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Preclinical antitumor activity of orally administered platinum (IV) complexes

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Abstract. Several novel platinum (IV) mixed ammine/amine dicarboxylate dichlorides of general structure [Pt(IV)Cl_2 $(OCOY)_2$ $NH_3(XNH_2)$], where Y is aliphatic or aromatic and X is alicyclic or aliphatic, known to be particularly well absorbed following oral administration, were evaluated by that route for their antitumor activity. Testing of the Pt(IV) derivatives took place concomitantly with i.v. administered cisplatin and carboplatin in two s.c. staged tumor models, the murine M5076 sarcoma and human A2780 ovarian carcinoma. Based upon repetitive experiments which included an evaluation of different vehicles and treatment schedules, each of the orally administered Pt(IV) dicarboxylates was reproducibly active in the M5076 tumor, producing mean maximum gross log cell kill (LCK) values of between 1.5 and 2.0, and lifespan increases, reflected by mean maximum treated/control median survival (T/C) values, of 139–151%. Cisplatin and carboplatin given i.v. yielded mean maximum LCK of 3.5 and 2.5, respectively, as well as mean maximum T/C values of 166% and 164%, respectively, in the same tumor model. The best of the derivatives in the M5076 experiments, JM-216 [ammine/cyclohexylamine diacetato dichloride Pt(IV)], produced LCK values that averaged only 0.5 lower than that of carboplatin, and increases in lifespan not significantly different than that of carboplatin. Against the A2780 tumor, the Pt(IV) dicarboxylates produced individual best effects of between 0.8–1.1 LCK, based on data from two or three experiments. The mean maximum LCK values for cisplatin and carboplatin were 1.8 and 2.2 LCK, respectively. JM-225, ammine/cyclopentylamine diacetato dichloride Pt(IV), was active in two of three experiments, including one result comparable to that of carboplatin. The Pt(IV) mixed ammine/amine dicarboxylate dichlorides represent a novel class of Pt derivative capable of expressing oral antitumor activity in both murine and human tumor models.

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Introduction

Cisplatin and carboplatin represent two platinum (Pt) coordination compounds which have well-established efficacy in certain human neoplastic diseases. Numerous other Pt derivatives are currently being evaluated clinically in an attempt to demonstrate either a broader spectrum of antitumor activity [1, 5] and/or activity particularly in tumors with developed resistance to one or both of the above-mentioned marketed Pt compounds [2–4, 8, 11, 16].

Another form of advancing cancer chemotherapy is to facilitate its administration and enhance patient acceptability. If a Pt derivative were available which had clinical activity when administered orally, it would provide both clinician and patient with a valid alternative to currently available Pt drug therapy. Such a compound could routinely be given on an outpatient basis (with concomitant reduction in hospital costs). Although both cisplatin and carboplatin are often active when given orally to mice bearing tumors, the degree of activity is less than that obtained when they are given parenterally ([7, 9, 10, 14]; unpublished observations), and much higher doses are required due to their relatively limited bioavailability [7, 9, 10, 12].

The problem of poor oral bioavailability associated with cisplatin and carboplatin may have been circumvented via the application of some novel chemistry. A new class of Pt(IV) coordination compounds, having properties especially suitable for oral administration, were described recently by investigators from the Institute of Cancer Research (ICR) and the Johnson-Matthey Technology Centre, both in England, as well as our own laboratories [7, 9, 10, 12, 13]. These novel Pt derivatives have a general structure corresponding to [Pt(IV)Cl_2 $(OCOY)_2$ $NH_3(XNH_2)$], where Y is aliphatic or aromatic and X is alicyclic or aliphatic. They have been synthesized specifically to circumvent the poor gastrointestinal absorption of other Pt complexes [10, 12] and have the general characteristics of being relatively low in molecular weight, neutral, kinetically inert, acid stable, and lipophilic [7]. Several of these Pt(IV) mixed ammine/amine dicarboxylate dichlorides

have undergone initial antitumor [9, 10], absorption [10, 12], and toxicological [10, 13] assessments. Based upon those preliminary investigations and chemistry considerations, and as a part of a continuing collaborative program between the ICR, Johnson-Matthey Co. and ourselves, four Pt derivatives were selected for more intensive evaluation. We report herein the results of experimental antitumor tests involving the four selected derivatives evaluated concomitantly with both cisplatin and carboplatin.

Materials and methods

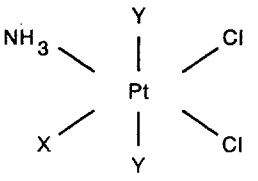
Mice. Female C57BL/6, (C57BL/6 × DBA/2) F_1 (BDF_1) hybrids, and BALB/c background athymic mice, 6–7 weeks old, were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, Ind.). They were provided food and water ad libitum.

