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BIODISTRIBUTION ANALYSIS OF CISPLATIN IN LIPOSOMAL FORM IN ANIMALS WITH CISPLATIN-RESISTANT AND CISPLATIN-SENSITIVE CARCINOMA

M.M. Nosko, V.M. Pivnyuk, G.I. Solyanik, G.I. Kulik, I.N. Todor, V.Ya. Momot, O.R. Melnikov, O.V. Ponomareva, V.F. Chekhun*

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv 03022, Ukraine

Aim: **To analyze the relation between pharmacokinetics of cisplatin in liposomal form and antitumor efficacy toward cisplatin-resistant and cisplatin-sensitive variants of Guerin carcinoma.** ***Methods:*** **Concentration of platinum was measured by atomic absorption spectrophotometry (C115M1 "Selmi", Ukraine). Elimination constant was calculated based on the dynamics of cisplatin concentration in time period between 1 h to 24 h using nonlinear regression analysis. Area under curve (AUC_{24}) was calculated by the trapezium method.** ***Results:*** **It was shown that for liposomal form of cisplatin (LCp) AUC_{24} in tumor practically didn't depend on the level of the tumor sensitivity, while in animals with the resistant variant (CpRGC), AUC_{24} for free cisplatin (FCp) decreased by 70% less ($p < 0.001$) as compared to the sensitive tumor strain (CpSGC). Significant decrease of elimination constant of LCp compared to FCp in blood serum of rats bearing either CpRGC or CpSGC tumors favors cisplatin accumulation in tumor tissues with low vascularization level. The dynamics of cisplatin concentration in CpRGC variant was characterized by 90% higher level in 24 h after administration of LCp as compared to FCp ($p < 0.05$). This fact may explain increased antitumor efficacy of LCp compared to FCp toward CpRGC variant. In the study of kidney function, AUC_{24} index for LCp was by 68.6% ($p < 0.01$) and 50.7% ($p < 0.05$) lower than AUC_{24} index for FCp in rats with CpRGC and CpSGC variants, respectively. No significant differences have been found in biodistribution of cisplatin in both pharmaceutical forms in liver and lung in CpRGC- or CpSGC-bearing rats.** ***Conclusion:*** **The results suggest that cisplatin in liposomal form possesses higher specificity of antitumor action than free cisplatin.**

Key Words: **cisplatin, liposomal cisplatin, pharmacokinetics, biodistribution, cisplatin-resistance.**

The problem of resistance of malignant tumors to platinum compounds remains a challenge of modern clinical and experimental oncology. Among the main causes of low susceptibility of tumor cells to platinum-containing agents are the decreased intracellular accumulation, the elevated glutathione S-transferase and metallothionein activities, the activation of DNA repair system [1–4]. The development of the novel pharmaceutical forms of platinum-containing agents providing their increased accumulation in tumor cells has been considered to be useful to overcome cisplatin-resistance [5]. The development of liposomal forms of cisplatin may be regarded as an advantageous approach [6, 7].

The parameters of pharmacokinetics and bioavailability of cisplatin after intravenous and oral administration have been assessed in the clinical studies [8]. The experimental studies have demonstrated the elevated bioavailability of liposomal forms of various antitumor drugs [9–11].

Earlier we have shown the different therapeutic activity of free and liposomal cisplatin *in vivo* (cisplatin-sensitive and cisplatin-resistant Guerin carcinoma) [12, 13]. Our data have revealed the higher efficacy of liposomal form of cisplatin for treatment of resistant form of Guerin carcinoma.

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*Correspondence: E-mail: mykhailo.nosko@gmail.com

Abbreviations used: AUC – area under curve; C_{max} – maximal concentration of cisplatin; CpRGC – animals with transplanted strain of Guerin carcinoma resistant to cisplatin; CpSGC – animals with transplanted strain of Guerin carcinoma sensitive to cisplatin; FCp – free cisplatin; k_e – elimination constant of cisplatin; LCp – liposomal form of cisplatin; TGI – tumor growth inhibition.

