Comparative anti-tumor efficacy of two orally administered platinum(IV) drugs in nude mice bearing human tumor xenografts

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The oral anti-tumor activity of a novel platinum(IV) complex, coded as LA-12, with a bulky adamantylamine ligand was evaluated and compared with another platinum(IV) complex satraplatin. The human carcinoma xenografts of colon HCT116, prostate PC3, and ovarian A2780 and A2780/cisR (resistant to cisplatin) were used to evaluate the in-vivo anti-tumor activity. The daily × 5 repeated dose regimen in equimolar doses of LA-12 and satraplatin, administered in 2 cycles, was selected for this evaluation. All doses of LA-12 and satraplatin were significantly effective in comparison with the control. The activities of LA-12 in all doses and all used tumor xenografts were higher than equimolar doses of satraplatin. The highest effect was reached with LA-12 at a dose of 60 mg/kg. The shapes of growth curves of ovarian carcinoma A2780 and its subline resistant to cisplatin after therapy with LA-12 were very similar. This shows that LA-12 is able to overcome resistance to cisplatin. *Anti-Cancer Drugs* 17:201–206 © 2006 Lippincott Williams & Wilkins.

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Introduction

Currently, all approved platinum drugs which play an important role in cancer therapy (cisplatin, carboplatin, oxaliplatin) are complexes of platinum oxidative number II [1]. Serious side-effects and resistance to treatment are the main disadvantages of these complexes. For these reasons, new platinum complexes are being developed with special regard to improvement of the effectiveness and toxicology profile [2,3].

In our studies described previously [4,5] a novel platinum(IV) complex, coded LA-12, with a bulky adamantylamine ligand (Fig. 1a) demonstrated promising in-vitro anti-tumor activity. This paper follows up the previous results [6] and continues the assessment of anti-tumor activities *in vivo* on human tumor xenografts. The acute toxicity of LA-12 after oral administration is relatively low (maximum tolerated dose in mice was 1000 mg/kg) [6]. Repeated toxicity studies were performed on rodents and non-rodents (results not shown). It could be stated that the toxicity of LA-12 was relatively low. High doses of LA-12 produced hematological toxicity, which resulted in leukocytopenia and thrombocytopenia. No signs of hepato- or nefrotoxicity were found, and no influence on the respiratory and cardiovascular system and motor functions was observed. An emetogenic effect was recorded in dogs only and it was significantly lower in comparison to cisplatin.

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LA-12 is currently in phase I clinical trials and it is believed phase II will be opened in the near future.

The analog molecule, satraplatin (Fig. 1b), with a cyclohexylamine ligand has been investigated in a number of preclinical and clinical studies [7–10], and has now been introduced in phase III clinical evaluation in the second-line treatment of hormone-refractory prostate cancer [11]. Therefore, we decided to compare the anti-tumor activity of both complexes, which could be introduced in clinical practice to improve anti-tumor treatment.

Materials and methods

Tested compounds

The platinum complex (*OC*-6-43)-bis(acetato)(1-adamantylamine)amminedichloroplatinum(IV), coded as LA-12 (Fig. 1a), was synthesized from cisplatin according to the procedure detailed previously [12]. The platinum complex satraplatin (Fig. 1b) (*OC*-6-43)-bis(acetato)amminedichloro(cyclohexylamine)platinum(IV) was prepared similarly according to the literature [13].

Human tumor lines

Four human tumor lines (colon carcinoma HCT-116, prostate carcinoma PC-3, ovarian carcinoma A2780 and resistant ovarian carcinoma A2780/cisR) were used for the evaluation of anti-tumor efficacy. The tumor lines were

purchased from the European Collection of Cell Cultures (Salisbury, UK). The lines were multiplied in tissue culture RPMI 1640/2 mmol/l glutamine/10–15% FTS and then were administered s.c. at 1×10^7 per mouse together with 0.1 ml of Matrigel (BD Biosciences, Heidelberg, Germany) onto the right side of all animals.

