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Preclinical report

Antitumor activity of dextran derivatives immobilizing platinum complex (II)

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The *in vivo* antitumor activity and toxicity of a newly synthesized polymeric prodrug of cisplatin was investigated and also compared with plain cisplatin. The prodrug included a dicarboxymethyl-dextran conjugate of cisplatin (DCM-Dex/CDDP). DCM-Dex/CDDP was i.v. injected in mice bearing s.c. Colon 26 mouse colon cancer cells. The tissue distribution of platinum was thereafter determined by flameless atomic absorption spectrophotometry. The platinic concentration of the organs showed a high rate of retention at 24 h after injection in the DCM-Dex/CDDP-treated mice. No biochemical or hematologically adverse effects were observed. In addition, DCM-Dex/CDDP showed a significantly higher antitumor activity than cisplatin alone. These results indicate that DCM-Dex/CDDP may therefore be a potentially effective cancer chemotherapy. [© 2000 Lippincott Williams & Wilkins.]

Key words: Antitumor activity, cisplatin, dextran, drug delivery system, polymeric drug.

Introduction

cis-Dichlorodiamine platinum (II) (cisplatin, CDDP) is widely used for cancer chemotherapy, but one major drawback is its toxicity, mainly renal damage, which thus limits its use in cancer therapy.[1] To overcome such toxicity, the pharmacokinetic behavior this group of drugs must be changed. Recently, various polymeric anticancer drugs have been developed in an attempt to both improve the pharmacokinetic behavior and to decrease the adverse effects.[2–5] In addition to modifying the pharmacokinetic behavior of these drugs, these polymeric drugs have to maintain their slow release from the conjugate and also maintain a high concentration in tumorous tissue.[6] Based on such factors, some authors synthesized dextrans carrying platinum complexes.[7,8] Recently, we synthesized a prodrug of cisplatin by immobilizing cisplatin via a chelate-type coordination bond to dicarboxymethyl-dextran (DCM-Dex/CDDP). The *in vitro* characteristics and *in vivo* plasma disposition of DCM-Dex/CDDP have been previously reported.[9] The *in vitro* release behavior of cisplatin derivatives from DCM-Dex/CDDP was found to be very slow. The area under the plasma concentration curve (AUC) for DCM-Dex/CDDP was greater than that of cisplatin. In the present study, we investigated the *in vivo* antitumor activity and toxicity of DCM-Dex/CDDP and also compared the findings with cisplatin.

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Materials and methods

Chemicals

Dextran was purchased from Wako Pure Chemical Industries (Osaka, Japan) and had an average molecular weight (MW) of 60 000. Cisplatin was purchased from Sigma (St Louis, MO). DCM-Dex/CDDP was prepared according to the method reported previously[9] and its structure is shown in Figure 1.

Animals and tumors

Nine-week-old male BALB/c mice and 6-week-old male Wistar rats were obtained from Charles River Japan (Yokohama, Japan). Colon 26 mouse colon cancer cells were maintained in RPMI 1640 culture media containing 10% FCS and 2×10^5 cells were transplanted s.c. into the dorsum of the BALB/c mice. Wistar Rats and Colon 26 tumor-transplanted mice were maintained under constant room temperature

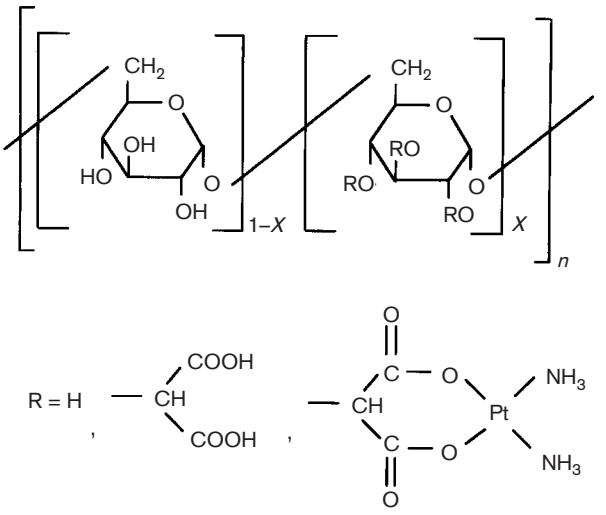

Figure 1. Structure of DCM-Dex/CDDP. The weight-average molecular weight is about 30 000. The degree of introduction of the dicarboxymethyl group is 35.5 mol% per sugar unit. The degree of introduction of the platinum complex is 4.66 mol% per sugar unit.