The present study was aimed to analyze the relation between pharmacokinetics of cisplatin in liposomal form and its efficacy toward cisplatin-resistant and cisplatin-sensitive variants of Guerin carcinoma. Cisplatin in the basic pharmaceutical form was used as a reference preparation.

MATERIALS AND METHODS

100 female Wistar rats with body weight of 150 ± 5 g were used. All experiments performed according to the international guidelines of work with laboratory animals were approved by Bioethics Committee of R.E. Kavetsky IEPOR NASU (Kyiv, Ukraine).

Cisplatin resistance in tumors was generated by 12 sequential transplantations of tumor cells in animals treated with cisplatin. Tumors were transplanted subcutaneously into interscapular region by injection of 0.5 ml of tumor cell suspension (2×10^6 cells per animal). Cisplatin for infusions (Ebewe, Austria), and its liposomal form as lyophilized powder for preparation of infusion solution (by 0.01 g, CAS Biolik, Kharkiv, Ukraine) were used.

Animals with transplanted cisplatin sensitive (CpSGC) and cisplatin resistant (CpRGC) strains of Guerin carcinoma, were housed in the following groups: 1) animals with CpSGC treated with liposomal form of cisplatin (LCp); 2) animals with CpSGC treated with free cisplatin (FCp); 3) animals with CpSGC not treated with cytastatics — control group for CpSGC; 4) animals with CpRGC treated with LCp; 5) animals with CpRGC treated with FCp; 6) animals with CpRGC not treated with cytostatics — control group for CpRGC.

When the transplanted tumors reached the volume of 0.3 cm^3, the animals from respective groups with

CpSGC and CRGC received 5 injections of LCp and FCp at a dose of 1.2 mg/kg body weight (every two days, 5 injections in total). The samples of blood serum, liver, kidneys, lungs, and tumor tissue were taken in 0.25 h; 0.5 h; 1 h; 3 h; 6 h, and 24 h after LCp and FCp administration.

Assessment of cisplatin concentration. Concentration of cisplatin after its single administration in liposomal or basic pharmaceutical forms was assessed in blood serum, tissues of tumor, kidneys, liver, and lungs of rats with CpSGC and CpRGC. For this purpose, tissue samples were mineralized by the method of dry cineration [14]. The concentration of platinum was measured by atomic absorption spectrophotometry (C115M1 "Selmi", Ukraine) by calibrating curve for platinum standards (Fluka, Switzerland) at the range of studied concentrations.

Analysis of cisplatin biodistribution in rat body. Biodistribution of cisplatin (in different pharmaceutical forms) in body of animals with CpSGC and CpRGC variants was characterized by the following pharmacokinetic parameters: *AUC-24* — area under pharmacokinetic curve determined in one day after administration of the drug; C_{max} — maximal concentration of cisplatin during observation period; k_e — elimination constant of cisplatin calculated based on the dynamics of cisplatin concentration in time period between 1 h to 24 h using nonlinear regression analysis. Area under pharmacokinetic curve was calculated by the trapezium method according to the formula:

$$AUC = \sum_{i=1}^{i=5} (C_i + C_{i+1}) \times (t_{i+1} - t_i) / 2$$

wherein t_i — time point of measuring cisplatin concentration in animal's body counted from the moment of drug administration; C_i — concentration of cisplatin at t_i.

Statistical analysis was performed with the use of descriptive statistics as well as Student's and Whitney tests and Statistica program.

RESULTS AND DISCUSSION

Cisplatin in both pharmaceutical forms has been shown to inhibit effectively the growth of the original variant of Guerin carcinoma. As shown in Fig. 1, even on the first day after the beginning of cisplatin administration, the suppression of tumor growth was similar for both forms (FCp and LCp). The LCp efficacy assessed upon termination of the treatment was very high with tumor growth inhibition (TGI) indices being 99.0% and 99.4% for LCp and FCp, respectively (Table 1).

