

THE CONCENTRATION OF CERULOPLASMIN IN BLOOD OF TUMOR-BEARING RATS AFTER ADMINISTRATION OF A DIRHENIUM(III) COMPOUND AND CISPLATIN

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Contemporary investigations of mechanisms of resistance to platinides showed the key role of copper metabolism in cancer patients and proposed possible methods to attenuate the resistance by modulation of copper transporting mechanisms. In this vein, investigation of ceruloplasmin (Cp) levels – the main copper-containing protein in blood, in experiments with tumor-containing animals upon cytostatics administration is topical and has great importance. The concentration of Cp was measured in the serum of tumor-bearing rats with ordinary (T8) and resistant to cisplatin (T8*) Guerin's carcinoma upon administration of cisplatin and quadruple bonding dirhenium(III) compound dichlorotetra- μ -isobutyrate dirhenium(III) (I) in different medicamental forms. It was shown that development of tumor in T8 group led to increasing of concentration of Cp in 3.7 times and in T8* group – more than in 8 times in comparison to control, confirming the essential role of Cp in the formation of resistance phenomenon. Administration of cisplatin together with I led to effective inhibition of tumor in groups with T8 and T8*, indicating decreased resistance in the group T8*. Greater reduction of Cp levels was observed in the groups with T8* upon administration of the rhenium-platinum antitumor system, than in groups with T8, that underlines the importance of further investigations of the dirhenium(III) compounds in the resistance to cytostatics cancer models. Some mechanisms concerning the regulation of copper homeostasis and properties of nano-composites are discussed.

Key words: ceruloplasmin, cluster rhenium(III) compound, Guerin's carcinoma, resistance to cisplatin.

Ceruloplasmin (Cp) is a copper-containing protein that includes 40-70% of the whole plasma copper. It possesses the enzymatic activity as ferroxidase and takes part in oxidizing of bivalent iron. Cp belongs to proteins of acute phase and serves as a marker of inflammation [1].

Changing of Cp concentrations in blood was shown under different diseases. The reducing of Cp level was noticed under Wilson's, Menon's diseases, copper deficiencies and aceruloplasminemia [2]. Also, the Cp level is changing under cancer development: concentration of Cp in blood was increased under oncological diseases of different etiologies and

increased Cp level was also found in the tissues of onco-patients [3].

Contemporary knowledge about Cp functions in cancerogenesis includes its role as a prognostic marker of some types of cancer [4]; as an adipokine which is overexpressed in adipose tissue of obese subjects [5]; as a substance that promotes tumor progressions through influence on the glycolytic process in cancer cells [6].

It is well-known that resistance to platinides, especially to cisplatin (cPt), is a negative phenomenon in the oncological practice and includes some complicated mechanisms, among which blockade

of the transport of platinum compounds is considered to be the most ultimate. The expression of the human copper transporter 1 (hCtr1), which transports also platinum compounds, was found to be significantly reduced in cisplatin-resistant cancer cells. Moreover, one of the recently found tools to overcome cPt resistance was to introduce cPt together with copper - chelating agents [7-9].

In our previous works, the anticancer and antioxidant properties of cluster rhenium(III) compounds which were administered to tumor-bearing animals separately, with cPt, (Re - Pt system), in liposomal forms and in the forms of solid lipid nanoparticles in the model of ordinary (T8) and resistant to cPt (T8*) Guerin's carcinoma was shown [10-12, 17]. But the concentration of Cp in these works was not investigated. Thus, the aim of the work was to investigate the influence of the introduction of cPt and a cluster rhenium(III) compound in the form of nanoliposomes and solid lipid nanoparticles on the concentration of Cp in the blood of tumor-bearing rats under development of T8 and T8*.

Materials and Methods

In our research, we studied the cluster compound dichlorotetra- μ -isobutyrate dirhenium(III) $\text{Re}_2(\text{i-C}_3\text{H}_7\text{COO})_4\text{Cl}_2$ - (**I**) synthesized in the Ukrainian State University of Chemical Technology, Dnipro [13]. Nanoliposome [I] nl forms of **I** and the Re-Pt system with 4:1 ratio of the components inside lipid [I+cPt (4:1)] nl, [I+cPt (4:1)] np were prepared *ibid* according to [14]. The experimental part was described in detail in [11, 17, 18].

