Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

• Փորձարարական և տեսական հոդվածներ •Экспериментальные и теоретические статьи•
•Experimental and theoretical articles•

Biolog. Journal of Armenia, 3 (69), 2017

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

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The content of total phospholipids and neutral lipids as well as their individual fractions in nuclear matrix preparations from rat kidney cells after *in vivo* action of the antitumor drug cisplatin was investigated. It was revealed that cisplatin treatment has reduced the total quantity of nuclear matrix phospholipids and neutral lipids by about 25 % and 27 % 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 nuclear matrix lipids metabolism to antitumor drug cisplatin action. It was supposed that the cisplatin antitumor action also can be performed via quantitative changes of internuclear lipids, which are able to regulate the principal functions of cell nuclei.

Cisplatin – kidney – nuclear matrix – phospholipids-neutral lipids

Հետազոտվել է ընդհանուր ֆոսֆոլիպիդների և չեզոք լիպիդների, ինչպես նաև դրանց առանձին ֆրակցիաների քանակն առնետի երիկամների բջիջներից ստացված կորիզային մատրիքսի պատրաստուկներում հակաուռուցքային դեղամիջոց ցիսպլատինի *in vivo* ազդեցությունից հետո: Ցույց է տրվել, որ ցիսպլատինով մշակելու արդյունքում կորիզային մատրիքսի ֆոսֆոլիպիդների և չեզոք լիպիդների ընդհանուր քանակը նվազում է համապատասխանաբար՝ 25% և 27%-ով: Լիպիդների ընդհանուր պարունակության նվազումն ուղեկցվում է ֆոսֆոլիպիդների և չեզոք լիպիդների առանձին ֆրակցիաների քանակական փոփոխությամբ: Տվյալները ցույց են տալիս քրոմատինի լիպիդների մետաբոլիզմի բարձր զգայնությունը հակաուռուցքային դեղամիջոց ցիսպլատինի նկատմամբ: Ենթադրվում է, որ ցիսպլատինի հակաուռուցքային ազդեցությունն իրականացվում է նաև այն ներկորիզային լիպիդների քանակական փոփոխությունների շնորհիվ, որոնք պատասխանատու են բջջի կորիզի հիմնական ֆունկցիաների կարգավորման համար:

Ցիսպլատին – երիկամ – կորիզային մատրիքս – ֆոսֆոլիպիդներ – չեզոք լիպիդներ

Исследовалось содержание общих фосфолипидов и нейтральных липидов, а также их индивидуальных фракций в препаратах ядерного матрикса из клеток почек крыс при *in vivo* воздействии противоопухолевого препарата цисплатина. Показано, что при введении цисплатина сокращается общее количество фосфолипидов и нейтральных липидов ядерного матрикса соответственно на 25% и 27%. 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 также посредством количественных изменений внутриядерных липидов, ответственных за регуляцию основных функций клеточного ядра.

Цисплатин – почки – ядерный матрикс – фосфолипиды – нейтральные липиды

Our previous results showed the reliable changes in phospholipids and neutral lipids quantities in rat kidney chromatin preparations after the *in vivo* action of cisplatin. It was supposed that those changes may have a comprehensive influence which on the whole must promote the antineoplastic effects of the chemotherapeutic agent [9]. Taking into consideration that the nuclei, especially the chromatin, are the main targets for cisplatin action it was occurred to study the lipid quantity and quality changes in another intranuclear structure – in nuclear matrix. It is well known that the nuclear matrix is a salt-extracted biochemical fraction of the nucleus. Usually, the nuclear matrix is composed predominantly of non-histone proteins and small amounts of DNA, RNA, phospholipids and neutral lipids [15] and its participation in basic functions of nucleus is well known [15,17]. It is noted also that the phospholipids play an important role in tight binding of nuclear matrix with nucleic acids directly or via association with matrix non-histone proteins [1,17]. So, any alterations in lipid content in nuclear matrix may be significant performing of basic functions of nucleus: in the organization of nuclear DNA, DNA replication, transcription and RNA processing.

At the same time it was showed that the kidney cells can accumulate the higher effective concentration of cisplatin, than any other organ. It seems impossible to exclude the significance of nuclear lipids quantitative alterations for cisplatin antitumor effects. Simultaneously the cisplatin accumulation preferentially causes either apoptosis or necrosis, depending on exposure time and concentration. 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 [10, 12, 14].

In this paper the alterations of quantities of total phospholipids and neutral lipids as well as changes of their individual fractions content in nuclear matrix 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 1000 g animal weight. Rats were decapitated after 24 hours of cisplatin injection. Rat kidney nuclei were isolated by the method of Blobel and Potter [4]. Nuclear matrix preparations were isolated from purified nuclei by the method of Berezney and Coffey [2]. Lipid extraction was carried out by Bligh and Dayer [3]. The fractioning of both phospholipids and neutral lipids was carried out by micro thin layer chromatography (micro TLC) using L silicagel, 6x9 sm^2 plates with the thickness of layer 5-7 mcm, using chloroform – methanol – water in ratio 65:25:4 (in case of phospholipids) and diethyl ester – petroleum ester – formic acid in ratio 40:10:1 (in case of neutral lipids) as a dividing mixture. After the chromatography the plates were dried up at 20^0 C and were treated by 15.6 % $CuSO_4$ in 8 % phosphoric acid (in case of phospholipids) and by 10 % H_2SO_4 (in case of neutral lipids). Then the elaborated plates were heated at 180^0 C for 15 min. The quantitative estimation of separated and specific died phospholipids was carried out by special computer software FUGIFILM Science Lab 2001 Image Gauge V 4.0, which was destined for densitometry. Obtained results were treated by statistics.

