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Molecular-weight-dependent pharmacokinetics and cytotoxic properties of cisplatin complexes prepared with chondroitin sulfate A and C

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Abstract

In order to screen out an optimum complex for reducing the nephrotoxicity of cisplatin (CDDP), we investigated and compared CDDP–chondroitin sulfate complexes to CDDP in terms of in vivo pharmacokinetics and in vitro cytotoxicity. The polymeric carriers used in the study were chondroitin sulfate A (CSA, 4-sulfate) with mean molecular weights of 10 kDa (CSA-1) and 23 kDa (CSA-2), and chondroitin sulfate C (CSC, 6-sulfate) with mean molecular weights of 8 kDa (CSC-1) and 25 kDa (CSC-2). The resultant complexes (CDDP–CSA-1, CDDP–CSA-2, CDDP–CSC-1 and CDDP–CSC-2) were administered intravenously to rats. The obtained plasma concentration–time curves during the 3 h period studied for all complexes are biphasic. The plasma dispositions of complexes were dependent on the molecular sizes with urinary excretion as main elimination pathway. CDDP–CSA-1 and CDDP–CSC-1 were unable to effectively increase the plasma retention of platinum due to rapid renal excretion. Furthermore, CDDP–CSA-1 disappeared from plasma more quickly than CDDP–CSC-1. CDDP–CSA-2 and CDDP–CSC-2, with similar urinary excretion as CDDP, gave rise to approximately five and four-fold increase in $AUC_{0-3\ h}$ values, respectively, than that was achieved with native CDDP treatment. Biodistribution was compared between CDDP–CSA-2 and CDDP–CSC-2. Both complexes effectively suppressed the extensive distribution of CDDP into most tissues, especially kidney. However, CDDP–CSC-2 showed less reduction effect than CDDP–CSA-2. In addition, a significantly higher accumulation in tumor tissue was found with the administration of CDDP–CSA-2 than CDDP. Moreover, CSA complexes displayed an IC_{50} of 6 μM Pt-equivalents against SW4800 human colon cancer cells, similar to that of CDDP, whereas CSC complexes were less active than CDDP. These studies indicate that the complex prepared with CSA, which is greater than 20 kDa of molecular size, is superior to that of CSC, exhibiting improved pharmacokinetics and similar pharmacological activity to the native drug. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Cisplatin; Chondroitin sulfate A; Chondroitin sulfate C; Complex; Macromolecular carrier; Pharmacokinetics

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1. Introduction

Cisplatin (CDDP) is a very effective anticancer drug used against solid tumors such as testicular, ovarian, non-small lung, head and neck tumor (Rosenberg, 1985; Smith and Talbot, 1992; Lebwohl and Canetta, 1998; Steward, 1998). However, its clinical application is limited by several side effects, such as renal, gastrointestinal and neurological toxicity. Among these toxicities, nephrotoxicity is the most common dose-limiting effect (Anand and Bashey, 1993; Cvitkovic, 1998).

With an i. v. administration, CDDP follows a flow-limited transport, freely traverses compartment barriers, and distributes ubiquitously in all compartments of the body (Farris et al., 1985). In order to improve the therapeutic index of CDDP, it is necessary to increase the efficacy of the drug distribution through more effective targeting methods. Polymeric drug conjugation plays an important role in providing a polymer-based drug delivery system (Duncan et al., 1996). Polymer prodrugs are expected to be able to maintain high level of active species in the circulation, to selectively accumulate more drug in the tumor via the enhanced permeability and retention effect (EPR effect) of tumor, and then to reversibly liberate the parent drug at the site of action (Maeda and Matsumura, 1989; Takakura and Hashida, 1996). Several natural and synthesized polymers such as carboxylmethyl dextran (Schechter et al., 1986), hyaluronic acid (Schechter et al., 1989), alginate (Imai et al., 1997) and *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer (Gianasi et al., 1999) have been employed to form complexes with CDDP. These studies have shown that the formed complexes could retain the same activity as the parent drug, presumably due to reversible release of active platinum complexed with carboxylate groups of polymers. However, the relative affinity between platinum and a polymer seems much more important, because it determines the stability of the resulting complex in the circulation and the activity in tumor site.

