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Pharmacokinetics and tissue distribution of platinum in rats following single and multiple oral doses of LA-12 [(*OC*-6-43)-bis(acetato)(1-adamantylamine)amminedichloroplatinum(IV)]

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Abstract

The pharmacokinetics of total and free plasma platinum (Pt) and Pt tissue distribution were investigated in rats after oral administration of (*OC*-6-43)-bis(acetato)(1-adamantylamine)amminedichloroplatinum(IV) (LA-12). Plasma and ultrafiltrate were sampled until 48 h and tissue samples were taken at 24 and 48 h after single doses of 38.6 or 540 mg LA-12/kg, and after once-a-day dosing of 4.3 or 38.6 mg kg^{-1} LA-12 over 14 consecutive days. Total plasma Pt concentrations increased less than proportionally to the 14-fold increase in the single dose. The mean C_{max} values of 1.5 and 6.3 mg L^{-1} were observed at 0.5 and 1 h, respectively, and the mean AUC values achieved were 29 and 144 mg h L^{-1}. The highest tissue Pt concentrations were found in the liver and kidneys. Platinum was undetectable in the brain while in other tissues (muscle, skin, heart, lungs), the concentrations were lower (after single dose) or similar (after multiple doses) when compared to the plasma C_{max} values. Plasma Pt concentrations after once-a-day dosing of 38.6 mg kg^{-1} were two- to three-fold less than that after a single dose while Pt concentrations in various tissues rose two- to four-fold. Accumulation of Pt was even higher in the kidneys (seven-fold) and spleen (nine-fold). After once-a-day dosing, tissue Pt levels increased proportionally with the dose within the range from 4.3 to 38.6 mg kg^{-1}. At the same time, the increase in total plasma Pt concentrations was 40% less than proportional. Concentrations of Pt in the plasma ultrafiltrate decreased rapidly with the initial half-life of 1 h.

Keywords: Platinum cytostatics; Pharmacokinetics; Tissue distribution; Rat

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1. Introduction

Platinum compounds are very effective anticancer drugs used against solid tumours, such as testicular, ovarian, lung, head and neck cancers (Lebwohl and Canetta, 1998). Research has focused on the development of new platinum drugs for oral administration, which could further increase the therapeutic use of platinum anticancer drugs due to flexible and convenient dosage. Satraplatin [(*OC*-6-43)-bis-(acetato)amminedichloro(cyclohexylamine)platinum (IV)] (JM216) is the first orally administered platinum complex with documented efficacy and acceptable safety in patients with hormone-refractory prostate cancer (Kelland, 2000) and small-cell lung cancer (Fokkema et al., 1999). LA-12 (Fig. 1), [(*OC*-6-43)-bis(acetato)(1-adamantylamine)amminedichloroplatinum(IV)], is a lipophilic platinum(IV) complex structurally similar to satraplatin, which contains 1-adamantylamine instead of cyclohexylamine non-leaving ligand. LA-12 has shown higher cytotoxicity in vitro than that of cisplatin, and no cross-resistance (Zak et al., 2004). The drug has entered the Phase I of clinical trials.

The aim of the present study was to describe total and ultrafiltered platinum pharmacokinetics in plasma and platinum tissue distribution in rats after both single and multiple oral doses of LA-12. Platinum cytostatics rapidly form a variety of reactive intermediates, which bind irreversibly to various constituents of blood and plasma. Investigating the pharmacokinetics of the intact parent compound and its metabolites is, therefore, technically difficult due to problems concerning sensitivity and specificity of analytical methods. Monitoring of total and free platinum plasma pharmacokinetics and platinum tissue concentrations using atomic absorption spectrometry as performed in the present study is a suitable strategy generally accepted for the investigation of platinum complexes (Graham et al., 2000; Lebwohl and Canetta, 1998; Liu et al., 2002).

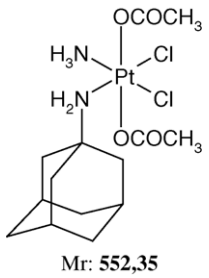

Fig. 1. Chemical structure of (*OC*-6-43)-bis(acetato)(1-adamantylamine)amminedichloroplatinum (IV) (LA-12).

2. Materials and methods

2.1. Chemicals and solutions

LA-12 was synthesized in PLIVA-Lachema a.s. by a method described previously (Zak et al., 2004). Nitric acid and phosphoric acid were Suprapur grade from Merck (Darmstadt, Germany). Magnesium nitrate was obtained from Lachema (Brno, Czech Republic). Triton X-100 was purchased from Sigma–Aldrich (Prague, Czech Republic). Double deionized water of 18 M cm^{-1} specific resistivity, obtained in a Milli-Q Plus Millipore system, was used to prepare all the solutions. A 10 mg Pt mL^{-1} primary standard solution was Titrisol Merck. A stock standard solution of 0.1 mg Pt mL^{-1} 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.

