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An orally active antitumor cyclohexanediamine–Pt(IV) complex: *trans,cis,cis*-bis(*n*-valerato)(oxalato)(1*R*,2*R*-cyclohexanediamine)Pt(IV)

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In order to develop orally active antitumor platinum complexes, several cyclohexanediamine–Pt(IV) complexes of a general formula *trans,cis,cis*-[Pt(IV) $(OCOC_nH_{n+1})_2$ (oxalato)(1*R*,2*R*-cyclohexanediamine)] were synthesized by derivatizing oxaliplatin [Pt(II)(oxalato)(1*R*,2*R*-cyclohexanediamine), *l*-OHP], which is a potent antitumor cyclohexanediamine–Pt(II) complex we have prepared and now undergoing clinical trials. The *l*-OHP derivatives were found to be stable, lipophilic and reduced to yield *l*-OHP, an active species, quantitatively by ascorbate *in vitro*. All the derivatives were antitumor active against mouse lymphocytic leukemia L1210 when given i.p. In particular, *trans*-bis-valerato-oxalato-1*R*,2*R*-dach–Pt(IV), C5-OHP, showed markedly high activity. C5-OHP also exhibited significant antitumor activity against L1210 when orally administered. C5-OHP was considered to be a suitable candidate for the oral cancer chemotherapy agent to be developed.

Key words: Antitumor activity, cyclohexanediamine–Pt(IV) complex, oral agent, oxaliplatin derivative, physicochemical property.

Introduction

Cisplatin [*cis*-Pt(II)$Cl_2(NH_3)_2$)] is a highly antitumor active metal complex and it is one of the most widely used antitumor agents in cancer chemotherapy. However, it showed severe toxicities.[1] Carboplatin [Pt(II)(cyclobutanedicarboxylato)$(NH_3)_2$] had been introduced as a second-generation platinum complex, but it showed severe myelosuppression and cross-resistance with cisplatin.[2,3] The authors have been developing antitumor Pt(II) complexes containing 1,2-cyclohexanediamine (1,2-dach) as a carrier ligand and have prepared 'Oxaliplatin' [Pt(II)(oxalato)(1*R*,2*R*-dach), *l*-OHP] as a superior antitumor agent of the next generation (Figure 1).[4–9] *l*-OHP is now undergoing clinical trials and has been found to be efficacious against melanoma, ovarian, testicular, lung, stomach and colorectal cancers, showing no nephrotoxicity, no cardiotoxicity, no hematotoxicity and no mutagenicity. Another important feature is that cisplatin-resistant cell lines are sensitive to *l*-OHP.

The quality of life of cancer patients is now an important problem in cancer chemotherapy. The existing antitumor platinum drugs have been i.v. administered by long-term infusion methods in terms of antitumor activity, toxic side effects and bioavailability, and have required hospitalization. The development of orally active antitumor platinum agents will be a significant benefit and advantage for cancer outpatients to undergo treatments conveniently without hospitalization, and for terminal patients to be treated at hospices and homes. Thus, the next generation of antitumor platinum complexes should be oral agents.

Johnson Matthey Inc. are developing oral antitumor platinum complexes in cooperation with the Institute of Cancer Research (UK) and Bristol-Myers Squibb (USA). They synthesized various ammine/amine mixed ligand complexes of a general formula *trans,cis,cis*-[Pt(IV)(carboxylato)$_2$$Cl_2$$(NH_3)$(cyclohexylamine)] and tested them against ADJ/PC6 plasmacytoma. Among them, JM216, *trans,cis,cis*-[Pt(IV)(acetato)$_2$$Cl_2$$(NH_3)$(cyclohexylamine)] and JM221, *trans,cis,cis*-[Pt(IV)(butyrato)$_2$$Cl_2$$(NH_3)$(cyclohexylamine)] were found to