Animals

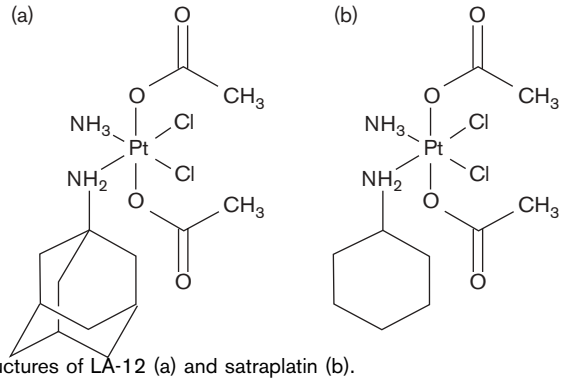
CD-1 strain female outbred nude mice–male for the prostate PC-3 carcinoma–(AnLab, Prague, Czech Republic) were used in the experiments. All the animals were maintained according to OECD guidelines. Mice were kept in an air laminar flow box for small laboratory animals KAT-F-SZ/1 provided with radiation-sterilized bedding SAWI-Research Bedding (AnLab) and fed with a radiation-sterilized ST-1 diet (Bergman, Kocanda, Czech Republic) with free access to water *ad libitum*.

To facilitate as good absorption of the administered product doses as possible, the diet was withdrawn from the mice 5 h prior to and provided 2 h after the oral administration of the substances tested.

Administration of the tested compounds

LA-12 and satraplatin were suspended in 0.6% solution of methylcellulose, and administered p.o. by gastric gavage in a volume of 5 ml/kg/day for 5 subsequent days on days 1–5 and 16–20 (2 cycles) at daily dose levels of 20, 40 and 60 mg/kg/day. Satraplatin was administered in equimolar doses of 18, 36.2 and 54.4 mg/kg/day on the same schedule.

Fig. 1



Structures of LA-12 (a) and satraplatin (b).

Evaluation of the experiment

The study was evaluated comparing growth data and curves of tumors in particular experimental groups with those in controls; the percentage of tumor growth inhibition (%TGI) and the percentage of tumor weight inhibition (%TWI) were calculated.

The therapy was started when the tumors achieved volumes of 0.18–0.25 cm^3. Mice bearing comparably sized tumors were randomized into treatment groups of eight animals including control groups. In the course of the experiment, the mice were weighed each third day and the tumor diameters were measured with a caliper. The tumor volume was calculated from the formula $V = \text{length} \times \text{width}^2 \times \pi/6$

On the 30th day of the experiment, the mice were sacrificed, and the tumors were removed and weighed.

Median tumor volumes at 1-day intervals were calculated from the measured values by means of linear approximation. The average median values for the first 15 days $Ø^{1-15}$ and for the second 15 days $Ø^{16-30}$ were calculated and applied for calculation of 'optimal percentage T/C' according to the procedure described elsewhere [14]. The results based on comparison of treated tumor increase (ΔT) and the control (ΔC) served for the evaluation of anti-tumor efficacy in the course of treatment and these values were used to calculate a percent T/C as follows:

$$\%T/C = (\Delta T/\Delta C) \times 100 \quad \text{where } \Delta T > 0$$
$$\%T/C = (\Delta T/T_1) \times 100 \quad \text{where } \Delta T > 0$$

and T_1 is the median tumor volume at the start of treatment. The results of these calculations are presented in Tables 1–3, together with other experimental data.

The %TGI and %TWI were calculated using the formulas:

$$\%TGI = [1 - (V_{treat}/V_{contr})] \times 100$$
$$\%TWI = [1 - (m_{treat}/m_{contr})] \times 100$$

Table 1 **Anti-tumor activity of LA-12 and satraplatin on human colon HCT116 carcinoma xenografts**

Dose (mg/kg/day)	%T/C $Ø^{1-15}$	%T/C $Ø^{16-30}$	%T/C V_{30}	%TGI	%TWI
LA-12 daily × 5 in 2 cycles					
20	2.5	8.1	10.8	82.8	88.1
40	−10.0	5.2	11.2	82.4	83.2
60	−45.0	−15	2.3	90.7	94.7
Satraplatin daily × 5 in 2 cycles					
18	37.5	37.9	40.3	53.4	49.5
36.2	22.5	24.1	26.9	67.7	65.3
54.4	5.0	11.5	15.4	78.5	82.1