(25°C), and provided with free access to standard chow and tap water throughout the study, which was carried out at our animal facility (Laboratory Animal Center for Biomedical Research, Nagasaki University) in accordance with the our ethical code about the research use of animals.

Tissue distribution of platinum

The tissue distribution of platinum was analyzed in the tumor-bearing mice injected i.v. with either free cisplatin or DCM-Dex/CDDP both at doses of 3 mg/kg equivalent cisplatin (each group n=6). The mice were killed 1, 4 and 24 h after drug injection though the tail vein, and thereafter the liver, spleen, kidney, heart, lung and any tumors were excised and weighed immediately. All excised tissue specimens were rinsed with saline and stored at 4°C for a later analysis of the platinum concentrations in the tissue specimens.

Urinary excretion of DCM-Dex/CDDP

A polyethylene tube (internal diameter 0.5 mm; Natsume-Seisakusyo, Tokyo, Japan) was inserted and fixed in the urinary bladder of the rat. The urethra was ligated in order to precisely collect the urine. The rats were given an i.v. injection though the jugular vein of either cisplatin or DCM-Dex/CDDP at a dose of 1.5 mg/kg equivalent cisplatin (each group n=6). Urine samples were collected at various times (15–240 min). All samples were stored at 4°C in the same way as for the tissue specimens in the previous experiment.

Antitumor effect

Mice with tumors measuring about 5 mm in diameter were given three repeated i.v. injections though the tail vein of either free cisplatin, DCM-Dex/CDDP, each at a dose of 3 mg/kg equivalent cisplatin, or saline as a control at 4 day intervals, respectively. To evaluate the tumor growth inhibitory effect of free or DCM-Dex/CDDP, the size of the tumor was recorded every 4 days after the first injection. The tumor weight was estimated based on the following formula: length $\times$ width$^2 \times 1/2$. The results are expressed as the relative mean tumor weights (W_1/W_2), where W_1 is the estimated tumor weight at a given time and W_2 is the initial tumor weight. The mice were killed at 16 days after the first injection and the transplanted tumors were then excised to compare the tumor weight among the three groups (each group n=6).

Biochemical and hematological analyses

Wistar rats were i.v. given injections though the tail vein of either DCM-Dex/CDDP at a dose of 4.5 mg/kg equivalent cisplatin or saline (each group n=6). Blood samples were taken from the tail vein. The serum levels of glutamic pyruvate transaminase (GPT) and lactate dehydrogenase (LDH) were monitored on days 3, 7, 12 and 15 days after injection. The blood cell counts were thereafter monitored from the same samples.

Platinum concentrations in the tissue specimens

The platinum concentration in the samples was determined by flameless atomic absorption spectrophotometry (Z-8000; Hitachi, Tokyo, Japan) according to a previously described procedure.[10]

Statistical analyses

Any significant difference in the relative tumor weights was assessed by the one-way analysis of variance (ANOVA) and Fisher's PSLD *post hoc* test. Any differences in the concentration of platinum in the tissues specimens were analyzed by Student's t-test, while differences in the tumor weight were analyzed by the generalized Wilcoxon test. All p values were two-tailed and $p < 0.05$ was considered to indicate statistical significance.

Results

Tissue distribution of platinum

The tissue concentration of platinum was compared in mice injected with free cisplatin or DCM-Dex/CDDP (Table 1). After the i.v. injection of free cisplatin, the platinic concentration in the tissue was rapidly decreased in all tissue specimens. On the other hand, the platinum concentration in the tissue specimens remained high 24 h after administration in the DCM-Dex/CDDP-treated group. Regarding the tumors, the DCM-Dex/CDDP-treated group maintained a high level of platinum at 24 h after administration.

Urinary excretion of DCM-Dex/CDDP

The excretion rate of platinum (μg/min) excreted in the urine within 3 h after the i.v. injection of either free cisplatin or DCM-Dex/CDDP is shown in Figure 2. The platinum in the urine was rapidly excreted within 1 h after administration in the free cisplatin-injected rats. However, in the DCM-Dex/CDDP administered group, a small quantity of platinum was constantly found in the urine.

