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ORIGINAL ARTICLE

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A new orally active antitumor 1*R*,2*R*-cyclohexanediamine-platinum(IV) complex: *trans*-(*n*-valerato)chloro(1*R*,2*R*-cyclohexanediamine) (oxalato)platinum(IV)

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Abstract *Purpose*: The authors have previously reported that *trans*-bis(*n*-valerato)(1*R*,2*R*-cyclohexanediamine) (oxalato)platinum(IV) (C5-OHP), an oxaliplatin derivative, is an orally active antitumor agent in an intraperitoneal (i.p.) L1210 murine leukemia model. In this study, several oxaliplatin derivatives of the general formula *trans*-(carboxylato)chloro(1*R*,2*R*-cyclohexanediamine)(oxalato)platinum(IV) were synthesized in order to find new derivatives with greater oral activity than C5-OHP in a clinically predictive tumor model. In the formula, the carboxylate and chloride ligands are situated in axial positions. *Methods*: Four complexes with the axial carboxylate ligands *n*-butyrate, *n*-valerate, *n*-caproate or *n*-heptanoate were synthesized and designated C4-OHP-Cl, C5-OHP-Cl, C6-OHP-Cl and C7-OHP-Cl, respectively. The oral antitumor activity of the complexes was evaluated against the murine reticulosarcoma M5076 implanted subcutaneoulsy (s.c.) in to male BDF_1 mice. The complexes were administered orally daily for 5 days in two cycles initiated on days 5 and 12 postimplantation. The physicochemical properties were examined by measuring the concentrations of the complexes in test solutions at intervals by HPLC. The pharmacokinetic behaviors of C5-OHP-Cl, C6-OHP-Cl and C5-OHP following a single oral administration were studied in non-tumor-bearing male BDF_1 mice. *Results*: Of the complexes synthesized in this study, C5-OHP-Cl, which exhibited high activity in the i.p. L1210 model, was found to be orally active in the s.c. M5076 model while C5-OHP was not. The in vitro reduction of the complexes by ascorbate was much more rapid than that of C5-OHP, while the complexes were more stable than C5-OHP in HCl-acidic and alkaline solutions. Pharmacokinetic study showed that C_{max} and $AUC_{0–24h}$ values of plasma total and filterable platinum of C5-OHP-Cl were four to six times greater than those of C5-OHP, indicating that C5-OHP-Cl was absorbed more than C5-OHP. *Conclusion*: C5-OHP-Cl was found to be a superior l-OHP derivative C5-OHP, exhibiting significant oral antitumor activity in the s.c. M5076 model. The enhanced activity of C5-OHP-Cl was considered to be due in part to increased susceptibility to reduction and increased gastrointestinal absorption. C5-OHP-Cl is a suitable candidate for further study as an oral cancer chemotherapy agent.

Key words Antitumor platinum complex · Orally active agent · Oxaliplatin derivative

Abbreviations $AUC_{0–24\ h}$ area under the platinum concentration–time curve up to 24 h post-administration · C_{max} peak platinum concentration in plasma · t_{max} time of peak platinum concentration in plasma

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Introduction

Platinum complexes are now a well-established class of cancer chemotherapy agents. Cisplatin [*cis*-diammine-dichloroplatinum(II)] and carboplatin [diammine(1,1-cyclobutanedicarboxylato)platinum(II)] are currently used in the treatment of a number of human malignancies [1, 16, 32]. However, their usefulness is limited by severe toxic side effects such as nephrotoxicity,

gastrointestinal toxicity and myelosuppression, and by resistance to them. To ameliorate these disadvantages, much effort has been put into developing new antitumor platinum complexes that possess greater activity and/or less toxicity and are efficacious against tumors resistant to cisplatin and carboplatin [6, 9].

Platinum complexes of 1,2-cyclohexanediamine (1,2-dach) have been described and have been reported to show non cross-resistance with cisplatin [3, 4]. Among the three optical isomers, 1*R*,2*R*-dach, 1*S*,2*S*-dach and *R*,*S*-dach, 1*R*,2*R*-dach has been found to have the highest activity [11, 12] and oxaliplatin [(1*R*,2*R*-dach)(oxalato) platinum(II); l-OHP] has been successfully developed as a second generation platinum agent [17–19, 29–31]. It is efficacious against melanoma, ovarian, testicular, lung, stomach and colorectal cancers, and it shows no nephrotoxicity, cardiotoxicity, hematotoxicity, or mutagenicity, and very low myelosuppression. It also shows no cross-resistance with cisplatin [23, 25, 31]. Because of these excellent features, l-OHP was approved as a new antineoplastic drug in France in 1996.

The quality of life (QOL) of cancer patients is an important problem in cancer treatment. The existing platinum complexes have the disadvantages that they are administered by parenteral routes including long-term intravenous infusion and intra-arterial injection and that hospitalization is required. Development of orally active platinum agents could offer cancer patients a significant benefit and improvement in QOL by enabling them to undergo platinum agent therapy at home or in a hospice. Platinum complexes of the next generation should be orally active agents.

