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CISPLATIN *IN VIVO* ACTION ON LIPID CONTENT IN CHROMATIN FROM RAT KIDNEY CELLS

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The content of total phospholipids and neutral lipids as well as of their individual fractions in chromatin preparations from rat kidney cells after *in vivo* action of the antitumor drug cisplatin was investigated. It was established that cisplatin treatment reduced the total quantity of chromatin phospholipids and neutral lipids by about 25% and 23% correspondingly. The diminution of total lipids quantity was accompanied by alterations in quantities of individual fractions of phospholipids and neutral lipids. These data demonstrate the high sensitivity of chromatin lipids metabolism to antitumor drug cisplatin action. It was supposed that the cisplatin antitumor action can be realized via quantitative changes of chromatin lipids, which are able to regulate the principal functions of cell nuclei.

Cisplatin – kidney – chromatin – phospholipids – neutral lipids

Հետազոտվել է հակաուռուցքային դեղամիջոց ցիսպլատինի in vivo ազդեցությունն առնետի երիկամի բջիջներից ստացված քրոմատինի պատրաստուկներում ֆոսֆոլիպիդների և չեզոք լիպիդների ընդհանուր քանակի, ինչպես նաև դրանց առանձին ֆրակցիաների պարունակության վրա: Ցույց է տրվել, որ ցիսպլատինի ազդեցության արդյունքում քրոմատինի ֆոսֆոլիպիդների և չեզոք լիպիդների ընդհանուր քանակը նվազում է համապատասխանաբար՝ 25% և 23%-ով: Լիպիդների ընդհանուր պարունակության նվազումն ուղեկցվում է ֆոսֆոլիպիդների և չեզոք լիպիդների առանձին ֆրակցիաների քանակական փոփոխությամբ: Տվյալները վկայում են հակաուռուցքային դեղամիջոց ցիսպլատինի նկատմամբ քրոմատինի լիպիդների մետաբոլիզմի բարձր զգայունության մասին: Ենթադրվում է, որ ցիսպլատինի հակաուռուցքային ազդեցությունն իրականացվում է քրոմատինի այն լիպիդների քանակական փոփոխությունների շնորհիվ, որոնք պատասխանատու են բջջի կորիզի հիմնական ֆունկցիաների կարգավորման համար:

Ցիսպլատին – երիկամ – քրոմատին – ֆոսֆոլիպիդներ – չեզոք լիպիդներ

Исследовалось содержание общих фосфолипидов и нейтральных липидов, а также их индивидуальных фракций в препаратах хроматина из клеток почки крыс при *in vivo* воздействии противоопухолевого препарата цисплатина. Установлено, что при обработке цисплатином сокращается общее количество фосфолипидов и нейтральных липидов хроматина соответственно на 25% и 23%. Убывание количества тотальных липидов сопровождается изменениями в содержании индивидуальных фракций фосфолипидов и нейтральных липидов. Результаты указывают на высокую чувствительность метаболизма липидов хроматина к действию противоопухолевого препарата цисплатина. Предполагается, что противоопухолевое действие цисплатина осуществляется посредством количественных изменений липидов хроматина, ответственных за регуляцию основных функций клеточного ядра.

Цисплатин – почка – хроматин – фосфолипиды – нейтральные липиды

Cisplatin (cis-diaminedichloroplatinum II) is widely used as an effective chemotherapeutic agent for treatment of various malignancies [6]. Cisplatin induces cytotoxicity by alterations of transcription, DNA replication processes, via induction of all pathways of apoptosis [4, 6]. However, it should be noted that cisplatin damages tumor cells as well as normal ones. The effectiveness of cisplatin is dose-depended, although its use in higher concentrations is limited because of several side effects, such as nephrotoxicity, neurotoxicity and others [9, 11,14].

It has been established, that the kidney cells can accumulate the higher effective concentration of cisplatin, than any other organ. This accumulation preferentially causes either apoptosis or necrosis, depending on exposure time and concentration. As in case of cisplatin induced neurotoxicity, nephrotoxicity is due to the production of reactive oxygen species (ROS), which interact with DNA, lipids and proteins [11, 14]. These interactions lead to lipid peroxidation, DNA molecule damages and eventually renal cell injury and death [9, 14].