Tumors. The M5076 murine sarcoma and A2780 human ovarian carcinoma were maintained in vivo via serial s.c. passage in C57BL/6 and athymic mice, respectively.

Compounds. Cisplatin from clinical vials was prepared in sterile normal saline. Bulk carboplatin was prepared in sterile distilled water. The four Pt(IV) ammine/amine dicarboxylates intended for oral administration, JM-216, -225, -251, and -269, are described in Fig. 1. These derivatives were synthesized (>98% purity), at Johnson-Matthey Co. and provided to us. They were each prepared as suspensions in two different vehicles: peanut oil or sterile distilled water adjusted to about pH 6.0 with 0.1N HCl plus 0.1% Tween 80 (W/T80). Suspensions were homogenized for 10 min, using a vortex shaker, and kept in amber bottles; in addition, vessels containing JM-251 were wrapped in foil to minimize further exposure to light (e.g., during vortex stirring). Cisplatin and carboplatin were administered i.v., and the other derivatives were given p.o.; all compounds were administered within 2 h (1 h for JM-251) of their preparation in an injection volume equivalent to 0.01 ml/g of body weight. Mice were weighed individually prior to each injection. Deaths caused by drug toxicity were defined as those occurring prior to the mice bearing tumors of 1 g. Any treatment producing 15% or more drug-induced lethality was defined as excessively toxic and was excluded from consideration. A maximum tolerated dose (MTD) was defined as one whose toxicity approached but did not attain the degree of lethality just described as being excessive. Operationally, when a particular dose of a compound caused excessive lethality, the MTD would be typically assigned the nearest concomitantly evaluated lower dose which was not excessively lethal. No control mice died bearing tumors less than 1 g.

Antitumor testing. Detailed experimental procedures describing our antitumor testing protocols have been published for both the M5076 [14] and A2780 [15] tumor models. Briefly, for mice implanted s.c. with M5076 tumor fragments, treatments were begun on day 5 post-implant. For mice implanted s.c. with A2780 tumor fragments, treatments were begun when their tumors were estimated (as determined by caliper measurements) to have grown to between 40 and 100 mg. In the three A2780 experiments described herein, treatment initiation corresponded from between day 7 to day 13 post-implant; but, in any given experiment, all of the mice included were selected within 24 h of each other. Groups consisted of eight to ten mice. Treatment schedules varied and are described individually in the Results section. In all the experiments, each compound was evaluated using three or four closely titrated (e.g., 33–50% increments) doses.

Antitumor activity was based upon treatment-induced delays in primary tumor growth, expressed as gross log cell kill (LCK), or increases in median lifespan, expressed as %T/C, as described previously [14, 15]. Statistical comparisons were made using Gehan's generalized Wilcoxon test [6].



Derivative	X (Amine Ligand)	Y (Axial Ligand)
JM - 216	Cyclohexylamine	$OCOCH_3$
JM - 225	Cyclopentylamine	$OCOCH_3$
JM - 251	Cyclohexylamine	OCOH
JM - 269	Cycloheptylamine	$OCOCH_3$

Fig. 1. Structures of platinum (IV) mixed ammine/amine dicarboxylate dichlorides

Results

M5076 antitumor experiments

Four experiments were performed in which orally administered Pt(IV) ammine/amine dicarboxylates were compared to parenterally administered carboplatin and cisplatin versus staged s.c. M5076 sarcoma. A summary of the optimal effects observed in those experiments in presented in Table 1.

In the first experiment, cisplatin and carboplatin were administered using two treatment schedules, every fourth day for six injections (q4dx6) and every seventh day for four injections (q7dx4), of nearly identical duration. JM-216, -225, and -251 were administered orally in peanut oil on a q7dx4 schedule. Cisplatin caused greater than 4 LCK and T/C values of 164% and 176% at the optimal doses (OD) used on the two different schedules evaluated. Carboplatin yielded 2.7 and 3.2 LCK, as well as maximum T/C values of 157% and 162% at the OD used on the same two treatment schedules. The greater inhibition of primary tumor growth obtained with cisplatin was also to be observed, to varying degrees, in subsequent M5076 experiments, but differences in maximum increased lifespan were never of statistical significance.