A novel series of ammine/amine platinum(IV) dicarboxylate complexes is the first class of complexes to be prepared as oral agents [8]. Among them, JM216 [*trans*-bis(acetato)-*cis*-(ammine)(cyclohexylamine) dichloroplatinum(IV)] has been found to show significant antitumor activity via the oral route and has been chosen for full clinical development [8, 10, 28]. Phase I trials of JM216 have been completed [21, 24] and it is currently in phase II trials.

The present authors are seeking to prepare orally active l-OHP derivatives, since l-OHP is an excellent antitumor drug as described above. If an l-OHP derivative could be properly designed to be absorbable and stable in the digestive tract and serve as the l-OHP prodrug, it would be an orally active antitumor platinum complex with similar biological features to those of l-OHP. In our previous study, several l-OHP derivatives of the general formula *trans*-bis(carboxylato)(1*R*,2*R*-dach)(oxalato)platinum(IV) (described simply as bis-carboxylato complexes below) were synthesized and *trans*-bis(*n*-valerato)(1*R*,2*R*-dach)(oxalato)platinum(IV) (C5-OHP) was found to be orally active in an intraperitoneal (i.p.) L1210 murine leukemia model [14]. However, C5-OHP failed to exhibit significant antitumor activity in a subcutaneous (s.c.) M5076 murine reticulosarcoma model as described in this report.

The physicochemical properties and pharmacokinetic behavior of C5-OHP suggest that an l-OHP derivative with increased susceptibility to reduction and increased water solubility would exhibit greater antitumor activity than C5-OHP [14, 15]. With this in mind, the authors designed a new complex with the formula *trans*-(carboxylato)chloro(1*R*,2*R*-cyclohexanediamine)(oxalato) platinum(IV) and synthesized several complexes (described simply as carboxylato/chloro complexes below) in order to find new l-OHP derivatives which are orally active in tumor models predictive of activity against human tumors. The synthesis, oral antitumor activity, physicochemical properties and pharmacokinetic behavior of carboxylato/chloro complexes, in comparison with C5-OHP, are described in this report. The complexes synthesized in this study have an axial carboxylate ligand of *n*-butyrate, *n*-valerate, *n*-caproate or *n*-heptanoate and were designated C4-OHP-Cl, C5-OHP-Cl, C6-OHP-Cl and C7-OHP-Cl, respectively.

Materials and methods

Chemicals

C5-OHP was synthesized as reported previously [14]. l-OHP was obtained from Tanaka Kikinzoku Kogyo (Hiratsuka, Japan) and used as received. Methanol from Katayama Chemicals (Osaka, Japan) was distilled once prior to use. Other chemicals were of reagent grade or better and used without further purification.

Animals

Female C57BL/6, male CDF_1 (BALB/c × DBA/2) and male BDF_1 (C57BL/6 × DBA/2) mice, 6–7 weeks of age, were purchased from Japan SLC (Hamamatsu, Japan). All animal experiments were carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University.

Tumors

Murine leukemia L1210 and murine reticulosarcoma M5076 cell lines were obtained from the Japanese Foundation for Cancer Research (Tokyo, Japan). L1210 cells were maintained in RPMI-1640 (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal bovine serum (Intergen, Purchase, N.Y.), 50 mg/l streptomycin and 5000 U/l penicillin at 37 °C in a humidified atmosphere containing 5% CO_2. M5076 cells were maintained in vivo by serial s.c. passage in female C57BL/6 mice.

Synthesis of carboxylato/chloro complexes

The carboxylato/chloro complexes were synthesized by the two pathways of Method 1 and Method 2 shown in Fig. 1. The procedures are described below briefly.

Method 1: Bis-carboxylato complexes (axial carboxylate ligand *n*-butyrate, *n*-valerate, *n*-caproate or *n*-heptanoate) were prepared as previously described [14]. The complexes were dissolved in 1 *M* HCl/40% aqueous methanol and incubated at 37 °C for 15 h to replace an axial carboxylate ligand with chloride. The solutions were then neutralized with sodium hydroxide to pH 5 to 6 to stop the reaction. The C4-, C5-, C6- and C7-OHP-Cl produced were isolated and purified by reverse-phase HPLC.