All animal experiments were performed in accordance with the rules of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986). Rats without tumor were control group (control, No 1). Tumor growth was modeled by transplantation of 20% suspension of T8 or T8* to healthy rats (groups T8, No 2 and T8*, No 5 respectively).

A single intraperitoneal injection of cPt in solution (sl) or nanoliposomal (nl) forms at a dose 1.75 $\mu\text{mol/kg}$ was performed on the 9th day after tumors transplantation, groups: T8+cPt sl, No 3; T8*+cPt sl, No 6; T8+cPt nl, No 4; T8*+cPt nl, No 7. The Re-Pt antitumor system was introduced with separately introductions of the components when cPt was introduced in the same mode and quantities as in groups 3, 6 and 4, 7 and on the 3rd day after tumor transplantation **I** was administrated ten

times in liposomes in the dose of 7 $\mu\text{mol/kg}$, groups: T8+cPt+[I] nl, No 8; T8*+cPt+[I] nl, No 10; and in the form of mixed nanoliposomes and nanoparticles (np), where both cytostatics were inside lipid nanocapsules, in the same quantities and ratios, groups: T8+[I+cPt(4:1)] nl, No 9; T8+[I+cPt(4:1)] np, No 11; T8*+[I+cPt (4:1)] nl, No 12; T8*+[I+cPt(4:1)] np, No 13. Quantity of experimental animals in each group was 6-8.

Experimental animals were decapitated under chloroform anesthesia on the 21st day. Blood was collected in test tubes. The serum was obtained by natural plasma coagulation. The content of the Cp was investigated in the serum using the Ravin method and the standard reagent set (Private Joint-Stock Company Reagent, Ukraine). The degree of reduction of oxidative stress in plasma of experimental animals was evaluated as the ratio of the concentration of MDA in plasma of experimental animals to the concentration of MDA in plasma of rats with Guerin's carcinoma without any administration (ordinary or, respectively, resistant) [11, 17]. The results were statistically processed using the Student's *t*-test. Differences in values were considered statistically significant at $P \leq 0.05$.

Results and Discussion

Development of ordinary Guerin's carcinoma T8 led to increasing of concentration of Cp in 3.7 times in comparison to control (groups No 2 and No 1), Table 1.

At the same time, Cp concentration in serum of T8* - bearing rats increased in 8.5 times in comparison to control. Thus, development of a cPt-resistant tumor T8* led to increasing of Cp concentration in 2.3 times more intensively in comparison to ordinary tumor T8. These findings may support the upper cited works about essential role of Cp in the formation of resistance phenomenon in cancer cells [7-9].

Introduction of cPt solutions to tumor-bearing rats showed different directions of changing of Cp levels in T8 and T8* groups of animals: if introduction of cPt to T8 group led to increasing of Cp level on 22.9% in comparison to T8 group, the same introduction to T8* group led to decreasing of Cp on 27.0% in comparison to T8* group of animals. To our knowledge, such an essential difference in elevation or dropping of Cp levels as a reply to cisplatin solution introductions is first demonstrated.

Introductions of liposomes loaded with cPt led to higher efficacy of tumor inhibition in T8* group

Table 1. The concentration of Cp in serum, tumors inhibition and reducing of plasma MDA concentration in comparison to T8 and T8* groups under cPt introduction

Number of the group	Characteristic of the group	Cp, mg/l	Tumors inhibition, % [10, 17]	Reducing of plasma MDA concentration, % [10, 17]
1	Control	30.8 ± 1.5	–	–
2	T8	112.6 ± 3.7*	–	–
3	T8+cPt sl	138.4 ± 6.9*#	72.0 ± 3.6	35.5 ± 1.6
4	T8+cPt nl	261.4 ± 12.9*#	65.0 ± 3.3	55.9 ± 3.1
5	T8*	261.4 ± 18.5*	–	–
6	T8*+cPt sl	190.8 ± 9.5*##	36.0 ± 1.8	32.4 ± 1.6
7	T8*+cPt nl	260.5 ± 13.0*	52.3 ± 2.6	79.2 ± 3.6

