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ORIGINAL ARTICLE

A comparative study of pharmacokinetics, urinary excretion and tissue distribution of platinum in rats following a single-dose oral administration of two platinum(IV) complexes LA-12 (*OC*-6-43)-bis(acetato)(1-adamantylamine)amminedichloro platinum(IV) and satraplatin (*OC*-6-43)-bis(acetato)amminedichloro(cyclohexylamine)platinum(IV)

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Abstract
Purpose This study compared the pharmacokinetics, tissue distribution, and urinary excretion of platinum in rats after single oral doses of LA-12 and satraplatin.
Methods Both platinum derivatives were administered to male Wistar rats as suspensions in methylcellulose at four equimolar doses within the range of 37.5–300 mg LA-12/kg body weight. Blood sampling was performed until 72 h, and plasma and plasma ultrafiltrate were separated. Moreover, urine was collected until 72 h, and kidney and liver tissue samples were obtained at several times after administration. Platinum was measured by atomic absorption spectrometry. The pharmacokinetics of platinum was analyzed by population modelling and post hoc Bayesian estimation as well as using non-compartmental pharmacokinetic analysis of the mean concentration–time curves.
Results Platinum was detected in all plasma and ultrafiltrate samples 15 min after oral administration of both compounds and peaked between 3–4 h and 1–3 h, respectively. Similar for LA-12 and satraplatin, the C_{max} and AUC values of plasma and ultrafiltrate platinum increased less than in proportion to dose. The mean C_{max} and AUC values of plasma platinum observed after administration of LA-12 were from 0.84 to 2.5 mg/l and from 20.2 to 75.9 mg h/l. For ultrafiltrate platinum, the corresponding ranges were 0.16–0.78 mg/l and 0.63–1.8 mg h/l, respectively. The AUC of plasma platinum was higher after satraplatin ($P < 0.001$). However, administration of LA-12 resulted in significantly higher AUC values of ultrafiltrate platinum after the doses of 150 mg and 300 mg/kg ($P < 0.01$), respectively, and the C_{max} values were significantly higher starting from the dose of 75 mg/kg LA-12 and upward ($P < 0.01$). Cumulative 72-h urinary recovery of platinum dose was below 5% for both compounds, and it decreased with the dose of satraplatin ($P < 0.01$), while a numerical decrease was observed after administration of LA-12 that did not reach statistical significance ($P = 0.41$). The renal clearance of free platinum was similar regardless of the dose and compound administered. Platinum concentrations in the liver homogenate exceeded those in the kidney. Distribution of platinum to tissues was higher after LA-12 compared to satraplatin. The difference in kidney platinum increased with dose and was twofold after 350 mg/kg LA-12. Liver platinum was twofold higher after LA-12 across all four doses.
Conclusions In conclusion, this first comparative pharmacokinetic study with LA-12 and satraplatin shows that characteristics of platinum exposure evaluated in the plasma, plasma ultrafiltrate and kidney and liver tissues increase less than in proportion to dose following a single-dose administration of 37.5–300 mg/kg to Wistar rats. These findings together with the dose-related elevation in the pharmacokinetic characteristics *V*/*F* and CL/*F* of

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platinum and ultrafiltrate platinum as well as a drop in platinum urinary recovery are consistent with a dose-related decrease in the extent of oral bioavailability most likely due to saturable intestinal absorption.

Keywords Platinum anti-cancer drugs · Pharmacokinetics · Wistar rat

Introduction

Platinum compounds are very effective anti-cancer drugs used against solid tumours such as testicular, ovarian, lung, head and neck cancers [1]. Research has focused on new platinum drugs with improved efficacy, which can overcome resistance to currently used drugs. Another objective is to develop platinum compounds suitable for oral administration which could further increase the therapeutic use of platinum anti-cancer drugs due to flexible and convenient dosing [2]. Satraplatin [(*OC*-6-43)-bis(acetato)amminedichloro(cyclohexylamine) platinum(IV)] (also known as JM216) is the first orally administered platinum complex with documented efficacy and acceptable safety in patients with hormone-refractory prostate cancer and small-cell lung cancer [3].

LA-12 (Fig. 1), [(*OC*-6-43)-bis(acetato)(1-adamantylamine)amminedichloroplatinum(IV)], is a novel platinum(IV) complex with a bulky adamantylamine ligand which has demonstrated higher cytotoxicity in vitro than that of cisplatin and no cross-resistance [4, 5] and also higher anti-tumour efficacy than satraplatin on human tumour xenografts [6]. Importantly, LA-12 proved role in apoptosis, which is not fully dependent on protein p53. The results confirm its therapeutic potential as a very promising anti-cancer agent targeting both p53+ and p53− cells [7]. Nowadays, LA-12 undergoes clinical development.

The aim of this study was to compare the pharmacokinetics, urinary excretion and tissue distribution of platinum in Wistar rats after single oral doses of LA-12 or satraplatin. Both platinum derivatives were administered to male Wistar rats as suspensions in methylcellulose at four equimolar doses within the range of 37.5–300 mg LA-12/kg body weight.