Chondroitin sulfates (CS), natural polysaccharides, are typically composed of differently sulfated residues of D-acetyl-galactosamine and D-glucuronic acid(Kelly, 1998). Chondroitin 4-sulfate (chondroitin sulfate A, CSA) and chondroitin 6-sulfate (chondroitin sulfate C, CSC) were employed as to form complexes with CDDP. It was observed that the sulfate groups were also highly favored to react with platinum species (Hausheer et al., 1998). Our purpose of the study is to apply both carboxylate and sulfate groups to form a complex via displacement of two labile chlorides of CDDP. Our previous study found that CSA with a mean molecular weight of 23 kDa was capable of increasing the plasma retention of CDDP while reducing its nephrotoxicity (Zhang et al., 2000). Moreover, Albertini et al. (1999) found that CSA and CSC exhibited completely different physiological role against copper-dependent oxidation of lipoprotein, probably due to the different Cu^{2+} binding ability of CSA and CSC.

In order to obtain further information about applying chondroitin sulfates as polymeric carriers of CDDP, we examined the molecular size-dependent pharmacokinetics of the CSA and CSC complexes of CDDP and their in vitro cytotoxicity. Based on the comparison study, differences in pharmacokinetic behavior and cytotoxicity between CSA and CSC complexes were found in this study.

2. Materials and methods

2.1. Materials

CDDP was purchased from Sigma Chemical Company (St. Louis, MO). Chondroitin 4-sulfates (CSA) with mean molecular weights of 10 kDa (CSA-1) and 23 kDa (CSA-2), and chondroitin 6-sulfates (CSC) with mean molecular weights of 8 kDa (CSC-1) and 25 kDa (CSC-2) were obtained from Seikagaku Corporation (Kanagawa, Japan). CDDP–CSA and CDDP–CSC complexes were prepared by dissolving CDDP (1 mg/ml) and CS (8.4 mg/ml) in deionized water. The mixture was shaken for 2 days until reaching the reaction equilibrium of 80–85% CDDP forming complex with chondroitin sulfates, as determined by detection of uncomplexed CDDP on a CE990/990-10 type capillary electrophoresis system (Lauerlads, PRINCE, The Netherlands) with a

UV spectrophotometer (CE-970, Jasco Co, Japan). All other chemicals were of analytical grade.

Wistar male rats (7 weeks) were used after 1-week acclimation with food and water available ad libitum.

SW4800 human colon cancer cells were obtained as a kind gift from Dr Hiroshi Maeda (Department of Microbiology, School of Medicine, Kumamoto University, Japan).

2.2. In vivo pharmacokinetic evaluation

2.2.1. Plasma disposition and urinary accumulation studies

After fasting overnight, rats were anesthetized with ether, and the femoral vein and artery were cannulated using polyethylene tubing (PE50). In addition, the bladder was exposed through a short abdominal mid-line incision and catheterized with polyethylene casting tubing (outer: PE100 and inner: PE10). The animals were allowed to stabilize for 30 min prior to administration. CDDP was dissolved in saline at a concentration of 1 mg/ml; the prepared complexes (containing 1 mg/ml CDDP) were made iso-osmotic by the addition of glucose. Freshly prepared solution, CDDP or its complexes, was then administered by a dose of 2 mg/kg via the femoral vein, respectively. Blood samples (0.1–0.2 ml) were collected from the femoral artery at 0.5 (only for CDDP), 1, 3, 5, 10, 15, 30, 45, 60, 90, 120, and 180 min into heparinized microtubes followed by centrifugation to isolate the plasma. In order to determine the changes of protein free drug concentrations in plasma with CDDP treatment, aliquots of plasma were added to an equal volume of cold mixture of 10% TCA and 5% HCl. The supernatant was then analyzed for platinum. At sampling times of 30, 60, 120 and 180 min, the bladder was washed with 1.0 ml of saline via the tube inside and the urine was washed out via the outside tube into graduated syringe, and the volume of urine was measured. These samples were then analyzed for platinum content.

