Preclinical anti-tumor activity of a new oral platinum(IV) drug LA-12

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A novel anti-tumor platinum(IV) complex, coded as LA-12, with a bulky adamantylamine ligand displaying oral activity was prepared and its oral activity was evaluated. The murine ADJ/PC6 plasmacytoma and human A2780 ovarian carcinoma tumor model were used to evaluate the *in vivo* anti-tumor activity of a single dose and also of repeated doses with comparison to the activity of cisplatin and of the platinum(IV) complex satraplatin. The acute toxicity of LA-12 in mice is relatively low (maximum tolerated dose 1000 mg/kg), and the effective dose is comparable to that of cisplatin and higher than that of satraplatin. The therapeutic index derived from this is very high (250). In the human tumor model, two repeated dose schedule regimens were evaluated. LA-12 exerted a significantly higher anti-tumor activity than other substances, i.e. cisplatin and satraplatin, in repeated doses on the murine ADJ/PC6 plasmacytoma tumor model. The daily × 5 repeated dose regimen was selected for further evaluation. *Anti-Cancer Drugs* 16:653–657 © 2005 Lippincott Williams & Wilkins.

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Introduction

Some peroral cytostatics have been used as standard treatment for many years, e.g. melfalane (Alkeran), cyclophosphamide (Cytosar) and etoposide (Vepesid), while others have been launched to clinical practice recently, e.g. capecitabine (Xeloda) and idarubicine (Zavedos).

About 10 years ago, a novel platinum(IV) complex [1], i.e. satraplatin, was prepared [2] for oral administration, and later introduced into *in vitro* [3,4] and *in vivo* [5,6] preclinical and clinical testing [7–10]. The advantage of this new drug in comparison with well-known platinum(II) complexes (e.g. cisplatin and carboplatin) is that it broadens the clinical spectrum of platinum drug activities, circumvents their resistance [11] and facilitates patient comfort during chemotherapy.

The present article describes anti-tumor properties of a novel cytotoxic molecule—platinum(IV) complex with a bulky adamantylamine ligand (Fig. 1), coded as LA-12, currently in phase I trials. This compound was evaluated in the panel of preclinical studies including *in vitro* and *in vivo* anti-tumor efficacy, and toxicologic and pharmacokinetics studies [12,13]. In the course of anti-tumor testing this complex showed high *in vitro* anti-tumor efficacy [14–16].

In this article we present the *in vivo* anti-tumor efficacy of this compound on the murine ADJ/PC6 plasmacytoma and on the human A2780 ovarian adenocarcinoma in various dose regimens in comparison with the anti-tumor efficacy of cisplatin and satraplatin.

Materials and methods

Tested compounds

The platinum complex (*OC*-6-43)-bis(acetato)(1-adamantylamine)amminedichloroplatinum(IV), coded as LA-12, was synthesized from cisplatin through Cossa's salt and its substitution with adamantylamine to platinum complex in oxidation number II with subsequent oxidation with hydrogen peroxide and acetylation of the arising dihydroxo complex with acetic anhydride to the desired complex LA-12 (Fig. 1). This synthesis was described in detail earlier [14]. The platinum complex satraplatin was prepared similarly according to literature data [2]. Cisplatin was synthesized in PLIVA-*Lachema* (Brno, Czech Republic).

Cancer cell cultures

The mice plasmacytoma line ADJ/PC6 was obtained from Dr Phylis Goddard (Institute of Cancer Research, Sutton, UK). Tumor pieces of 1 mm^3 were s.c. transplanted onto the right side of the mice and when the size of tumors reached 1.0–1.3 cm^3, the therapy was started.

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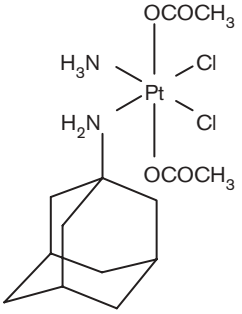
Fig. 1



Structural formula of LA-12.