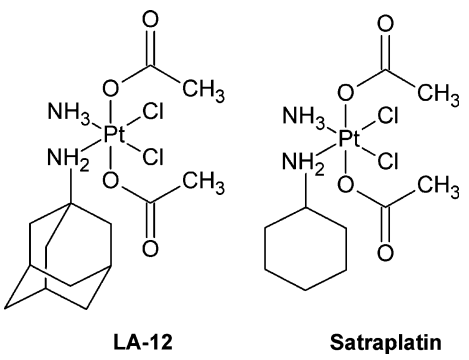

Fig. 1 Chemical structure of (*OC*-6-43)-bis(acetato)(1-adamantylamine)amminedichloroplatinum(IV) (LA-12) and (*OC*-6-43) bis(acetato)amminedichloro(cyclohexylamine)platinum(IV) (satraplatin)

Materials and methods

Chemicals and solutions

LA-12 and satraplatin (Fig. 1) were prepared in PLIVA-Lachema a.s. using methods described previously [4, 8]. Nitric acid and phosphoric acid were Suprapur grade from Merck (Darmstadt, Germany). Magnesium nitrate was obtained from Lachema (Brno, The Czech Republic). Triton X-100 was purchased from Sigma–Aldrich (Prague, The Czech Republic). Double-deionized water of 18 MΩ cm specific resistivity, obtained in a Milli-Q Plus Millipore system, was used to prepare all the solutions. A 10 mg/ml primary standard solution of platinum was Titrisol Merck. A stock standard solution of 0.1 mg/ml was prepared by dilution of the primary standard solution with 100 ml water after the addition of 0.1 ml nitric acid. Modification solution contained 2 ml nitric acid, 2 ml phosphoric acid, 3 ml Triton X-100 and 5 g magnesium nitrate in 1 l of water. Diluted modification solution was a mixture of 100 ml of the modification solution and 100 ml water.

Animals and dosing

Male albino Wistar–Hahn rats (6–8 weeks) weighing 235–268 g were kept under a 12-h light/dark cycle with free access to water and the pelletized standard diet for 13 days prior to experiments for acclimatization. All animal protocols were approved by the Institute's Animal Experimentation Ethics Committée, and animals were treated according to OECD guidelines. In the morning after overnight fast, the platinum compounds under study were administered by a gastric gavage in a volume of 1 ml/kg of body weight as suspensions in a 0.6% solution of methylcellulose in water. Rats were given four doses of LA-12 from the range of 37.5–300 mg LA-12/kg body weight or four equimolar doses of satraplatin.

Sampling for pharmacokinetics

Rats were randomly assigned to eight dosing groups of 18 rats each and, furthermore, to two of 12 blood sampling intervals (pre-dose and at 15 min, 0.5, 1, 1.5, 2, 3, 4, 8, 24, 48 and 72 h). Moreover, rats from eight additional groups of four animals each were kept at metabolic cages, and

urine was collected until 72 h. Two blood samples of 2 ml each were taken from retroorbital plexus under ether anaesthesia. Blood was collected into polypropylene test tubes containing 0.75% K_3EDTA (20 µl/ml of blood), K_3EDTA and cooled in a water bath (8°C) for 5 min. The samples were then centrifuged in a cooled centrifuge (8°C) at 3,000*g* for 10 min. The first aliquot (0.1 ml) of the supernatant was immediately frozen at −18°C. The remaining volume was transferred into an ultrafiltrate filter (Ultrafree-CL, 30 kD, Millipore, Prague, Czech Republic) and centrifuged in a cooled centrifuge (8°C) at 2,000*g* for 30 min. Ultrafiltrate was pipetted into a polypropylene test tube and immediately frozen at −18°C. After the second blood sampling interval, rats were killed by withdrawing whole blood from the abdominal artery. Liver and kidney were rinsed with 0.9% NaCl, blotted on filter paper, weighed, and immediately frozen at −18°C.

Platinum analysis

Platinum concentration was determined by a validated method based on electrothermal atomic absorption analysis with Zeeman background correction. An AAnalyst 800 spectrometer (Perkin-Elmer, Norwalk, CT, USA) with longitudinal AC Zeeman-effect background correction with transversely heated graphite tube (THGA^{TM}) and autosampler AS 800 was used. The tubes were coated with pyrolytic graphite and equipped with an integrated L'vov platform. The graphite furnace temperature programme for platinum was as follows: 110°C for 30 s, 130°C for 30 s, 1,300°C for 20 s, 2,200°C for 6 s (peak area absorbance reading), and 2,450°C for 3 s.

Samples of biological fluids (plasma, ultrafiltered plasma, urine) were diluted with 0.15% modification solution prior analysis. Solid samples (kidney, liver) were homogenized and weighted into PTFE vessel. Then, nitric acid (6 ml), hydrochloric acid (2 ml), hydrogen peroxide (1 ml) and hydrofluoric acid were added. Vessels were fixed into high pressure rotor bodies and digested by temperature programme: 10-min increasing up to 200°C, 20-min holding at 200°C. After cooling down, solutions were transferred into volumetric flasks and diluted with water.

The linearity of the calibration lines for biological fluids was confirmed ($r^2 > 0.999$) over the range of 0.01–0.50 mg/l. Based on the assayed concentrations of spiked quality control samples (0.06, 1.0, 5.0 mg/l diluted to linear calibration range of 0.02, 0.20, 0.20 mg/l), the inter-day accuracy and precision were adequate. The relative errors were between −2.4 and 16.7%, and the coefficients of variation were less than 6.4%. No effect was observed of the storage of plasma, ultrafiltrate, and urine at −18°C for up to 3 months and of four freeze/thaw cycles on the accurate determination of platinum (relative differences less than ±15%). Similarly, storage of biological fluids for 3 h at room temperature resulted in acceptable differences from −0.37 to −6.1%. Calibration standards of platinum tissue concentrations were prepared using standard addition method. The assay was linear in the range of 0.02–0.50 mg/l. Accuracy of liver platinum determination at three concentrations (0.05, 0.075, and 0.10 mg/l) was characterized by the relative errors from −10.4 to −2.2%. Repeated analyses of liver samples spiked with 0.05 mg/l platinum resulted in the coefficient of variation less than 2%.