* $P < 0.001$ relative to control, # $P < 0.05$ relative to group T8, ## $P < 0.001$ relative to group T8*

and to higher levels of Cp in both T8 and T8* groups (up to 261 mg/l). These differences in tumor inhibition in our experiments with free and encapsulated cPt in T8* group of animals coincide by numerous examples of successful application of liposomal formulations in attempts to overcome resistance [15]. So high Cp levels in groups' No 4 and No 7 has no explanation at the moment and require more detailed investigations. But differences in Cp levels between group's No 3 – No 4 and No 6 – No 7, i.e. in the influence of free cisplatin and liposomal cisplatin introductions on Cp concentrations may take place due to that known fact that liposomes have another transporting properties and mechanism of interaction with cancer cells than free forms of medicines. In fact, liposomal forms of drugs enter the cytoplasm, bypassing active import.

Reducing of tumors always was accompanied by the decreasing of oxidative stress, especially under application of the liposomal drugs [12, 17], that is also confirmed by data presented in Table 1. In

both kinds of tumors, introductions of liposomal forms of cisplatin led to more essential decreasing of the intensity of peroxide oxidation of lipids (POL).

Introduction of the Re-Pt system in liposomal forms (groups 8, 9, 10) led to very effective inhibition of T8 (Table 2) that was also shown earlier [11, 17].

Effective inhibition of tumor in groups with T8 (No 8, No 9, No 10) and T8* (No 11, No 12, No 13 that is an example of resistance overcoming) coincided with relatively low concentrations of Cp (68.5–204.0% to control) and (70.0–135.0% to control) respectively. Also, the decreasing of POL intensity in blood of experimental animals is observed.

Comparing data of Tables 1 and 2 we may conclude that introducing of the rhenium compound I in any form together with cisplatin led to the lowering of Cp together with effective inhibition of the tumors as ordinary, as resistant to cisplatin.

Especially interesting is to compare groups No 3 and No 8; No 6 and No 11. In all these ex-

Table 2. The concentration of Cp in serum, inhibition of tumors and reducing of plasma MDA concentration in comparison to T8 and T8* after Re-Pt system introduction

Number of the group	Characteristic of the group	Cp, mg/l	Tumors inhibition, % [11, 17]	Reducing of plasma MDA concentration, % [11, 17]
8	T8+[I] nl+cPt sl	93.5 ± 4.6*#	96.9 ± 4.8	81.8 ± 4.0
9	T8+[I+cPt(4:1)] nl	71.0 ± 3.5*##	97.8 ± 4.9	85.8 ± 4.0
10	T8+[I+cPt(4:1)] np	51.8 ± 2.6*##	96.2 ± 4.8	76.0 ± 3.7
11	T8*+[I] nl+cPt sl	72.3 ± 4.1*	98.0 ± 4.9	52.2 ± 2.1
12	T8*+[I+cPt(4:1)] nl	52.3 ± 2.6*##	70.8 ± 3.5	80.7 ± 3.5
13	T8*+[I+cPt(4:1)] np	56.1 ± 1.3##	46.5 ± 0.3	23.1 ± 1.0

* $P < 0.001$ relative to control, # $P < 0.05$ relative to group T8, ## $P < 0.001$ relative to group T8*

perimental groups the equal quantities of cisplatin in solution were introduced to tumor-bearing animals, but in groups No 8 and No 11 it was introduced after pretreatment with the rhenium compound in the liposomal form [I] nl. Efficacy of tumor inhibition was higher in these groups, and the Cp level was lower in 2.5–3 times in comparison to groups, where [I] nl was absent (No 3 and No 6).

Thus, according to the aim of the work, we have shown that development of a cPt-resistant tumor T8* led to increasing of Cp concentration in 2.3 times more intensively in comparison to ordinary tumor T8; introduction of cPt solutions to tumor-bearing rats showed different directions of changing of Cp levels in T8 and T8* groups of animals; introductions of liposomes loaded with cPt led to higher levels of Cp in both T8 and T8* groups in comparison to groups with free cPt introductions; introducing of the rhenium compound I in any form together with cisplatin led to the lowering of Cp in comparison to groups with cPt introductions.

If also to compare the average Cp level between groups of tumor-bearing rats shown in Table 3, it is evident that in the groups No 8, No 9 and No 10 there were level reduction in average by 36.0% in comparison to T8 (Table 1, group No 2).