2.2.2. Distribution kinetic studies

In the group for the determinations of tissue distribution, CDDP, CDDP–CSA-2 and CDDP–CSC-2 (equivalent to 2 mg/kg CDDP) were administered to rats via the jugular vein under ether anesthesia. Blood was withdrawn for plasma and whole blood samples at 10 min and 3 h, and the rat was immediately sacrificed by cutting the inferior vena cava. Kidney, liver, spleen and lung tissues were isolated, washed in isotonic saline, dried, and weighed. The samples were stored below -20 °C until analyzed for their platinum content.

2.2.3. Tumor tissue distribution

Male ddY mice of 30–35 g (6 weeks) were used in this study. Sarcoma 180 (S-180) tumor cells were kindly supplied (Department of Microbiology, School of Medicine, Kumamoto University). S-180 tumor cells were implanted s. c. into the dorsal skin of mice with an inoculum size of 2×10^6 cells. After 7–10 days, the mice bearing a tumor of 5–7 mm in diameter were selected for the tissue distribution study. 5 mg/kg CDDP or CDDP–CSA-2 was administered to a mouse via the tail vein. After 24 h, blood was withdrawn immediately before the rat was sacrificed by cutting the inferior vena cava. Then tumor tissue was collected, weighed, and stored at -20 °C until analyzed.

2.2.4. Sample analysis

The total platinum was detected by a flameless atomic absorption spectrophotometer as described by Imai et al., (1997). Briefly, plasma samples were diluted 8–200-fold with 0.25% Triton X-100, and the urine diluted 50–500-fold with deionized water prior to analysis. Tissue samples (about 0.5 g) were digested with 3.0 ml of nitric acid by a microwave digestion system. The digested samples were diluted to 5–100 ml with deionized water for analysis.

2.3. Cytotoxicity measurement

In vitro cytotoxicities of CDDP and its complexes were determined by measuring the inhibitions against SW4800 human colon cancer cells

using the MTT assay. An aliquot of 100 μl of cell suspension at a density of 3×10^4 cells per ml was added to each well of a 96-well plate (3×10^3 cells per well), and kept at 37 °C under a 5% CO_2 atmosphere over night. Eleven microlitres drug solution with the concentration range of 0.3–3000 μM was used to treat SW4800 cells. After culture for 72 h at 37 °C under a 5% CO_2 atmosphere, 90 μl MTT solution (0.55 mg/ml) was added after the removal of 90 μl of medium, and the culture was continued for additional 4 h. After an aliquot of 200 μl PBS was added to each well and kept for 1 min, the above medium was removed away. The cells were dissolved in 200 μl 0.04 M HCl–isopropylalchohol by shaking for 10 min. Then the absorbance at 570 nm was measured.

2.4. Data analysis

The plasma concentration–time data were fitted to a two-compartment model with i.v. injection with a nonlinear least-squares computer program (MULTI) with the reciprocal of concentration as the weighting factor.

Statistical analyses were performed by STATVIEW 4.02 MICROSOFT in MACINTOSH, and evaluated for statistically significant differences by a one-way analysis of variance (ANOVA).

3. Results

3.1. Molecular-weight-dependent pharmacokinetics

3.1.1. Plasma disposition of CDDP–CS complexes

The plasma concentration–time profiles of the complexes detected as the total platinum were compared with that of CDDP in terms of both total and free drug in Fig. 1. After 90 min, the free drug was undetectable in the case of CDDP administration. Our previous study found that the complex prepared with CSA (MW 23 kDa) effectively inhibited the protein binding of platinum, resulting over 80% of complex as protein-free species by 3 h in blood after i.v. administration. So in this experiment, analysis to the total drug concentration was selected, as it is an easy and quick method. The plasma concentration–time curve of all CDDP–CS complexes were fitted well to two-phase courses throughout the studied period for all CDDP–CS complexes. The pharmacokinetic parameters are listed in Table 1. The volume data for the central compartment show that all of the complexes were effective in suppressing the immediately extensive distribution of CDDP. The first two phases of total platinum obtained with CDDP administration represent a very rapid distribution and an equally rapid elimination. The complex effectively prolonged the half-lives of both the distribution phase ($t_{1/2\alpha}$) and the elimination phase ($t_{1/2\beta}$). The effect on reducing the rapid loss of drug from plasma was also reflected by 1.5–5.0-fold higher $AUC_{0-3\ h}$ values of the complexes than that of total drug of CDDP and 2.5–9.0-fold as compared with free drug. CDDP–CSA-2 exhibited prolonged half-life for distribution and elimination, and resulted in higher plasma levels when compared with CDDP–CSC-2.