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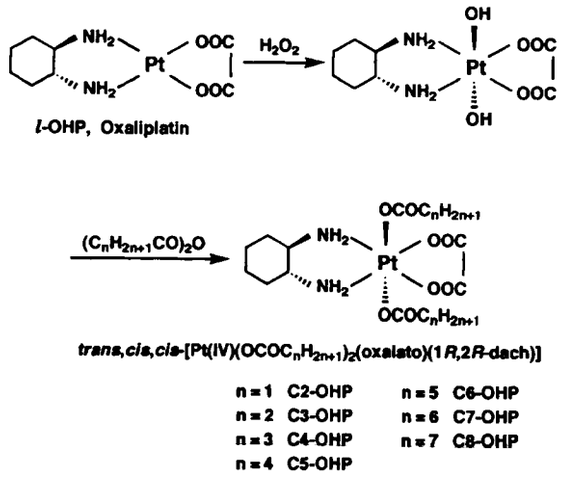

Figure 1. Synthesis of *l*-OHP derivatives of a general formula *trans,cis,cis*-[Pt(IV)($OCOC_nH_{2n+1}$)$_2$(oxalato)(1*R*,2*R*-dach)].

be orally antitumor active, and JM216 is currently undergoing phase I study.[10–14]

As described above, the *l*-OHP we prepared is a superior complex. If an *l*-OHP derivative which is lipophilic, stable and absorbable in the digestive tract and serves as the prodrug of *l*-OHP could be prepared, it would become a new orally active antitumor platinum complex with similar biological characteristics to those of *l*-OHP. With these points in mind, the authors synthesized several *l*-OHP derivatives of a general formula *trans,cis,cis*-[Pt(IV)($OCOC_nH_{2n+1}$)$_2$(oxalato)(1*R*,2*R*-dach)], including C2-OHP ($n=1$), C3-OHP ($n=2$), C4-OHP ($n=3$), C5-OHP ($n=4$), C6-OHP ($n=5$), C7-OHP ($n=6$) and C8-OHP ($n=7$). This paper describes the synthesis, physicochemical properties and antitumor activities of these complexes, in comparison with JM216 and JM221.

Material and methods

Chemicals

JM216 and Jm221 were synthesized in the manner of Giandomenico *et al.*[10] with minor modifications, being identified by elemental analysis. The results of elemental analyses are listed in Table 1. *l*-OHP was obtained from Tanaka Kikinzoku Kogyu (Hiratsuka, Japan) and used without any further purification. Distilled and deionized water was used throughout. Other chemicals were of reagent grade or better and used as received.

Synthesis of *l*-OHP derivatives

The *l*-OHP derivatives were synthesized by the pathway shown in Figure 1. *l*-OHP was initially oxidized with hydrogenperoxide and then the resulting *trans,cis,cis*-[Pt(IV)$(OH)_2$(oxalato)(1*R*,2*R*-dach)] was reacted with acid anhydrides.Reaction conditions were almost the same with all the *l*-OHP derivatives synthesized in this study. Conditions of synthesis of C5-OHP are described below briefly as an illustration. To 2 g of *l*-OHP (5.03 mmol) suspended in 50 ml of water was added 10 ml of 30% H_2O_2. The resulting solution was heated at 70°C for 15 min and then refrigerated for 2 h, this generating white crystalline *trans,cis,cis*-[Pt(IV)(O-H)$_2$(oxalato)(1*R*,2*R*-dach)] (1.6 g, 3.7 mmol). Then 1 g of *trans,cis,cis*-[Pt(IV)$(OH)_2$(oxalato)(1*R*,2*R*-dach)] (2.3 mmol) and 14 ml of valeric anhydride (70 mmol) were reacted in DMF for 24 h at 75°C. After the reaction, hexane was added to the mixture to afford crystalline precipitates, which were then collected and recrystallized from ethylacetate/hexane to give crystals at a yield of 34% (0.49 g,