Data analysis

Pharmacokinetic modelling was performed using the Kinetica software, version 4.0 (InnaPhase Corporation, Thermo Fisher Scientific Inc. Waltham, MA, USA). In the first step, population values for parameters of compartmental models were estimated using plasma platinum (ultrafiltrate platinum) concentrations of all animals. In the second step, individual estimates of pharmacokinetic parameters were obtained according to the maximum a posteriori Bayesian fitting method. Non-compartmental pharmacokinetic analysis (NCA) was also performed. For each compound and dose level, data from 18 animals were combined into a concentration–time profile using naive data pooling (three animals per sampling interval). Maximum concentration in plasma (C_{max}) and time to maximum concentration (T_{max}) were determined directly from the observed data. The area under the plasma (ultrafiltrate) platinum concentration–time curve from zero up to the last concentration above the quantification limit (AUC_{0-t}) was calculated by the linear trapezoidal method. The area under the plasma (ultrafiltrate) platinum concentration–time curve from zero up to infinity ($AUC_{0-\infty}$) was determined as the sum of the AUC_{0-t} and of the extrapolated part AUC_{extra}, i.e. the ratio of the concentration predicted at the time interval t and the terminal rate constant λ_z. The terminal rate constant was estimated by linear regression analysis of ln C vs. time data using the last three concentrations above the lower limit of quantification. Oral clearance (CL/F) was calculated by dividing the dose of platinum with the $AUC_{0-\infty}$. Apparent volume of distribution at equilibrium after oral administration (V_{ss}/F) was calculated using the formula: $V_{ss}/F = (\text{dose}\cdot\text{AUC})/\text{AUMC}$, where AUMC is the area under the first moment of the concentration–time curve from zero up to infinity. Apparent volume of distribution during terminal phase after oral administration (V_Z/F) was obtained as follows: $V_Z/F = \text{dose}/(AUC_{0-\infty}\cdot\lambda_z)$.

Statistical analysis was performed using GraphPad Prism, version 5.0 (GraphPad Software, San Diego, California). Platinum concentrations and all pharmacokinetic

characteristics were log transformed before testing. Dose-related changes in pharmacokinetic characteristics and their comparison between LA-12 and satraplatin were evaluated using two-way analysis of variance with compound, dose and compound × dose as fixed effects. Tissue concentrations after corresponding doses were compared between compounds using analysis of variance for repeated measures. When a significant effect was found, the Tukey test was used to compare the means. For all statistical procedures, P values < 0.05 were taken as significant.

Results

The pharmacokinetics of plasma platinum and ultrafiltrate platinum

The mean concentration–time profiles of plasma platinum and ultrafiltrate platinum are shown in Fig. 2. Platinum was detected in all plasma samples 15 min after oral administration of both compounds. The concentration–time curves were flat and peaked between 1.5 and 4 h for both compounds after all doses with the exception of 75 mg/kg satraplatin (8 h). After 72 h, plasma platinum decreased to a value in the range of 10–20% of the maximum concentration. Maximum concentrations of ultrafiltrate platinum were observed between 1 and 3 h. Ultrafilterable platinum decreased more rapidly than plasma platinum. Within 8 h, its concentration dropped below 10% of the maximum concentration, or it was less than the lower limit of quantification (0.01 mg/l) (Fig. 2).

Based on the statistical evaluation and graphical inspection of goodness-of-fit plots, plasma platinum concentrations were best described with an open two-compartment model with first-order absorption and elimination from the central compartment. Individual predicted concentrations of both compounds agreed well with assayed values (data not shown). Pharmacokinetic parameters of plasma platinum obtained by population modelling and post hoc Bayesian estimation are summarized in Table 1. Ultrafiltrate platinum concentrations were well described with an open one-compartment model with first-order absorption and elimination from the central compartment (data not shown). The pharmacokinetic characteristics are listed in Table 2. Pharmacokinetic parameters estimated using non-compartmental analysis of the mean concentration–time profiles of plasma and ultrafiltrate platinum are given in Table 3.

After administration of both compounds, concentrations of plasma platinum and derived characteristics of systemic platinum exposure (C_{max} and AUC) increased less than in proportion to the eightfold increase in the dose (Tables 1, 3). Similar for LA-12 and satraplatin, the pharmacokinetic parameters CL/F and V_d raised with the dose ($P < 0.001$, one-way ANOVA). In the whole range of doses, the AUC of plasma platinum was higher after satraplatin