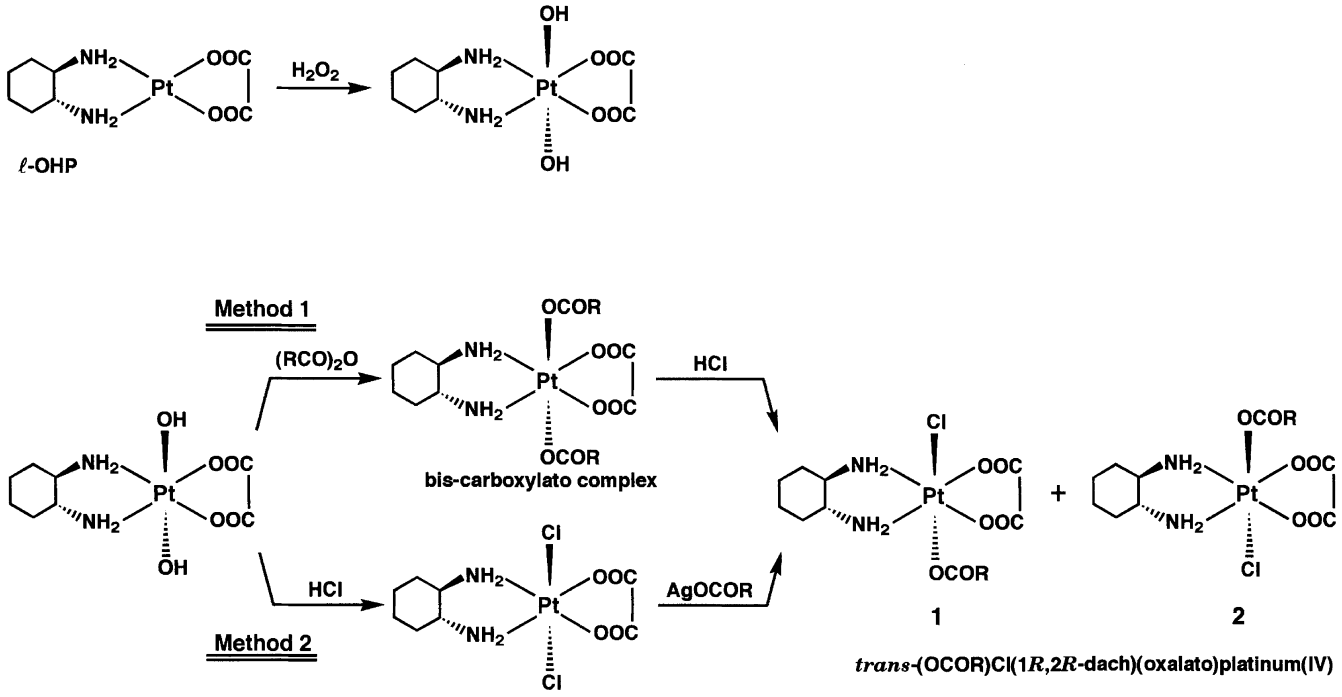

Fig. 1 Synthetic pathways of the carboxylato/chloro complexes

Method 2: l-OHP was oxidized by hydrogen peroxide and then treated with HCl to yield *trans*-dichloro(1*R*,2*R*-dach)(oxalato)platinum(IV). The complex produced was reacted with an equimolar amount of silver *n*-butyrate, silver *n*-valerate, silver *n*-caproate or silver *n*-heptanoate in methanol at room temperature (about 20 °C) for 2 days in the dark, thus generating C4-, C5-, C6- and C7-OHP-Cl, respectively. The silver chloride precipitate was filtered off and the carboxylato/chloro complexes were isolated and purified by silica gel column chromatography. Products were identified as the expected complexes by elemental analyses. Platinum was analysed colorimetrically [2].

Evaluation of antitumor activity

The carboxylato/chloro complexes were screened in an i.p. L1210 model coupled with i.p. drug injection. Briefly, male CDF_1 mice (five or six mice per group) were implanted i.p. with 1×10^5 L1210 cells on day 0. The mice were given i.p. a complex prepared freshly in a sterile saline on days 1, 5 and 9 days at a dose of 12.5, 25, 50 or 100 mg/kg per injection. Oral antitumor activity of the complexes was tested in an s.c. M5076 model coupled with oral drug treatment, according to the methods of Tashiro et al. [31] and Rose [26, 27]. Briefly, male BDF_1 mice (six mice per group) were inoculated s.c. with an M5076 tumor fragment (a 2-mm cube) on day 0. The mice were given daily a complex prepared freshly in sterile water by oral gavage (5 ml/kg) for 5 days in two cycles initiated on days 5 and 12 postinoculation at a dose of 12.5, 25, 50 or 100 mg/kg per administration.

Antitumor activity is presented in terms of increase in life-span reflected by the relative mean survival time (days) of complex-treated (T) and control (C) groups of mice (T/C% value). In the screening test, mice alive on day 30 were evaluated as cured and the survival time of 30 days was used in T/C% calculation. The activity criterion was a T/C% value of ≥125. The dose of a complex that yielded the maximum T/C% value was termed the optimal dose.

HPLC

In analysis of the carboxylato/chloro complexes, the complexes were chromatographed at 40 °C on a Shim-pack ODS-H column (4.6 mm i.d. × 25 cm; Shimadzu, Kyoto, Japan) with methanol/50 m*M* phosphate buffer (pH 4.5) eluent and detected at 210 nm. Methanol concentrations (v/v%) were as follows: 5% for C4-OHP-Cl and C5-OHP-Cl, 10% for C6-OHP-Cl, 20% for C7-OHP-Cl. In l-OHP analysis, l-OHP was chromatographed at 40 °C on a TSKgel SCX column (4.6 mm i.d. × 25 cm; Tosoh, Tokyo, Japan) with a 20% methanol/20 mM NaH_2PO_4 eluent and detected at 210 nm.

In vitro reduction by ascorbate

Solutions of the complexes (100 μ*M*) and ascorbate (5, 10, 30 and 100 mM) were freshly prepared with 20 m*M* HEPES buffer (pH 7.5) solution, and the pH values of these solutions were adjusted to 7.5 if necessary. The complex and ascorbate solutions were mixed in equal volumes to initiate the reaction, followed by incubation at 37 °C in the dark for up to 8 h. A portion of the reaction mixture was withdrawn at intervals and subjected to HPLC to determine the intact complex and l-OHP produced.