The human ovarian adenocarcinoma A2780 was obtained from the European Collection of Cell Cultures (Salisbury, UK). The tumors were s.c. transplanted to mice in amounts of 1×10^7 cells with 0.1 ml of BD Matrigel matrix HC (BD Biosciences, Heidelberg, Germany). The tumors were grown to a volume 0.10–0.15 cm^3.

Animals

All the animals were maintained according to OECD guidelines. For the studies on murine ADJ/PC6 adenocarcinoma, female BALB/c mice with body weights of 18–20 g were used. For the studies on human ovarian adenocarcinoma A2780, CD-1 strain female outbred nude mice with body weights of 18–20 g were used (AnLab, Prague, Czech Republic). BALB/c mice were kept in a specific pathogen-free facility for animals, nude mice CD-1 in air laminar flow boxes for small laboratory animals KAT/F-SZ-1. All the mice were provided with radiation-sterilized bedding (SAWI Research Bedding; AnLab) and fed with radiation-sterilized ST-1 diet (Bergman, Kocanda, Czech Republic) with free access to autoclaved water.

Administration of the compounds

Both satraplatin and LA-12 were suspended in 0.6% solution of methylcellulose and administered p.o. by gastric gavage. Cisplatin was diluted in 0.9% solution of saline and administered i.p.

Acute toxicity

For testing of the acute toxicity of LA-12, albino NMRI mice (body weight 20–25 g) were used in groups of five mice. Mice were weighed every 3 days. At the end of the experiment the mice were sacrificed and histologically examined. The maximum tolerated dose (MTD) after single-dose oral administration was defined as the dose that caused significant body weight loss (more than 5%).

Anti-tumor efficacy

Murine ADJ/PC6 plasmacytoma

For the studies on murine ADJ/PC6 plasmacytoma, female BALB/c mice were used, 10 animals in each group. LA-12 and satraplatin were administered p.o. in single doses of 1, 2, 5, 10, 20 and 40 mg/kg or daily in the doses of 2, 4 and 8 mg/kg/day for 9 consecutive days. Cisplatin was administered i.p. in single doses of 2, 4, 6 and 10 mg/kg.

The effective dose ED_{90} was evaluated on the 25th day of the experiment. The curves of the dependence between the administered dose of the drugs and tumor weights (in percent of control values) were plotted, using non-linear regression. Based on the curves obtained by non-linear regression, the effective dose was determined as the dose resulting in a 90% inhibition of tumor growth.

The therapeutic index (TI) was calculated as a quotient (ratio) of MTD and ED_{90}: $TI = MTD/ED_{90}$. For calculating TI, the MTD [11] and ED_{90} [5] literature values were used for satraplatin (see Discussion).

Human ovarian adenocarcinoma A2780

For the studies on human ovarian adenocarcinoma A2780, CD-1 strain female outbred nude mice were used, six animals per group. LA-12 was administered in two regimens: a single dose of 40 and 60 mg/kg every 7 days in 4 cycles and once a day for 5 consecutive days in the doses of 10, 20 and 30 mg/kg/day.

Satraplatin was administered in the second mentioned schedule in doses of 9, 18 and 27 mg/kg/day. The doses of satraplatin were equimolar to doses of LA-12.

In the course of the treatment, the tumors were measured every 3–4 days with a caliper. The tumor volumes were calculated from the formula: $V = \text{length} \times \text{width}^2 \times \pi/6$.

The tumor growth inhibition (%TGI) was calculated from the formula: $\%TGI = [1 - (V_{treat}/V_{contr})] \times 100$, where V_{treat} is the median tumor volume in the treated group and V_{contr} is the median tumor volume in the controls group.

Statistical evaluation

The evaluation of the efficacy of particular substances was based on measuring the tumor size in the course of the experiment by plotting tumor growth curves and statistical comparison of the curves obtained for particular groups and controls group. The non-paired two-sided Student's *t*-test was used for statistical evaluation and $p \leq 0.05$ was regarded as significant.

Results and discussion

In this study the hydrophilic formulation (suspension of the drugs in aqueous solution of methylcellulose) was selected for p.o. administration as being more convenient with respect to the formulation intended for use in

clinical trials. In the previously study of pharmacokinetics and excretion in pigs, in which the aqueous formulation was used, LA-12 showed rapid and efficient oral absorption [13].