Table 1. Elemental analyses of the Pt(IV) complexes synthesized in this study

Complex	Formula	Calculated (%)				Found (%)			
		C	H	N	Pt	C	H	N	Pt
C2-OHP	$C_{12}H_{20}N_2O_8Pt$	27.97	3.91	5.44	35.90	27.80	4.53	5.37	35.5
C3-OHP	$C_{14}H_{24}N_2O_8Pt$	30.94	4.45	5.15	37.85	31.10	4.16	5.26	37.5
C4-OHP	$C_{16}H_{28}N_2O_8Pt \cdot H_2O$	32.60	5.13	4.75	33.09	32.39	4.93	4.76	32.9
C5-OHP	$C_{18}H_{32}N_2O_8Pt \cdot H_2O$	35.01	5.55	4.54	31.59	35.30	5.49	4.66	32.2
C6-OHP	$C_{20}H_{36}N_2O_8Pt$	38.28	5.78	4.46	31.08	38.13	5.99	4.48	31.5
C7-OHP	$C_{22}H_{40}N_2O_8Pt \cdot 0.5H_2O$	39.76	6.22	4.21	29.35	39.77	6.32	4.25	29.1
C8-OHP	$C_{24}H_{44}N_2O_8Pt$	42.16	6.49	4.10	28.53	42.19	6.79	4.00	28.0
JM216	$C_{10}H_{22}N_2O_4Cl_2Pt$	24.01	4.43	5.60	39.00	24.43	4.75	5.40	38.3
JM221	$C_{14}H_{30}N_2O_4Cl_2Pt$	30.22	5.43	5.03	35.06	30.02	5.65	5.03	34.6

0.79 mmol). Products were identified as the expected *l*-OHP derivatives by elemental analyses. The results of elemental analyses are listed in Table 1.

HPLC

In the analysis of intact Pt(IV) complexes, sample solutions were chromatographed on an Inertsil ODS column (4.6 mm i.d. × 25 cm, 40°C)(GL Sciences, Tokyo, Japan) with methanol/50 mM phosphate buffer (pH 4.5) eluents and the complexes were spectrophotometrically detected at 210 nm. Methanol concentrations (v/v%) in eluents were as follows: 10% for C2-OHP, 25% for C3-OHP, 35% for C4-OHP and JM216, 50% for C5-OHP and JM221, 60% for C6-OHP, 70% for C7-OHP, and 75% for C8-OHP. In *l*-OHP analysis, sample solutions were chromatographed on a TSKgel SCX column (4.6 mm i.d. × 25 cm, 40°C) (Tosoh, Tokyo, Japan) with a 20% methanol–20 mM NaH_2PO_4 eluent and *l*-OHP was spectrophotometrically detected at 210 nm.

1-Octanol/water partition coefficient

The complexes were dissolved in 1-octanol-saturated water at concentrations of 10 and 50 μM; 1 ml of the platinum complex solution was vigorously shaken with 0.25, 0.5, 0.75 or 1 ml of water-saturated 1-octanol on a vortex mixer for 10 min at room temperature (about 20°C). Then, the platinum complex concentration in the aqueous phase was determined by means of HPLC. The partition coefficients were calculated from the following equation.

$$\text{Partition coefficient} = (C_i - C_w)/(C_w \times V_o)$$

where C_i is the initial platinum complex concentration in the aqueous phase, C_w is the platinum complex concentration in the aqueous phase after extraction and V_o is the volume of 1-octanol used for extraction.

Water solubility

To 1 ml of water was added 25 mg of a complex. The resulting solution was stirred vigorously at 50°C for 5 h followed by resting at room temperature (about 20°C) for 1 h and then the undissolved complex was filtered off with a membrane filter (pore size 0.45 μm). The platinum concentration in filtrate was determined by the colorimetric method.[17]

Stability

Stabilities of the complexes were evaluated in HCl solutions of 0.05. 0.25, 0.5, 0.75 and 1 N, and in buffers with pH values of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 Buffers used were 40 mM acetate buffer (pH 4.0), 40 mM phosphate buffer (pH 5.0 and 6.0), 40 mM HEPES buffer (pH 7.0 and 8.0) and 40 mM Bicine buffer (pH 9.0). A complex and HCl or buffer solutions were mixed in equal volumes to initiate the reaction followed by incubation at 37°C in the dark up to 8 h. A portion of the reaction mixture was withdrawn at adequate intervals and subjected to HPLC to determine the intact complex.

Reduction by ascorbate

Solutions of the Pt(IV) complexes (100 μM) and ascorbate (0.01 and 0.2 M) were freshly prepared with 20 mM HEPES buffer (pH 7.5) solution and the pH values of those solutions were adjusted to 7.5 if necessary. Complex and ascorbate solutions were mixed in equal volumes to initiate the reaction followed by incubation at 37°C in the dark up to 8 h. A portion of the reaction mixture was withdrawn at adequate intervals and subjected to HPLC to determine the intact complex and *l*-OHP.

