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Antitumor activity of a new series of platinum complexes: *trans*(±)-1,2-cyclohexanediammineplatinum(II) conjugated to acid polysaccharides

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Complexes with *trans*(±)-1,2-cyclohexanediammineplatinum(II) conjugated to acid polysaccharides were synthesized and their antitumor activities were tested in female CDF_1 mice with intraperitoneal leukemia L1210 cells. Platinum was released from the polymers under physiological conditions, with half-lives from 3.3 to 19.3 h. A hyaluronic acid-supported complex was the most effective against the tumors (all six mice survived for 60 days). The group given a chondroitin polysulfate-supported complex had five survivors, the chondroitin sulfate A group also had five, the chondroitin sulfate C group had three and the heparan sulfate group had two. Part of the antitumor activity was due to increased efficacy of the polymers. The bioavailability of these complexes is high. Therefore, acid polysaccharides should be a good system for delivering antitumor platinum complexes.

Key words: Acid polysaccharides conjugated, antitumor activity, drug delivery system, Pt(II) complexes.

Introduction

Since the discovery that *cis*-diamminedichloroplatinum(II) (cisplatin) is useful against tumors,[1] various related complexes have been synthesized by changing the leaving ligands[2–4] or the carrier ligand(s),[5–8] with a view to developing complexes with stronger antitumor activity and minimal host toxicity. However, only a few complexes, such as carboplatin,[9,10] have been found to be clinically superior to cisplatin. Some of the complexes are less toxic, some have a higher therapeutic ratio and some are more soluble in water than cisplatin. Recently, we found that liposoluble Pt(II) complexes have excellent antitumor activity when used with a lipophilic vehicle such as lipiodol (iodinated fatty-acid ethyl ester of poppy seed, used for X-ray diagnosis of uterine, lymph node and ovarian duct diseases).[11,12]

Here we describe the synthesis and testing of a new group of Pt(II) complexes. Antitumor activity in female CDF_1 mice with L1210 cells was the primary assay. The complexes had acid polysaccharides (APS), chondroitin sulfate A (chondroitin 4-sulfate, CSA), chondroitin sulfate C (chondroitin 6-sulfate, CSC), chondroitin polysulfate (further-sulfated chondroitin sulfate C, CPS), hyaluronic acid (HA) or heparan sulfate (Hep) as the leaving ligand and also as the rigid support portion of the complexes (Table 1). They all had *trans*(±)-1,2-diaminocyclohexane (DACH) as the carrier ligand. APS were also used as a kind of drug delivery system.

trans(±) - 1,2 - Cyclohexanediamminedichloroplatinum(II) (*t*-DACHPCl_2) was converted to the corresponding diaqua form by reaction with two equivalent moles of silver nitrate,[13] which was reacted with the sodium salt of the APS in an aqueous solution overnight (about 18 h) at room temperature in the dark. The reaction mixture was passed through a membrane filter (pore size 0.2 μm) to remove a small amount of insoluble material. If several complexes precipitated during the reaction, this procedure was omitted. The filtrate or the reaction mixture was dialyzed against distilled water at 5°C for 18–20 h and the resulting solution was lyophilized. This gave a white powder, which was an APS conjugated to *trans*(±)-1,2-cyclohexanediammineplatinum(II) (APS–DACHP). The per-

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Table 1. Physical properties of APS (glycosaminoglucanes) used as the polymer support for the synthesis of platinum complexes

APS	MW[a]	S (%)	$[\alpha]_D(c = 1, H_2O)$[b]	ACA (APTT)[c]
HA1	10000	—	−65.69	>10000
HA3	30000	—	−70.20	>10000
HA15	150000	—	−70.00	>10000
HA100	1000000	—	−70.00	>10000
CSA1	30000	6.10	−28.00	7600
CSC1	15000	6.31	−25.00	7600
CSC2	30000	6.36	−20.44	>10000
CSC3	60000	6.36	−20.67	>10000
CPS1	17640	10.88	−12.79	100
CPS2	19200	13.0	−14.01	10
CPS3	4000	13.0	−13.98	50
CPS4	10000	13.0	−13.89	12.5
Hep1	15000	12.0	+60.00	2.5

[a]The values indicate average molecular weight of each APS.
[b]Optical rotations were measured at 25°C in water.
[c]APTT, activated partial thromboplastin time. The values shown are 50% anticoagulant activity of sulfated polysaccharides, in μg/ml.