2.2. Animals

Male albino Wistar–Hahn rats (6–8 weeks) weighing 211–262 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.

2.3. Pharmacokinetic and tissue distribution studies

Pharmacokinetics in plasma was investigated by non-compartmental methods using naive pooling of data. Rats were randomly assigned to four dosing groups of 20 rats each and, furthermore, to two of eight post-dose sampling intervals ($N = 16$ rats in each dosing group) and one pre-dose interval ($N = 4$ control

rats). Tissue samples were taken from four control rats and from all rats in each of the dosing groups at 24 h ($N = 8$ rats) and 48 h ($N = 8$ rats) after the single and last-multiple administration, respectively. Samples of approximately 1 g wet weight from the liver, kidneys, spleen, lungs, heart, skin, skeletal muscle and brain were rinsed with 0.9% NaCl, weighed, and immediately frozen at −18 °C.

Rats were assigned to two single doses of either 38.6 or 540 mg kg^{-1} BW LA-12. Multiple dose study was performed with once-a-day dosing of either 4.3 or 38.6 mg kg^{-1} BW LA-12 over 14 consecutive days. After fasting overnight, rats were administered orally by gastric gavage, a freshly prepared suspension of LA-12 in 0.6% methylcellulose. Blood samples were obtained from the retroorbital plexus under slight ether anesthesia pre-dose and at 0.5, 1, 1.5, 2, 4, 8, 24 and 48 h and collected into polypropylene test tubes containing 40 μL of 0.75% disodium edetate. Two blood samples of 2 mL were taken from each animal. The samples were centrifuged at 3000 × *g* for 15 min and 0.1 mL of the supernatant were immediately frozen at −18 °C. The remaining volume was transferred into an ultrafiltrate filter (Ultrafree-CL, 30 kD cutt-off) and centrifuged at 2000 × *g* for 20 min. Ultrafiltrate was pipetted into a polypropylene test tube and immediately frozen at −18 °C.

2.4. Platinum analysis

Samples were analysed using an atomic absorption spectrometry. The system consisted of model 3030 atomic absorption spectrometer, with electrothermal atomization in an atomizer HGA-500, and a sampler AS-40 (Perkin-Elmer, Norwalk, CT, USA). Pyrolytically coated graphite tubes were used throughout. The absorbance of platinum was measured at 265.9 nm. Experiments were run with 20 and 40 μL samples and measurements were made based on peak height absorbance. The graphite furnace temperature programme for platinum was as follows: 100 °C for 30 s, increase to 110 °C in 30 s, 1600 °C increase in 20 s and hold for 10 s, 2700 °C for 4 s (peak height absorbance reading), and 2700 °C for 3 s.

2.4.1. Sample preparation procedures

Plasma (50 μL or less) samples were diluted with modification solution in four dilutions ratios (two-, four-, 10- and 25-fold). Each sample was analysed with a blank sample obtained from an untreated animal and three calibration solutions in the range of 0.05–0.5 μg Pt mL^{-1} of blank plasma.

Whole tissue samples (0.5–1.5 g) were acid-digested for 2 h at 148 °C in tightly closed 17 mL glass vials after addition of 0.4 mL 65% nitric acid and 0.4 mL water. The digest was quantitatively transferred into a 5 mL volumetric flask and volume made up with diluted modification solution.

2.4.2. Validation of the assay

A full pre-study validation was performed and involved the system suitability test, the limit of quantitation, linearity, intra- and inter-assay precision and accuracy, selectivity, and stability studies. The lower limit of quantification (LLQ) was 0.03 μg Pt mL^{-1} of plasma. The linearity was proven within the range of 0.03–0.5 μg Pt mL^{-1} of plasma. Intra- and inter-assay imprecision, expressed as the relative standard deviations, were less than 6% at the 3 × LLQ level and less than 3% at the levels of 5 and 1 μg Pt mL^{-1} of plasma, respectively. Intra- and inter-assay inaccuracies were less than 12% at the 3 × LLQ level and less than 4% at the levels of 5 and 1 μg Pt mL^{-1} of plasma. Based on the analysis of blank samples of rat plasma, ultrafiltrate and tissue, the method was proved specific. The stability tests indicated no significant Pt loss during three repeated thawing and freezing cycles. Long-term stability at −18 °C was proven over 12 weeks as a minimum and short-term stability was shown at the laboratory temperature for 3 h as a minimum.