Antitumor activity when given i.p.

Antitumor activities of the complexes when given i.p. were tested according to the protocol for the routine screening at the National Cancer Institute (Bethesda, MD). This assay system is called the 'i.p. assay system'. Mouse lymphocytic leukemia L1210 cells (10^5 cells) were transplanted i.p. into male CDF_1 mice on day 0. The complexes were i.p. injected on days 1, 5 and 9. T/C% values were calculated from the mean survival times (day) of treated (T) and control (C) mice. Cured mice were not included in the T/C% calculation.

Antitumor activity when orally administered

Antitumor activities of C5-OHP and JM216 when orally administered were tested as follows. This

assay system is called the 'p.o. assay system'. L1210 cells (10^5 cells) were i.p. transplanted into male CDF_1 mice on day 0. The complexes were suspended in 0.2 ml of olive oil and orally administered on days 1, 2, 3, 4 and 5 (Q01D × 5) or on days 1, 3, 5, 7 and 9(Q02D × 5), with a stomach catheter. T/C% values were calculated from the mean survival times (day) of treated (T) and control (C) mice.

Results

Lipophilicity and water solubility

Lipophilicity and water solubility are important physicochemical properties in the gastrointestinal absorption of drugs. In order to evaluate the lipophilicities of the *l*-OHP derivatives synthesized in this study, their 1-octanol/water partition coefficients were measured and compared with those of *l*-OHP, JM216 and JM221. As can be seen in Table 2, the partition coefficient of *l*-OHP derivatives increased approximately by a factor of 10 with an increase of one carbon atom of the axial ligand, and C7-OHP and C8-OHP showed coefficients as high as more than 1000, The partition coefficients of the *l*-OHP derivatives, except C2-OHP, were larger than that of *l*-OHP, 0.023. The partition coefficients of JM216 and JM221 measured in our laboratory were 1.8 and 140, respectively, while values in the parentheses are those reported by Giandomenico *et al.*[10] C4-OHP, C5-OHP and C6-OHP were found to have partition coefficients generally comparable to those of JM216 and JM221.

Then, water solubilities of the complexes were measured and are listed in Table 2. The water solubilities of C5-OHP to C8-OHP decreased in this order and were lower than that of *l*-OHP. Unexpectedly, the solubilities of C2-OHP, C3-OHP and C4-OHP, which were in the range 57–70 mM, were higher than that of *l*-OHP, 24 mM, which is consistent with the solubility reported by Kidani *et al.*[18] The solubilities of JM216 and JM221 measured in our laboratory were 2.0 and 0.9 mM, respectively, and are similar to those reported by Giandomenico *et al.*[10] The *l*-OHP derivatives, except C7-OHP and C8-OHP, showed similar or greater water solubilities than JM216 and JM221.

Table 2. 1-Octanol/water partition coefficients and water solubilities of the Pt(IV) complexes and *l*-OHP

Complex	Partition coefficient	Water solubility (mM)
l-OHP	2.3×10^{-2}	24
C2-OHP	1.4×10^{-2}	61
C3-OHP	9.2×10^{-2}	57
C4-OHP	1.0	70
C5-OHP	8.6	6.8
C6-OHP	1.0×10^{2}	1.0
C7-OHP	1.3×10^{3}	2.3×10^{-1}
C8-OHP	$>2.0 \times 10^{3}$	4.5×10^{-2}
JM216	1.8 (0.1*)	2.0 (9.2×10^{-1}*)
JM221	1.4×10^{2} (40*)	9.0 (3.8×10^{-1}*)

