
Vd_t (ml/kg)	315 $\pm$ 91	—
Vd_{ss} (ml/kg)	426 $\pm$ 108	—
CL (ml/min/kg)	1.5 $\pm$ 0.2	—
AUC_{free}/AUC_{total} ratio	0.38	—

Each value represents the mean $\pm$ S.D. of results from 4 experiments. AUC is the area under the platinum concentration-time curve; MRT is the mean residence time; K_{12} and K_{21} are the distribution rate constants; K_{el} is the elimination rate constant; Vd_c and Vd_t are the distribution volumes of central and tissue compartments, respectively; Vd_{ss} is the steady state distribution volume; CL is the total body clearance.

ically and the results are shown in Table 1. The ratio of the AUC for free platinum to that for total platinum was 0.38, so 62% of the CDDP administered was bound to plasma proteins.

Table 2 summarizes pharmacokinetic parameters for the total and the free platinum in the plasma after administration of the dextran conjugated CDDP. The values of the total platinum AUC and MRT for DCM-Dex/CDDP were 11.2 times and 4.8 times, and thus significantly higher than with OX-Dex/CDDP, respectively. Although the value of the free platinum AUC for DCM-Dex/CDDP was almost equal to that for OX-Dex/CDDP, the free platinum MRT value for DCM-Dex/CDDP was 4.6 times significantly higher than that for OX-Dex/CDDP. The ratio of the AUC for free platinum to that for total platinum in OX-Dex/CDDP and DCM-Dex/CDDP was 0.19 and 0.02, so 81% and 98% of the platinum complex existed as conjugated or bound form in the plasma after administration of the conjugates, respectively. DCM-Dex/CDDP showed a significantly lower value in the total clearance of the total platinum compared with that in OX-Dex/CDDP.

DISCUSSION

CDDP is the most active antitumor agent, but major dose-limiting toxic effects, mainly on kidney, have limited its clinical application.¹³⁾ CDDP is virtually insoluble, not only in water but also in lipid. Moreover, resistance to CDDP frequently appears in many tumor cell lines.¹²⁾ Therefore, many approaches have been used to synthesize novel CDDP derivatives, having a broader spectrum of antitumor activity, greater solubility, reduced toxicity, and a lack of cross-resistance. We recently developed 4-pyridoxate diammine hydroxy platinum, a novel CDDP derivative, to improve the efficiency of drug delivery to brain tumors.¹⁸⁾

The conjugation of highly active antitumor drugs to dextran derivatives seems to offer a promising way to optimize their delivery. Dextran, the polymer of glucose, has been used as a plasma volume expander for many years in the clinical field. However, it has recently been suggested as a macromolecular carrier for drug delivery, a targeting drug to specific sites of action.²⁾ Physicochemical and physiological properties of dextran, such as its high degree of water solubility, availability in different molecular weights, the presence of numerous reactive hydroxyl groups available for drug fixation, and relatively low *in vivo* toxicity, are beneficial for a drug carrier.¹⁰⁾ Accordingly, macromolecular conjugates using dextran derivatives as drug carriers have been tested for several antitumor drugs: daunomycin,⁵⁾ mitomycin C,^{6,8,9)} and adriamycin.⁷⁾ Dextran conjugated CDDP may have the potential to decrease the rapid association of CDDP with plasma components or normal tissues, thus prolonging its retention in blood circulation and availability to tumor cells. This may result in decreased renal distribution of CDDP and reduce its toxicity. Schechter *et al.*²⁰⁾ reported the use of carboxymethylated dextran as a drug carrier to increase availability of CDDP to the tumor cells. We have synthesized several kinds of dextran conjugates of Dach-Pt, an antitumor platinum complex, and showed that the conjugation of Dach-Pt to oxidized and dicarboxymethylated dextrans led to a higher stability of Dach-Pt and prolongation of its antitumor activity compared with the conjugation of Dach-Pt to car-

Table 2. Pharmacokinetic Parameters for Total Platinum (as Conjugated and Released Platinum Complex) and Free Platinum (as Unbound Platinum Complex Released) in Plasma after Intravenous Administration of Dextran Conjugated CDDP (3 mg/kg Equivalent CDDP) to Rats

Parameter	OX-Dex/CDDP		DCM-Dex/CDDP	
	Total platinum	Free platinum	Total platinum	Free platinum
AUC (min- μ g/ml)	1129 $\pm$ 264	219 $\pm$ 5	12616 $\pm$ 5196*	254 $\pm$ 72
MRT (min)	185 $\pm$ 118	72 $\pm$ 10	888 $\pm$ 317*	330 $\pm$ 75*
K_{12} (min ⁻¹)	0.036 $\pm$ 0.018	0.020 $\pm$ 0.014	0.006 $\pm$ 0.004*	0.002 $\pm$ 0.002*
K_{21} (min ⁻¹)	0.029 $\pm$ 0.010	0.033 $\pm$ 0.016	0.017 $\pm$ 0.008	0.003 $\pm$ 0.001*
K_{el} (min ⁻¹)	0.021 $\pm$ 0.005	0.022 $\pm$ 0.002	0.008 $\pm$ 0.001*	0.002 $\pm$ 0.001*
Vd_c (ml/kg)	101 $\pm$ 14	—	120 $\pm$ 38	—
Vd_t (ml/kg)	117 $\pm$ 24	—	56 $\pm$ 43*	—
Vd_{ss} (ml/kg)	218 $\pm$ 13	—	176 $\pm$ 74	—
CL (ml/min/kg)	2.0 $\pm$ 0.4	—	0.2 $\pm$ 0.1*	—
AUC_{free}/AUC_{total} ratio	0.19		0.02	