The ADJ/PC6 murine plasmacytoma was chosen for the evaluation of the tumor growth inhibition *in vivo* because it has been used as a suitable model for anti-tumoral testing of platinum cytostatics for more than 20 years [17].

Table 1 Comparison of the efficacy of LA-12, satraplatin and cisplatin

Substance administered	MTD (mg/kg)	ED_{90} (mg/kg)	TI
LA-12	1000	4.0	250
Satraplatin	200[a]	5.8[a]/14.3	34.5
Cisplatin[b]	7	4	1.75

[a]Administration in arachis oil [5].
[b]i.p. administration.

Table 2 Median initial tumor volumes (%) in particular experimental groups—repeated dose of LA-12 and satraplatin/ 9 consecutive days in ADJ/PC6 plasmacytoma

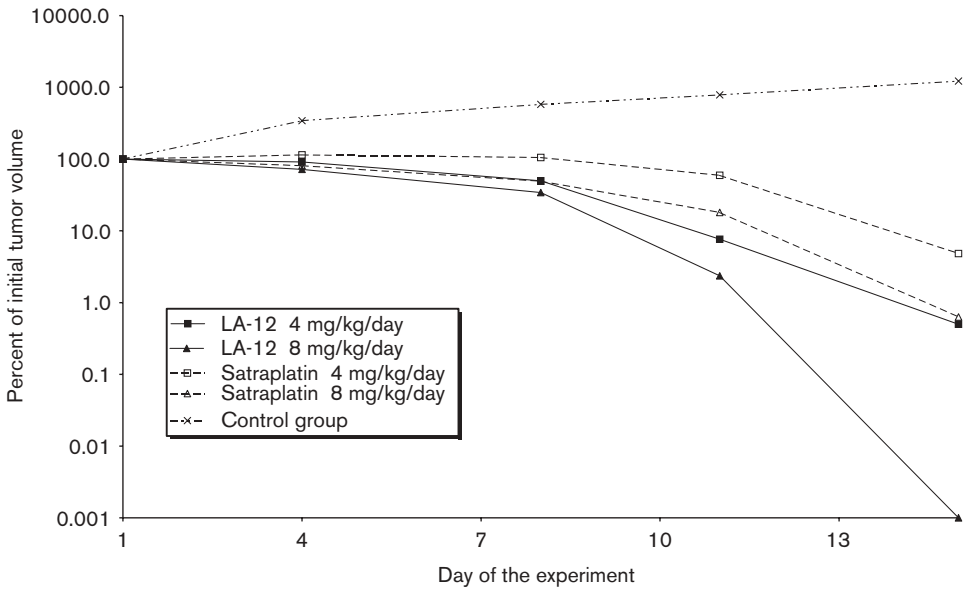
Substance administered	Dose (mg/kg)	Day of experiment				
		1	4	8	11	15
LA-12	2	100	98.9	70.9	8.0	3.9
LA-12	4	100	91.2	49.8	7.6	0.5
LA-12	8	100	72.0	34.2	2.4	0.0
Satraplatin	2	100	124.7	135.0	65.8	12.9
Satraplatin	4	100	114.5	105.5	59.3	4.8
Satraplatin	8	100	81.1	49.2	18.0	0.6
Cisplatin	2	100	193.9	157.6	100.9	33.4
Cisplatin	4	100	276.3	259.9	161.5	25.3
Controls	0	100	343.1	577.7	786.2	1222.6

The MTD of LA-12 was found at 1000 mg/kg. This dose caused reversible weight loss, about 10%. According to the histological examination the main toxicity was myelosuppression. At the doses over the MTD, an influence on the gastrointestinal and genitourinary tract was found.

The results of the single-dose evaluation of the toxicity and anti-tumor activity presented above indicate that the acute toxicity of the new platinum complex LA-12 is relatively low (Table 1). The effective dose ($ED_{90} = 4.0$ mg/kg) is considerably lower than that of satraplatin ($ED_{90} = 14.3$ mg/kg) and is nearly comparable to the ED_{90} value for cisplatin (4 mg/kg). These results are somewhat different from those described in the literature [5] (ED_{90} of satraplatin 5.8 mg/kg). These differences probably result from various methods used in studies of the anti-tumor efficacy, especially from the use of different formulations for p.o. administration of anti-tumor agents (arachis oil versus solution of methylcellulose).