Antitumor effect

The growth inhibitory effect of DCM-Dex/CDDP on Colon 26 cells was investigated in mice by the i.v. injection of this drug. Figure 3 shows the growth inhibition curve of saline, free cisplatin and DCM-Dex/CDDP against Colon 26 transplanted s.c. In the control group, tumor growth was rapid and the ratio of the tumor volume at 14 days to the initial volume was more than 300. Three i.v. injections of free cisplatin relatively suppressed the tumor growth to some extent. On the other hand, a more marked growth inhibition was obtained after three injections of DCM-Dex/CDDP and the tumor volume ratio to the initial volume at 14 days was less than 60. Figure 4 shows the transplanted Colon 26 tumors excised at 16 days after the first treatment. The actual weights of the excised tumors were: saline, 1037 ± 104 (SE) mg; free cisplatin, 584 ± 47 mg; DCM-Dex/CDDP, 314 ± 39 mg. The actual weight of the excised tumors of the DCM-Dex/

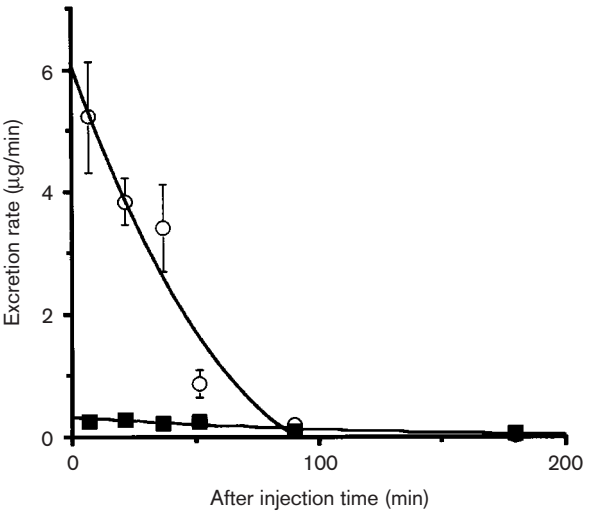

Figure 2. The excretion rate of platinum in the urine. All data indicate the mean ± SD: (○) cisplatin and (■) DCM-Dex/CDDP.

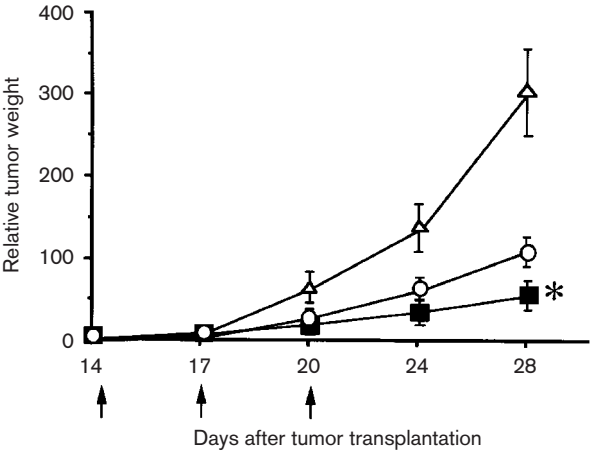

Figure 3. The therapeutic effect of cisplatin or DCM-Dex/CDDP on growth in transplanted tumors. All data indicate the mean ± SD: (△) saline, (○) cisplatin and (■) DCM-Dex/CDDP. *$p < 0.05$ versus cisplatin by a one-way ANOVA and Fisher's PLSD *post hoc* test. The injection schedules of the drugs is shown as the arrows.

Table 1. Tissue distribution of platinum in tumor-bearing mice after i.v. injection

Tissue	Concentration of platinum (μg/g tissue)					
	Cisplatin			DCM-Dex/CDDP		
	1 h	4 h	24 h	1 h	4 h	24 h
Tumor	2.70	2.15	1.45	1.51[a]	3.35[a]	2.08[a]
Liver	1.76	1.30	0.75	2.96[b]	5.09[a]	5.04[a]
Spleen	0.53	0.41	0.16	0.63[b]	1.48[a]	1.45[a]
Kidney	2.51	2.22	1.03	1.07[a]	2.07[b]	2.27[a]
Lung	0.50	0.83	0.22	1.04[a]	1.19[b]	0.83[b]
Blood	1.22	0.59	0.05	2.02[a]	1.63[b]	0.51[a]

All data indicate the mean.
Student's *t*-test: [a]$p < 0.01$ versus cisplatin; [b]$p < 0.05$ versus cisplatin.