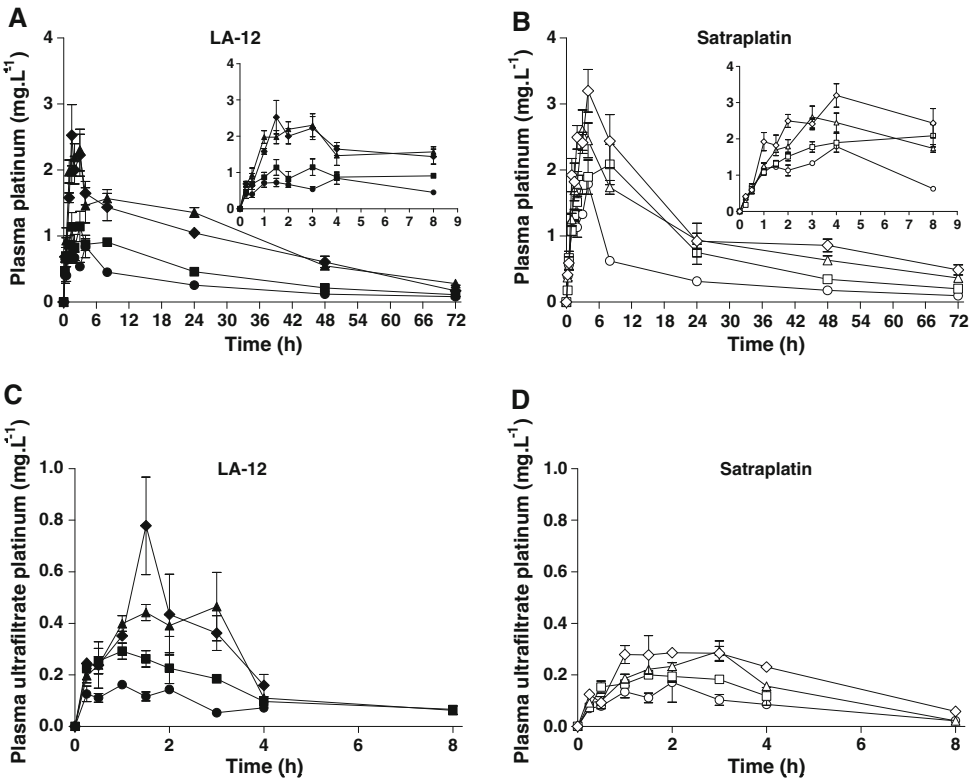

Fig. 2 Mean (SEM) concentrations of plasma platinum (**a**, **b**) and plasma ultrafiltrate platinum (**c**, **d**) after a single-dose oral administration of LA-12 or satraplatin to Wistar rats ($N = 3$ for each sampling interval). Platinum drugs were administered at four doses equimolar to 37.5 (*circles*), 75 (*squares*), 150 (*triangles*) and 300 mg/kg LA-12 (*diamonds*)

Table 1 Pharmacokinetic parameters of plasma platinum obtained by population modelling and post hoc Bayesian estimation following single oral doses of LA-12 and satraplatin

Compound	Dose (mg/kg)	k_a (1/h)	k_e (1/h)	$t_{1/2}$ (h)	CL/*F* (ml/min/kg)	AUC (mg h/l)	k_{12} (1/h)	k_{21} (1/h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	*V*/*F* (l/kg)
LA-12	37.5	0.58 ± 0.05	0.12 ± 0.008	5.6 ± 0.37	12.1 ± 2.33	19.0 ± 4.2	0.48 ± 0.03	0.092 ± 0.003	1.0 ± 0.09	41 ± 1.7	5.8 ± 1.1
	75	0.56 ± 0.03	0.12 ± 0.01	5.6 ± 0.45	16.2 ± 4.82	29.4 ± 8.6	0.49 ± 0.04	0.096 ± 0.004	1.0 ± 0.08	40 ± 1.5	7.8 ± 2.2
	150	0.50 ± 0.06	0.095 ± 0.009	7.3 ± 0.69	12.5 ± 2.77	74.1 ± 17.3	0.52 ± 0.03	0.13 ± 0.01	0.99 ± 0.06	39 ± 3.0	8.6 ± 2.6
	300	0.72 ± 0.12	0.048 ± 0.008	14 ± 2.4	21.4 ± 3.70	84.8 ± 16.5	0.27 ± 0.05	0.17 ± 0.008	1.5 ± 0.28	45 ± 2.2	27 ± 0.70
P value		0.30	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Satraplatin	37.5	0.76 ± 0.03	0.065 ± 0.003	11 ± 0.33	6.63 ± 0.60	33.4 ± 3.1	0.15 ± 0.04	0.10 ± 0.007	2.1 ± 0.51	35.8 ± 3.8	6.2 ± 0.49
	75	0.40 ± 0.02	0.070 ± 0.004	9.9 ± 0.57	8.16 ± 0.79	54.5 ± 6.3	0.17 ± 0.02	0.16 ± 0.008	1.9 ± 0.22	22.9 ± 1.2	7.0 ± 0.39
	150	0.32 ± 0.05	0.064 ± 0.003	11 ± 0.34	9.36 ± 0.65	92.8 ± 6.4	0.26 ± 0.03	0.086 ± 0.003	1.7 ± 0.20	50.2 ± 1.8	8.7 ± 0.63
	300	0.17 ± 0.03	0.078 ± 0.004	8.9 ± 0.34	12.0 ± 0.71	147 ± 8.9	0.26 ± 0.02	0.052 ± 0.003	1.9 ± 0.31	120 ± 6.5	9.2 ± 0.53
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0076	<0.0001	<0.0001

Data are mean ± SD of 18 animals

P values refer to the effect of the dose

Table 2 Pharmacokinetic parameters of plasma ultrafiltrate platinum obtained by population modelling and post hoc Bayesian estimation following single oral doses of LA-12 and satraplatin

Compound	Dose (mg/kg)	k_a (1/h)	C_{max} (mg/l)	T_{max} (h)	AUC (mg h/l)	k_e (1/h)	$t_{1/2}$ (h)	CL/*F* (ml/min/kg)	CL_R[a] (ml/min/kg)	*V*/*F* (l/kg)
LA-12	37.5	1.10[b]	0.175 ± 0.061	1.2 ± 0.03	0.565 ± 0.187	0.67 ± 0.05	1.0 ± 0.06	423 ± 116	14.0 ± 5.2	38.1 ± 10.0
	75	1.2 ± 0.31	0.325 ± 0.063	1.1 ± 0.23	1.05 ± 0.200	0.65 ± 0.12	1.1 ± 0.16	440 ± 103	14.7 ± 2.8	40.5 ± 3.70
	150	0.79 ± 0.18	0.433 ± 0.080	1.4 ± 0.23	1.60 ± 0.366	0.69 ± 0.14	1.1 ± 0.23	576 ± 118	11.6 ± 4.2	52.1 ± 1.63
	300	0.54 ± 0.19	0.545 ± 0.195	1.5 ± 0.25	2.22 ± 0.454	0.86 ± 0.08	0.81 ± 0.11	821 ± 140	17.6 ± 5.9	56.8 ± 6.89
P value		–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.48	<0.0001
Satraplatin	37.5	1.10[b]	0.146 ± 0.023	1.5 ± 0.08	0.736 ± 0.209	0.34 ± 0.05	2.0 ± 0.28	315 ± 59.6	12.4 ± 3.8	53.6 ± 5.40
	75	0.68 ± 0.10	0.213 ± 0.030	1.7 ± 0.20	0.982 ± 0.158	0.53 ± 0.07	1.3 ± 0.13	460 ± 74.4	14.9 ± 1.7	51.7 ± 1.68
	150	0.43 ± 0.05	0.240 ± 0.040	1.9 ± 0.27	1.26 ± 0.357	0.67 ± 0.12	1.1 ± 0.27	710 ± 140	15.8 ± 5.3	65.5 ± 4.29
	300	0.31 ± 0.07	0.288 ± 0.056	1.9 ± 0.20	1.69 ± 0.109	0.81 ± 0.03	0.85 ± 0.10	1,040 ± 60.8	15.8 ± 7.3	77.4 ± 3.31
P value		–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.73	<0.0001