Each of the orally administered Pt derivatives was active in this first experiment. At the highest dose tested for each of them, 150 mg/kg per adm, maximum primary tumor growth delays reflecting 1.4 to 2.5 LCK were achieved, with JM-216 yielding the greatest effect. Although dose escalation to obviously toxic amounts was not achieved in this initial experiment, subsequent testing, as well as historical data, would support the contention that the highest dose tested of each Pt derivative was, in fact, proximal to the MTD levels. A statistical comparison made between the maximum tumor inhibition produced by carboplatin (the less active of the two reference drugs), versus the best inhibitory effect caused by each of the orally administered derivatives, yielded significant ($P < 0.01$) differences. There was, however, much less distinction between the maximum increases in lifespan caused by carboplatin and the orally administered derivatives. Carboplatin's maximum T/C value of 162% was not statistically

Table 1. Antitumor activity of orally administered platinum derivatives compared to intravenously administered cisplatin and carboplatin versus staged subcutaneous M5076 sarcoma

Compound	Treatment[a]		Range of doses evaluated (mg/kg/adm)	Maximum antitumor effect (optimal dose, mg/kg/adm)							
	Schedule, route	Vehicle		Expt. 1		Expt. 2		Expt. 3		Expt. 4	
				LCK[b]	%T/C[b]	LCK	%T/C	LCK	%T/C	LCK	%T/C
Cisplatin	q7dx4, i.v.	saline	3.5–8.0	4.6 (8)	176 (8)	3.6 (8)	168 (8)	–	–	–	–
	q4dx6, i.v.	saline	2.0–4.8	4.3 (4.5)	164 (4.5)	2.5 (4.5)	148 (3)	2.7 (4.5)	161 (3)	3.2 (4.8)	160 (4.8)
Carboplatin	q7dx4, i.v.	water	40–90	3.2 (60)	157 (60)	2.1 (60)	151 (40)	–	–	–	–
	q4dx6, i.v.	water	27–60	2.7 (40)	162 (40)	2.0 (60)	144 (60)	2.2 (60)	166 (27)	2.5 (40)	176 (40)
JM-216	q7dx4, p.o.	oil	66–180	2.5 (150)	156 (100)	1.5 (120)	137 (80)	2.1 (120)	147 (120)	–	–
	q7dx4, p.o.	W/T80	80–180	–	–	–	–	1.7 (180)	151 (180)	–	–
	q4dx6, p.o.	W/T80	44–100	–	–	–	–	0.5 (44)	129 (44)	–	–
JM-225	q7dx4, p.o.	oil	66–180	1.8 (150)	167 (150)	1.3 (120)	131 (80)	1.7 (180)	154 (180)	–	–
	q7dx4, p.o.	W/T80	80–180	–	–	–	–	0.8 (120)	142 (80)[c]	–	–
	q4dx6, p.o.	W/T80	44–100	–	–	–	–	1.5 (100)	144 (100)	–	–
JM-251	q7dx4, p.o.	oil	66–240	1.4 (150)	152 (100)	1.6 (180)	132 (180)	–	–	1.4 (180)	140 (180)
	q7dx4, p.o.	W/T80	80–180	–	–	–	–	–	–	0.9 (120)	134 (180)
	q4dx6, p.o.	W/T80	53–120	–	–	–	–	–	–	1.1 (120)	139 (120)
JM-269	q7dx4, p.o.	oil	270–600	–	–	1.4 (400)	131 (270)	–	–	1.9 (400)	136 (270)
	q7dx4, p.o.	W/T80	270–600	–	–	–	–	–	–	0.8 (400)	145 (270)
	q4dx6, p.o.	W/T80	120–400	–	–	–	–	–	–	1.4 (270)	146 (270)
Control to 1 g (TVDT)[d]				19.8 (3.3)		20.0 (4.7)		18.8 (4.8)		17.8 (4.3)	
Control MST[d]				44.5		49.5		46.5		47.0	

[a] Treatments were begun 5 days following s.c. implantation of tumor fragments. There were 9 or 10 mice/group. W/T80 = water plus 0.1% Tween 80 adjusted to about pH 6

[b] Delays in tumor growth are given in terms of gross log cell kill (LCK), and increases in lifespan are reflected as %T/C, according to calculations described in the Materials and methods section

[c] A laboratory accident prevented a determination of %T/C at the next highest dose tested and probable optimal dose, 120 mg/kg per adm

[d] Median time for control tumors to reach 1 g in size and the tumor volume doubling time (TVDT) in days. Median survival times (MST), in days, of control mice

different than the maximum T/C values of the derivatives, which ranged from 152% to 167%. It should also be noted that the greatest increases in lifespan for two of the three derivatives, JM-216 and -251, occurred at dose levels less than those associated with maximum primary tumor growth inhibition; the failure to obtain increased lifespan at higher doses further supports the likelihood of our having sufficiently escalated dose titrations to include MTD levels.