Table 1. Tumor weight and tumor growth inhibition indices after treatment with cisplatin in different pharmaceutical forms on Day 20 after tumor transplantation

Variant of Guerin carcinoma	Control animals		LCp		TGI (%)	FCp		TGI (%)
	Tumor weight (g)					Tumor weight (g)		
	M	m	M	m		M	m	
Sensitive	35.3	5.8	0.4	0.1	99.0	0.2	0.08	99.4
Resistant	50.4	4.2	13.8	2.1	72.6	35.3	4.7	30,0

The inhibition of CpRGC growth by both pharmaceutical forms of cisplatin was significantly lower as compared with the inhibition of the original variant of tumor (see Fig. 1, Table 1). As far as cisplatin is a cycle-specific anticancer drug, its lower efficacy toward CpRGC is in part associated with lower growth rate of the tumor compared to the original (CpSGC) tumor strain.

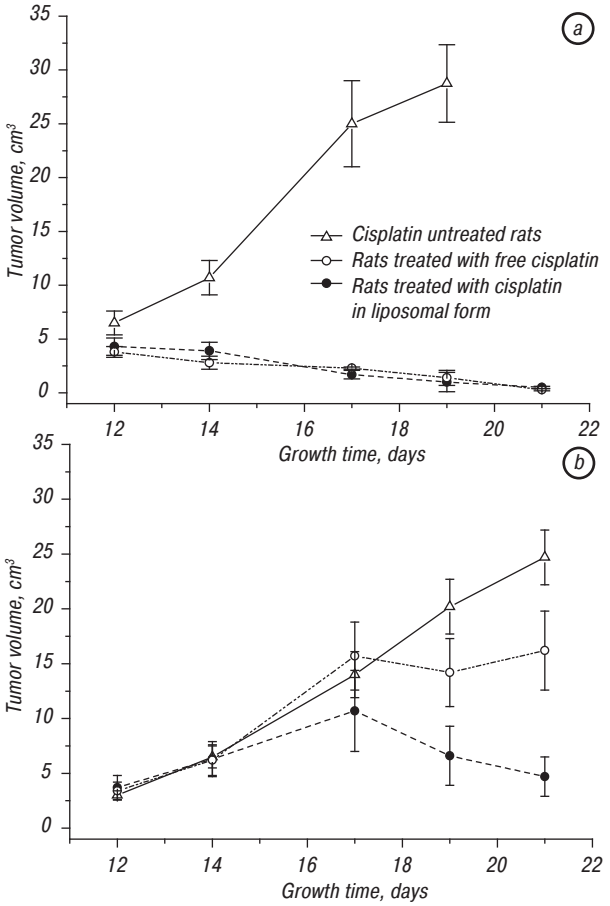

Fig. 1. Growth kinetics of CpSGC (*a*) and CpRGC (*b*) variants with and without therapy with cisplatin in different pharmaceutical forms

Analysis of CpRGC growth kinetics has shown that growth inhibition of resistant tumor variant by cisplatin both in free and liposomal forms became evident only one week after the initiation of the treatment. The effects of LCp were more pronounced as compared with FCp. At the end of LCp therapy, tumor volume was 3 times as less as that upon FCp treatment (see Fig. 1), with TGI indices being 72.6% and 30% for LCp and FCp, respectively. Therefore, an antitumor efficacy of cisplatin in liposomal form toward CpRGC variant exceeded significantly that of FCp. In contrast, both LCp and FCp demonstrated the similar efficacy in CpSGC variant of Guerin carcinoma.

The pharmacokinetic studies have shown that such differences in sensitivity of resistant variant to cisplatin in different pharmaceutical forms are largely attributed to the differences in cisplatin biodistribution in rat body. Fig. 2 shows three-phase pattern of the dynamics of cisplatin concentrations (in both pharmaceutical forms) in blood serum of rats bearing CpSGC or CpRGC variants after intraperitoneal administration of the drug. After absorption of the drug (the first phase with duration <1 h), the relatively short phase of cisplatin distribution followed by the prolonged phase of its elimination are observed. The area under pharmacokinetic curve of LCp one day after its administration practically doesn't depend on the degree of sensitivity of Guerin

carcinoma. AUC_{24} for LCp in rats with CpRGC or CpSGC variant doesn't differ significantly (Table 2). In contrary, the area under pharmacokinetic curve of FCp in animals with CpRGC variant was by 70% less ($p < 0.001$) as compared to the sensitive strain.