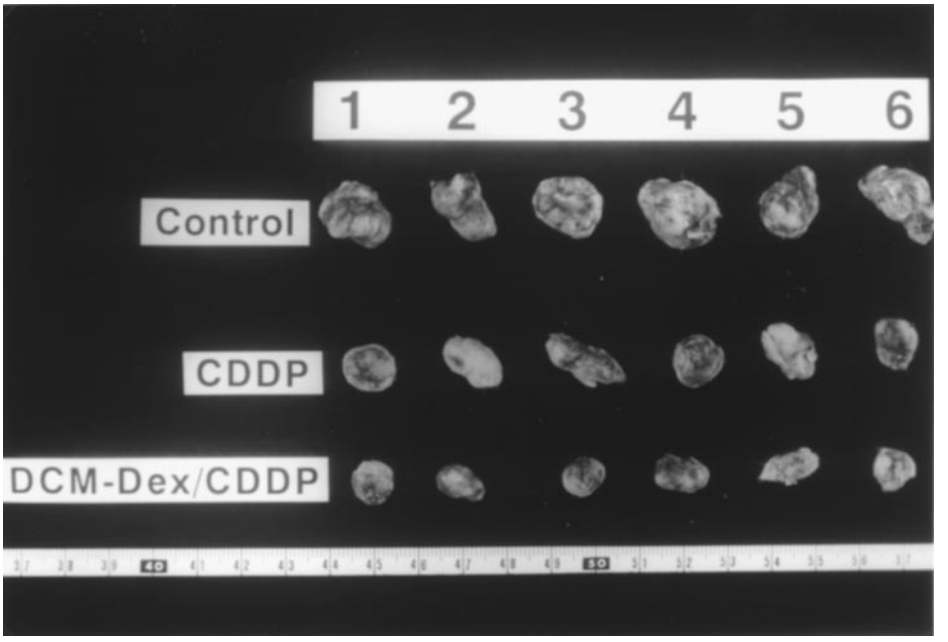

Figure 4. Photograph of whole xenotransplanted tumors excised from mice.

CDDP-treated group was thus smaller than the other two groups and the difference was significant ($p < 0.01$).

Biochemical and hematological analyses

The serum chemistry findings and blood profile are shown in Figures 5 and 6. The levels of GPT and LDH after the injection of DCM-Dex/CDDP showed almost no change at 15 days after injection. In addition, no change was observed in the blood profile after the i.v. injection of the same drug in rats as well.

Discussion

Although cisplatin is widely used for cancer chemotherapy, its utility is limited due to its adverse side effects, consisting mainly of renal and gastrointestinal damage.[1] These adverse effects must be overcome before this treatment can be safely used in cancer chemotherapy. One possible way to achieve these goals might be to conjugate the drug to such high molecular weight compounds as chitin and dextran.[4,5] Scechter *et al.*[11] reported the use of carboxymethylated dextran as a drug carrier in order to minimize the adverse effects associated with cisplatin. In the present study, we synthesized a polymeric prodrug of cisplatin by immobilizing cisplatin via a chelate-type coordina-