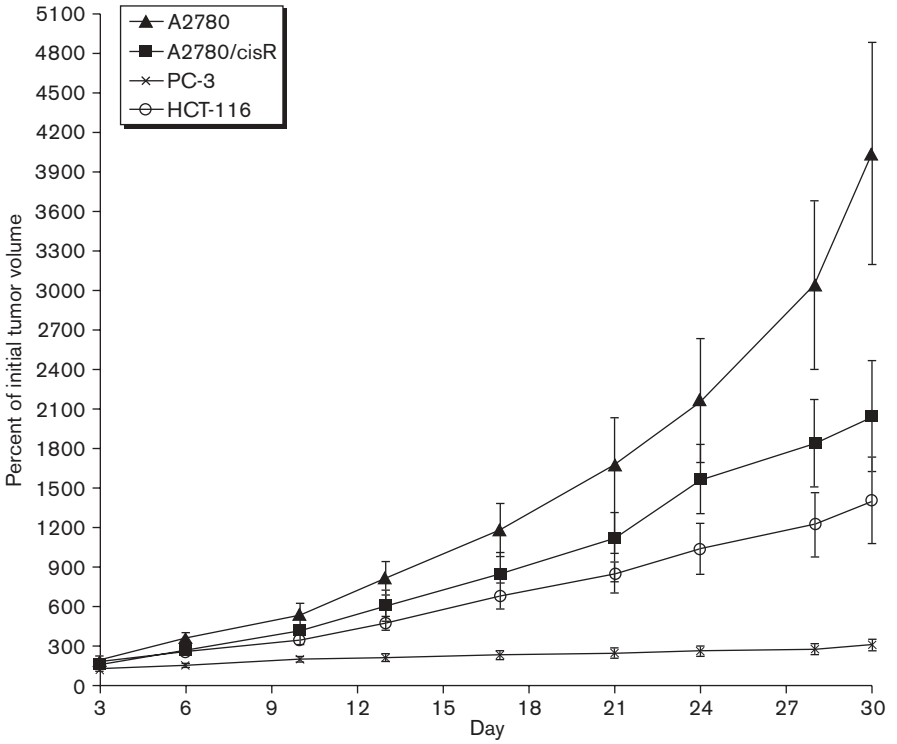
Table 2 Anti-tumor activity of LA-12 and satraplatin on human prostate PC3 carcinoma xenografts

Dose (mg/kg/day)	%T/C Ø$^{1-15}$	%T/C Ø$^{16-30}$	%T/C V_{30}	%TGI	%TWI
LA-12 daily × 5 in 2 cycles					
20	69.2	51.5	45.0	37.7	42.0
40	30.8	21.2	25.0	51.4	53.1
60	−14.3	−19.0	−23.8	76.2	87.7
Satraplatin daily × 5 in 2 cycles					
18	76.9	69.7	65.9	23.0	24.1
36.2	15.4	27.8	43.2	39.0	44.4
54.4	15.4	18.2	25.0	50.9	56.2

Table 3 Anti-tumor activity of LA-12 and satraplatin on human ovarian A2780 and A2780/cisR carcinoma xenografts