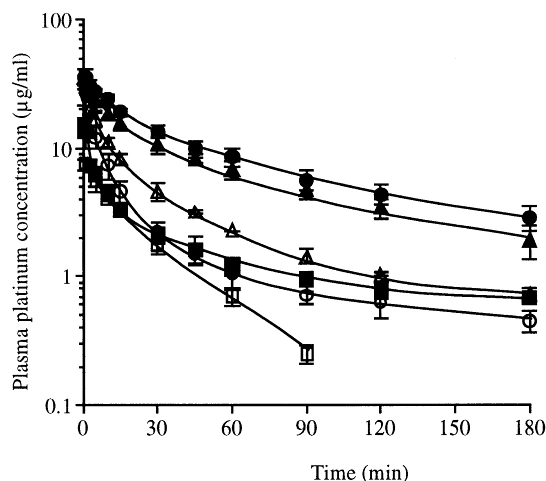

Fig. 1. Time-concentration courses following a 2 mg/kg bolus i.v. administration of CDDP and its complexes to rats. CDDP was detected as free drug (□) and total platinum (■). The total platinum levels were detected following administrations of CDDP–CSA-1 (○), CDDP–CSA-2 (●), CDDP–CSC-1 (△), and CDDP–CSC-2 (▲). All values are means ± S.D. ($n = 4$).

Table 1
Pharmacokinetic parameters of total platinum following bolus i.v. administration of CDDP and its complexes to rats

Parameters	CDDP		CDDP–CS complex			
	Free	Total	CSA-1	CSA-2	CSC-1	CSC-2
($t_{1/2\alpha}$) (min)	3.1 ± 1.0	1.7 ± 0.3	4.2 ± 0.8	10.3 ± 3.4	4.37 ± 0.8	5.81 ± 0.7
($t_{1/2\beta}$) (min)	21.5 ± 0.4	27.7 ± 5.8	61 ± 5.8	78 ± 12.3	44.8 ± 6.9	60.8 ± 6.9
V (ml/kg)	212.5 ± 23.4	138.5 ± 5.1	75.0 ± 6.43[a]	56.5 ± 2.3	62.7 ± 4.6[a]	54.5 ± 10.4[a]
AUC(0–180 min) (μg h/ml)		4.7 ± 0.05	6.9 ± 1.0	24.5 ± 4.3[a]	11.2 ± 0.6[a]	20.7 ± 3.2[a]
CLR (ml/min per kg)	4.47[b]		3.51 ± 0.99	0.69 ± 0.15[a]	2.28 ± 0.28[a]	0.76 ± 0.07[a]

[a] Significantly different from the corresponding result of CDDP group ($P<0.05$).
[b] CLR of CDDP was estimated by mean cumulative excretion of platinum in urine and mean AUC value of free drug within 60 min.

3.1.2. Urinary excretion

The cumulative excretions via urine are shown in Fig. 2. Rats treated with CDDP–CSA-1 and CDDP–CSC-1 excreted nearly 60% of the dose within 3 h. Excretions of CDDP–CSA-2 and CDDP–CSC-2 were about 45–50%, which showed no significant difference when compared with CDDP treatment. The biliary excretions for all complexes were very low, approximately 0.2% of the dose within 3 h (data not shown). The renal clearances of the complexes were calculated from the equation: renal clearance, excretion in urine in 3 h/$AUC_{0-3\ h}$. For CDDP administration, the renal clearance was obtained by using the data of cumulative excretion within 1 h (an average of 34.6%) and $AUC_{0-1\ h}$ of the free drug (an average of 2.58 μg h/ml). As shown in Table 1, the renal clearances for the complexes significantly decreased with the increase in molecular size. CDDP–CSA-2 and CDDP–CSC-2 showed similar renal clearances which are six-fold lower than the renal clearance free CDDP obtained in our study and much lower than 12.3 ml/min per kg reported by Siddik et al. (1987). This result illustrates that complexes with the molecular size of over 20 kDa extensively controlled the rapid renal clearance of free CDDP.