In the second M5076 experiment, the oral doses for the three previously tested Pt derivatives were slightly escalated, and JM-269 was also included for the first time. The tumor volume doubling time (TVDT) slowed to 4.7 days in this study, compared to 3.3 days in the first experiment. Cisplatin caused 3.6 LCK at its OD on the q7dx4 schedule, and 2.5 LCK on the q4dx6 schedule. The advantage of the weekly dosing schedule was also reflected in the lifespan assay. The maximum effect on the q7dx4 schedule was a T/C of 168%, compared to 148% T/C obtained at the OD on the q4dx6 schedule. Both schedules, however, yielded similar maximum LCK (of 2.0 and 2.1) and lifespan increases (144% T/C and 151% T/C) when carboplatin was administered. The best effect of carboplatin was, again, slightly inferior to the optimum cisplatin effect.

Oral administration of each of the four Pt derivatives in M5076 expt. no. 2 was performed using a q7dx4 treatment schedule; all but JM-251 reached obvious MTD levels. At their ODs, or, in the case of JM-251, the highest dose tested, the Pt derivatives produced 1.3–1.6 LCK. These maximum effects were significantly ($P < 0.01$) less than the tumor growth delay caused by the less active of the two marketed Pt drugs, carboplatin. Regarding lifespan increases, the maximum T/C values associated with oral Pt derivative administration ranged from 131% to 137%, which were less (but not significantly) than the 151% T/C value caused by carboplatin on its most effective schedule.

In the third M5076 experiment performed, we compared two of the orally administered derivatives, JM-216 and -225, in a W/T80 vehicle using two different treatment schedules, q7dx4 and q4dx6, and also included the familiar peanut oil vehicle on the q7dx4 schedule. Cisplatin and carboplatin were also included; they were given i.v. on a q4dx6 schedule.

Cisplatin caused a delay in primary tumor growth equivalent to 2.7 LCK. The TVDT in this experiment, 4.8 days, was almost the same as in the previous study. Carboplatin yielded 2.2 LCK at its OD. Maximum increases in lifespan were also similar for both marketed drugs, 161% T/C for cisplatin and 166% T/C for carboplatin. Interestingly, the doses associated with maximum %T/C for both these compounds were less than the OD needed for maximum primary tumor growth delays.

JM-216 given in oil on a q7dx4 schedule achieved a maximum LCK of 2.1 at its OD of 120 mg/kg per adm.