Water solubility

To 1 ml of water was added 0.2 g of a complex. The resulting solution was stirred vigorously at room temperature (about 20 °C) for 24 h and then the undissolved complex was filtered off with a membrane filter (pore size 0.45 μm). The platinum concentration in the filtrate was determined colorimetrically [2].

1-Octanol/water partition coefficient

One milliliter aliquots of the complex solutions prepared in 1-octanol-saturated water at concentrations of 10 and 50 μM were vigorously shaken with 0.25, 0.5 or 1 ml of water-saturated 1-octanol on a vortex mixer for 10 min at room temperature (about 20 °C). The complex concentration in the aqueous phase was then determined by HPLC. The partition coefficients were calculated according to the equation: partition coefficient $= (C_i - C_w)/(C_w \times V_o)$, where C_i is the initial complex concentration in the aqueous phase, C_w is the complex concentration in the aqueous phase after extraction and V_o is the volume of 1-octanol.

Table 1 Elemental analyses of the carboxylato/chloro complexes synthesized in this study

Complex	Formula		Content of element (%)			
			C	H	N	Pt
C4-OHP-Cl	$C_{12}H_{21}N_2O_6Pt$-$Cl \cdot 2H_2O$	Calculated	25.93	4.53	5.04	35.10
		Found	26.04	4.37	5.05	35
C5-OHP-Cl	$C_{13}H_{23}N_2O_6Pt$ $Cl \cdot H_2O$	Calculated	28.29	4.57	5.08	35.35
		Found	28.15	4.37	5.06	36
C6-OHP-Cl	$C_{14}H_{25}N_2O_6Pt$-$Cl \cdot 0.5H_2O$	Calculated	30.19	4.71	5.03	35.03
		Found	29.93	4.62	5.14	36
C7-OHP-Cl	$C_{15}H_{27}N_2O_6PtCl$	Calculated	32.06	4.84	4.99	34.72
		Found	32.26	4.79	4.82	34

Stability

Stabilities of the complexes were evaluated in HCl solutions of 0.05, 0.25, 0.5, 0.75 and 1 *M*, and in buffers with pH values of 4.0, 5.0, 6.0, 7.2 and 8.0. The buffers used were 40 m*M* acetate (pH 4.0), 40 m*M* phosphate (pH 5.0 and 6.0) and 40 m*M* HEPES (pH 7.2 and 8.0). A complex and an HCl or buffer solution were mixed in equal volumes to initiate the reaction, followed by incubation at 37 °C in the dark for up to 8 h. A portion of the reaction mixture was withdrawn at intervals and subjected to HPLC to determine the intact complex.

Pharmacokinetic behavior

Male BDF_1 mice were given C5-OHP-Cl, C6-OHP-Cl or C5-OHP prepared freshly in sterile water by oral gavage (5 ml/kg) at a dose of 50 μmol/kg. About 0.6 ml of blood was collected at various times for upto 24 h after administration from the jugular vein into ice-cooled heparinized tubes under light anesthesia with ether. The blood was immediately centrifuged at 10 000 rpm for 2 min at 4 °C to separate the plasma. An aliquot (0.15 ml) of the plasma was immediately subjected to nitric acid/hydrogen peroxide digestion followed by determination of the platinum concentration by graphite furnace atomic absorption spectrometry (GFAAS) [13]. Another aliquot (0.1 ml) of the plasma was immediately placed in an Amicon micropartition starter kit (MPS-1) fitted with an Amicon YMT membrane (Danvers, Mass.) and centrifuged at 3000 rpm for 15 min at 4 °C to obtain an ultrafiltrate. The plasma ultrafiltrate was immediately analyzed for platinum concentration by GFAAS without the nitric acid/hydrogen peroxide digestion. The $AUC_{0-24\ h}$ values of total and filterable platinum in the plasma were calculated by the trapezoidal rule. The $AUC_{0-24\ h}$ calculation was based on the mean concentration at each sampling time, since only one blood sample was obtainable from a mouse.

Statistical analysis

Statistical analyses were carried out using the unpaired Student's *t*-test and the log-rank test using StatView 4.0 for Macintosh computers (Nankodo, Tokyo, Japan), *P*-values < 0.05 were considered significant.

Results

Synthesis of the carboxylato/chloro complexes

The four carboxylato/chloro complexes, C4-OHP-Cl, C5-OHP-Cl, C6-OHP-Cl and C7-OHP-Cl, were synthesized by the two pathways shown in Fig. 1. In Method 1, bis-carboxylato complexes were first prepared and then hydrolyzed in the presence of hydrochloric acid to replace an axial carboxylate ligand with chloride. In Method 2, *trans*-dichloro(1*R*,2*R*-dach)(oxalato)platinum(IV) was first prepared and then treated with a silver carboxylate to remove an axial chloride and simultaneously introduce carboxylate to the axial position. Table 1 shows the results of elemental analyses of the products prepared by Method 2 as an illustration. The results of the analyses of the products of both methods were in good agreement with the calculated values, and consequently the products were identified as the expected complexes. The overall yields from l-OHP to the carboxylato/chloro complexes were approximately 20% by Method 1 and more than 70% by Method 2. Method 2 was therefore used in subsequent syntheses of the carboxylato/chloro complexes.