Stability

Concentrations of the *l*-OHP derivatives, *l*-OHP, JM216 and JM221 were traced in HCl solutions and buffers with pH values of 4.0–9.0 at 37°C to obtain fundamental knowledge on their stabilities in biological milieu such as the stomach (HCl acidic) and body fluids (slightly alkaline). The stabilities in HCl solutions were evaluated at concentrations of 0.05, 0.25, 0.5, 0.75 and 1 N. All the complexes disappeared monoexponentially with time and their half-life periods were calculated from the pseudo-first-order rate constants. The half-lives in 0.05 N and 1 N HCl solutions are presented in Table 3. As *l*-OHP is known to be unstable in the presence of chloride ions,[19] it degraded very rapidly in HCl solutions. Its half-life period was 0.7 h in 0.05 N HCl solution. Although the *l*-OHP derivatives also disappeared, they were unexpectedly much more stable than *l*-OHP. The stability of the *l*-OHP derivatives was higher for a complex with a greater axial carboxylate ligand and their disappearance rates increased proportionally to the HCl concentration over a range of 0.05–1 N. JM216 and JM221 in HCl solutions also disappeared in the kinetically the same fashion as the *l*-OHP derivatives. The half-lives of JM216 and JM221 in 1 N HCl solution were 3.6 and 8.6 h, respectively, and comparable to those of C3-OHP to C7-OHP.

Next, stabilities of the *l*-OHP derivatives, *l*-OHP, JM216 and JM221 were examined in the pH range of 4.0–9.0. No significant changes in concentration were observed with all the complexes in the pH 4–6 region. At pH 7.0, 8.0 and 9.0, some of the Pt(IV) complexes showed monoexponential decay and their half-lives are listed in Table 3, whereas *l*-OHP was stable. The stability of *l*-OHP derivatives was higher for a complex with a greater axial carboxylate ligand. The decline rates were higher at pH 9.0

Table 3. Stabilities of the pt(IV) complexes and *l*-OHP in HCl and alkaline solutions

Complex	Half-life period (h)				
	0.05 N HCl	1 N HCl	pH 7.0	pH 8.0	pH 9.0
l-OHP	0.7	<0.1	>50	ND	ND
C2-OHP	33	2.0	>50	8.7	0.9
C3-OHP	>50	3.1	ND	17	1.5
C4-OHP	>50	4.4	ND	23	2.3
C5-OHP	>50	5.5	ND	35	3.3
C6-OHP	ND	7.1	ND	>50	5.3
C7-OHP	ND	9.4	ND	>50	12
C8-OHP	ND	11	ND	ND	16
JM216	>50	3.6	>50	5.9	0.6
JM221	ND	8.6	>50	>50	13

ND, significant decrease in concentration was not observed.

than 8.0. JM216 and JM221 disappeared in a similar manner.

Reduction by ascorbate

The reduction of *l*-OHP derivatives, JM216 and JM221 by ascorbate was studied *in vitro*. The concentrations of the complexes decreased monoexponentially in the presence of a large excess of ascorbate. Their half-lives calculated from the pseudo-first-order rate constants are listed in Table 4. The reduction of the *l*-OHP derivatives was slower for a complex with a greater axial carboxylate ligand and their reduction rates were almost proportional to the ascorbate concentration, as can be seen for C2-OHP to C5-OHP. JM216 and JM221 decayed in a kinetically similar manner.

Next, a reduced product yield in *l*-OHP derivatives–ascorbate reaction mixtures was identified by means of HPLC. Chromatographic conditions were first investigated to resolve and detect *l*-OHP in the reaction mixture since *l*-OHP was considered to be the most prospective reduced product. On a cation exchange column (TSKgel SCX, 4.6 mm i.d. × 25 cm, 40°C) with a eluent of 20% methanol–20 nM NaH_2PO_4, *l*-OHP and *l*-OHP derivatives were eluted at retention times of 22 and about 7 min, respectively, and resolved from coexisting components. *l*-OHP and *l*-OHP derivatives were quantitated by spectrophotometric detection at 210 nm. Figure 1 represents a chromatogram of C5-OHP–ascorbate reaction mixture incubated at 37°C for 8 h in the dark as an illustration. A *l*-OHP peak was observed in chromatograms of *l*-OHP derivatives–ascorbate reaction mixtures, revealing that *l*-OHP was produced by the *in vitro* reduction with ascorbate. Then, changes in *l*-OHP concentration in the reaction mixtures were followed. Time courses of *l*-OHP and C5-OHP are shown in Figure 3 as an illustration. While the *l*-OHP concentration increased and the parent complex concentration decreased with time, the sum of *l*-OHP and its parent complex concentrations was almost constant up to 8 h (Figure 2). The *l*-OHP derivatives were found to be reduced by ascorbate and to yield *l*-OHP quantitatively *in vitro*.