The TI for LA-12 is very high (250); the TI of satraplatin was calculated from literature values of MTD [18] and ED_{90} [5], because these values were obtained by the same method (oral administration of suspension in arachis oil). Degenerative changes of the genitourinary and gastrointestinal tract were observed in doses over the

Fig. 2



Medians of tumor volumes after administration of different doses of LA-12 and satraplatin and control. Orally administered once a day for 9 consecutive days on murine ADJ/PC6 plasmacytoma.

Table 3 Anti-tumor activity of LA-12 and satraplatin on the human 2780 ovarian adenocarcinoma

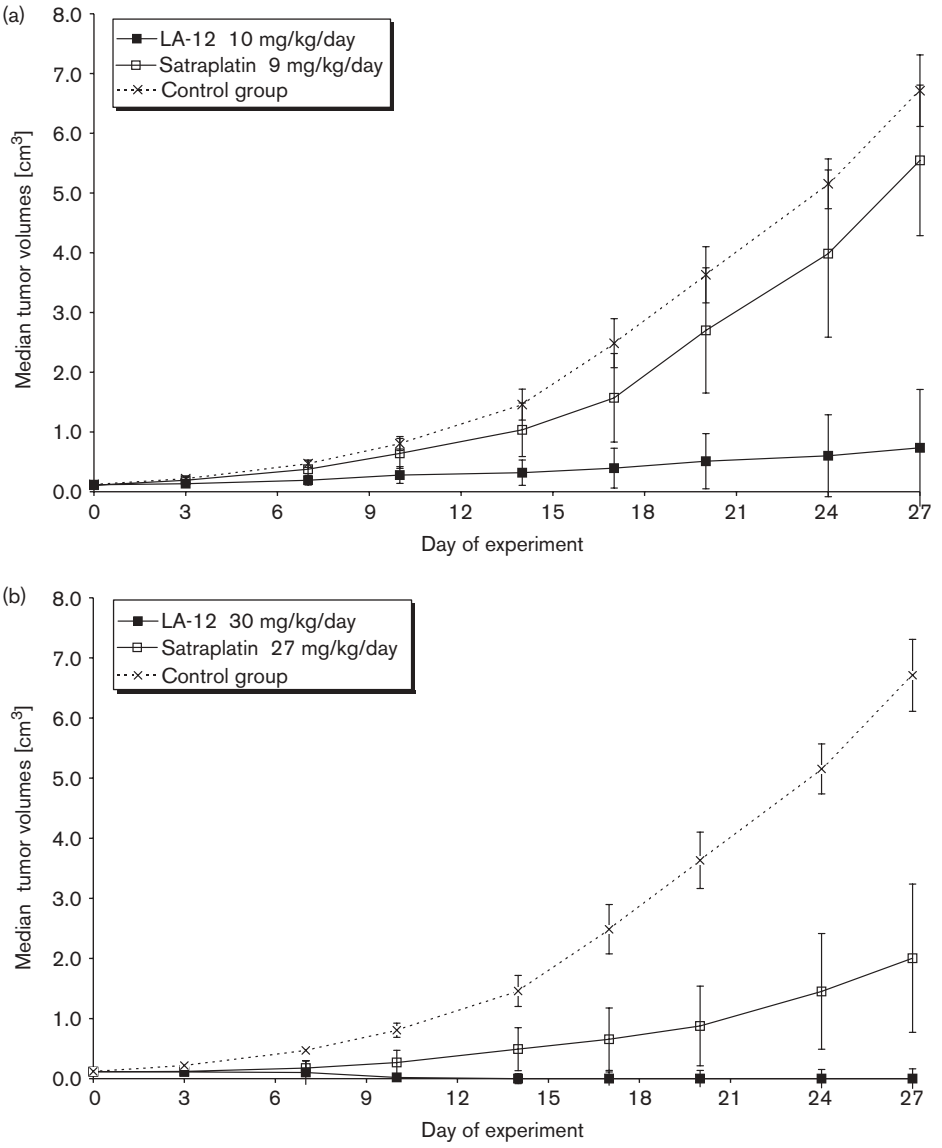
Schedule	Level (mg/kg)	Total (mg/kg)	%TGI			
			Day 21	*p*	Day 28	*p*
LA-12, single dose q7 days[a]	40	160	87.94	<0.01	85.73	<0.01
	60	240	82.66	<0.01	85.77	<0.01
LA-12, daily × 5[b]	10	50	85.96	<0.01	89.05	<0.01
	20	100	90.64	<0.01	92.89	<0.01
	30	150	100.00	<0.01	100.00	<0.01
Satraplatin, daily × 5[b]	9	45	25.66	<0.01	17.36	<0.05
	18	90	46.32	<0.01	34.90	<0.01
	27	135	75.88	<0.01	70.14	<0.01