centage of platinum (3–25%) depended on the added molar ratio of the diaqua complex. The solubility of APS–DACHP complexes varied from 3 to 30 mg/ml of water and depended on the number of DACHP molecules bound to the polymer. The optical rotations tended to be lower when platinum contents were higher (Table 2). These *trans*(±)-DACHP conjugated APS were studied by cellulose acetate membrane electrophoresis (0.1 M pyridine-formic acid, pH 3.0), high performance liquid chromatography with a gel filtration column (G4000PWXL, 0.1 M NaCl, A210 nm), elemental analysis of platinum (by inductively coupled plasma spectroscopy), quantitative analysis of glycosaminoglucanes (phenol–sulfuric acid method), ^{1}H-nuclear magnetic resonance spectroscopy and infrared spectrophotometry. These data supported the proposed structure. The schematic structure of *trans*-(±) DACHP conjugated to chondroitin sulfate is shown in Figure 1.

Platinum release experiments were done on APS–DACHP complexes under physiological conditions *in vitro* (phosphate saline buffer, pH 7.18) at 37°C. The rate constants were calculated by plotting the time against the percentage of a filtrable

Table 2. Physical properties of synthesized *t*-DACHP-conjugated APS complexes

Complex	Composition (%)[a]			MW of original APS[b]	$[\alpha]_D$ $(c = 1, H_2O)$[c]
	Pt	GAG	S		
HA1–Pt10S	24.5	61.2	—	10000	−40.24
HA1–Pt11S	17.2	72.7	—	10000	−46.39
HA3–Pt11S	17.1	72.9	—	30000	−48.13
HA15–Pt11S	17.0	73.0	—	150000	−46.76
HA100–Pt11S	17.0	73.0	—	1000000	−47.00
CSA1–Pt1	1.03	91.47	6.08	30000	−26.40
CSA1–Pt2	1.80	90.08	6.05	30000	−26.20
CSC1–Pt10S	23.0	63.5	4.06	15000	−15.13
CSC1–Pt11S	14.0	77.8	5.00	15000	−19.06
CSC2–Pt11S	14.3	77.3	4.92	30000	−13.00
CSC3–Pt11S	14.1	77.8	5.03	60000	−11.58
CPS1–Pt10S	19.5	69.1	7.01	17640	−11.40
CPS1–Pt11S	12.5	80.2	8.72	17640	−8.47
CPS2–Pt10S	18.9	70.0	9.25	19200	−4.54
CPS3–Pt10S	18.8	70.4	9.28	4000	−10.75
CPS4–Pt10S	18.8	70.4	9.20	10000	−0.56
CPS4–Pt11S	11.4	81.9	10.72	10000	−7.80
Hep1–Pt10S	19.1	70.4	8.01	15000	+36.18
Hep1–Pt11S	10.4	83.5	8.56	15000	+50.95

[a]Platinum contents were measured by inductively coupled plasma spectrometry in 1N HNO_3. Glycosaminoglucane (GAG) quantitative analysis was done by the phenol–sulfuric acid method. Sulfur contents were analyzed by elemental analysis.
[b]The values indicate average molecular weight of original APS.
[c]Optical rotations were measured at 25°C in water.

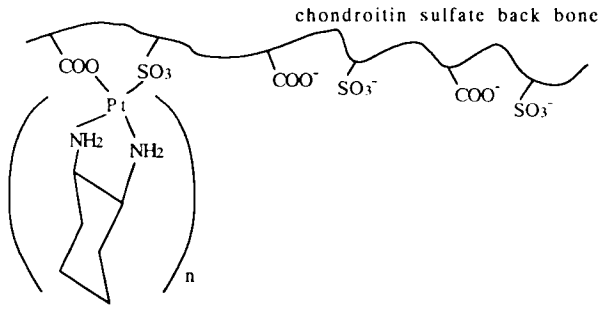

Figure 1. Schematic mode of binding of *t*-DACHP to chondroitin sulfates.

platinum in the total (Centricon was used, cut off molecular weight above 1000). Half-lives of the CSC complexes were 3.3 and 19.3 for CSC–DACHP1 (platinum content 14.5%) and CSC–DACHP2 (platinum content 12.5%), respectively. Half-lives of the HA complexes were 12.8 and 17.8 h for HA–DACHP4 (platinum content 4.9%) and HA–DACHP5 (platinum content 3.7%), respectively. The APS–DACHP complexes had more platinum and had higher releasing rate constants in CSC–, CPS– and HA–DACHP complexes. The half-lives of HA–DACHP complexes, which have only a carboxylate residue as an acid, are short; however, those of CSC–DACHP complexes, which have both carboxylate and sulfate anionic residues, are long.