As shown in Fig. 1, a pair of structures is possible for a carboxylato/chloro complex in connection with the structure of the cyclohexanediamine carrier ligand. These isomers are enantiomers and are presumably produced as a racemate. In this study, the carboxylato/chloro complexes were used as enantiomeric mixtures.

Antitumor screening test

An antitumor screening test of the carboxylato/chloro complexes was first conducted using a conventional i.p. L1210 model coupled with i.p. drug injection. The complexes were given i.p. on days 1, 5 and 9 postimplantation. The antitumor activities expressed as T/C% values are listed in Table 2. The complexes were all found to show antitumor activity, with T/C% values greater than 125. Among them, C5-OHP-Cl and C6-

Table 2 Antitumor activities (in terms of T/C%) of the carboxylato/chloro complexes following i.p. injection into the i.p. L1210 leukemia model

Complex	Dose (mg/kg/injection)			
	100	50	25	12.5
C4-OHP-Cl	63	195	145	130
C5-OHP-Cl	88	283	168	–
C6-OHP-Cl	80	280	173	–
C7-OHP-Cl	114	176	137	122

Table 3 Antitumor activities of C5-OHP-Cl, C6-OHP-Cl and C5-OHP following oral administration to the s.c. M5076 reticulosarcoma model

Complex	Dose (mg/kg/administration)	Survical time (days)		
		Range	Mean	T/C%
Control		24–27	25.7	
C5-OHP	12.5	25–29	27.3	106
	25	27–32	29.5	115
	50	27–30	28.5	111
	100	25–29	26.8	104
C5-OHP-Cl	12.5	28–33	30.3	118
	25	31–37	34.3	134
	50	34–39	36.8	143*
	100	27–30	27.8	108
C6-OHP-Cl	12.5	28–33	30.0	117
	25	29–34	31.7	123
	50	28–33	29.8	116
	100	27–30	28.3	110

* $P < 0.05$, vs survival curve for C5-OHP at 25 mg/kg/administration, log-rank test

OHP-Cl showed particularly high maximum T/C% values of 283 and 280, respectively. These two complexes were selected for oral activity evaluation.

Oral antitumor activity

Oral antitumor activities of C5-OHP-Cl and C6-OHP-Cl were examined in an s.c. M5076 reticulosarcoma model together with C5-OHP. The complexes were given to mice daily for 5 days in two cycles initiated on days 5 and 12 postimplantation, according to the dosing schedule used by Rose [27]. Table 3 lists the results of the oral activity tests. C5-OHP-Cl was judged to be orally active in the s.c. M5076 model, with T/C% values greater than 125. C5-OHP-Cl achieved a maximum T/C% value of 143 at a dose of 50 mg/kg per administration. C6-OHP-Cl and C5-OHP failed to yield T/C% values exceeding 125 and consequently were judged not to be orally active. The difference between the survival curves of C5-OHP-Cl and C5-OHP at their optimal doses was then analyzed using the log-rank test. The test showed that the survival time of the group receiving C5-OHP-Cl was significantly longer than that of the group receiving C5-OHP. The carboxylato/chloro complex, C5-OHP-Cl, therefore exhibited enhanced oral antitumor activity compared with C5-OHP.

Since C5-OHP-Cl showed significant oral activity at doses of 25 and 50 mg/kg per administration, its toxicity was evaluated using the criterion of [27], that is treated mice dying before any deaths in the control group were considered to have died of drug toxicity. In the oral activity test, no mice died earlier than the control mice at any dose. Non-tumor-bearing BDF_1 mice were given C5-OHP-Cl at a dose of 50 or 100 mg/kg per administration according to the same dosing schedule as in the oral activity test. With both doses, mice were all alive on day 39 after starting the drug treatment, which was the longest survival time in the oral activity test, indicating that a regimen of at least 100 mg/kg per administration of C5-OHP-Cl is tolerable.

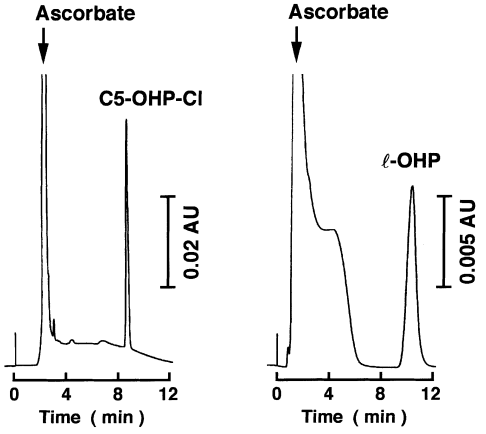

Fig. 2 Typical chromatograms from the analysis of C5-OHP-Cl (*left*) and l-OHP (*right*) in a C5-OHP-Cl-ascorbate reaction mixture. The reaction mixture of C5-OHP-Cl (50 μ*M*) and ascorbate (5 m*M*) was incubated at 37 °C for 2 h in the dark

In vitro reduction by ascorbate

Since the carboxylato/chloro complexes were expected to be reduced more rapidly than C5-OHP, their susceptibilities to reduction were evaluated in vitro using ascorbate as a reducing agent. The left-hand chromatogram in Fig. 2 was obtained during the analysis of C5-OHP-Cl in the C5-OHP-Cl-ascorbate reaction mixture. All the complexes disappeared monoexponentially in the presence of ascorbate. The half-lives of the complexes at ascorbate concentrations of 5 and 100 m*M* were calculated from the pseudo-first-order rate constants and are listed in Table 4. As expected, the reduction of the carboxylato/chloro complexes was much more rapid than that of C5-OHP and comparable to that of JM216 [14]. The rates of reduction of the carboxylato/chloro complexes decreased with an increase in the carbon number of the axial ligand and their rates were approximately proportional to the ascorbate concentration.