Nowadays it is well known that nuclear lipids are important for regulating many essential cellular processes such as DNA replication, transcription and gene expression [1, 8, 13]. Recent advances demonstrated the involvement of nuclear lipids in remodeling of chromatin and epigenetic regulation of gene expression [5, 18].

Changes of nuclear lipid levels can induce the recruitment of chromatin remodeling proteins (histones and non-histone) which in turn may induce a conformational change of the proteins and their interactions with other molecules [5].

It was established that the quantity of nuclear lipids, including the chromatin bound ones, altered in dependence on nuclei functional activity [1, 5, 18]. Furthermore it was shown that lipids of chromatin can regulate the chromatin structure via alteration of some enzymes activity, which accomplished histone modifications as well as inverse processes [5]. It is impossible to exclude the significance of chromatin lipids quantitative alterations for cisplatin antitumor effects realization.

In this paper the alterations of quantities of total phospholipids and neutral lipids as well as changes of their individual fractions content in chromatin preparations from rat kidney cells after the cisplatin *in vivo* action were investigated.

Materials and methods. The experiments were carried out on albino rats (120-150 g weight). Cisplatin was injected peritoneal in concentration of 5 mg per 1000g animal weight. Rats were decapitated after 24 hours of cisplatin injection. Rat kidney nuclei were isolated by the method of Blobel and Potter [3]. The chromatin fraction from rat kidney cells nuclei was isolated by method of Umansky [17]. Lipid extraction was carried out by Bligh and Dyer [2]. The fractionation of phospholipids was carried out by micro thin layer chromatography (microTLC) using 6x9 sm^2 plates with L silicagel, and chloroform-methanol-H_2O in ratio 65:5:4 as development mixture. The same plates and diethyl ester-petroleum ester-formic acid in ratio 40:10:1 as a dividing mixture were used for the fractionation of chromatin neutral lipids by microTLC method.

After the chromatography the plates with fractionated lipids were dried up at 20^{o}C. Then, the plates with fractionated phospholipids were treated by solution of 15,6% $CuSO_4$ in 8% phosphoric acid and the plates with fractionated neutral lipids were treated by 10% H_2SO_4. The elaborated plates were heated at 180^{o}C for 15 minutes.

The quantitative estimation of separated and specific dyed phospholipids and neutral lipids was carried out by special computer program FUGIFILM Science Lab.2001 Image Gauge V 4, 0 which was destined for densitometry. Obtained data were treated by statistics.

Results and Discussion. Cisplatin *in vivo* action reliably decreases the total amounts of both phospholipids and neutral lipids in chromatin preparations from rat kidney cells by 25% and 22,6% correspondingly (tabl. 1, fig.1). Taking into consideration that

metabolism of chromatin lipids is regulated of that of the nuclei and the metabolism of nuclear lipids is regulated of that of the cytoplasm [1] one may conclude that antitumor agent leads to appreciable repression of whole lipid metabolism in kidney of rat cells.

Table 1. Total phospholipids and neutral lipids content (in mcg/g of tissue) in chromatin preparation of rat kidney cells in baseline and after *in vivo* treatment of cisplatin.

*-p < 0,05

Variants	Phospholipids of chromatin from rat kidney cells (mcg/g of tissue)	Neutral lipids of chromatin from rat kidney cells (mcg/g of tissue)
Baseline	288,00±6,35	190,00±4,20
Cisplatin	*216,00±5,48	*147,00±3,70

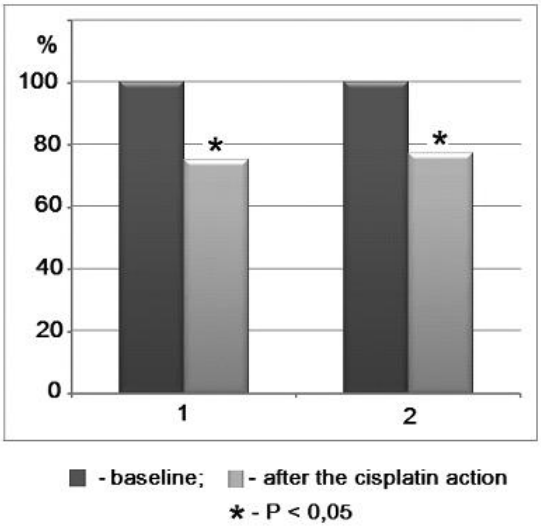

Fig. 1. Changes in of total phospholipids (1) and neutral lipids content (2) in chromatin preparation of rat kidney cells in baseline and after *in vivo* treatment of cisplatin.