3.2. Cytotoxicity

The antitumor activity of the prepared complexes was examined by measuring the growth inhibition of SW4800 human colon cancer cells in response to 3-day exposure to these complexes. The drug concentration that produced 50% inhibition of cell growth (IC_{50}) was calculated by stimulating the concentration–inhibition curve. The IC_{50} values are listed in Table 2. The IC_{50} values were the same for CDDP and CDDP–CSA complexes. Higher IC_{50} values reflect CDDP–CSC complexes exhibited lower activities than CDDP.

3.3. Tissue distribution

CDDP–CSA-2 and CDDP–CSC-2 were selected to examine the rapid tissue distribution.

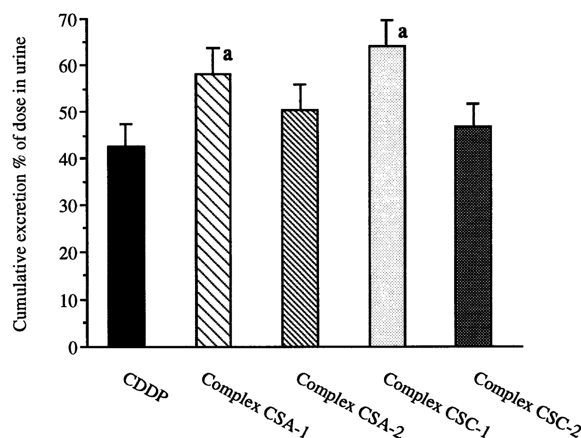

Fig. 2. Cumulative urinary excretion percent of dose determined at 3 h after a 2 mg/kg bolus i.v. administration of CDDP or its complexes to rats. All values are mean ± S.D. ($n = 4$). (a) Significantly different from the corresponding result of CDDP group ($P < 0.05$).

Table 2
Growth inhibition of cancer cell lines by CDDP or CDDP–CS complexes

Cell line	IC_{50} (μM)				
	CDDP	CDDP–CS complex			
		CSA-1	CSA-2	CSC-1	CSC-2
SW 4800	6.56 ± 1.5	6.83 ± 0.8	6.41 ± 0.56	8.1 ± 0.81	8.7 ± 1.4

The tissue–plasma partition ratios at 10 min for main tissues are shown in Fig. 3. At 10 min, CDDP was found to perfuse into the main tissues at much higher levels in comparison with the complexes. The highest partition ratio of 6 was found in kidney in the case of CDDP treatment, for which CDDP–CSA-2 and CDDP–CSC-2 reduced it to 1.5 and 2.5, respectively. The result also shows that CDDP–CSA-2 is superior to CDDP–CSC-2 in suppressing the rapid distribution of platinum into kidney. Both complexes showed similar efficacy to suppress the rapid uptake of CDDP by other tissues, such as liver, spleen and lung. The tissue–plasma partition ratios at 3 h were shown in Fig. 4. Within longer duration, both complexes significantly reduced accumulation in the kidneys, lung and erythrocytes. However, CDDP–CSC-2 was found to distribute in liver, spleen and erythrocyte significantly much more than CDDP–CSA-2.

The above results have shown that CDDP–CSA-2 complex is safer than CDDP–CSC-2 in respect to decreased distribution to normal tissues. In order to investigate the drug accumulation of in tumor tissue following CDDP–CSA-2 administration, we measured the tumor platinum contents at 24 h after a 5 mg/kg i.v. administration of CDDP or CDDP–CSA-2. The results were expressed as a ratio of platinum content in tumor tissue to that in plasma, as shown in Fig. 5. By 24 h following CDDP–CSA-2 administration, platinum contents exceeded plasma concentration by a factor of 5.0, comparing to 3.89 for CDDP administration. The results show that complexation of CDDP with CSA-2 effectively enhances accumulation of platinum species in tumor.