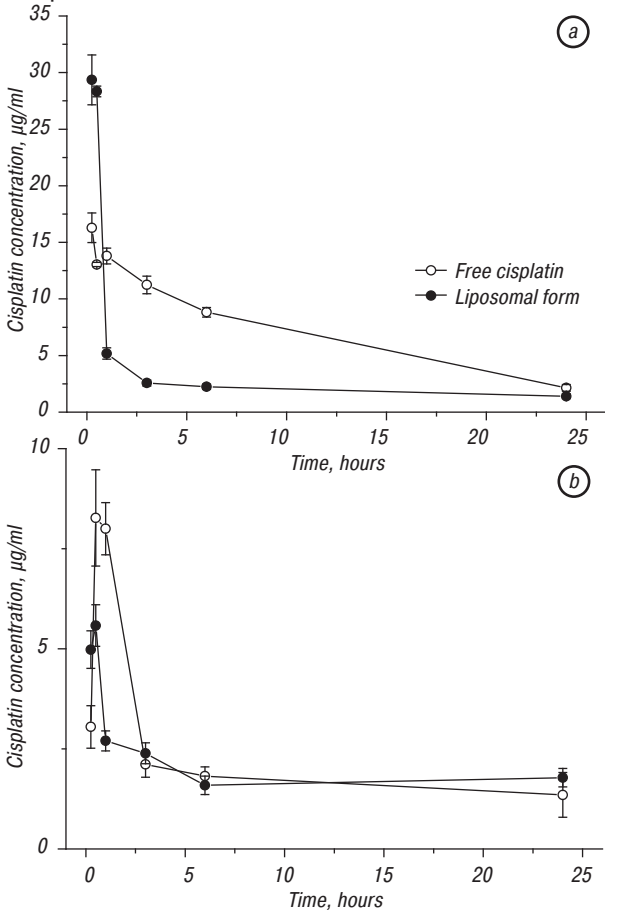

Fig. 2. Dynamics of cisplatin concentration in blood serum of rats with CpSGC (*a*) and CpRGC (*b*)

Table 2. Pharmacokinetic parameters of cisplatin (FCp and LCp) in blood serum of rats with CpSGC and CpRGC

Pharmacokinetic parameters	Sensitive variant of Guerin carcinoma		Resistant variant of Guerin carcinoma	
	LCp	FCp	LCp	FCp
AUC_{24} (μg × h/L)	63.3 ± 10.2*	163.9 ± 30.8	45.1 ± 7.1	49.8 ± 8.9
C_{max} (μg/L)	29.4 ± 2.2*	16.3 ± 1.3	5.6 ± 0.5	8.3 ± 2.2
K_{el} (h^{-1})	0.03 ± 0.004*	0.08 ± 0.002	0.11 ± 0.02*	0.28 ± 0.1

*The differences are significant ($p < 0.05$) as compared with FCp.

For CpRGC-bearing rats, LCp and FCp are bioequivalent: there is no statistically significant difference between area under pharmacokinetic curve and maximal concentration of the drugs. For CpSGC-bearing rats, AUC_{24} for LCp is less by 61%, and C_{max} — higher by 80% as compared with respective FCp data.

One should pay attention to the significant decrease of elimination constant of cisplatin in liposomal form as compared with free one from blood serum of rats bearing either CpRGC or CpSGC tumors. Also the half-life of liposomal cisplatin in animals' blood increases as well as the ratio between the area under pharmacokinetic curve (calculated at $t > 1$ day) for liposomal cisplatin ($AUC_t(LCp)$) to $AUC_t(FCp)$ of free form, so:

$$\frac{AUC_t(LCp)}{AUC_t(FCp)} > \frac{AUC_{24}(LCp)}{AUC_{24}(FCp)}$$

This fact seems to be important because half-life increase of liposomal cisplatin in blood provides for its accumulation in tissues with low level of vascularization, including the tumor itself. And the data on dynamics of cisplatin concentrations in tumor tissue have in fact demonstrated the trend (regarding the average values) for higher AUC_{24} values of LCp compared with FCp (Table 3).