Data are mean ± SD of 18 animals

P values refer to the effect of the dose

[a] CL_R was evaluated in four animals in each group

[b] The interindividual variability was impossible to estimate

Table 3 Non-compartmental pharmacokinetic analysis of the mean concentration versus time profiles of plasma platinum and ultrafiltrate platinum following single oral doses of LA-12 and satraplatin

Compound	Sample	Dose (mg/kg)	C_{max} (mg/l)	T_{max} (h)	AUC_{0-t}[a] (mg h/l)	AUC (mg h/l)	λ_z (1/h)	$t_{1/2}$ (h)	CL/F (ml/min/kg)	V_z/F (l/kg)	V_{ss}/F (l/kg)
LA-12	Plasma	37.5	0.844	4.0	17.4	20.2	0.027	25.8	10.9	24.4	22.8
		75	1.15	3.0	29.3	33.2	0.029	24.1	13.3	27.6	26.3
		150	2.30	3.0	67.7	75.9	0.033	21.1	11.6	21.2	23.5
		300	2.53	1.5	60.7	67.1	0.032	21.4	26.3	48.9	51.4
Satraplatin	Plasma	37.5	1.79	4.0	25.3	29.4	0.024	28.4	7.52	18.4	15.3
		75	2.08	8.0	53.3	60.5	0.027	25.5	7.28	16.0	14.2
		150	2.60	3.0	66.4	85.5	0.020	35.2	10.3	31.4	29.4
		300	3.20	4.0	81.5	99.3	0.025	27.7	17.8	43.8	44.7
LA-12	Ultrafiltrate	37.5	0.162	1.0	0.427	0.625	0.30	2.3	352	70.1	73.4
		75	0.292	1.0	1.15	1.41	0.22	3.1	313	84.7	87.8
		150	0.465	3.0	1.72	1.91	0.30	2.3	462	91.4	99.2
		300	0.778	1.5	1.48	1.78	0.57	1.2	995	104	154
Satraplatin	Ultrafiltrate	37.5	0.171	2.0	0.658	0.720	0.33	2.1	303	55.0	67.2
		75	0.200	1.5	0.644	1.27	0.20	3.4	347	102	113
		150	0.292	3.0	1.16	1.21	0.51	1.4	730	85.9	139
		300	0.286	2.0	1.53	1.71	0.33	2.1	1030	191	249

[a] The last sampling interval was 72 h, ultrafiltrate platinum was below the quantification in all samples collected at 10 h

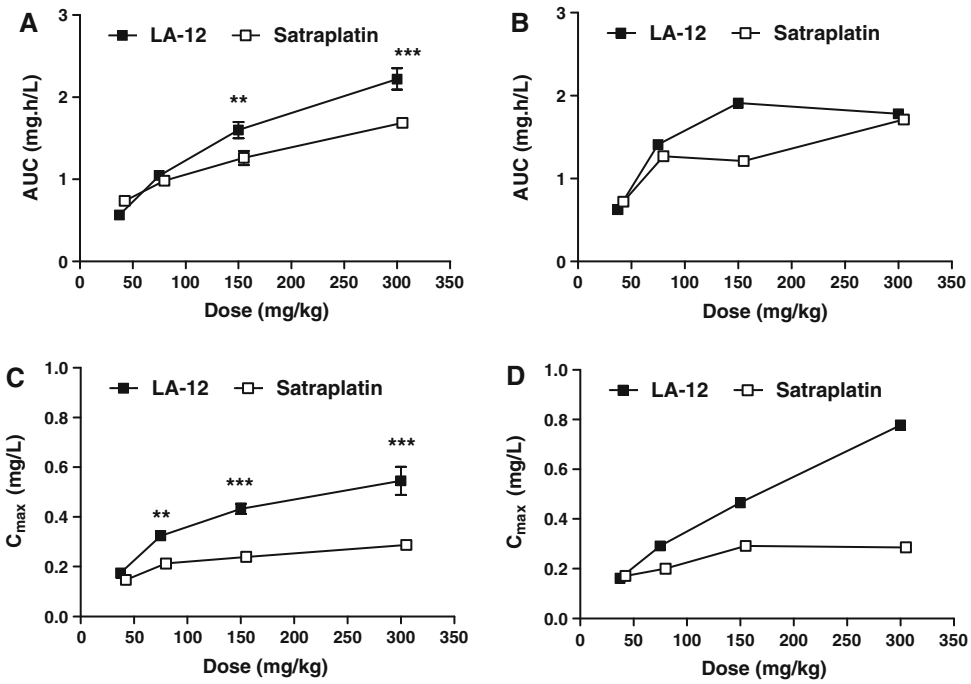

Fig. 3 Mean (SD) values of the area under the concentration–time curve (AUC) and of the maximum concentration (C_{max}) of plasma ultrafiltrate platinum after a single-dose oral administration of LA-12 or satraplatin. The AUC and C_{max} values estimated by population modelling (**a**, **c**) and using the non-compartmental pharmacokinetic analysis of the mean concentration versus time profiles (**b**, **d**). $^{**}P < 0.01$, $^{***}P < 0.001$ in comparison with satraplatin