4. Discussion

We investigated the dependency of pharmacokinetics of CDDP complexes on the mean molecular weight of CS by analyzing platinum concentrations in plasma and urine. The total plasma platinum–time curves obtained with the treatment of the complexes were compared with those measured as total and free drug after a similar dose administration of CDDP (Fig. 1). After introduction to circulation, CDDP binds irreversibly to low-molecular-weight nucleophiles and macromolecules (such as protein). The free drug, which is therapeutically effective, could not be detected beyond 90 min after a 2 mg/kg i.v.

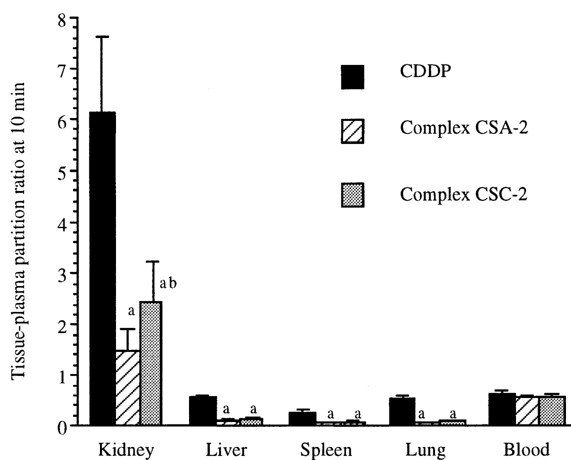

Fig. 3. Tissue–plasma partition ratios determined at 10 min following a 2 mg/kg i.v. administration of CDDP and CDDP–CSA-2 and CDDP–CSC-2 complexes to rats. All values are means ± S.D. ($n = 4$). (a) Significantly different from the corresponding result of CDDP group ($P < 0.05$); (b) Significantly different from the corresponding result of CDDP–CSA-2 group ($P < 0.05$).

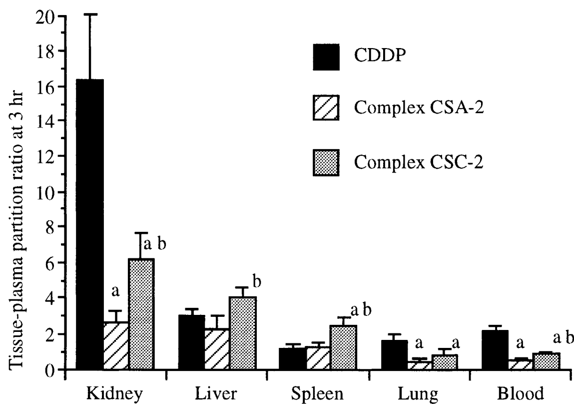

Fig. 4. Tissue–plasma partition ratios determined at 3 h following a 2 mg/kg i.v. administration of CDDP and CDDP–CSA-2 and CDDP–CSC-2 complex to rats. All values are means ± S.D. ($n = 4$). (a) Significantly different from the corresponding result of CDDP group ($P < 0.05$); (b) Significantly different from the corresponding result of CDDP–CSA-2 group ($P < 0.05$).

administration. Thereafter, platinum species mainly existed as protein bound compounds, similar to what was reported by Farris et al. (1985). Previous study found that the CS complex was effective in inhibiting protein binding of platinum in the circulation (Zhang et al., 2001). The plasma levels of the complexes were elevated with the

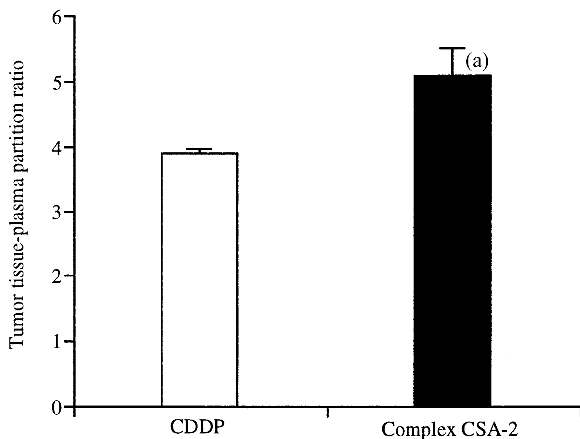

Fig. 5. Tumor tissue–plasma partition ratios at 24 h following a 5 mg/kg i.v. administration of CDDP and CDDP–CSA-2 complex to mice. Values are means ± S.D. ($n = 3$). (a) Significantly different from the corresponding result of CDDP group ($P < 0.05$).