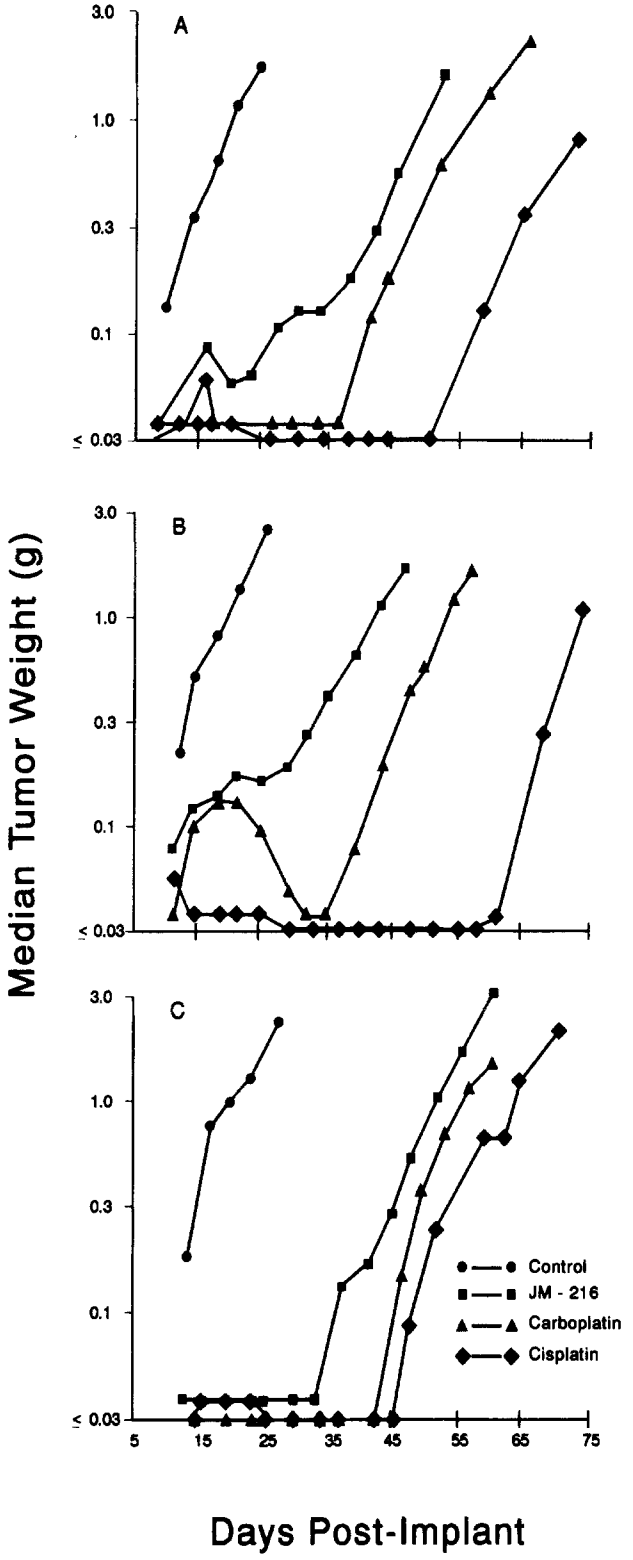


Fig. 2 A–C. Maximum tumor growth delays caused by orally administered (in oil) JM-216 concomitantly evaluated in three experiments with i.v. cisplatin and carboplatin versus staged s.c. M5076 sarcoma. All treatments began day 5 post-implant. **A** JM-216 150 mg/kg per adm, cisplatin 8 mg/kg per adm, carboplatin 60 mg/kg per adm, all treatments q7dx4. **B** JM-216 120 mg/kg per adm, cisplatin 8 mg/kg per adm, carboplatin 60 mg/kg per adm, all treatments q7dx4; **C** JM-216 120 mg/kg per adm q7dx4, cisplatin 4.5 mg/kg per adm q4dx6, carboplatin 60 mg/kg per adm q4dx6. Saline-treated control (●); JM-216 (■); cisplatin (◆); and carboplatin (▲)

This result was almost the same as the best inhibitory effect associated with carboplatin's administration, and represents the only time we observed an orally administered Pt derivative causing an inhibition of M5076 tumors comparable to either of the two marketed drugs. A summary of the best antitumor effects of JM-216, compared to carboplatin versus M5076 in each of the three experiments performed, is shown in Fig. 2.

Using the same q7dx4 treatment schedule, but suspending JM-216 in W/T80, we observed that the highest dose tested, 180 mg/kg per adm, was tolerated and caused a 1.7 LCK. Thus, on a q7dx4 schedule, JM-216 was less toxic orally when administered in W/T80. Its antitumor activity in this vehicle was similar to that observed when it was given in peanut oil, although a more definitive determination must await dose escalation in the aqueous vehicle. JM-216 was also given in W/T80, using a q4dx6 treatment schedule. It was unexpectedly toxic on this schedule, and in this vehicle, and achieved only 0.5 LCK at the MTD of 44 mg/kg per inj. The therapeutic advantage seen, when using the q7dx4 schedule as opposed to the q4dx6 schedule, was also reflected in the lifespan data. When administered q7dx4, in oil or W/T80, maximum T/C values of 147% and 151%, respectively, were obtained; these values were in contrast to the maximum T/C of 129% obtained in W/T80 using a q4dx6 schedule.

JM-225 underwent the same sort of vehicle and schedule evaluation as did JM-216, but, with a slightly different outcome. With the previously used oil vehicle and a q7dx4 treatment schedule, JM-225 achieved a 1.7 LCK at the highest dose tested, 180 mg/kg per adm. This effect was significantly less ($P < 0.01$) than the 2.2 LCK produced using carboplatin. When the vehicle was changed to W/T80, q7dx4 oral dosing of JM-225 yielded only a 0.8 LCK at an MTD of 120 mg/kg per adm. Unlike JM-216, the mice tolerated less JM-225 on the q7dx4 schedule when oil was replaced with W/T80 as the vehicle. Yet, when a q4dx6 schedule was tried using W/T80 as the vehicle, 100 mg/kg per adm, the highest dose evaluated, was tolerated and produced a 1.5 LCK, and T/C of 144%. A laboratory accident prevented a lifespan reading of the q7dx4 treatment with JM-225 in W/T80 at its MTD; but, at the next lower dose, 80 mg/kg per adm, a T/C of 142% was obtained. In comparison, q7dx4 treatment with JM-225 in oil yielded a maximum T/C of 154%. These increases in lifespan were not statistically different than the maximum lifespan improvements caused by either carboplatin or cisplatin.

In the final M5076 experiment, no. 4, the same vehicle and schedule evaluations made in the previous study were applied to JM-251 and -269. The TVDT of 4.3 days was within the scope of the three previous experiments. Cisplatin achieved 3.2 LCK at its OD on a q4dx6 schedule, and a maximum T/C of 160%. Carboplatin, on the same schedule, caused a 2.5 LCK at its OD, and a maximum T/C of 176%. The LCK associated with carboplatin was statistically greater ($P < 0.01$) than any of the effects against primary tumor growth caused by the various oral treatments involving JM-251 and -269.