($P < 0.001$). The values of ultrafiltrate platinum CL/F and V_d showed a similar dose dependency as those of plasma platinum (Tables 2, 3). However, administration of LA-12 when compared to satraplatin resulted in significantly higher AUC values of ultrafiltrate platinum after the doses of 150 mg and 300 mg/kg, respectively. The C_{max} values were significantly higher starting from the dose of 75 mg/kg LA-12 and upward (Fig. 3).

Urinary excretion of platinum

Excretion of platinum in urine is shown in Fig. 4. After all doses, highest amounts of platinum were found in the first of the three 24-h intervals of collection. The mean 72-h urinary recovery of platinum in urine was less than 5% regardless of the dose and compound. After administration of satraplatin, the 72-h urinary recovery of platinum

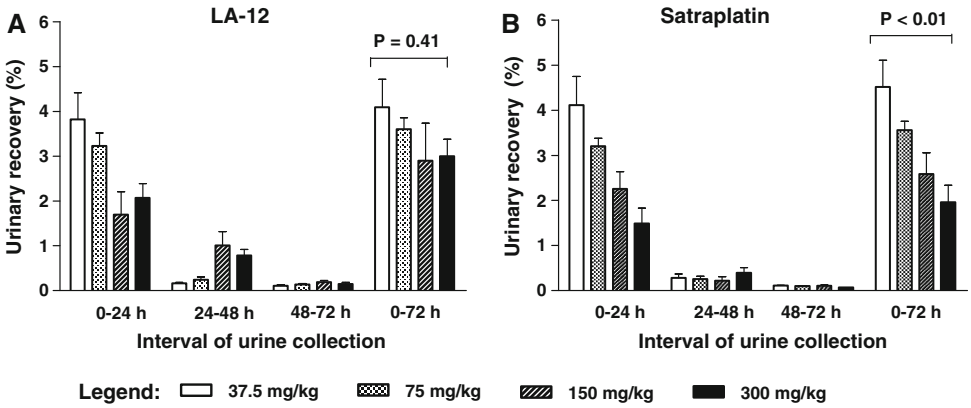

Fig. 4 Mean (SD) urinary recovery of the platinum dose (%) after a single-dose oral administration of **a** LA-12 or **b** satraplatin to Wistar rats ($N = 4$). Platinum drugs were administered at four doses equimolar to 37.5, 75, 150 and 300 mg/kg LA-12. *P* values show the effect of the dose

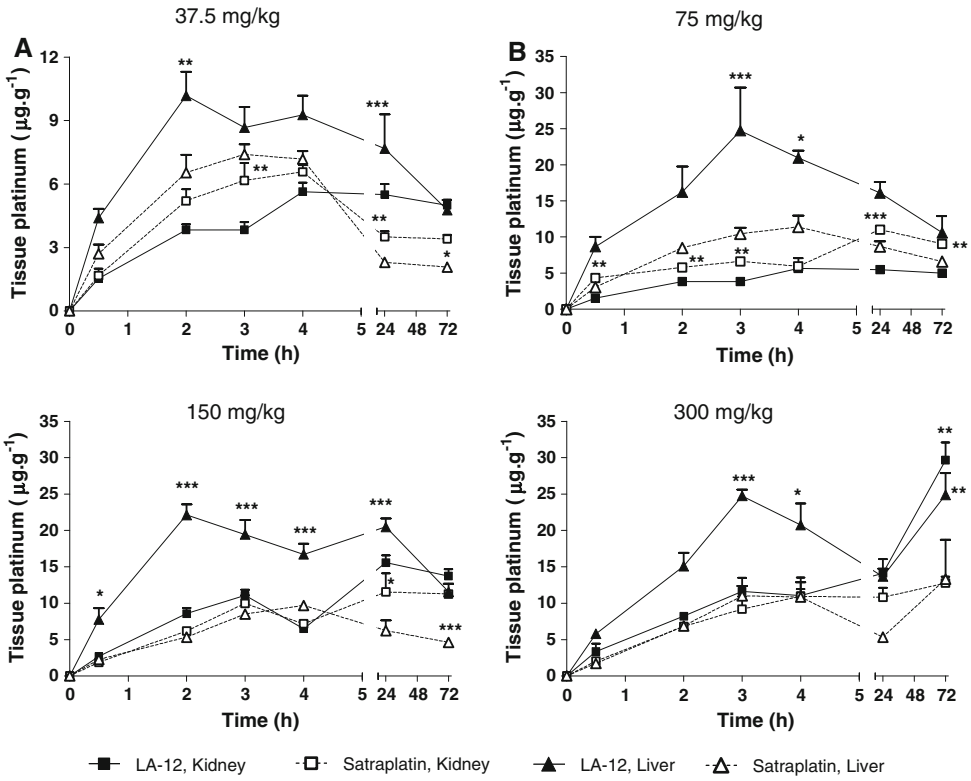

Fig. 5 Mean (SEM) platinum concentration versus time curves in the kidney and liver tissue homogenates after a single-dose oral administration of LA-12 or satraplatin to Wistar rats ($N = 3$ for each sampling interval). Platinum drugs were administered at four doses equimolar to **a** 37.5, **b** 75, **c** 150 and **d** 300 mg/kg LA-12. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ in comparison with satraplatin

decreased with the dose ($P < 0.01$). For LA-12, a numerical decrease was observed that did not reach statistical significance ($P = 0.41$) (Fig. 4). Renal clearance of platinum showed no dose dependency and was comparable ($P > 0.05$) for both derivatives (Table 2).