Table 4. Reduction of the Pt(IV) complexes by ascorbate *in vitro*

Complex	Half-life period (h) at ascorbate concentration	
	5 mM	100 mM
C2-OHP	26	1.5
C3-OHP	37	2.2
C4-OHP	46	2.6
C5-OHP	50	2.8
C6-OHP	>50	3.2
C7-OHP	>50	3.3
C8-OHP	>50	3.6
JM216	0.8	<0.1
JM221	1.4	0.1

Antitumor activity in the i.p. assay system

Antitumor activities of the *l*-OHP derivatives *in vivo* were initially tested against mouse lymphocytic leukemia L1210 cells according to the protocol use for routine screening at the National Cancer Institute, and compared with those of *l*-OHP, JM216 and JM221. Antitumor activities of the complexes were

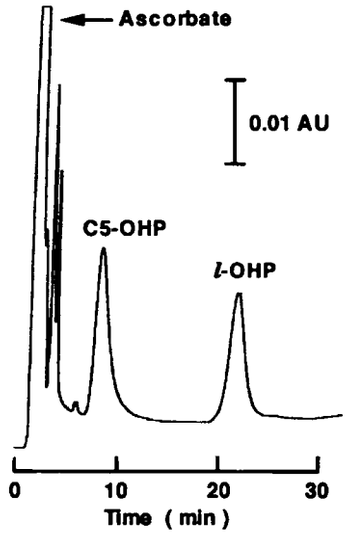

Figure 2. A chromatogram of C5-OHP–ascorbate reaction mixture incubated at 37°C for 8 h. The reaction mixture was prepared to contain 50 μM C5-OHP and 0.1 M sodium ascorbate with 50 mM HEPES–NaOH buffer (pH 7.5) and incubated at 37°C in the dark. An aliquot (20 μl) of the reaction mixture was withdrawn at 8 h and subjected to HPLC with a TSKgel SCX column. HPLC conditions are the same as in the text.

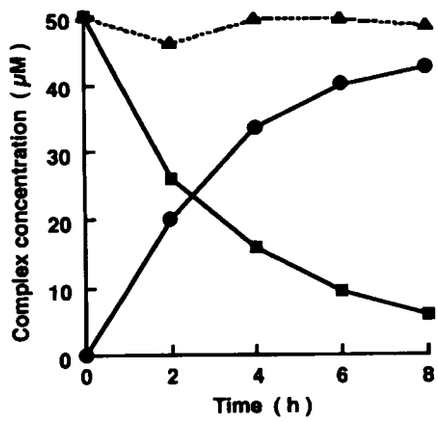

Figure 3. Time courses of C5-OHP and *l*-OHP in the C5-OHP–ascorbate reaction mixture. The reaction conditions were the same as in the Figure 1. An aliquot (20 μl) of the reaction mixture was withdrawn at 1 h intervals and subjected to HPLC with a TSKgel SCX column. HPLC conditions are the same as in the text. Symbols: ■, C5-OHP; ●, *l*-OHP; ▲, C5-OHP + *l*-OHP.

evaluated by T/C% values calculated from the mean survival times (day) of treated (T) and control (C) mice. T/C% values greater than 125 were judged to be positive in this assay system. T/C% values of the complexes are listed in Table 5. As reported previously,[7,19] *l*-OHP exhibited high antitumor activity, showing a maximum T/C% of 308. All the *l*-OHP derivatives exhibited T/C% values greater than 125 and were consequently found to be antitumor active. Among them, C4-OHP, C5-OHP and C6-OHP gave significantly high maximum T/C%, which were 271 with one cured mouse, 309 with two cured mice and 286 with three cured mice, respectively. These results were comparable to those of *l*-OHP and revealed that C4-OHP, C5-OHP and C6-OHP had remarkably higher antitumor activities. Although JM216 and JM221, used as positive controls, were antitumor active, their maximum T/C% values were 222 with JM216 and 145 with JM221. C4-OHP, C5-OHP and C6-OHP were more active than JM216 and JM221 in the present system.