JM-251 given q7dx4 in peanut oil achieved a 1.4 LCK at its OD of 180 mg/kg per adm. This result was very

similar to the maximum antitumor effects observed in the previous two experiments. When W/T80 was used as the vehicle in place of oil, q7dx4 treatment yielded only 0.9 LCK at an OD of 120 mg/kg per adm; a higher dose of 180 mg/kg per adm was tolerated, but unaccompanied by an improvement in antitumor efficacy. When the treatment schedule was changed to q4dx6, JM-251 given in W/T80 yielded a maximum LCK of 1.1, but the dose of 120 mg/kg per adm used to achieve this result was possibly not a MTD.

On a q7dx4 schedule, using peanut oil as the vehicle, optimal treatment with 400 mg JM-269/kg per adm caused a 1.9 LCK. The same dose on the same schedule was an OD when W/T80 was substituted as the vehicle, but only 0.8 LCK was achieved. A better result in W/T80, 1.4 LCK, was obtained on a q4dx6 schedule at an OD of 270 mg JM-269/kg per adm.

In the four experiments performed, using the most efficacious treatment evaluated, cisplatin caused a mean maximum LCK of 3.5 and a mean maximum T/C of 166%. A similar evaluation of the collective data pertaining to carboplatin finds a mean maximum LCK of 2.5 and 164% T/C. In comparison, the maximum mean LCK and %T/C values for each of the four orally administered Pt derivatives, using the best result available per experiment, were as follows: JM-216 – 2.0 LCK and 148% T/C; JM-225 – 1.6 LCK and 151% T/C; JM-251 – 1.5 LCK and 141% T/C; and JM-269 – 1.7 LCK and 139% T/C.

A2780 antitumor experiments

Three experiments were performed in which the four orally administered Pt derivatives were compared to parenterally administered carboplatin and cisplatin versus staged s.c. A2780 human ovarian carcinoma xenografts. A summary of the optimal effects observed in those experiments is presented in Table 2.

In the first experiment, cisplatin achieved a maximum LCK of only 0.6 at the highest dose tested, 4.5 mg/kg per adm. Using the same treatment schedule, none of the four dicarboxylate derivatives administered orally in peanut oil yielded an active result at the dose levels tested. Although both JM-216 and -269 were evaluated at doses which were sufficiently escalated to include lethal levels, JM-225 and -251 were not, and possibly greater amounts of these derivatives could have been tolerated. The best effect obtained with any of the derivatives, 0.8 LCK, was achieved using JM-216, and this tumor growth delay was similar to that found using cisplatin.

In the next A2780 experiment, no. 2, the TVDT decreased to 2.5 days, compared to the 3.3 day value of the first study. On a q4dx6 schedule, carboplatin achieved 3.6 LCK at 60 mg/kg per adm, and cisplatin caused 1.4 LCK at 4.8 mg/kg per adm. Cisplatin was also evaluated on a more consolidated treatment regimen, q4dx4, which allowed for greater escalation of individual doses. Using this schedule, 2.4 LCK was achieved at 6 mg/kg per adm. The dicarboxylate derivatives in peanut oil were evaluated on a q7dx4 schedule, the same regimen which was applied success-

Table 2. Antitumor activity of orally administered platinum derivatives compared to intravenously administered cisplatin and carboplatin versus staged subcutaneous human A2780 ovarian carcinoma xenografts

Compound	Treatment[a]		Range of doses evaluated (mg/kg/adm)	Maximum LCK (optimal dose, mg/kg/adm)		
	Schedule, route	Vehicle		Expt. 1	Expt. 2	Expt. 3
Cisplatin	q4dx6, i.v.	saline	2.0–4.8	0.6 (4.5)	1.4 (4.8)	–
	q4dx4, i.v.	saline	4.8–7.5	–	2.4 (6)	2.3 (7.5)
Carboplatin	q4dx6, i.v.	water	27–60	1.6 (60)	3.6 (60)	–
	q4dx4, i.v.	water	60–90	–	–	1.3 (90)
JM-216	q4dx6, p.o.	oil	44–100	0.8 (44)	–	–
	q7dx4, p.o.	oil	80–180	–	0.4 (80)	–
	q7dx3, p.o.	oil	120–240	–	–	0.6 (240)
	q7dx3, p.o.	W/T80	180–300	–	–	0 (300)
JM-225	q4dx6, p.o.	oil	44–133	0.2 (100)	0.1 (100)	–
	q7dx4, p.o.	oil	80–180	–	1.0 (180)	–
	q7dx3, p.o.	oil	180–300	–	–	1.1 (180)
	q7dx3, p.o.	W/T80	120–240	–	–	0 (240)
JM-251	q4dx6, p.o.	oil	44–150	0.2 (66)[c]	0.9 (150)	–
	q7dx4, p.o.	oil	93–210	–	0.3 (210)	–
JM-269	q4dx5, p.o.	oil	180–400	0 (180)	–	–
	q7dx4, p.o.	oil	180–600	–	0.8 (400)	–
Control to 1 g (TVDT)[d]				22.5 (3.3)	19.0 (2.5)	22.0 (2.5)