Platinum in the kidney and liver

After the doses less than 150 mg/kg, platinum in the kidney and tissue homogenates peaked between 2 and 4 h and then decreased slowly. After higher doses, tissue distribution of platinum was delayed and secondary peaks appeared on the concentration–time curves at 24 or 72 h (Fig. 5). Kidney tissue concentrations of platinum and derived characteristics AUC_{0-72h} and C_{max} (Fig. 6) were comparable between LA-12 and satraplatin after doses of 37.5–150 mg/kg, but the highest dose of LA-12 resulted in markedly higher concentrations. Platinum concentrations and AUC_{0-72h} and C_{max} values in the liver homogenate were approximately twofold higher after LA-12 across all four doses. In both organs, the characteristics of platinum exposure increased in proportion to dose only after the doses of 37.5 and 75 mg/kg, respectively. With further dose escalation, they augmented less (Fig. 6).

Discussion

This first direct comparison of LA-12 to satraplatin shows that in the range of doses under study (37.5–300 mg/kg),

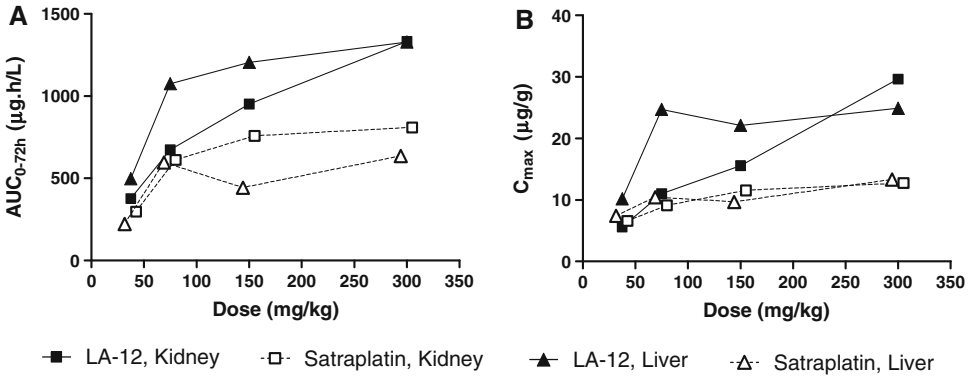

Fig. 6 The relationships between the dose and **a** the area under the platinum concentration versus time curve in the interval 0–72 h ($AUC_{0-72\ h}$) and **b** the maximum concentration of platinum in the kidney and liver homogenates. Pharmacokinetic characteristics were obtained by non-compartmental analysis

the pharmacokinetics of plasma and ultrafiltrate platinum, urinary excretion and tissue distribution of platinum are similar after a single oral administration of both compounds to Wistar rats. For both platinum derivatives, the characteristics of platinum exposure (C_{max} and AUC) evaluated in the plasma, plasma ultrafiltrate and tissues increased less than in proportion to dose. Urinary recovery of platinum expressed as a fraction of the dose decreased with increasing dose of satraplatin. For LA-12, it displayed a decreasing trend, but the differences did not reach statistical significance. Moreover, the pharmacokinetic characteristics *V*/*F* and CL/*F* of plasma and ultrafiltrate platinum displayed similar dose-dependent increases. These findings are consistent with a dose-related decrease in the extent of oral bioavailability (*F*) of LA-12 and satraplatin most likely due to saturable intestinal absorption. Nonlinear pharmacokinetics of plasma and ultrafiltrate platinum has been previously reported in mice for oral satraplatin after doses ranging from 9.5 to 200 mg/kg and in a phase I study after doses from 60 to 700 mg/m^2, respectively [9, 10].

LA-12 molecule was designed with the purpose of its enhanced penetration and higher accumulation in cancer cells due to increased lipophilicity caused by incorporation of bulky hydrophobic ligand—adamantylamine. This presumption was confirmed by the higher cytotoxicity and increased platinum levels in cancer cells in comparison with other platinum based anti-tumour drugs [5, 11, 12]. The range of doses investigated is therapeutically relevant as shown in the study directly comparing anti-tumour activities of LA-12 and satraplatin in vivo in mice using the human carcinoma xenografts of colon HCT116, prostate PC3, and ovarian A2780 and A2780/cisR (resistant to cisplatin). The daily ×5 repeated dose regimen of equimolar doses of LA-12 and satraplatin, administered in two cycles, was selected for this evaluation. The highest effect was reached with LA-12 at a dose of 60 mg/kg. LA-12 was able to overcome resistance to cisplatin of the ovarian carcinoma A2780 subline. The activities of LA-12 in all doses and all used tumour xenografts were higher than equimolar doses of satraplatin [6]. In another study, 135 mg/kg satraplatin p.o. (q7dx4 schedule) exhibited comparable activity to that observed for i.v. cisplatin and carboplatin across four human ovarian carcinoma mice xenografts of widely differing sensitivity to cisplatin and carboplatin [13].