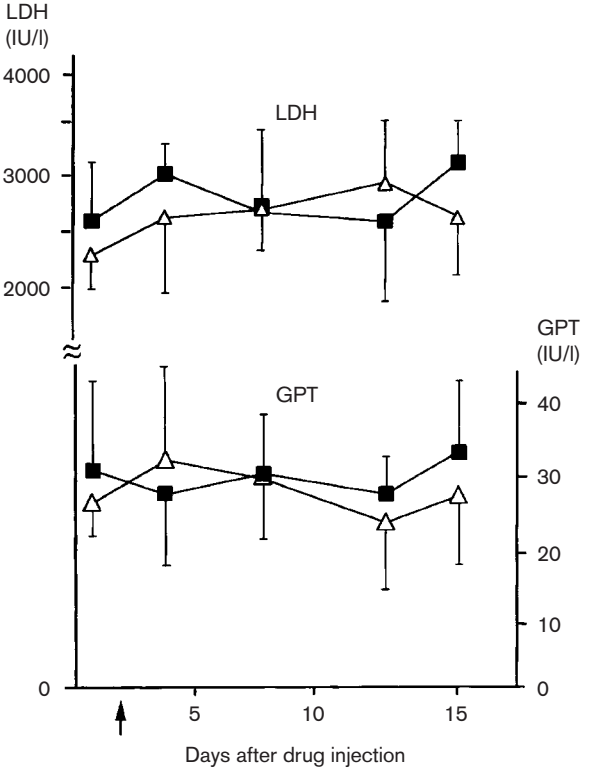

Figure 5. Serum chemistry findings. All data indicate the mean ± SD: (△) saline and (■) DCM-Dex/CDDP. GPT, glutamic pyruvate transaminase; LDH, lactate dehydrogenase. The injection schedule of the drugs is shown as the arrow.

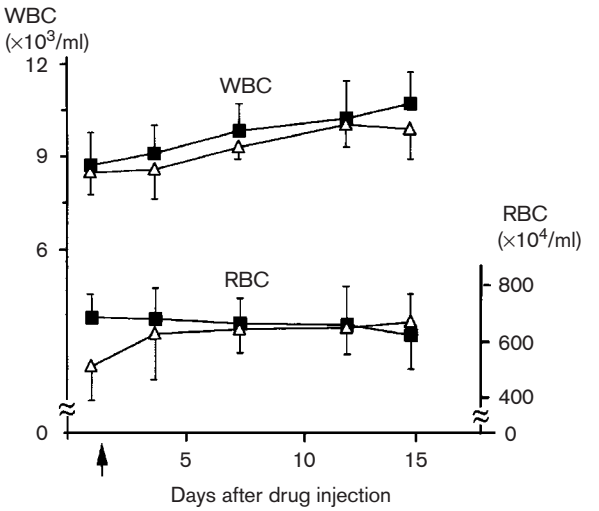

Figure 6. Blood profile findings. All data indicate the mean ± SD: (△) saline and (■) DCM-Dex/CDDP. RBC, red blood cells; WBC, white blood cells. The injection schedule of the drugs is shown as the arrow.

tion bond to dicarboxymethyl-dextran (DCM-Dex/CDDP). The *in vitro* release half-life of DCM-Dex/CDDP has been reported to be 45 h.[9] The tissue distribution of platinum from cisplatin and DCM-Dex/CDDP was determined in rats. The platinum clearance of the tissue closely paralleled the serum clearance in cisplatin. However, the platinum concentration of the tissue specimens from the DCM-Dex/CDDP-treated group remained high 24 h after i.v. injection. Our *in vivo* study showed DCM-Dex/CDDP to release cisplatin derivatives slowly.[9] These findings suggest that a high blood platinum concentration is thus retained in the DCM-Dex/CDDP-treated group. As a result, a high level of platinum remains in the blood stream of the DCM-Dex/CDDP group. A significant difference was observed in the level of platinum released in the urine and that returned in the uridiferous tubules in the kidneys.[12] The release of platinum in the urine in the DCM-Dex/CDDP-treated group was slower than in the cisplatin group. DCM-Dex/CDDP is thus not considered to induce any major adverse drug reactions in the kidneys. DCM-Dex/CDDP, which is a polymeric drug, was found to have a higher *in vivo* antitumor effect than cisplatin in the present study. Such polymeric antitumor drugs have a larger accumulation at the tumor site than low molecular weight drugs due to their enhanced permeability and retention (EPR) effects.[13] A longer *in vivo* circulation has also been reported after the administration of polymeric drugs.[9,14] For these reasons, DCM-Dex/CDDP is considered to have a strong antitumor effect. Polymeric antitumor drugs cannot strengthen the direct cytotoxicity against tumor cells by themselves, but they can reduce the number of adverse effects in comparison to low molecular antitumor drugs.[15] Figures 5 and 6 show that DCM-Dex/CDDP had hardly any influence on the blood bone marrow and liver function in our experiments. In conclusion, these observations suggest the high molecular weight carrier-medicated delivery of cisplatin to be a potentially effective cancer chemotherapeutic method.

Acknowledgments

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