2.5. Data analysis

The pharmacokinetic parameters of total and ultrafiltered platinum were estimated by use of standard non-compartmental methods. Maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were determined directly from the observed data. The area under the plasma concentration–time curve from zero up to the last sampling time 48 h ($AUC_{0–48h}$) was calculated by the linear trapezoidal method. The area under the plasma concentration–time curve from zero up to infinity (AUC) was determined as the sum of the $AUC_{0–48h}$ and of the extrapolated part of the AUC (AUC_{extra}, the ratio of the predicted concentration at

Table 1
Non-compartmental pharmacokinetic analysis of total platinum in the plasma and plasma ultrafiltrate after single oral doses of LA-12 to rats

	Plasma	Plasma	Ultrafiltrate
Dose (mg kg^{-1})	38.6	540	540
C_{max} (mg L^{-1})	1.54	6.25	1.21
t_{max} (h)	0.5	1	1
$AUC_{0–48h}$ (mg h L^{-1})	23.9	109	5.88
AUC (mg h L^{-1})	29.0	144	6.24
$\%AUC_{extra}$ (%)	17.5	24.4	5.8
λ_z (h^{-1})	0.0356	0.0284	0.0488
$t_{1/2}$ (h)	19.5	24.4	14.2
CL/F (L h^{-1} kg^{-1})	1.33	3.74	86.6
V_z/F (L kg^{-1})	37.5	131	1770
V_{ss}/F (L kg^{-1})	36.0	127	1170

48 h to the terminal rate constant λ_z estimated using the last three concentrations above the LLQ). Apparent total plasma clearance (CL/F) of platinum was calculated by dividing the dose with the AUC. The per cent peak-trough fluctuation (%PTF) was calculated using the formula $100 \times (C_{max} - C_{min})/C_{average}$.

3. Results

The pharmacokinetic parameters of total and free Pt are summarized in Tables 1 and 2. The mean platinum concentration versus time profiles are shown in Figs. 2 and 3 and Pt tissue concentrations are presented in Figs. 4 and 5, respectively.

Table 2
Non-compartmental pharmacokinetic analysis of total plasma platinum after once-a-day oral dosing of LA-12 over 14 consecutive days

	Plasma	Plasma
Dose (mg kg^{-1})	4.3	38.6
Tau[a] (h)	24	24
AUC_{ss} (mg h L^{-1})	1.87	11.7
CL/F (L h^{-1} kg^{-1})	2.30	3.30
C_{min} (mg L^{-1})	0.0343	0.340
C_{max} (mg L^{-1})	0.184	0.950
t_{max} (h)	0.5	0.5
$C_{average}$ (mg L^{-1})	0.0781	0.486
%PTF[b] (%)	191	125

[a] The dosing interval.
[b] The percent peak–trough fluctuation.

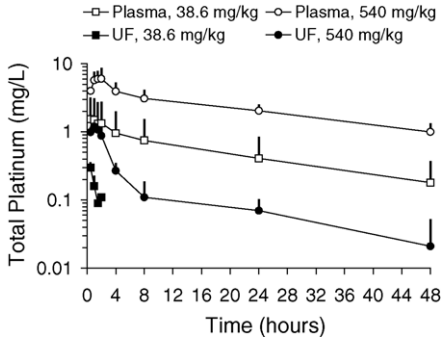

Fig. 2. Mean (S.D., $N = 4$ rats) concentrations of total platinum in the plasma and plasma ultrafiltrate after a single-dose administration of LA-12.

3.1. The pharmacokinetics of total plasma platinum

The maximum plasma concentrations of total Pt were observed within 1 h after administration of both single doses. Total plasma Pt concentrations increased less than proportionally to the 14-fold increase of the dose. After a single dose of 540 mg kg^{-1}, the mean C_{max} of total Pt and mean AUC value were four- and five-fold higher than that after the lower dose (Table 1). No accumulation of total plasma Pt occurred after once-a-day dosing of 38.6 mg kg^{-1} LA-12. Conversely, the mean C_{max} and the mean AUC value after the 14th dose were 1.6- and 2.5-fold less than those after a single-dose administration. The between-dose comparison of the mul-

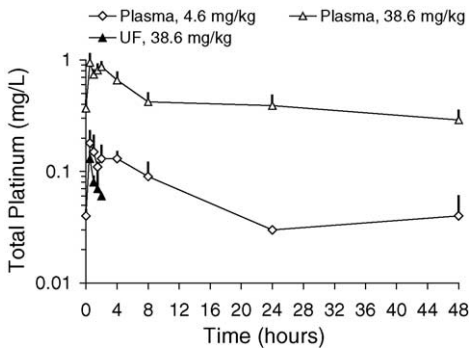

Fig. 3. Mean (S.D., $N = 4$ rats) concentrations of total platinum in the plasma and plasma ultrafiltrate after once-a-day oral dosing of LA-12 over 14 consecutive days.