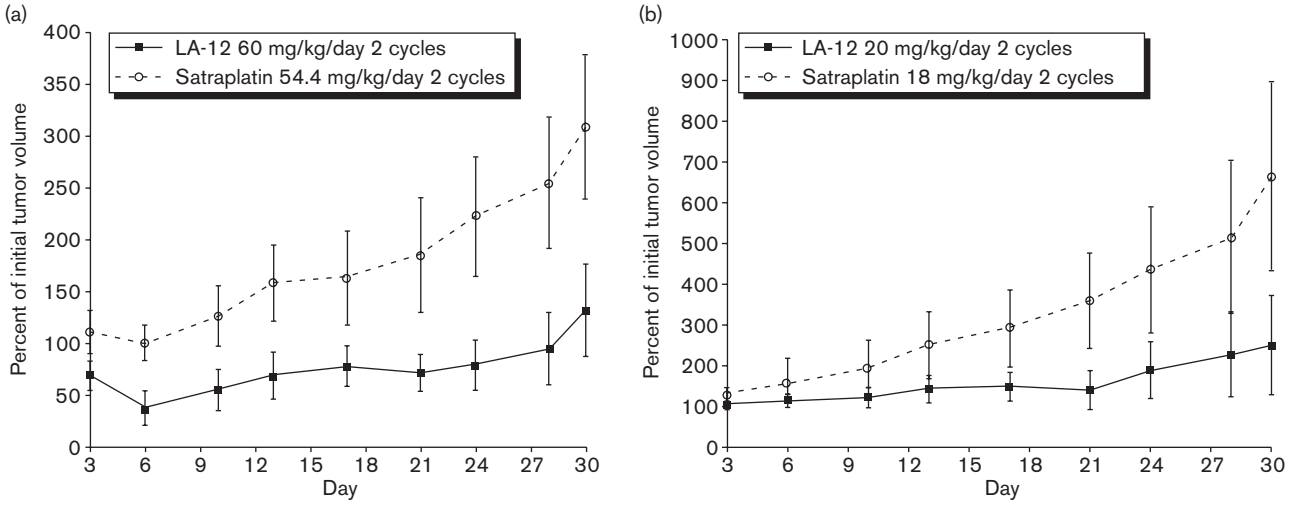
Dose (mg/kg/day)	%T/C Ø$^{1-15}$	%T/C Ø$^{16-30}$	%T/C V_{30}	%TGI	%TWI
Ovarian A2780					
LA-12 daily × 5 in 2 cycles					
20	29.9	32.4	32.7	65.4	65.1
40	19.5	19.4	14.6	83.1	81.2
60	1.3	9.5	10.7	86.9	84.9
Satraplatin daily × 5 in 2 cycles					
18	45.5	46.1	53.8	45.1	40.3
36.2	40.3	38.3	32.1	66.1	63.6
54.4	35.1	27.7	25.2	72.7	66.2
Ovarian A2780/cisR (resistant to cisplatin)					
LA-12 daily × 5 in 2 cycles					
20	43.8	50.4	32.2	64.8	65.4
40	22.9	35.7	28.5	68.4	68.4
60	8.3	13.0	11.3	84.6	87.7
Satraplatin daily × 5 in 2 cycles					
18	50.0	52.9	51.3	46.4	43.9
36.2	41.7	60.9	59.3	38.9	35.3
54.4	25.0	32.8	31.3	65.6	63.3

Fig. 2



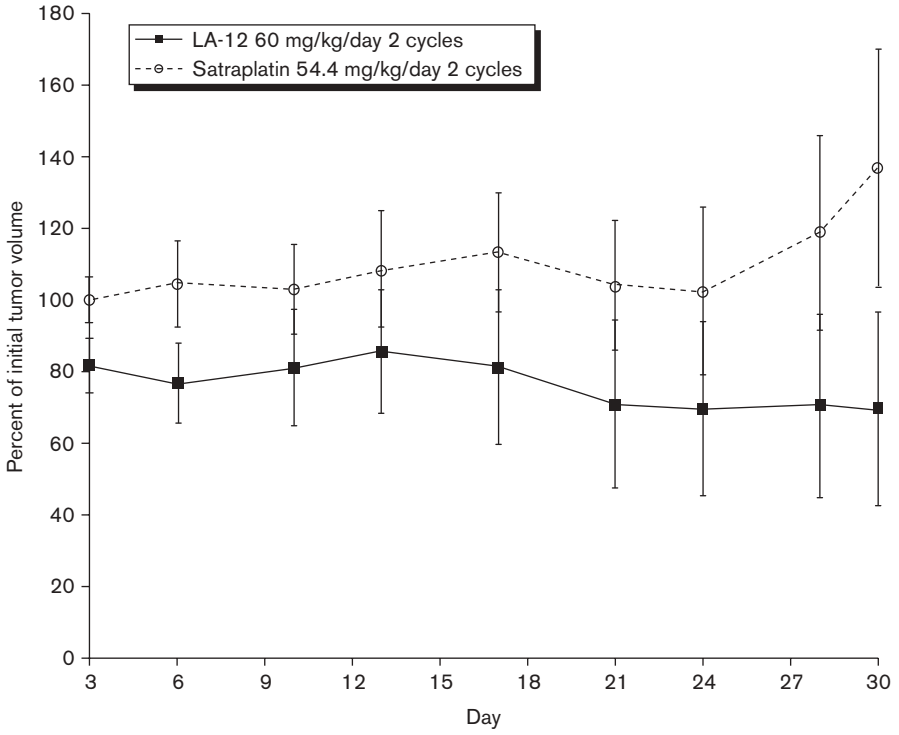
Comparison of growth curves of the tumor xenografts used in this study in control groups.