The fractionation of chromatin phospholipids by the microTLC method revealed five individual phospholipids in baseline as well as after the cisplatin action. (tab. 2). Sphingomyelin, phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine and cardiolipin were obtained among the phospholipids of rat kidney cells chromatin preparations (tab. 2).

The relative content of revealed phospholipid fractions testified that phosphatidylethanolamine and phosphatidylcholine were the major components and formed about 58% of total phospholipids in rat kidney chromatin preparations (tab. 2). The percentage of sphingomyelin, phosphatidylinositol and cardiolipin was correspondingly 14,5%, 12% and 15% (tab. 2).

The fractionation of neutral lipids from chromatin of rat kidney cells disclosed four individual fractions both in baseline and after the cisplatin action. Cholesterol and free fatty acids together composed more than 70% of total neutral lipids, while the cholesterol esters and triglycerides were presented in approximately equal quantities (12,5% and 13,17% correspondingly) (tab. 3).

Table 2. The relative content (in percentage) of individual phospholipid fractions in chromatin preparations of rat kidney cells before and after the cisplatin action

*-p < 0,05

N	Phospholipids	Baseline	Cisplatin
		%	%
1	Sphingomyelin	14,50±0,35	15,70±0,60
2	Phosphatidylinositol	12,00±0,30	12,40±0,38
3	Phosphatidylcholine	32,50±0,58	30,80±0,44
4	Phosphatidylethanolamine	26,00±0,40	24,40±0,73
5	Cardiolipin	15,00±0,28	16,70±0,50
Total content		100	100

Table 3. The relative content (in percentage) of individual neutral lipid fractions in chromatin preparations of rat kidney cells before and after the cisplatin action.

*p < 0,05

N	Neutral lipids	Baseline	Cisplatin
		%	%
1	Cholesterol	37,63±0,74	36,70±0,48
2	Cholesterol esters	12,50±0,46	11,30±0,33
3	Free fatty acids	36,70±0,57	37,40±0,60
4	Triglycerides	13,17±0,37	14,60±0,39
Total content		100	100

The negligible changes were obtained in relative content of phospholipids individual fractions as well as that of neutral lipids fractions in rat kidney chromatin preparations after the cisplatin *in vivo* action (tab. 2 and 3). It means that cisplatin expressed rather universal affect on various metabolic pathways of lipids in chromatin and perhaps in whole nuclei. In order to discuss at length the revealed changes the absolute quantities of individual lipid fractions expressed in micrograms per gram of tissue were studied (tab. 4 and 5).

Table 4. The quantities (in micrograms per gram of tissue) of individual phospholipid fractions in chromatin preparations of rat kidney cells before and after the cisplatin action

*p < 0,05

N	Phospholipids	Baseline	Cisplatin
1	Sphingomyelin	41,76±1,00	*33,90±1,30
2	Phosphatidylinositol	34,56±0,86	*26,85±0,82
3	Phosphatidylcholine	93,60±1,07	*66,55±1,00
4	Phosphatidylethanolamine	74,88±1,15	*52,70±1,58
5	Cardiolipin	43,20±0,81	*36,00±1,10
Total content		288,00±6,35	*216,00±5.48

The quantities of all lipids (phospholipids and neutral lipids) fractions of rat kidney chromatin preparations were decreased reliably after the *in vivo* action of cisplatin (tab. 4 and 5). The most diminution of content among phospholipid fractions was observed in case of phosphatidylethanolamine and phosphatidylcholine: by 29,6% and 28,9% correspondingly (fig.2), which was more than the decrease of total phospholipid content (25.0%). The decreases of phosphatidylinositol, sphingomyelin and cardiolipin content (by 22,3%, 18,8% and 16,7% correspondingly) were less than those for chromatin total phospholipid (fig.2).

Table 5. The quantities (in micrograms per gram of tissue) of individual neutral lipid fractions in chromatin preparations of rat kidney cells before and after the cisplatin action.