Each value represents the mean $\pm$ S.D. of results from 4 to 5 experiments. * p <0.05, compared with OX-Dex/CDDP.

boxymethylated dextran *in vitro*.^{14,15)} The oxidized and dicarboxymethylated dextrans were able to form double coordination bonds with the platinum complex, but the carboxymethylated dextran formed only a single coordination bond. Accordingly, the coordination bond of Dac-Pt with the oxidized and dicarboxymethylated dextrans was stronger than that with the carboxymethylated dextran, and the oxidized and dicarboxymethylated dextran conjugates of Dac-Pt protected the cytotoxic activity of immobilized platinum complex from deactivating factors. In the present study, therefore, we newly synthesized OX-Dex/CDDP and DCM-Dex/CDDP, and tested *in vitro* characteristics and *in vivo* plasma disposition in comparison with CDDP.

The sustained release of platinum complex from these dextran conjugated CDDPs was achieved *in vitro*. However, the drug release behaviors for the two conjugates were different. DCM-Dex/CDDP was stable for a significantly longer period in PBS. The difference in the drug release behavior between the two conjugates is probably related to the strength of chelate-type coordination bonds in the conjugates. The coordination bond of the platinum complex with the dicarboxymethylated dextran must be stronger than that with the oxidized dextran.

We measured *in vitro* drug cytotoxic activity against colon 26, using the MTT assay method. This is a simple colorimetric test of cell proliferation and survival, and is now being adopted for routine use in clinical practice worldwide. It seems that two dextran conjugated CDDP acted as a prodrug-type derivative, considering CDDP showed the highest cytotoxic activity. The difference in the cytotoxic activity between OX-Dex/CDDP and DCM-Dex/CDDP is due to amounts of the platinum complex released from the conjugates during 48 h incubation before the measurement of the cytotoxic activity. Because approximately 90% of the platinum complex was released during incubation, OX-Dex/CDDP showed a similar cytotoxic activity to CDDP. However, in DCM-Dex/CDDP approximately 50% of the platinum complex remained in conjugated form before the cytotoxic activity measurement, so that DCM-Dex/CDDP showed a lower cytotoxic activity than OX-Dex/CDDP and CDDP.

It is well known that the cytotoxic activity of CDDP gradually decreases in the bloodstream because of ligand exchange reactions with compounds having amino groups in

the blood, such as proteins, amino acids, and so on.²¹⁾ The macromolecular derivatives of CDDP are expected to keep the platinum complex away from these deactivating factors and thus assure continuation of its high cytotoxic activity during circulation in the bloodstream. Thus, we investigated the effect of preincubation with medium containing serum on the *in vitro* drug cytotoxic activity. DCM-Dex/CDDP retained residual cytotoxic activity at a markedly higher level than OX-Dex/CDDP or CDDP in the medium containing serum. This suggests that the dicarboxymethylated dextran kept the cytotoxic activity of immobilized platinum complex by protecting it from deactivating factors in the medium containing serum. The resistance of platinum complex against deactivating factors in serum is due to both the strength of the coordination bond of the platinum atom with the carrier polymer and the steric hindrance of the carrier polymer. The strength of the chelate-type coordination bonds in DCM-Dex/CDDP was higher than that in OX-Dex/CDDP, as shown in Fig. 2. This is the reason DCM-Dex/CDDP showed longer maintenance of high cytotoxic activity in the medium containing serum.

We investigated plasma disposition of the drugs following intravenous administration to rats to determine the *in vivo* fate of dextran conjugated CDDP. Although the total platinum plasma concentration-time profile for OX-Dex/CDDP was similar to that for CDDP, a markedly higher profile was achieved when DCM-Dex/CDDP was administered. DCM-Dex/CDDP showed the largest AUC and MRT values and the lowest total clearance of the total platinum. Moreover, 98% of platinum complex of DCM-Dex/CDDP existed as conjugated or bound form in plasma. These results show that retention of DCM-Dex/CDDP in blood circulation was much greater than that of OX-Dex/CDDP, and that drug was released from DCM-Dex/CDDP more slowly than from OX-Dex/CDDP *in vivo*.