Table 4 Reduction of the carboxylato/chloro complexes by ascorbate in vitro. Values are mean (n = 3) half-lives (h). Relative standard deviations were below 5%

Complex	Ascorbate concentration	
	5 mM	100 mM
C4-OHP-Cl	1.0	< 0.1
C5-OHP-Cl	1.2	< 0.1
C6-OHP-Cl	1.8	0.1
C7-OHP-Cl	2.3	0.2

The reduced products of the carboxylato/chloro complexes were then studied. As C5-OHP are known to yield l-OHP as the reduced product [14], the reaction mixtures of the carboxylato/chloro complexes and ascorbate were analyzed for l-OHP by means of HPLC. The right-hand chromatogram in Fig. 2 was obtained during the analysis of l-OHP in the C5-OHP-Cl-ascorbate reaction mixture. l-OHP was detected in the carboxylato/chloro complex-ascorbate reaction mixtures and was found to be produced by reduction of the carboxylato/chloro complexes. Figure 3 shows the changes with time of the concentrations of C5-OHP-Cl and l-OHP produced in the reaction mixtures. While the concentration of a carboxylato/chloro complex decreased, the l-OHP concentration increased. The sum of the concentrations of carboxylato/chloro complex and l-OHP produced remained almost constant. The carboxylato/chloro complexes were therefore found to be reduced by ascorbate to yield l-OHP quantitatively in vitro.

Water solubility and 1-octanol/water partition coefficient

The carboxylato/chloro complexes were expected to be more water-soluble than C5-OHP. The water solubilities and 1-octanol/water partition coefficients of the complexes were determined. The results are shown in Table 5. While the water solubility decreased in the order from

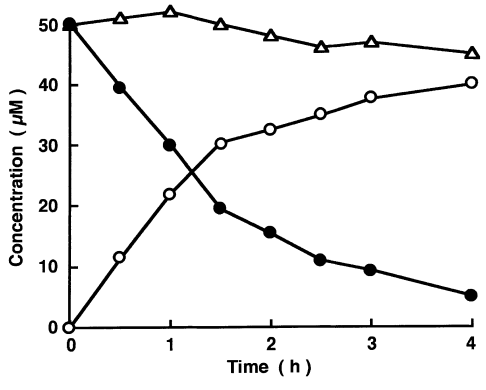

Fig. 3 Time courses of C5-OHP-Cl and l-OHP concentrations in C5-OHP-Cl-ascorbate reaction mixture. The reaction mixture of C5-OHP-Cl (50 μM) and ascorbate (5 mM) was incubated at 37 °C in the dark (● C5-OHP-Cl, ○ l-OHP, △ C5-OHP-Cl + l-OHP)

Table 5 Water solubilities and l-octanol/water partition coefficients of the carboxylato/chloro complexes. Values are means (n = 3). Relative standard deviations were below 5%

Complex	Solubility (mM)	Partition coefficient
C4-OHP-Cl	147	5.6×10^{-2}
C5-OHP-Cl	41	2.4×10^{-1}
C6-OHP-Cl	9.6	8.4×10^{-1}
C7-OHP-Cl	2.2	2.7

C4-OHP-Cl to C7-OHP-Cl, the increasing carbon number of the axial carboxylate ligands, C4-OHP-Cl, C5-OHP-Cl and C6-OHP-Cl was associated with increasing water solubilities compared with C5-OHP. On the other hand, the 1-octanol/water partition coefficients of the complexes were all smaller than that of C5-OHP, and increased in the order from C4-OHP-Cl to C7-OHP-Cl.

Stability

The stabilities of the carboxylato/chloro complexes were examined in HCl solutions and buffers with pH values of 4.0 to 9.0 at 37°C to obtain fundamental information on the behavior in HCl-acidic (equivalent to conditions in the stomach) and slightly alkaline (equivalent to body fluids such as blood and lymph) conditions. Decreases in concentrations of the carboxylato/chloro complexes were observed in HCl solutions and buffers with pH values of 7.2, 8.0 and 9.0 but not in buffers with pH values of 4.0, 5.0 and 6.0. Degradation of the complexes proceeded monoexponentially and the half-lives were calculated from the pseudo-first-order rate constants. Table 6 shows the half-lives of the complexes in 0.05 M and 1 M HCl solutions and buffers with pH values of 7.2 and 8.0. The complexes were found to be more stable than C5-OHP in these solutions, showing longer half-lives [14].