The antitumor activity of these platinum complexes was tested in female CDF_1 mice after leukemia L1210 cells were implanted intraperitoneally (1×10^5 cells per mouse). Platinum complexes were dissolved or suspended in water, and given intraperitoneally on days 1 and 5 after tumor transplantation. The doses were calculated based on molar contents of DACHP in the polymers. The best results of antitumor activity in each complex are summarized with the administered doses in Table 3. The antitumor activity of CSC–DACHP complexes depended on the mean molecular weight of the chondroitin sulfate. CSA–DACHP complexes, in contrast to CSC–DACHP complexes, were very active against the tumor, even at very low doses (4.8 μmol Pt/kg). The 60-day survival rates of mice given CPS1–Pt complexes were fairly good. This shows that the polymers of low molecular weight are more active against tumors than those of high molecular weight. With 60-day survival rate as the criterion, the most effective platinum complex with HA was HA1–Pt11S,

Table 3. Antitumor activity of APS-supported *t*-DACHP complexes on murine leukemia L1210 in mice[a]

Complex[b]	Dose[c] (μmol Pt/kg)	Median survival time (day)	T/C[d] (%)	60-day survivors/total mice[e]
CSA1–Pt1	4.8	>60	>750	5/6
CSA1–Pt2	4.8	>45	>563	3/6
CSC1–Pt11S	12	29.0	363	1/6
CSC2–Pt11S	12	>42.5	>531	3/6
CSC3–Pt11S	12	27.0	338	1/6
CPS1–Pt10S	30	27.5	344	2/6
CPS2–Pt10S	30	28.5	356	2/6
CPS3–Pt10S	30	>60	>667	5/6
CPS4–Pt10S	30	22.0	275	2/6
CPS4–Pt11S	30	>56.5	>713	3/6
HA1–Pt11S	30	>60	>750	6/6
HA3–Pt11S	12	18.0	225	0/6
HA15–Pt11S	12	>42	>525	3/6
HA100–Pt11S	12	17.5	219	1/6
Hep–Pt10S	30	16.0	200	0/6
Hep–Pt11S	30	24.0	300	2/6
Cisplatin[f]	13.3	15.0	188	0/6
t-DACHPCl$_2$[f]	13.7	19.5	244	1/6

[a]L1210 cells (1×10^5) were transplanted intraperitoneally on day 0 into 6 week old female CDF$_1$ mice weighing 18.8 ± 0.9 g.
[b]Complexes were dissolved or suspended in water and given intraperitoneally on days 1 and 5 after transplantation of tumor cells.
[c]Doses were calculated based on platinum contents in the polymer.
[d]The ratio of median survival time of the treated group to that of the control group.
[e]The ratio of 60-day survivors versus total mice.
[f]Cisplatin and *t*-DACHPCl$_2$ were used as the positive controls.

followed by HA15–Pt11S, HA100–Pt11S and HA3–Pt11S. Complexes with HA of greater molecular weight were generally less active. These results suggest that the molecular weight of the polymer (APS) is important for antitumor activity, but it is not clear yet whether the mean molecular weight range of the polymer is always associated with high antitumor activity. Hep–Pt10s was less active than Hep–Pt11s. For APS complexes, the platinum content was related to the antitumor activity (data not shown) and complexes with more platinum were less active than those with less platinum. These APS-supported platinum complexes were at least as active as their parent complex, *t*-DACHPCl$_2$, and at lower doses. In particular, the CSA–DACHP complex series was very active at doses 2.5–7 times lower than the parent complex. This shows that the biological availability of these complexes is high, i.e. the active principle, released gradually from the polymers, is used efficiently.

However, the degree of antitumor activity of APS–Pt complexes is difficult to explain by high bioavailability alone. Furthermore, comparing doses with the same amounts of platinum, we find that the CSA and CSC complexes in which each complex has a small amount of platinum were more active than those with large amounts of platinum. This suggests that after the active complex is released, the polymer portion acts against the tumor. Some mice were given a combination of cisplatin or *t*-DACHPCl$_2$ and CSA at the same doses corresponding to the molar composition of the CSA–DACHP complex. The combination of CSA (500 mg/kg) and *t*-DACHPCl$_2$ (4.5 mg/kg, 11.84 μmol Pt/kg) was more active than the platinum alone (4/6 versus 5/6 of 60-day survivors), but CSA alone had no effect. Therefore, chondroitin sulfate increased the antitumor effect of platinum complexes. This phenomenon has been observed for both cisplatin and *t*-DACHPCl$_2$ in L1210 culture cells *in vitro*, but its mechanism is not yet clear. These results may be explainable in part by the findings of Murata *et al.*[14,15] that heparin or a sulfated chitin can inhibit lung metastasis of tumors in mice.