Table 3. Pharmacokinetic parameters of cisplatin in tumor tissue of rats with Guerin carcinoma

Pharmacokinetic parameters	Sensitive variant of Guerin carcinoma		Resistant variant of Guerin carcinoma	
	LCp	FCp	LCp	FCp
AUC_{24} (μg × h/L)	74.3 ± 7.3*	57.9 ± 7.1	40.5 ± 3.1*	33.2 ± 2.8

*The differences are significant ($p < 0.05$) as compared with FCp.

It's necessary to note that the dynamics of cisplatin concentration in tumor tissue of CpRGC-bearing rats is characterized by 90% higher level ($p < 0.05$) in 24 h after LCp administration as compared with FCp (Fig. 3). Being in line with such biodistribution of cisplatin in tumor tissue, continuous injections of the drug will result in the increased area under pharmacokinetic curve after LCp administration as compared with FCp, and this fact could explain higher antitumor activity of liposomal cisplatin compared to its free form toward CpRGC.

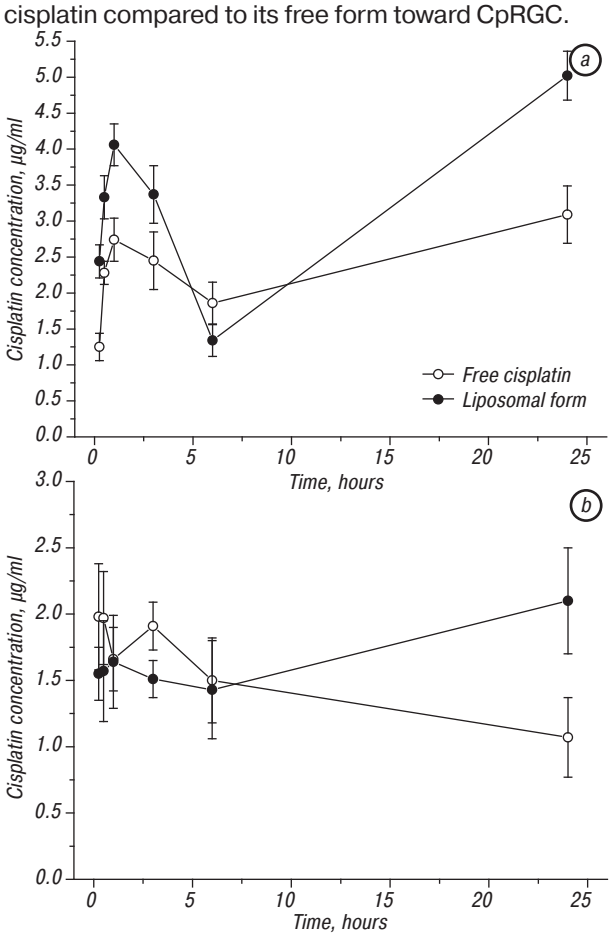

Fig. 3. Dynamics of cisplatin concentration in tumor tissue of rats with CpSGC (*a*) and CpRGC (*b*)

It is known that an effectiveness of antitumor activity of cisplatin is significantly limited by its nephrotoxicity, which is directly correlated with maximal concentration of cisplatin in the kidneys and the amount of the drug that passes through the kidneys. Analysis of cisplatin biodistribution in the kidneys has

shown that its concentration after FCp administration was significantly higher at all stages (independently of tumor type) compared with the respective indices of liposomal form (Fig. 4, Table 4). Along with this, for LCp, AUC_{24} indices were by 68.6% ($p < 0.01$) and 50.7% ($p < 0.05$) lower than AUC_{24} indexes for FCp in kidneys of rats with CpRGC and CpSGC, respectively.