Fig. 3



Tumor growth curves of human colon HCT116 carcinoma xenografts: (a) 60 mg/kg LA-12 and 54.4 mg/kg satraplatin once a day for 5 consecutive days repeated on days 16–20; (b) 20 mg/kg LA-12 and 18 mg/kg satraplatin once a day for 5 consecutive days repeated on days 16–20.

Fig. 4



Tumor growth curves of human prostate PC3 carcinoma xenografts: 60 mg/kg of LA-12 and 54.4 mg/kg of satraplatin once a day for 5 consecutive days repeated on days 16–20.

where V_{treat} is the median tumor volume and m_{treat} is the median of tumor weight in the treated group, and V_{contr} the median tumor volume and m_{contr} is the median tumor weight in the control group.

The tumor growth curves were constructed on the basis of median tumor volumes measured during the 30-day period of tumor growth (see above). The graphs were plotted using a percentage of the initial tumor

volumes calculated from median tumor volumes (see Figs 2–5).

Results and discussion

The rates of tumor growth in control groups were dependent on the type of tumor used. For example, the growth of prostate tumor (PC-3) was 5–10 times slower than that of other tumors (Fig. 2). The %TGI, %TWI and T/C were used for comparison of results on these different kinds of tumors.

All doses of LA-12 and satraplatin were significantly effective in comparison with the control, but LA-12 was more effective against the tumor xenografts at all concentration levels than satraplatin. The most effective was LA-12 at a dose of 60 mg/kg/day. This dose was statistically significantly more effective than the equimolar dose of satraplatin at $P < 0.01$, with exception of the A2780/cisR line ($P < 0.05$).

The anti-tumor activities of LA-12 and satraplatin were measured and compared with each other on the basis of tumor growth progress. The anti-tumor efficacies of both complexes against the human colon carcinoma HCT116 are summarized in Table 1 and Fig. 3(A and B).

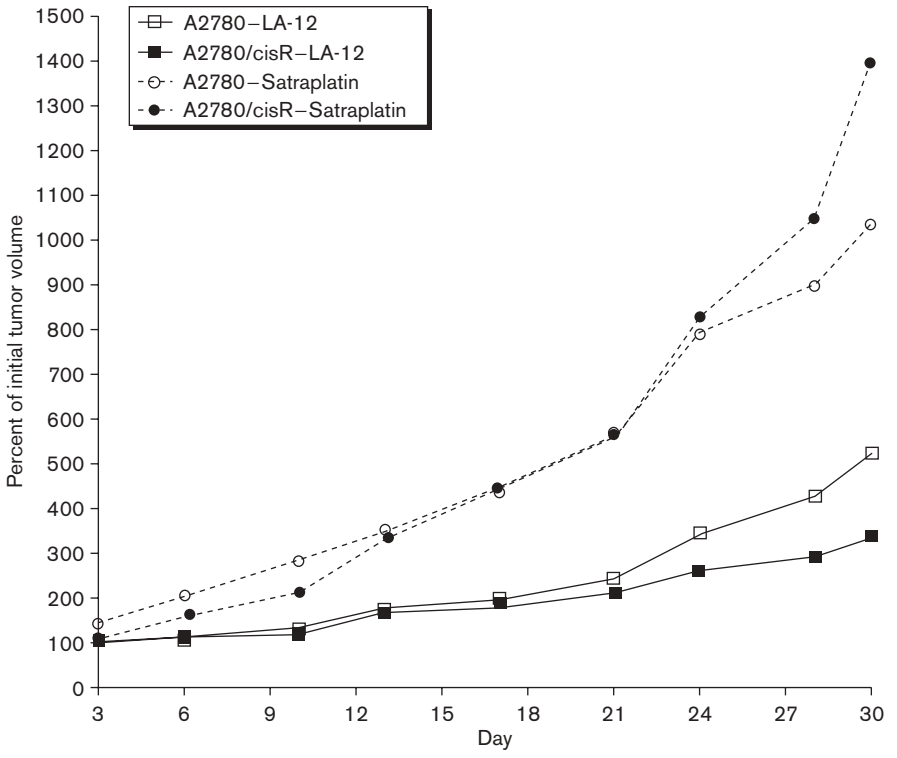
All doses of LA-12 and satraplatin were significantly more effective in comparison with the control ($P < 0.01$). The activity of LA-12 was higher than equimolar doses of satraplatin at all doses ($P < 0.01$). The highest effect was reached with LA-12 at a dose of 60 mg/kg/day with a decrease in tumor size of 45% in the first half and 15% in the second half of the experiment.