The rates of disappearance of the complexes increased in proportion to the HCl concentration in the range of 0.05 to 1 M in the HCl solutions and to the hydroxyl ion concentration in the buffers with pH values of 7.2, 8.0 and 9.0. The stability of the complexes was higher for those with a higher number axial carboxylate ligand.

Pharmacokinetic behavior

The pharmacokinetic behaviors of C5-OHP-Cl, C6-OHP-Cl and C5-OHP after a single oral administration were studied in non-tumor-bearing BDF_1 mice by monitoring the total and filterable platinum levels in the plasma. The time courses of total and filterable platinum concentrations in the plasma are shown in Fig. 4 and pharmacokinetic parameters are shown in Table 7. C5-OHP-Cl yielded higher C_{max} and $AUC_{0-24\ h}$ values of total and filterable platinum than C6-OHP-Cl and C5-OHP. The C_{max} and $AUC_{0-24\ h}$ values of plasma total and filterable platinum of C5-OHP-Cl were four

Table 6 Stability of the carboxylato/chloro complexes in HCl and slightly alkaline solutions. Values are mean ($n = 3$) half-lives (h). Relative standard deviations were below 5%. *ND* indicates that the exact half-life period was not determined since a significant decrease in concentration was not observed

Complex	0.05 *M* HCl	1 *M* HCl	pH 7.2	pH 8.0
C4-OHP-Cl	> 50	6.1	> 50	33
C5-OHP-Cl	ND	7.4	> 50	48
C6-OHP-Cl	ND	8.9	> 50	> 50
C7-OHP-Cl	ND	11	ND	ND

to six times higher than those of C6-OHP-Cl and C5-OHP, indicating that C5-OHP-Cl was absorbed more than C6-OHP-Cl and C5-OHP in the gastrointestinal tract.

The filterable-to-total platinum ratios of the three complexes were over 0.8 at 1 h postadministration and decreased with time thereafter. Significant differences in the filterable-to-total platinum ratio were not observed among the three complexes.

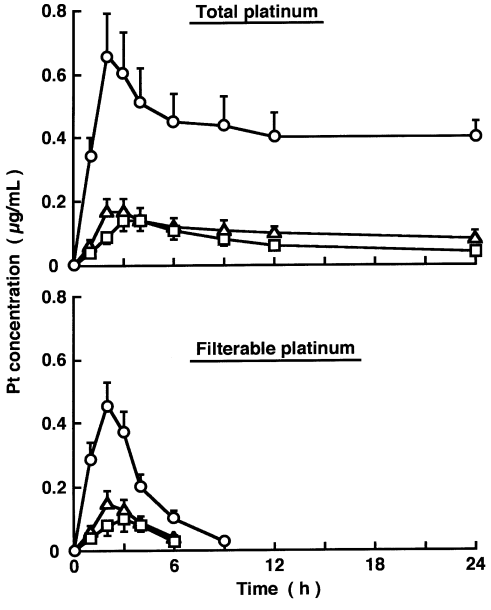

Fig. 4 Time courses of total (*top*) and filterable (*bottom*) platinum concentrations in plasma following a single oral administration of C5-OHP-Cl, C6-OHP-Cl and C5-OHP at a dose of 50 μmol/kg to mice (○ C5-OHP-Cl, △ C6-OHP-Cl, □ C5-OHP)

Discussion

In our previous study, we synthesized several lipophilic l-OHP derivatives, bis-carboxylato complexes, and found that C5-OHP was orally active in an i.p. L1210 model, which is sensitive to a number of platinum complexes [14]. However, C5-OHP did not exhibit significant oral activity in an s.c. M5076 model in subsequent studies (a part of the results is shown in Table 3). The M5076 model has been validated as a tumor model predictive of activity against human tumors [26]. In this study, several complexes with the new general formula *trans*-(carboxylato)chloro(1*R*,2*R*-dach)(oxalato)platinum(IV) were synthesized based on our ideas described below in order to find new l-OHP derivatives orally active in the M5076 model.

One of our ideas was to prepare complexes with an increased susceptibility to reduction compared with that of C5-OHP. Quadrivalent platinum complexes are kinetically inert and so it has been considered that the active species may be divalent platinum complexes produced by reduction in the biological milieu [7]. The in vitro reduction of C5-OHP by ascorbate is much slower than that of JM216. Some quadrivalent platinum complexes are known to be reduced rapidly. These include ormaplatin [tetrachloro(1,2-dach)platinum(IV), formerly called tetraplatin] [5, 10], JM216 [14], *trans*-dichloro(1*R*,2*R*-dach)(oxalato)platinum(IV) and *trans*-dichloro(1*R*,2*R*-dach)(malonato)platinum(IV) [22]. All these complexes contain a chloride ligand. The new formula was therefore designed to have a chloride ligand in an axial position.