increase in molecular size of CSA and CSC. Moreover, it was noted that CS complexes were mainly lost through urine after i.v. administration. This observation is identical to the pharmacokinetic feature of native CS polysaccharides (Palmieri et al., 1990). In addition, our results indicated that enzyme degradation did not seem to play an important role on the elimination of the complexes prepared by CS, although it was suggested by Guimaraes and Mourao (1997) that exogenous CS was degraded by enzymes in vivo. Urinary elimination is the main pathway determined by the molecular weights of the polymers. Guimaraes and Mourao. (1997) reported that the limit of renal filtration of CS was ≈ 30 kDa. Our results show that CDDP–CSA-1 and CDDP–CSC-1 were kept at significantly higher concentrations only for a short time period, and very high urinary excretions were found with these two complexes. The result suggests that molecular size around 10 kDa is too small to elevate the plasma retention. CDDP–CSA-2 and CDDP–CSC-2, with enlarged molecular size, effectively increased plasma retention and controlled the urinary excretion (Figs. 1 and 2). As a result, the AUCs obtained with the treatment of CDDP–CSA-2 and CDDP–CSC-2 were of four to five-fold higher than that of CDDP (Table 1).

Tissue distribution of platinum with the treatment of the complexes was evaluated by examining tissue–plasma partition ratios at 10 min and 3 h after a 2 mg/kg i.v. administration. Figs. 3 and 4 indicate that CDDP–CSA-2 and CDDP–CSC-2 effectively controlled distribution of CDDP to tissues, especially to kidney, within either early stage or longer duration after i.v. administration. However, higher partition ratios of tissue to plasma were found with CDDP–CSC-2. When taking account of urinary excretion, the loss of CDDP–CSC-2 from plasma did not correspond to the quantities excreted in urine when compared with CDDP–CSA-2. This evidence shows that CDDP–CSC-2 tends towards higher tissue accumulation than CDDP–CSA-2. We try to explain this finding by two possibilities: (a) CDDP–CSC-2 complex does not bind tightly with platinum in the circulation, therefore releasing free platinum species, which are responsible for the high tissue

accumulation. Or (b) as a carrier, CSC has higher affinity to tissues than CSA. In order to be clear on this question, we did a study to determine the complexation constants for reactions of CSA and CSC with CDDP by capillary electrophoresis. The results showed that both polymers complexed with CDDP via a moderate interaction, suggesting that the resulting complexes should be able to release platinum reversibly. Moreover, CSC showed much weaker linkage with platinum than CSA. These results support the first possibility. However, IC_{50} values obtained in the in vitro experiment indicate that CSC complex has lower activity than CSA complex. It seems that hydrated products in incubation medium has low contribution to the anticancer activity, presumably due to poor uptake by tumor cells; on the other hand, the complex effectively enters the cells by endocytosis and releases active species by the hydrolysis in lysosome. Further study will be required to prove this hypothesis.

In summary, this study provided the evidence that the sulfated position of chondroitin sulfates really affects the stability and the disposition of the CDDP complexes. The complex prepared with chondroitin sulfate A, with a molecular weight of over 20 kDa, is superior to that prepared by chondroitin sulfate C, because of improved pharmacokinetic properties and similar pharmacological activity to native drug.

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References

Albertini, R., Luca, G.D., Passi, A., Moratti, R., Abuja, P.M., 1999. Chondroitin-4-sulfate protects high-density lipoprotein against copper-dependent oxidation. Arch. Biochem. Biophys. 365, 143–149.

Anand, A.J., Bashey, B., 1993. Newer insights into cisplatin nephrotoxicity. Ann. Pharmacother. 27, 1519–1525.

Cvitkovic, E., 1998. Cumulative toxicities from cisplatin therapy and current cytoprotective measures. Cancer Treat. Rev. 24, 265–281.