Antitumor activity in the p.o. assay system

Next, antitumor activities of C5-OHP and JM216 by oral administration was tested against L1210. L1210 cells (10^5 cells) were transplanted i.p. into CDF_1 mice on day 0. C5-OHP was suspended in 0.2 ml of olive oil and administered with a stomach catheter to the starved mice on days 1, 2, 3, 4 and 5 (Q01D × 5) or on days 1, 3, 5, 7 and 9 (Q02D × 5). A T/C% greater than 140 is judged to be positive in this assay system. T/C% values obtained are listed in Table 6. C5-OHP showed a T/C% more than 140 on some dosing schedules—those were 148 at a dose of 20 mg/mouse with both the Q01D × 5 and Q02D × 5 schedules, 145 at 15 mg/mouse with the Q01D × 5 schedule and 143 at 10 mg/mouse with the Q01D × 5 schedule. JM216 was administered at a dose of 20 mg/mouse with the Q01D × 5 schedule. Although JM216 increased the survival time (day), its T/C% was 128. JM216 was not statistically judged to be antitumor active. C5-OHP was found to be orally antitumor active against L1210.

Discussion

While an oral platinum complex should be lipophilic enough to penetrate through the gut cell membranes and stable in the stomach of low pH and the small intestine, *l*-OHP itself is very hydrophillic and not stable in the presence of chloride ions.[19] So we designed Pt(IV) complexes of a formula *trans,cis,-cis*-[Pt(IV)$(OCOC_nH_{2n+1})_2$(oxalato)(1*R*,2*R*-dach)]. Since Pt(IV) complexes are known to be kinetically

Table 5. Antitumor activities of the Pt(IV) complexes and *l*-OHP against L1210 by i.p. injection

Complex	T/C% at dose (mg/kg)						
	200	100	50	25	12.5	6.25	3.12
l-OHP				T81	308(4)	253(1)	185
C2-OHP		214(1)	214	125	117	98	
C3-OHP		220	230(1)	150	138	117	
C4-OHP		160(1)	271(1)	221	156		
C5-OHP	112	309(1)	297(3)	258			
C6-OHP	166	286(3)	214(1)	188			
C7-OHP	130	207(1)	225(1)				
C8-OHP	150	112	116				
JM216		161(1)	222(1)	151	126	119	
JM221		109	103	142	119	145	

Numbers in the parentheses mean the cured mice in one group of six mice on 30-day observation. Cured mice were not included in the T/C% calculation. T indicates toxicity.

Table 6. Antitumor activities of C5-OHP against L1210 by oral administration

Complex	Dose (mg/mouse)	Dosing schedule	T/C%
C5-OHP	20	Q01D × 5	148
C5-OHP	20	Q02D × 5	148
C5-OHP	15	Q01D × 5	135
C5-OHP	15	Q02D × 5	145
C5-OHP	10	Q01D × 5	133
C5-OHP	10	Q02D × 5	143
JM216	20	Q01D × 5	128

exchange inert,[20] the complexes we designed would be much more stable than *l*-OHP in the digestive tract. Complexes with different lipophilicities could be prepared by changing the carbon number of the axial carboxylate ligand. In addition, our designed complexes are also expected to be reduced to yield *l*-OHP, similarly to other antitumor Pt(IV) complexes. If *l*-OHP is yielded as an active species, *l*-OHP derivatives would show excellent antitumor activities and low toxic side effects.

The lipophilicity and water solubility of a drug are important properties in gastrointestinal absorption. In general, a drug with higher lipophilicity is more permeable though the cell membrane and accordingly absorbed more efficiently in the digestive tract. On the other hand, some drugs show low absorption efficiency due to their poor water solubility since only the dissolved fraction of a drug in the digestive tract is absorbed. Compared with JM216, C4-OHP has comparable lipophilicity and higher water solubility. C5-OHP and C6-OHP have higher lipophilicity and water solubility than JM216 and JM221, respectively. C4-OHP, C5-OHP and C6-OHP are considered to be suitable for oral agents in terms of lipophilicity and water solubility.