[a]Orally administered every seventh day for 4 cycles.
[b]Orally administered once a day for 5 consecutive days.

MTD only. It is supposed that the therapeutic doses will be safe, with relatively good tolerability (Table 1).

Tumor growth inhibition by LA-12, satraplatin and cisplatin in ADJ/PC6 plasmacytoma after repeated dose schedules is summarized in Table 2 and Fig. 2. LA-12 demonstrated a significantly higher anti-tumor activity than satraplatin and cisplatin. At the end of the experiment the weights of tumors in all groups of mice were under the experimental error, but great differences were found in the rate of their reduction during the treatment.

Fig. 3



Efficacy of LA-12 and satraplatin on A2780 human ovarian adenocarcinoma. Daily × 5 schedule.

The tumor volumes on the 11th day after the start of LA-12 administration were significantly lower than in satraplatin ($p < 0.01$) for all dose levels. It means that the therapeutic effect of LA-12 was faster than of satraplatin.

When simultaneously comparing the efficacy of different doses of LA-12 with those of satraplatin, it is obvious that the dose of 4 mg/kg of LA-12 corresponds by its efficacy to 8 mg/kg of satraplatin and the dose of 2 mg/kg of LA-12 is more efficient than 4 mg/kg of satraplatin.

Only cisplatin at a dose of 8 mg/kg produced toxic symptoms—it resulted in a 24% decrease of the body weight after 15–22 days of the experiment with a simultaneous decrease of the animals treated.

Human xenografts

LA-12 in single-dose q7 days schedule in 4 cycles significantly inhibited the ovarian adenocarcinoma A2780 growth. Differences between dose levels of 40 and 60 mg/kg were not significant. Maximum %TGI (85.77) was reached at a dose level of 60 mg/kg. No influence on mice body weights was observed. LA-12 and satraplatin in daily × 5 schedules significantly inhibited A2780 growth in comparison with the control group for all doses ($p < 0.01$). Maximum %TGI (100.00) was reached for the dose of 30 mg/kg/day of LA-12. No influence on mice body weights was observed.

A comparable %TGI of the complex LA-12 was found at 50 mg/kg of total dose at the daily × 5 schedule and 240 mg/kg at the single-dose q7 days scheme, 85.77 and 89.05%, respectively. The anti-tumor efficacy at cumulative dose 150 mg/kg in the daily × 5 schedule was higher than that of 240 mg/kg at single-dose q7 days regimen (Table 3). This finding is in accordance with previously published data with satraplatin [6]. Comparison of the efficacy of LA-12 and satraplatin in the daily × 5 schedule provided in equimolar doses showed significantly higher anti-tumor efficacy of LA-12 (Table 3 and Fig. 3). The differences of efficacy of LA-12 versus satraplatin were statistically significant at all doses of 10, 20 and 30 mg/kg/day of LA-12 versus 8, 18 and 27 mg/kg/day of satraplatin ($p < 0.05$). The daily administration for 5 consecutive days showed good efficacy and it will be used in further studies including clinical evaluation.

Acknowledgments

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