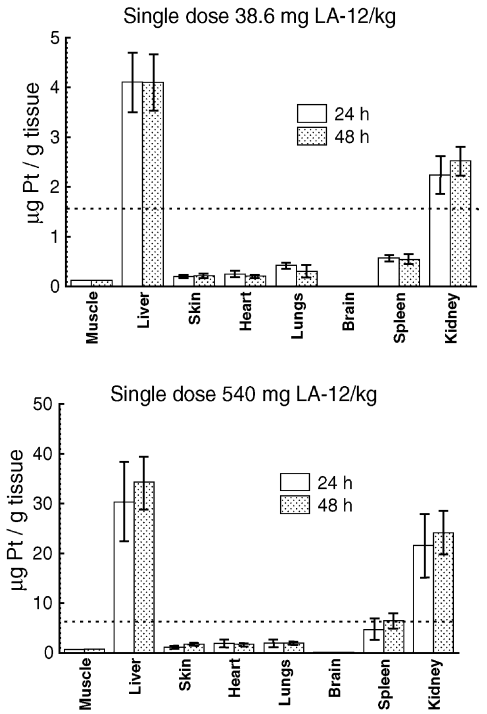

Fig. 4. Mean (S.D., $N = 4$ rats) tissue platinum concentrations after single-dose administration of 38.6 (upper graph) or 540 mg kg^{-1} (lower graph) LA-12. Tissues were obtained at 24 or 48 h after administration. Dotted line represents the mean maximum concentration of total plasma platinum.

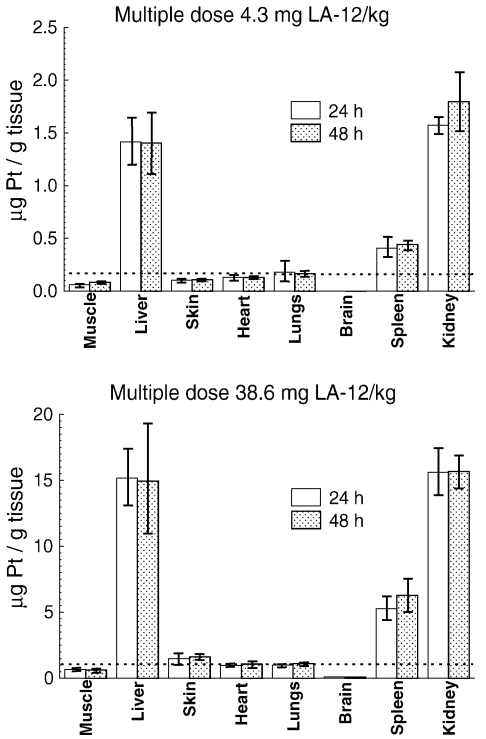

Fig. 5. Mean (S.D., $N = 4$ rats) tissue platinum concentrations after once-a-day dosing of 4.3 (upper graph) or 38.6 mg kg^{-1} (lower graph) LA-12 over 14 consecutive days. Tissues were obtained at 24 or 48 h after last administration. Dotted line represents the mean maximum concentration of total plasma platinum.

tiple doses of 4.3 and 38.6 mg kg^{-1}, respectively, provided the evidence of 40% less than proportional increases in both the mean C_{max} values and AUCs (Table 2).

3.2. The pharmacokinetics of free platinum

Concentrations of Pt in the plasma ultrafiltrate decreased rapidly below the quantification limit. The initial half-life was approximately 1 h or less (Figs. 2 and 3). This short half-life represents the kinetics of the free Pt distribution into tissues and elimination by the kidneys. Pharmacokinetic parameters of free Pt could reliably be estimated only after a single dose of 540 mg kg^{-1} (Table 1). Regardless of a dosage regimen, concentrations of free Pt accounted for 10–25% of total plasma Pt and, since 2 h after administration, for less than 10%.

3.3. Tissue concentrations of platinum

The tissue concentrations of Pt at 24 and 48 h after administration did not differ. This finding corresponds to the irreversible nature of Pt binding in tissues. The highest tissue Pt concentrations were found in the liver and kidneys. The concentrations found in other organs and tissues were less (after a single dose) or similar (after multiple doses) when compared to the mean C_{max} of total plasma Pt except for the brain where Pt was undetectable (Figs. 4 and 5).