The other idea was to prepare complexes with increased water solubility compared with that of C5-OHP. The bis-carboxylato complexes were synthesized to prepare lipophilic l-OHP derivatives, since a drug with higher lipophilicity is in general more permeable through cell membranes. However, the pharmacokinetic study on the bis-carboxylato complexes showed that the proportion absorbed in the gastrointestinal tract after a single oral administration of the same dose was greater for complexes with higher water solubility, indicating that water solubility is a more dominant factor than lipophilicity for gastrointestinal absorption of the bis-carboxylato complexes [15]. Some quadrivalent platinum complex with axial chloride ligands such as ormaplatin and *trans*-dichloro(1*R*,2*R*-dach)(oxalato)platinum(IV)

Table 7 Pharmacokinectic parameters of plasma total and filterable platinum of C5-OHP-Cl, C6-OHP-Cl and C5-OHP following a single oral administration in mice. Values are expressed as mean ($n = 3$) ± SD. * Significantly different from the values for C5-OHP-Cl by unpaired Student's *t*-test ($P < 0.01$).

Complex	T_{max} (h)		C_{max} (μg/ml)		AUC_{0-24} (μg · h/ml)	
	total	filterable	total	filterable	total	filterable
C5-OHP-Cl	2	2	0.66 ± 0.13	0.46 ± 0.07	10.2	1.76
C6-OHP-Cl	2	2	0.17 ± 0.04*	0.15 ± 0.04*	2.47	0.58
C5-OHP	3 ~ 4	3	0.14 ± 0.03*	0.10 ± 0.04*	1.69	0.42

are highly water soluble [22]. The carboxylato/chloro complexes are expected to be more water soluble than C5-OHP.

Among the carboxylato/chloro complexes synthesized in this study, C5-OHP-Cl exhibited significant oral antitumor activity in the s.c. M5076 model, while C5-OHP did not. Studies on the physicochemical properties showed that the carboxylato/chloro complexes were more susceptible to in vitro reduction by ascorbate than C5-OHP (Table 4), as expected. The increased susceptibility of C5-OHP-Cl to reduction would be responsible in part for the enhanced oral activity, although the in vitro reduction properties of C5-OHP-Cl may not necessarily be predictive of, the in vivo reduction properties. On the other hand, C5-OHP-Cl gave C_{max} and AUC_{0-24h} values of plasma filterable platinum three to six times greater than those of C6-OHP-Cl and C5-OHP in the pharmacokinetic experiments (Table 7). The plasma level of filterable platinum, in other words free-circulating platinum, has been considered to be a factor in the appearance of biological activity. The enhanced oral activity of C5-OHP-Cl may be due in part to the elevated level of plasma filterable platinum. The plasma platinum levels of C5-OHP-Cl, C6-OHP-Cl and C5-OHP indicate that C5-OHP-Cl was absorbed more than C6-OHP-Cl and C5-OHP in the gastrointestinal tract. The water solubility of C5-OHP-Cl was higher than those of C6-OHP-Cl and C5-OHP as shown in Table 5. The increased gastrointestinal absorptivity of C5-OHP-Cl may be due to its higher water solubility [15]. Thus, we were successfull in finding a carboxylato/chloro complex that exhibited significant oral antitumor activity in the s.c. M5076 model, based on our idea that an l-OHP derivative with high susceptibility to reduction and water solubility would exhibit significant oral activity.

In this study, the oral antitumor activity of C5-OHP-Cl was examined under the almost same conditions as in the study by Rose [27] on JM216 which is the first oral platinum agent and has already entered phase II clinical trials. Our results on C5-OHP-Cl were therefore compared with those of JM216 [27]. The maximum T/C% value and optimal dose were 143 and 50 mg/kg per administration for C5-OHP-Cl and are 153 and 20 mg/kg per administration for JM216. While the maximum T/C% value of C5-OHP-Cl is comparable to that of JM216, the optimal dose of C5-OHP-Cl is higher than that of JM216 by a factor of 2.5. This may appear to be a disadvantage of C5-OHP-Cl. On the other hand, toxicity is also an important aspect in the evaluation of the potency of a drug. Although only few experimental data on the toxicity of C5-OHP-Cl were obtained, significant toxicity was not observed in these investigations. The survival times of tumor-bearing and non-tumor-bearing mice receiving C5-OHP-Cl indicated that a regimen of 100 mg/kg per administration of C5-OHP-Cl was tolerable. The maximum tolerated dose of JM216 is 40 mg/kg per administration [27]. The ratio of the optimal dose to the maximum tolerated dose of C5-OHP-Cl was not less than 2. This value is almost same as or somewhat larger than that of JM216, suggesting that C5-OHP-Cl has potency comparable to that of JM216 and is a hopeful candidate as a new orally active platinum agent.

While degradation of C5-OHP-Cl was observed in HCl solutions and alkaline buffers, C5-OHP-Cl was more stable than C5-OHP and JM216 in these solutions [14]. As can be seen from the half-lives shown in Table 6, pH-dependent degradation of C5-OHP-Cl in the body was considered to be negligible, taking into account the acidity in the stomach, the time the complex stays in the stomach and the pH of the various parts of the body.

In conclusion, among the four carboxylato/chloro complexes synthesized in this study, C5-OHP-Cl was found to be a superior l-OHP derivative to C5-OHP, exhibiting significant oral antitumor activity in the s.c. M5076 model validated as clinically predictive. The enhanced activity of C5-OHP-Cl, compared with that of C5-OHP, was considered be due in part to increased susceptibility to reduction and increased gastrointestinal absorption. C5-OHP-Cl is a prime candidate for further study as an oral platinum drug.

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