When the *l*-OHP derivatives are administered orally, the complexes are first placed in HCl-acidic conditions (stomach) and then slightly alkaline conditions (body fluids such as blood and lymph). The *l*-OHP derivatives are desired to be stable under those conditions. The acidity of the gastric juice is expressed by the free HCl concentration (mEq/l) and normal acidity ranges from 20 to 40 mEq/l, which correspond to 0.02–0.04 N. HCl concentrations used in this study were 0.05, 0.25 0.5, 0.75 and 1 N because these hyperacidic conditions were required to trace the decrease in concentrations of the complexes exactly. The half-lives of *l*-OHP derivatives in the HCl solutions were much longer than that of *l*-OHP. The half-life of C2-OHP, whose disappearance was fastest among the *l*-OHP derivatives, was 33 h in 0.05 N HCl solution. HCl-mediated degradation of *l*-OHP derivatives in the stomach is considered to be negligible, taking into account the time a drug stays in the stomach. Although *l*-OHP derivatives degraded in alkaline conditions, their half-lives in the pH 7.0–9.0 region indicate that the *l*-OHP derivatives are stable enough under slightly alkaline conditions of the small intestine and body fluids. On the other hand, rates of decline of *l*-OHP derivatives in HCl solutions increased proportionally to the HCl concentration (HCl solution) and hydroxyl ion concentration (alkaline solution). The degradation was anticipated to result from the nucleophilic attack of chloride ions or hydroxyl ions to the platinum. The degra-

dation was slower for a complex with a bulky axial ligand. This may be due to the block effect of the axial ligand on the attack of nucleophiles.

Iproplatin [*cis*-dichloro-bis(isopropylamine)-*trans*-dihydroxoplatinum(IV)] and tetraplatin [tetrachloro(1,2-cyclohexanediamine)Pt(IV)] are typical Pt(IV)-based antitumor complexes. Since Pt(IV)-based antitumor platinum complexes are kinetically exchange inert[20] and iproplatin does not bind to DNA[21] and plasma proteins,[22] it has been considered that Pt(IV) complexes themselves are inactive and active species are divalent platinum complexes produced by reduction in biological milieu. The *l*-OHP derivatives were found to be reduced by ascorbate to yield *l*-OHP quantitatively *in vitro*. They are expected to serve as prodrugs of *l*-OHP *in vivo*.

The reduction of *l*-OHP derivatives by ascorbate *in vitro* was slower than those of JM216 and JM221. This may seem to be disadvantageous for *l*-OHP derivatives in exhibiting cytotoxicity. However, the *l*-OHP derivatives, except C8-OHP, gave the maximal T/C% values comparable to or greater than that of JM216, which was more active than JM221, as can be seen in Table 5. These results suggest that the active species of *l*-OHP derivatives, probably *l*-OHP, was much more cytotoxic than that of JM216, probably *cis*-ammine(cyclohexylamine)dichloroplatinum(II), or that amounts of the *l*-OHP derivatives incorporated into the tumor cells were more than that of JM216. On the other hand, C4-OHP, C5-OHP and C6-OHP showed markedly high activities comparable to that of *l*-OHP. C4-OHP, C5-OHP and C6-OHP are considered to become incorporated into cells to yield a sufficient amount of the active species, while the doses of C4-OHP, C5-OHP and C6-OHP giving the maximal T/C% are four to eight times higher than that of *l*-OHP.

Antitumor activities in the p.o. assay system were examined with C5-OHP and JM216. C5-OHP was used since it showed the highest T/C% values among the *l*-OHP derivatives in the i.p. assay system. The doses used in the p.o. assay system were five to 10 times the optimal dose of C5-OHP giving the maximal T/C% values in the i.p. assay system. C5-OHP showed a higher T/C% value than JM216. C5-OHP was judged to be antitumor active in p.o. assay system, too, but JM216 not. While increasing the dose of C5-OHP is inclined to increase the T/C%, the effect of the dose on antitumor effectiveness still remains unclear. Difference in T/C% values at a dose between two dosing schedules is also obscure. The effect of dose and dosing schedule must be studied further.

Conclusion

In the present study, we prepared *l*-OHP derivatives which were stable, lipophilic and reduced to yield *l*-OHP quantitatively by ascorbate *in vitro*. Among them, *trans*-bis-valerato-oxalato-1*R*,2*R*-dach-Pt(IV), C5-OHP, was found to have a higher antitumor activity than JM216 and JM221. Considering these results, C5-OHP is anticipated to be a suitable candidate for oral agents to be developed.

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