[a] Treatments were begun when sc-implanted tumor fragments had grown to between 40–100 mg in size (7–13 days post-implant). There were 8–10 mice/group. W/T80 = water plus 0.1% Tween 80 adjusted to about pH 6

[b] Log cell kill (LCK), as a reflection of delays in tumor growth, was calculated as described in Materials and methods section

[c] A laboratory accident prevented a determination of LCK at higher dose level of 100 mg/kg per adm

[d] Median time for control tumors to reach 1 gm in size and the tumor volume doubling time (TVDT) in days

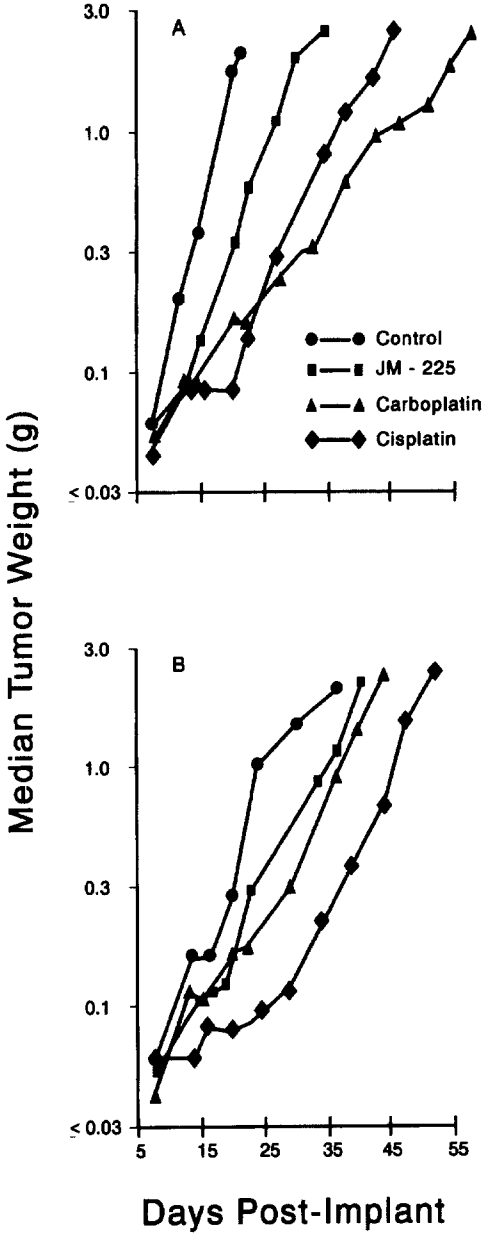


Fig. 3 A, B. Maximum tumor growth delays caused by orally administered (in oil) JM-225 concomitantly evaluated in two experiments with i.v. cisplatin and carboplatin versus staged s.c. human A2780 ovarian carcinoma xenografts. **A** JM-225 180 mg/kg per adm, q7dx4, cisplatin 6 mg/kg per adm q4dx4, carboplatin 60 mg/kg per adm q4dx6; all treatments began day 9 post-implant. **B** JM-225 180 mg/kg per adm q7dx3, cisplatin 7.5 mg/kg per adm q4dx4; carboplatin 90 mg/kg per adm q4dx4; all treatments began day 7 post-implant. Saline-treated control (●); JM-225 (■); cisplatin (◆); carboplatin (▲)

fully in the M5076 model. The OD of JM-216 was only 80 mg/kg per adm, somewhat less than had typically been observed in the M5076 experiments, and caused 0.4 LCK. JM-269 caused 0.8 LCK at its OD, 400 mg/kg per adm, a notably better result than had been obtained on the q4dx6 schedule used in the previous study. The highest dose tested of JM-251, 210 mg/kg per adm, did not cause any obvious compound-associated lethality while producing 0.3 LCK, but we were probably close to an MTD. JM-225 achieved an active result, 1.0 LCK, at its OD, 180 mg/kg per adm, but this was significantly ($P < 0.05$) less than the 2.4 LCK obtained with cisplatin. Both JM-225 and -251 were also retested on a q4dx6 schedule in expt. no. 2. Although JM-225 again did not do well on this schedule (0.1 LCK), JM-251 achieved 0.9 LCK at its OD of 150 mg/kg per adm.

In the final A2780 experiment performed, no. 3, JM-216 and -225 were evaluated on yet another schedule, q7dx3, using both peanut oil and W/T80 as vehicles. Cisplatin and carboplatin were likewise tested on a dose-intensified schedule, q4dx4, which had been used successfully for cisplatin in the previous study. The TVDT of 2.5 days was also the same as in expt. no. 2.