The systemic disposition characteristics of the dextran conjugates are significantly affected by their electric charges and molecular weights. A dextran conjugate with a positive charge and a low molecular weight (approximately 10000) showed poor tumor accumulation, because it was rapidly eliminated from the blood *via* extensive hepatic uptake and rapid urinary excretion.²²⁾ On the other hand, negative charge on the dextran conjugates decreased the nonspecific interaction with the body components, particularly with the liver, so

that a negatively charged dextran conjugate with a relatively large molecular weight remained in the blood circulation for a long time, and accumulated in the tumor.^{22,23)} Thus, DCM-Dex/CDDP was suggested to be a suitable dextran conjugated CDDP for passive tumor targeting; it is a negatively charged macromolecule, has a relatively large molecular weight, and persists for long periods of time in the circulating blood. DCM-Dex/CDDP is expected to concentrate the active platinum complex to the tumor site after administration and to exhibit a higher antitumor activity than CDDP. Tissue distribution characteristics of these conjugated CDDPs remains obscure, however. Further *in vivo* experiments using a tumor-bearing animal to investigate tissue distribution and therapeutic activity of the conjugates are necessary to determine the usefulness of DCM-Dex/CDDP.

This report shows that DCM-Dex/CDDP has potential as a macromolecular derivative of CDDP for passive targeting to a tumor.

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REFERENCES

- 1) Sezaki H., Hashida M., Muranishi S., "Gelatin Microspheres as Carriers for Antineoplastic Agents: Optimization of Drug Delivery," eds. by Bungaard H., Hansen A. B., Kofod H., Munksgaard, Copenhagen, 1982, pp. 316—332.
- 2) Sezaki H., Takakura Y., Hashida M., *Adv. Drug Deliv. Rev.*, **3**, 247—266 (1989).
- 3) Hashida M., Takakura Y., *J. Controlled Release*, **31**, 163—171 (1994).
- 4) Ohya Y., Nonomura K., Ouchi T., *J. Bioact. Compat. Polym.*, **10**, 223—234 (1995).
- 5) Hurwitz E., Wilchek M., Pitha J., *J. Appl. Biochem.*, **2**, 25—35 (1980).
- 6) Takakura Y., Matsumoto S., Hashida M., Sezaki H., *Cancer Res.*, **44**, 2505—2510 (1984).
- 7) Dillan R. O., Shawler D. L., Johnson D. E., Meyer D. L., Koziol J. A., Frincke J. M., *Cancer Res.*, **46**, 4886—4891 (1986).
- 8) Takakura Y., Kitajima M., Matsumoto S., Hashida M., Sezaki H., *Int. J. Pharm.*, **37**, 135—143 (1987).
- 9) Takakura Y., Atsumi R., Hashida M., Sezaki H., *Int. J. Pharm.*, **37**, 145—154 (1987).
- 10) Larsen C., *Adv. Drug Deliv. Rev.*, **3**, 103—154 (1989).
- 11) Maeda H., Oda T., Matsumura Y., Kimura M., *J. Bioact. Compat. Polym.*, **3**, 27—43 (1988).
- 12) Loehrer P. J., Einhorn L. H., *Ann. Int. Med.*, **100**, 704—713 (1984).
- 13) Von Hoff D. D., Schilsky R., Reichert C. M., Reddick R. L., Rozenweig M., Young R. C., Muggia F. M., *Cancer Treat. Rep.*, **63**, 1527—1531 (1979).
- 14) Ohya Y., Masunaga T., Baba T., Ouchi T., *J. Biomater. Sci. Polymer Edn.*, **7**, 1085—1096 (1996).
- 15) Ohya Y., Masunaga T., Baba T., Ouchi T., *Pure Appl. Chem.*, **A33**, 1005—1016 (1996).
- 16) Takakura Y., Kaneko Y., Fujita T., Hashida M., Sezaki H., *J. Pharm. Sci.*, **78**, 117—121 (1989).
- 17) Mosmann T., *J. Immunol. Methods*, **65**, 55—63 (1983).
- 18) Tokunaga Y., Nakashima M., Shibata S., Fujita H., Anda T., Khalid H., Kuzuya M., Sasaki H., Ichikawa M., *Pharm. Sci.*, **3**, 353—356 (1997).
- 19) Yamaoka K., Tanigawara Y., Nakagawa T., Uno T., *J. Pharmacobio-Dyn.*, **4**, 879—885 (1981).
- 20) Schechter B., Pauzner R., Arnon R., Wilchek M., *Cancer Biochem. Biophys.*, **8**, 277—287 (1986).
- 21) Takahashi K., Seki T., Nishimura K., Minamide S., Iwabuchi M., Ono M., Nagamine S., Horinishi H., *Jpn. J. Cancer Res.*, **76**, 68—74 (1985).
- 22) Takakura Y., Fujita T., Hashida M., Sezaki H., *Pharm. Res.*, **7**, 339—346 (1990).
- 23) Nishikawa M., Yamashita F., Takakura Y., Hashida M., Sezaki H., *J. Pharm. Pharmacol.*, **44**, 396—401 (1992).