*p < 0,05

N	Neutral lipids	Baseline	Cisplatin
1	Cholesterol	71,50±1,40	54,00±0,70*
2	Cholesterol esters	23,75±0,87	16,60±0,48*
3	Free fatty acids	69,75±1,08	55,00±1,75*
4	Triglycerides	25,00±0,70	21,40±0,57*
Total content		190,00 ±4,20	147,00±3,70*

In case of neutral lipids the most diminution of content was observed in cholesterol and its esters fractions (by 24.5% and 30,1% correspondingly) which was more than the decrease of total neutral lipids content (22,6%). The decreases of free fatty acids and especially of glycerides (21,1% and 14,4% correspondingly) were less than those for total neutral lipids (fig.2).

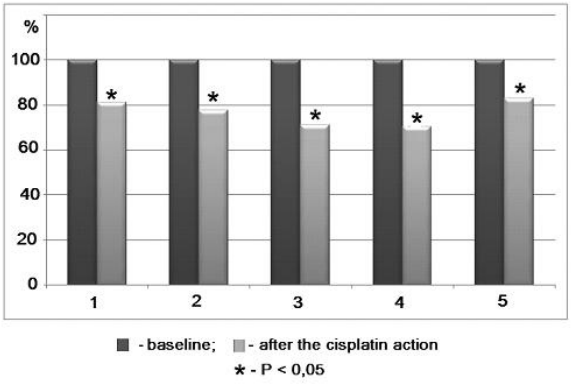

Fig. 2. Changes (in %) of absolute quantity of phospholipids individual fractions after the cisplatin action. 1 - sphingomyelin, 2 – phosphatidylinositol, 3 - phosphatidylcholine, 4 – phosphatidylethanolamine, 5 – cardiolipin

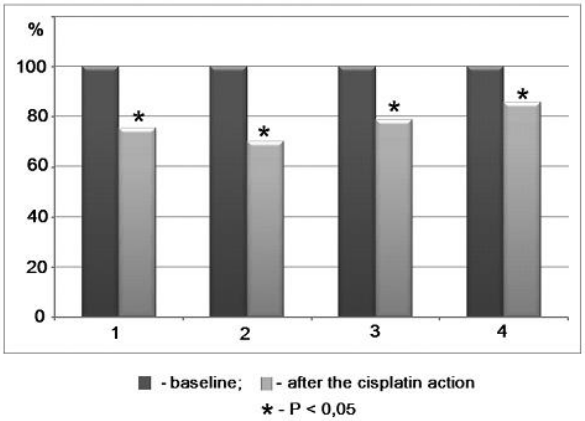

Fig. 3. Changes (in %) of absolute quantity of neutral lipids individual fractions after the cisplatin action. 1 – cholesterol, 2 - cholesterol esters, 3 - free fatty acids, 4 - triglycerides

Taking into consideration that cell nuclei is a site of lipids active metabolism one may conclude that these remarkable changes in absolute quantities of individual phospholipid fractions may be the consequence of cisplatin action on lipid metabolic

pathways in nuclei. These changes may offer some serious prerequisites for alteration the functioning those processes where these phosphilipids participate, regulate or act. It is known that phosphatidylethanolamine promotes the chromatin decondensation, induces transition of chromatin from solenoid to nucleosome, that DNA-bound cardiolipin can regulate DNA replication; alters the condensation of chromatin [12, 16]. It is known also that the quantitative ratio of sphingomyelin/phosphatidylcholine predicts the subsequent fate of cells [1, 8, 13] and the chromatin structure could be regulated by phosphoinositides via binding to the C-terminal tail of histone H1 [5]. Furthermore it is well known that sphingomyelin and phosphatidylinositol are the members of corresponding nuclear signaling systems [5, 16]. So, changes in quantities of phospholipid fractions in chromatin caused by cisplatin *in vivo* action may have a comprehensive influence which on the whole must promote the antineoplastic effects of the chemotherapeutic agent. One can draw a similar conclusion in case of alteration in content of individual neutral lipids.

Although the cisplatin action is specific in different tissues which is clearly seen in manifestations of various negative side effects including ototoxicity, gastrotoxicity, myelosuppression, allergic reactions [10, 15] and nephrotoxicity (as the main negative effect) [4, 9], the mention-above alterations of quantities of chromatin lipids in rat kidney as well as in rat liver, thymus [7, 8] and brain (unpublished data) cells, on the whole, are similar. This identity of cisplatin-depended DNA-bound lipids behavior in chromatin preparations from various tissues in all probability indicates that cisplatin displays its antitumor effect first of all via changes of chromatin lipids quantity.

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