The tumor volume and weight measurements at the end of the study led to the same conclusions.

The prostate PC-3 xenograft is distinguished from the others by the most prolonged rate of growth and also by the greatest response to LA-12 treatment.

The anti-tumor activity of LA-12 against human prostate carcinoma PC–3 was compared with that of satraplatin, and the results are presented in Table 2 and Fig. 4. The rate of tumor growth was slower than that of the colon and the ovarian tumor rates by about an order of magnitude. The other conclusions are similar to

Fig. 5



Tumor growth curves of human ovarian carcinomas A2780 and A2780/cisR: 60 mg/kg LA-12 and 54.4 mg/kg satraplatin once a day for 5 consecutive days repeated on days 16–20.

HCT116 carcinoma, i.e. the higher activity of LA-12 at equimolar doses of satraplatin and the most effective dose of 60 mg/kg/day with a reduction of about 20% of the tumor extent during the experiment in comparison to the initial volume. The weights and volumes at the end of the trial were mutually in accordance.

The growth of tumor human ovarian xenografts A2780 and A2780/cisR (resistant to cisplatin) was measured and compared. It was found that the growth of lines resistant to cisplatin in the control group was slower than that of the non-resistant line (see Fig. 5). The tumor volumes in the control groups at the end of experiment were 8.4 cm^3 for A2780 against 4.8 cm^3 for A2780/cisR. The shapes of growth curves of ovarian carcinoma A2780 and its subline resistant to cisplatin after therapy by LA-12 were very similar (see Fig. 5). This shows that no cross-resistance exists between LA-12 and cisplatin.

Conclusions concerning anti-tumor efficacy are analogous to the above-mentioned types of tumors, but the activity of LA-12 was much greater than the activity of satraplatin. The most efficient was LA-12 at a dose of 60 mg/kg/day (see Fig. 5 and Table 3). In comparison with our previous evaluation of anti-tumor efficacy [7] against the ovarian carcinoma xenograft A2780, the present values of TGI were lower. The initial different size of the tumors (0.1 against 0.2 cm^3) was the reason of this disparity.

There is a very good agreement between results of the volume and weight measurements of the tumors in the end of trial.

In the experimental group of mice administered with LA-12, there were body weight decreases comparable to that of satraplatin. Only the dose of 54.4 mg/kg of satraplatin, used in application against the ovarian A2780 xenograft, induced a steeper decrease in body weight in comparison to the corresponding dose of LA-12.

Two types of tumors (colon and prostate carcinoma) decreased their volumes in comparison with the initial tumor volume by 15–45% after administration of LA-12 at a dose of 60 mg/kg/day. When cisplatin-sensitive and cisplatin-resistant lines of ovarian carcinoma A2780 were compared, the conclusion from the in-vitro studies [4,5] that no cross-resistance exists between LA-12 and cisplatin was confirmed.

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