Duncan, R., Dimitrijevic, S., Evagorou, E.G., 1996. The role of polymer conjugates in the diagnosis and treatment of cancer. STP Pharm. Sci. 6, 237–263.

Farris, F.F., King, F.G., Dedrick, R.L., Litterst, C.L., 1985. Physiological model for the pharmacokinetics of cis-dichlorodiammineplatinum (II) (DDP) in the tumored rat. J. Pharmacokinet. Biopharm. 13, 13–39.

Gianasi, E., Wasil, M., Evagorou, E.G., Keddle, A., Wilson, G., Duncan, R., 1999. HPMA copolymer platinates as novel antitumour agents: in vitro properties, pharmacokinetics and antitumour activity in vitro. Eur. J. Cancer 35, 994–1002.

Guimaraes, M.A.M., Mourao, P.A.S., 1997. Urinary excretion of sulfated polysaccharides administered to Wistar rats suggests a renal permselectivity to these polymers based on molecular size. Biochim. Biophys. Acta 1335, 161–172.

Hausheer, F.H., Kanter, P., Cao, S., Haridas, K., Seetharamulu, P., Reddy, D., Petluru, P., Zhao, M., Murali, D., Saxe, J.D., Yao, S., Martinez, N., Zukowski, A., Rustum, Y., 1998. Modulation of platinum-induced toxicities and therapeutic index: mechanistic insights and first- and second-generation protecting agents. Semin. Oncol. 25, 584–599.

Imai, T., Fujii, K., Shiraishi, S., Otagiri, M., 1997. Alteration of pharmacokinetics and nephrotoxicity of cisplatin by alginates. J. Pharm. Sci. 86, 244–247.

Kelly, G.S., 1998. The role of glucosamine sulfate and chondroitin sulfates in the treatment of degenerative joint disease. Altern. Med. Rev. 3, 27–40.

Lebwohl, D., Canetta, R., 1998. Clinical development of platinum complexes in cancer therapy: an history perspective and an update. Eur. J. Cancer 34, 1522–1534.

Maeda, H., Matsumura, Y., 1989. Tumoritropic and lymphotropic principles of macromolecular drugs. Crit. Rev. Ther. Drug 6, 193–210.

Palmieri, L., Conte, A., Giovannini, L., Lualdi, P., Ronca, G., 1990. Metabolic fate of exogenous chondroitin sulfate in the experimental animal. Arzneim.-Forsch. Drug Res. 40, 319–323.

Rosenberg, B., 1985. Fundamental studies with cisplatin. Cancer 55, 2303–2316.

Schechter, B., Pauzner, R., Wilchek, M., Arnon, R., 1986. Cis-platinum (II) complexes of carboxymethyl-dextran as potential antitumor agents. II. In vitro and in vivo activity. Cancer Biochem. Biophys. 8, 289–298.

Schechter, B., Neumann, A., Wilchek, M., Arnon, R., 1989. Soluble polymers as carriers of cis-platinum. J. Control. Release 10, 75–87.

Smith, I.E., Talbot, D.C., 1992. Cisplatin and its analogues in the treatment of advamced breast cancer: a review. Br. J. Cancer 65, 787–793.

Siddik, Z.H., Newell, D.R., Boxall, F.E., Harrap, K.R., 1987. The comparative pharmacokinetics of carboplatin and cisplatin in mice and rats. Biochem. Pharmcol. 36, 1925–1932.

Steward, W.P., 1998. Combination studies with gemcitabine in the treatment of non-small-cell lung cancer. Br. J. Cancer 78 (Supplement 3), 15–19.

Takakura, Y., Hashida, M., 1996. Macromolecular carrier systems for targeted drug delivery: pharmacokinetic considerations on biodistribution. Pharm. Res. 13, 820–831.

Zhang, J.S., Imai, T., Otagiri, M., 2000. Effect of a cisplatin–chondroitin sulfate A complex in reducing the nephrotoxicity of cisplatin. Arch. Toxicol. 74, 300–307.

Zhang, J.S., Ma, S.F., Suenaga, A., Otagiri, M., 2001. Stability of a cisplatin–chondroitin sulfate A complex in plasma and kidney in terms of protein binding. Biol. Pharm. Bull. 24, 970–972.