Carboplatin achieved 1.3 LCK at an OD of 90 mg/kg per adm, and cisplatin's 2.3 LCK was nearly identical to that observed previously using the q4dx4 schedule. Using the peanut oil vehicle, JM-216 caused 0.6 LCK at the highest dose tested, 240 mg/kg per adm. It is unlikely that greater dose levels would have been tolerated. JM-225 in oil achieved a maximum 1.1 LCK at its OD, 180 mg/kg per adm. Neither dicarboxylate derivative administered orally in W/T80 caused any delay in tumor growth at any dose level evaluated on the q7dx3 schedule.

The average best effects per experiment for cisplatin and carboplatin were 1.8 LCK and 2.2 LCK, respectively. The individual best effects for the orally administered derivatives ranged narrowly between 0.8–1.1 LCK. Their activities varied greatly depending on the treatment schedule and vehicle used. JM-225, for example, was active in two experiments (comparable to carboplatin on one occasion) when administered in oil using a weekly interval between dosing (Fig. 3), but was inactive on a q4dx6 schedule, or when given in W/T80. JM-269 also yielded better activity when given on a weekly treatment schedule, but JM-251 did best on a q4dx6 schedule. JM-216 was inactive in all three experiments; yet, it achieved 0.8 LCK in the study in which cisplatin caused only a 0.6 LCK.

Discussion

The availability of the novel mixed ammine/amine Pt(IV) dicarboxylates [7], and demonstration of their particularly good absorption in mice following oral administration [10], had led to their preliminary evaluation as orally active antitumor compounds [9, 10]. Several examples of this class of Pt complex were selected for intensive comparative antitumor evaluations on the basis of favorable toxicity [10, 13] and chemistry synthesis considerations, and preliminary antitumor data [10] for one of them, JM-216, supported such efforts.

Based on repetitive, concomitant antitumor testing, which included vehicle and treatment schedule variations, each of the orally administered Pt(IV) dicarboxylates evaluated was found to have meaningful activity (≥ 1.5 LCK) in the murine M5076 sarcoma model and borderline effects (0.8–1.1 LCK) in the human A2780 ovarian carcinoma model. These activity levels were inferior to the effects generally obtained with both cisplatin and carboplatin, often with statistically significant differences involved. However, the extent of the differences observed between

the less active of the two reference Pt drugs and the most effective Pt(IV) derivative in any given experiment was often 0.7 LCK or less. We have traditionally defined superior activity as represented by a 10-fold improvement in tumor cell killing or reduction in tumor burden; conversely, two compounds which differed by less than 1 LCK would be considered operationally as having comparable antitumor activity. As such, orally administered JM-216, for example, can be considered comparable to i.v. administered carboplatin in each of three M5076 experiments in which they both appeared, and comparable to i.v. cisplatin in one A2780 experiment; similarly, orally administered JM-225 was comparable to i.v. carboplatin in one A2780 experiment. Furthermore, with regard to the label of comparability assigned to JM-216 and carboplatin on the basis of the M5076 tumor growth delay data, their similar maximum extensions of lifespan offer further support of this position. It should be noted too that orally administered cisplatin and carboplatin produced only borderline activity in the staged s.c. M5076 tumor model [14].

With but occasional exceptions, the maximum effects of each compound in both afore-mentioned tumor models, when using a particular treatment and vehicle, varied by 1.0 LCK or less between experiments. No obvious reason surfaced to explain the few outlying responses observed, even upon giving due consideration toward changing workforce, compound lot, tumor passage, etc., over the nearly year-long study, but their occurrence reinforces the need to include concomitant testing of reference agents during analog evaluations, and not to rely solely on historical data.

Although there exist some reasonable claims for comparability to the reference Pt drugs among these Pt(IV) dicarboxylates, their activities were often diminished when an aqueous based vehicle was substituted for peanut oil. For two of the derivatives, JM-225 and -269, the activity observed when they were given orally in W/T80 was improved upon by reducing the interval between administrations. This may indicate a pharmacokinetic difference between the above-mentioned derivatives and, for example, JM-216. Upon absorption, which is reported to be above 50% for both JM-216 and JM-225 [10, 12], the latter derivative may be more available (less irreversibly bound to protein) than JM-216, and eliminated more rapidly, thereby requiring more frequent administration to optimize therapy.

From preliminary data describing the broad spectrum oral activity of JM-216 against two human tumors, to a degree comparable to cisplatin [10], and our own data described herein, it is concluded that the Pt(IV) dicarboxylates are of potential utility as orally active antitumor agents. The findings of Harrap et al. [10] and Morgan et al. [13] pertaining to the relative emetic potentials of these compounds, and the particularly favorable results obtained with JM-225 and JM-269, further support consideration of derivatives of this class as clinical candidates.

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