HA-supported complexes were the most effective of the APS, but they have relatively low solubility in water. CPS complexes were more active and more soluble than the CS, HA or Hep complexes. However, CPS, which is generated after the release of the active platinum complex, is an anticoagulant.

We have studied Pt(II) complexes with altered leaving ligands (unpublished results), but there have been no reports of the synthesis and antitumor activity of polymer-conjugated Pt(II) complexes. There is one report on chondroitin sulfate–Pt(II) complexes as examples of anionic macromolecules, including others such as polyglutamic acid, polyaspartic acid and serum albumin.[16] These complexes have several advantages: (i) better bioavailability than DACHPCl$_2$, i.e. equal or greater antitumor activity with a lower dose of Pt(II), (ii) some selectivity of organ distribution (CSC–DACHP complexes accumulated more in the liver than in other organs if given intraperitoneally, data not shown), (iii) release of the active principle over an appropriate period at a suitable rate and (iv) some antitumor activity of chondroitin sulfate after the platinum is liberated.

These APS supported platinum complexes can be used in developing organ-targeted antitumor platinum complexes, and APS such as chondroitin sulfates and HA are good drug delivery systems for platinum complexes.

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References

1. Rosenberg B, VanCamp L, Trosko JF, *et al.* Pt compounds: a new class of potent antitumor agents. *Nature* 1969; **222**: 385–6.
2. Farrell N, Roberts JD, Hacker MP. Shikimic acid complexes of Pt. Preparation, reactivity, and antitumor activity of (*R*,*R*-1,2-diaminocyclohexane)-bis(shikimato)-Pt(II). Evidence for a novel rearrangement involving Pt–carbon bond formation. *J Inorg Biochem* 1991; **42**: 237–46.
3. Galeano A, Berger MR, Keppler BK. Activity of two Pt-linked phosphonic acids against autochthonous rat colorectal cancer as well as in two human colon-cancer cell lines. *Cancer Chemother Pharmacol* 1992; **30**: 131–8.
4. Maeda M, Suga T, Takasuka N, *et al.* Effect of bis(bilato) - 1,2 - cyclohexanediammineplatinum(II) complexes on lung metastasis of B16-F10 melanoma cells in mice. *Cancer Lett* 1990; **55**: 143–7.
5. Clear MJ, Hoeschele JD. Antitumor Pt compounds. Relationship between structure and activity. *Pt Metals Rev* 1973; **17**: 2–13.
6. Hacker MP, Douple EB, Krakoff IH (eds). *Pt coordination complexes in cancer chemotherapy*. Boston: Martinus Nijhoff 1983.
7. Nicolini M (ed.) *Pt and other metal coordination compounds in cancer chemotherapy*. Boston: Martinus Nijhoff 1987.
8. Howell SB (ed.). *Pt and other metal coordination compounds in cancer chemotherapy*. New York: Plenum Press 1991.

9. Lira-Puerto V, Silva A, Morris M, *et al.* Phase II trial of carboplatin or iproplatin in cervical cancer. *Cancer Chemother Pharmacol* 1991; **28**: 391–6.
10. Viñolas N, Daniels M, Estapé J, *et al.* Phase II trial of carboplatin and etoposide activity in pretreated breast cancer patients. *Am J Clin Oncol* 1992; **15**: 160–2.
11. Maeda M, Uchida NA, Sasaki T. Liposoluble Pt(II) complexes with antitumor activity. *Jpn J Cancer Res* 1986; **77**: 523–5.
12. Maeda M, Takasuka N, Suga T, *et al.* New antitumor Pt(II) complexes with both lipophilicity and water miscibility. *Jpn J Cancer Res* 1990; **81**: 567–9.
13. Connors TA, Jones M, Ross WCJ, *et al.* New Pt complexes with anti-tumor activity. *Chem-Biol Interact* 1972; **5**: 415–24.
14. Murata J, Saiki I, Matsuno K, *et al.* Inhibition of tumor cell arrest in lungs by antimetastatic chitin heparinoid. *Jpn J Cancer Res* 1990; **81**: 506–13.
15. Murata J, Saiki I, Nishimura S, *et al.* Inhibitory effect of chitin heparinoids on the lung metastasis of B16-BL6 melanoma. *Jpn J Cancer Res* 1989; **80**: 866–72.
16. Smith DG, Brown DS, Bernstein P, *et al.* Pt and palladium complexes. US patent 4,673,754 1987.

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