The non-linearity of total tissue Pt concentrations was less than that in the plasma. The Pt concentrations in different tissues were five- to seven-fold higher after the single dose of 540 mg kg^{-1} as compared to 38.6 mg kg^{-1} (Fig. 4). Importantly, the nine-fold difference between the once-a-day doses of 4.3 and 38.6 mg kg^{-1} was followed by proportional 9.9- to 12.9-fold increases in tissue concentrations reached

Table 3
Comparison of pharmacokinetics of total and free plasma platinum in the rat after single oral administration of LA-12 and in the BalbC^{-} mice after single oral administration of satraplatin

	LA-12			Satraplatin		
	Plasma	Plasma	Ultrafiltrate	Plasma	Plasma	Ultrafiltrate
Dose (mg kg^{-1})	38.6	540	540	50	200	200
AUC (mg h L^{-1})	29.0	144	6.24	113	338	4.99
C_{max} (mg L^{-1})	1.54	6.25	1.21	4.94	10.4	1.4
t_{max} (h)	0.5	1	1.0	0.5	2.0	0.5
$t_{1/2}$ (h)	19.5	24.4	14.2	29.7	31.6	2.05
CL/F (L h^{-1} kg^{-1})	1.33	3.74	86.6	0.44	–	40.1

in the muscle, liver, skin, and kidneys. The increase in the Pt concentration in the spleen was 13.5-fold and that in the heart and lungs were 7.4- and 5.6-fold, respectively (Fig. 5).

Contrary to total plasma Pt concentrations, tissue Pt concentrations after the 14th dose of 38.6 mg kg^{-1} LA-12 were two- to four-fold higher than after a single dose. Accumulation of Pt was even higher in the kidneys (seven-fold) and spleen (nine-fold) (Figs. 4 and 5).

4. Discussion

The present study has been conducted in accordance to the current good laboratory practice guidelines and investigated the total and free Pt pharmacokinetics in the plasma and Pt tissue distribution in rat. The once-a-day dosing of 4.3 and 38.6 mg kg^{-1} LA-12 over 14 consecutive days corresponds to cumulative doses of 60 mg kg^{-1} (10% of the maximum tolerated dose) and 540 mg kg^{-1} (90% of the maximum tolerated dose), respectively. Platinum was assayed by a well-validated atomic absorption spectrometry method. Free plasma Pt comprises non-protein bound parent drug, and its biotransformation products as well as platinum bound to small molecules. In general, only free plasma Pt is considered to be pharmacologically active. However, a recent human study with satraplatin described a correlation between the nadir of thrombocytopenia and the AUC of total plasma Pt while the AUC of free Pt was not predictive. The C_{max} values for total Pt and free plasma Pt were related to neutropenia and to thrombocytopenia (Vouillamoz-Lorenz et al., 2003).

The pharmacokinetic parameters CL/F and C_{max} of total plasma Pt observed in the present study indicate, approximately, three-fold lower availability of total plasma Pt in rats than after comparable dose of satraplatin to the BalbC^{-} mice (Table 3) (Barnard et al., 1999). This difference may be ascribed to different vehicles (solution of methylcellulose for LA-12 versus arachis oil for satraplatin) (Kelland et al., 1993; McKeage et al., 1994a,b) and different animal species used. Alternatively, the lower availability of plasma Pt may be caused by a more extensive tissue uptake and binding. The volume of distribution of total and free Pt is the pharmacokinetic characteristic, which most profoundly differs between various platinum drugs. Tissue Pt concentrations at 48 h after a single oral administration of 200 mg kg^{-1} satraplatin to BalbC^{-} mice achieved 6–19 and 2.8–12 μg g^{-1} in the liver and kidney, respectively, and were proportionally less than that after 540 mg kg^{-1} of LA-12 to the rat in the present study (Barnard et al., 1999).

Less than proportional increase of C_{max} and AUC values of total plasma Pt was seen in mice with dose escalation above 40 mg kg^{-1} satraplatin (McKeage et al., 1994b). The present study made a similar finding with LA-12 in rat. It is assumed that optimal antitumor activity necessitates once-a-day administration of LA-12 over several consecutive days. After once-a-day administration over 14 consecutive days, Pt accumulated in tissues and tissue Pt concentrations increased linearly with the dose within the range from 4.3 to 38.6 mg kg^{-1} while total plasma Pt levels rose 40% less than in proportion with the dose.

Acknowledgement

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