Greater reduction of Cp levels was observed in the groups No 11, No 12 and No 13 with T8*, where the Re-Pt system was introduced. There was a reduction of Cp levels in average on 77.0% in comparison to T8* (Table 1, group No 5). The comparison with appropriate groups where I was introduced together with cisplatin, also showed the larger reduction of the Cp concentration in groups with T8* than in groups with T8. It means that I influence on the copper homeostasis of both types of tumors, but in somehow different manner that underlines the importance of following investigations of the dirhenium(III) com-

pounds in the cancer models with resistance to cytostatics.

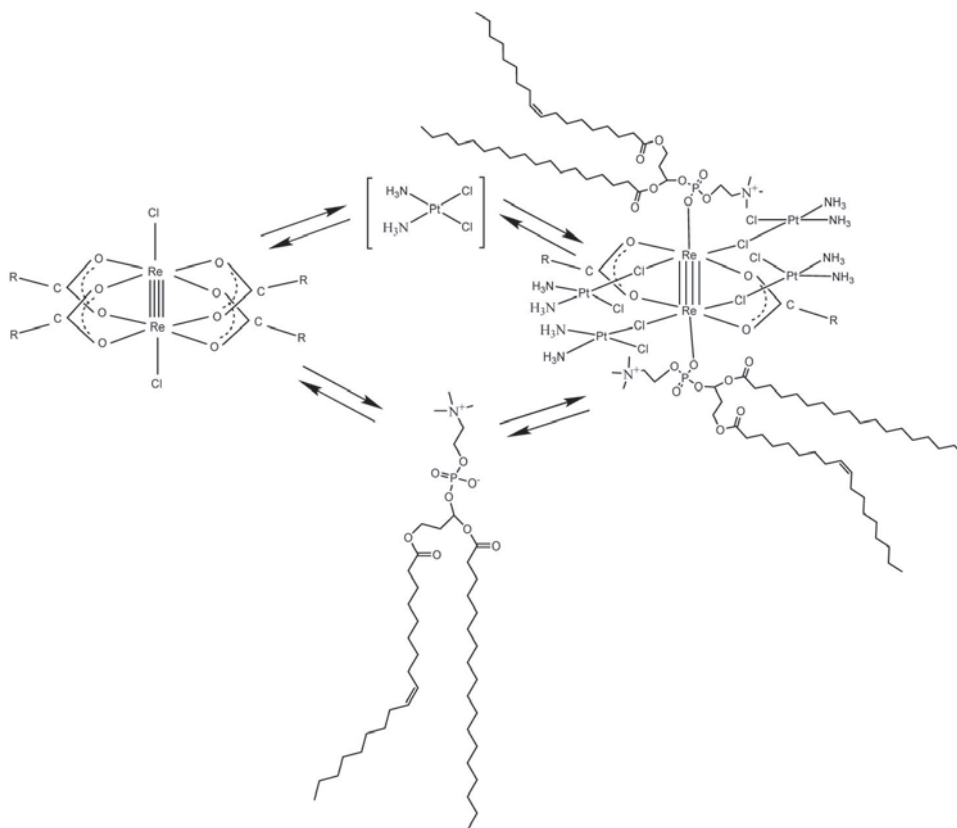
Recent findings revealed that hCtrl regulates intracellular copper homeostasis, which, in turn, controls hCtrl expression via a homeostatic feedback loop [7, 9]. The copper-lowering agents increased the expression of hCtrl, subsequently resensitizing tumor cells to platinum therapy. Does the rhenium compound I play somehow the role of the copper-lowering agent in these experiments? We have no any supplying facts for this now. But if so, the hypothesis that resistance to platinum therapy can be reversed through the use of a copper-lowering agent as a rhenium compound like I is very promising. But we know, that mechanism of platinum compounds transport are multifactorial and any stage of the mechanisms underlying cisplatin transport (both import and export) and retention in the tumor cells are important determinants and may be affected by I.

Introductions of nano-liposomes and solid lipid nanoparticles, loaded with cisplatin and I, was effective as in tumor-decreasing, as in POL inhibition and also was accompanied by low Cp levels. These nanoparticles also come to the cancer cell bypassing active import, fusing with the cell membranes of cancer cells [12]. The efficacy of such “nanobins” was explained by us by the existence of the equilibrium inside a nanoparticle [14] between I, phosphatidylcholine and cisplatin that is shown on the Figure.