Platinum anti-cancer drugs rapidly form a variety of reactive intermediates, which bind irreversibly to constituents of blood and plasma as well as in tissues. Investigating the pharmacokinetics of the parent compound and its metabolites is, therefore, technically difficult due to problems concerning sample handling and sensitivity and specificity of analytical methods. Efficient hyphenated bioanalytical techniques such as high-performance liquid chromatography combined with inductively coupled plasma mass spectrometry (HPLC–ICPMS) and HPLC–tandem mass spectrometry (HPLC–MS/MS) are increasingly used in speciation of platinum for assessing stability, biotransformation, and protein and red blood cell binding of platinum drugs both in vitro and in vivo [14, 15]. The analysis of samples from in vivo and in vitro studies with satraplatin with the help of high-performance liquid chromatography coupled with mass spectrometry (HPLC–MS) and HPLC-off-line AAS demonstrated that satraplatin underwent rapid and extensive biotransformations by reduction to the platinum(II) compound (JM118), and by hydrolysis to other metabolites (JM383, JM518, JM559). Using these techniques, very low (in mice) or undetectable (in patients) concentrations of the parent drug were described in the plasma ultrafiltrate after oral administration of satraplatin [16, 17]. According to results of HPLC–ICPMS analyses, satraplatin concentrations decrease rapidly with the initial half-life of 6.3 min during in vitro incubation at 37°C in human blood. Satraplatin-derived Pt becomes rapidly and irreversibly bound to the red blood cell membrane [18]. After oral administration of satraplatin, these processes most likely proceed to a significant extent before blood sampling. The parent drug is reduced by haemoglobin and possibly other haem proteins and its reduction products attack proteins and low-molecular-

weight compounds [19]. The stability of satraplatin and JM118 is significantly higher during incubation at 37°C in human plasma. The compounds disappear from plasma with half-lives of 2.5 and 1.1 h, and the rate of irreversible protein binding of platinum is characterized with the half-life of 5.2 and 1.1 h, respectively [20].

In this study, measurements of platinum in biological fluids and tissues were performed using flameless AAS. This strategy is generally accepted for the investigation of body exposure to platinum after administration of platinum complexes [1], and the same approach was used in several in vivo studies with satraplatin [9, 10, 21–23]. The concentration of plasma platinum is a sum of ultrafilterable platinum and platinum irreversibly bound to plasma proteins and other macromolecules. Plasma ultrafiltrate platinum comprises the parent drug, its biotransformation products and platinum bound to small molecules such as glutathione, methionine and cysteine. The pharmacokinetics of plasma ultrafiltrate platinum was approximately linear within the range of 37.5–150 mg/kg of LA-12, while no such relationship was observed after satraplatin. Higher doses of LA-12 (>75 mg/kg) resulted in a higher bioavailability of plasma ultrafiltrate platinum compared to satraplatin. Since it is generally accepted that plasma ultrafiltrate platinum is a better predictor of efficacy and/or toxicity, it potentially implies that higher and more predictable effects may be attainable after LA-12 compared to satraplatin. Hanada and coworkers have shown that cisplatin and its ultrafilterable metabolites (i.e. "mobile platinum") distribute rapidly into tissues and organs after i.v. administration to rats and are transformed to platinum bound to macromolecules (fixed platinum) which persist in tissues over long time [24]. In agreement with this view, the concentrations of platinum in the kidney and liver tissue increased less than proportionally to dose after the doses exceeding 75 mg/kg. Concentrations of platinum in the kidney homogenate were approximately twofold higher after 150 and 300 mg LA-12 compared to equimolar doses of satraplatin. The same difference was observed in the liver after all four doses. The characteristics of tissue exposure to platinum (C_{max} and $AUC_{0–72h}$) document extensive platinum distribution into the kidneys and liver after administration of both compounds. The pattern of tissue distribution of platinum after oral administration of LA-12 to Wistar rats has been described previously. The relative organ concentrations at 24-h post-dosing were liver > kidneys ≫ spleen > lungs > heart = skin > muscle. Platinum was undetectable in the brain [25].

The limitation of this study is that direct comparison of LA-12 and satraplatin with respect to the in vitro kinetics of platinum plasma protein binding and RBC uptake was not performed. Given the instability of satraplatin, LA-12 and their metabolites, the differences observed between the concentrations of plasma and plasma ultrafiltrate platinum after administration of both compounds could from a part be ascribed to changes occurring in vitro during sample processing and storage. To limit this possibility, the experiments with LA-12 and satraplatin ran in parallel, blood samples were immediately cooled in ice-water bath and processed at 8°C within a fixed time frame of 45 min: plasma was separated within 15 min and ultrafiltrate was collected within additional 30 min. In vitro investigations of platinum distribution in the blood for cisplatin and oxaliplatin found no significant changes in plasma and ultrafiltrate platinum after cooling and processing samples within an hour [26, 27]. Moreover, data on platinum urinary excretion obtained for both derivatives argue against the existence of artificial differences in concentrations of plasma ultrafiltrate platinum generated after sampling (i.e. ex vivo). The renal clearance of free platinum was similar regardless of the dose and compound administered. This indicates that the amount of platinum excreted in urine was directly proportional to the AUC of plasma ultrafiltrate platinum.

In conclusion, this study shows that platinum concentrations and characteristics C_{max} and AUC evaluated in the plasma, plasma ultrafiltrate and kidney and liver tissues increase less than in proportion to dose following a single-dose administration of 37.5–300 mg/kg of LA-12 and satraplatin to Wistar rats. The dose-related increases in the pharmacokinetic characteristics V/F and CL/F of platinum and ultrafiltrate platinum as well as a drop in platinum urinary recovery are consistent with a dose-related decrease in the extent of oral bioavailability of both compounds most likely due to saturable intestinal absorption. Regarding the extent of platinum exposure, plasma platinum concentrations were higher after satraplatin, while higher doses of LA-12 (150 and 300 mg/kg) resulted in higher plasma ultrafiltrate, and kidney platinum concentrations and approximately twofold higher concentrations of liver platinum were found after LA-12 across the whole range of doses.

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