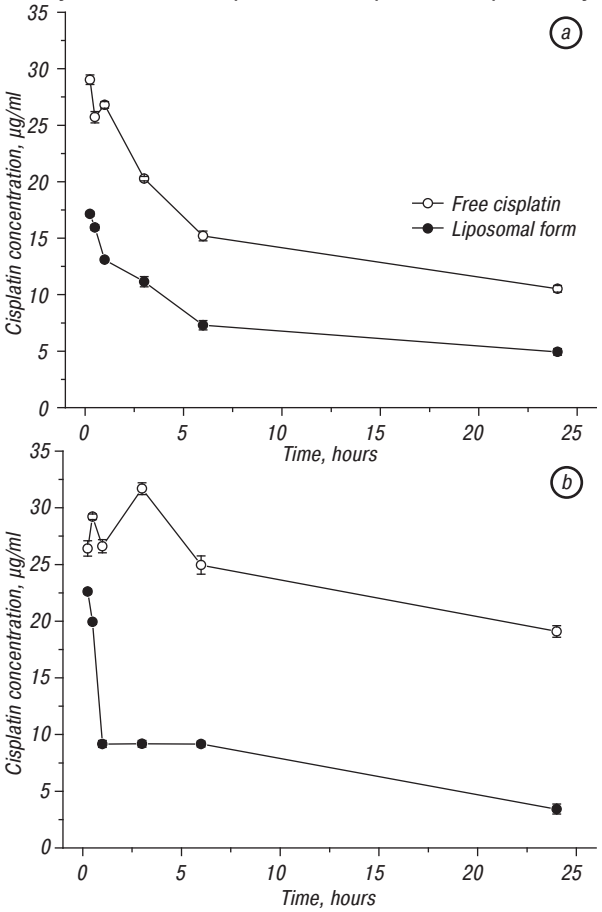

Fig. 4. Dynamics of cisplatin concentration in kidney tissue of rats with CpSGC (*a*) and CpRGC (*b*)

Table 4. Pharmacokinetic parameters of cisplatin in kidneys of rats with Guerin carcinoma

Pharmacokinetic parameters	Sensitive variant of Guerin carcinoma		Resistant variant of Guerin carcinoma	
	LCp	FCp	LCp	FCp
AUC_{24} (μg × h/L)	173.4 ± 26.0*	351.7 ± 52.8	176.0 ± 26.4*	560.5 ± 84.1
C_{max} (μg/L)	17.2 ± 0.21	29.0 ± 0.42	22.6 ± 0.20	26.4 ± 0.68

*The differences are significant ($p < 0.05$) as compared with FCp.

Therefore, cisplatin in liposomal form shows higher efficacy toward resistant variant of Guerin carcinoma and lower nephrotoxicity than the drug in free pharmaceutical form.

Analysis of biodistribution of cisplatin in both pharmaceutical forms in liver and lung tissues of rats with CpSGC and CpRGC did not reveal significant differences (Table 5). One could observe only 33% decrease ($p < 0.05$) of AUC_{24} of FCp in lung tissues of rats with CpRGC compared to that for liposomal form.

The results allow one to consider that cisplatin in liposomal form possesses higher specificity of antitumor action than free cisplatin. For cisplatin-sensitive tumors, such increased specificity of LCp preparation is specified by significant decrease of its nephrotoxicity, while in the case of cisplatin-resistant tumors — by significantly higher (compared to the basic form) efficacy of its antitumor action as well as lower nephrotoxicity.

Table 5. Pharmacokinetic parameters of cisplatin in liver and lungs of rats with Guerin carcinoma

Pharmacokinetic parameters	Sensitive variant of Guerin carcinoma		Resistant variant of Guerin carcinoma	
	LCp	FCp	LCp	FCp
		Liver		
AUC_{24} (μg × h/L)	14.29 ± 2.14	15.6 ± 2.3	16.7 ± 2.5	17.0 ± 2.5
		Lungs		
AUC_{24} (μg × h/L)	22.9 ± 3.4	28.3 ± 4.2	21.91 ± 3.3	18.9 ± 2.8

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