As it was shown for the dirhenium (III) compound with *cis*-dicarboxylate structure [14], phosphatidylcholine moieties from the lipid shell substituted axial positions and cisplatin may substitute carboxylic groups. In the case with I, this substitution may take place with the formation of several adducts mono-, di-, three- and even four-substituted products. Such equilibrium inside a liposome consisting from I, phosphatidylcholine and cisplatin (or

Table 3. Decreasing of the ceruloplasmin concentration in serum after Re-Pt system introduction in comparison to T8 and T8* groups without or with cPt applying

Number of the group	Characteristic of the group	To T8, %	To T8+cPt in appropriate form, %
8	T8+[I] nl+cPt sl	17.0 ± 2.4	32.4 ± 3.3
9	T8+[I+cPt(4:1)] nl	37.0 ± 5.4	85.5 ± 2.7
10	T8+[I+cPt(4:1)] np	54.0 ± 2.8	–
11	T8*+[I] nl+cPt sl	72.4 ± 2.2	62.1 ± 4.3
12	T8*+[I+cPt(4:1)] nl	80.0 ± 1.4	79.9 ± 3.1
13	T8*+[I+cPt(4:1)] np	78.5 ± 3.0	–



The reaction I with cisplatin and phosphatidylcholine which possibly may pass in the liposome

hydrolyzed cisplatin) gives to the inner complex additional chemical potential that facilitates interaction with DNA and results in synergistic or additive effect on DNA adducts formation [14].

In this work, we showed that cisplatin drug resistance might be overcome by the simultaneous introduction of a rhenium cluster compound and cisplatin in different nano-forms. Successful tumor growth inhibition was followed by decreasing of Cp level and POL intensity in plasma that opens new aspects of the influence of rhenium quadruple bonding compounds on the copper homeostasis of tumor-bearing animals. We have demonstrated that this type of resistance is highly bound with the level of Cp that shows possible intervention procedures. In light of the complexity of cPt drug resistance, to enhance the overall outcome in cancer chemotherapy, it is imperative that other resistance mechanisms have to be addressed as well.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

РІВЕНЬ ЦЕРУЛОПЛАЗМІНУ В КРОВІ ЩУРІВ-ПУХЛИНОНОСІЇВ ЗА ВВЕДЕННЯ СПОЛУКИ ДИРЕНІЮ (III) ТА ЦИСПЛАТИНУ

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Сучасні дослідження механізмів резистентності до платинидів показали вирішальну роль метаболізму міді в онкопацієнтів та обґрунтували методи подолання резистентності шляхом модуляції транспортних механізмів міді. Виходячи з цього, визначення рівня церулоплазміну (Cp) – основного мідьвмісного протеїну крові в експериментах з тваринами-пухлиноносцями за

введення цитостатиків є важливим і актуальним. Концентрацію Ср вимірювали в сироватці шурів-пухлиноносіїв зі звичайною (Т8) і резистентною до цисплатину (Т8*) карциномою Герена за введення цисплатину і сполуки диренію (III) з почверним зв'язком дихлоротетра- μ -ізобутиратодиренію (III) (I) у різних лікарських формах. Показано, що розвиток пухлин у тварин групи Т8 супроводжувався підвищенням концентрації Ср у 3,7 раза, а у тварин групи Т8* – більше ніж у 8 разів порівняно з інтактними тваринами, що підтверджує істотну роль Ср у формуванні явища резистентності. Введення цисплатину разом із I призводило до ефективного інгібування пухлинного росту в групах з Т8 і Т8*, що для групи Т8* є прикладом подолання резистентності до цисплатину. Зниження концентрації Ср у групах тварин Т8*, що отримували реній-платин, було істотнішим, ніж у групах Т8, що підкреслює важливість подальших досліджень диренієвих (III) сполук у моделях резистентного до цитостатиків канцерогенезу. Обговорюються деякі механізми регулювання гомеостазу міді та властивостей наноконструктив.

Ключові слова: церулоплазмін, кластерна сполука ренію(III), карцинома Герена, резистентність до цисплатину.

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