Results and Discussion. Cisplatin *in vivo* action reliably decreases the total amounts of both phospholipids and neutral lipids in nuclear matrix preparations from rat kidney cells by 25 % and 27 % correspondingly (tab. 1, fig.1). Taking into consideration that of the same kind changes were also demonstrated in kidney chromatin preparations [1] one may conclude that antitumor agent leads to appreciable repression of whole lipid metabolism in nuclei of rat kidney cells.

Table 1. Total phospholipids and neutral lipids content (mcg/g of tissue) in nuclear matrix preparation of rat kidney cells in baseline and after *in vivo* treatment of cisplatin (*-p < 0.05)

Variants	**Phospholipids in nuclear matrix from rat kidney cells (mcg/g of tissue)**	**Neutral lipids in nuclear matrix from rat kidney cells (mcg/g of tissue)**
Baseline	85.50±1.90	73.50±1.90
Cisplatin	*64.00±1.40	*54.00±1.50

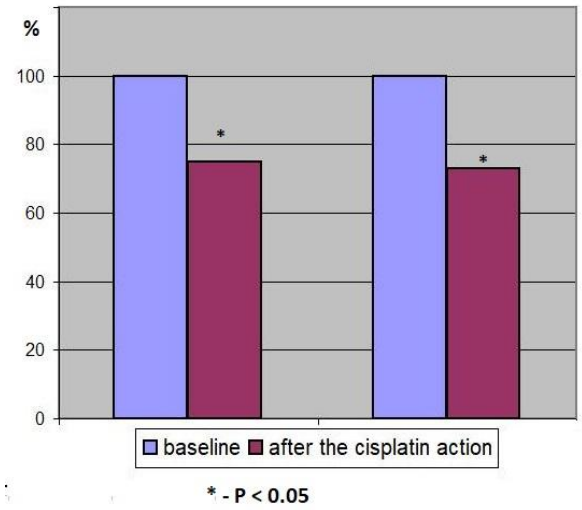

Fig. 1. Changes of total phospholipids (left diagrams) and neutral lipids content (right diagrams) in nuclear matrix preparation of rat kidney cells in baseline and after *in vivo* treatment of cisplatin.

The fractionation of nuclear matrix phospholipids by the microTLC method revealed five individual phospholipids in baseline as well as after the cisplatin action. 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 nuclear matrix preparations. The percentage of sphingomyelin, phosphatidylinositol and cardiolipin was correspondingly 18.20 %, 10.25 % and 13.30 % (tab. 2).

The fractionation of neutral lipids from nuclear matrix of rat kidney cells disclosed six individual fractions both in baseline and after the cisplatin action (tab. 3). In this sense the obtained results differ from those obtained in the other intranuclear structure – chromatin where only four fractions of neutral lipids were determined [9]. In nuclear matrix the main neutral lipid fractions are cholesterol and free fatty acids. They together composed more than 50 % of total neutral lipids, while the monoglycerides, cholesterol esters, triglycerides and diglycerides were presented in following quantities: 15,2 %, 13,4 %, 13,2 % and 8,00 % correspondingly (tab. 3).

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

N	Phospholipids	Baseline	Cisplatin
		%	%
1	Sphingomyelin	18.20±0.28	16.20±0.29
2	Phosphatidylinositol	10.25±0.37	12.50±0.26
3	Phosphatidylcholine	35.00±0.50	31.75±0.46
4	Phosphatidylethanolamine	23.25±0.66	28.25±0.54
5	Cardiolipin	13.30±0.40	11.30±0.62
Total content		100	100

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

N	Neutral lipids	Baseline	Cisplatin
		%	%
1	Cholesterol	27.60±0.86	30.00±0.35
2	Cholesterol esters	13.40±0.42	19.05±0.48
3	Free fatty acids	22.60±1.06	15.25±0.43
4	Triglycerides	13.20±0.62	10.70±0.19
5	Diglycerides	8.00±0.68	12.00±0.32
6	Monoglycerides	15.20±0.26	13.00±0.30
Total content		100	100

The perceptible changes were obtained in relative content of phospholipids individual fractions as well as that of neutral lipids fractions in rat kidney nuclear matrix 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 nuclear matrix 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).

The absolute quantities of all phospholipid individual fractions were decreased reliably after the *in vivo* action of cisplatin (tab 4). The most diminution of content among phospholipid fractions was observed in case of cardiolipin, sphingomyelin and phosphatidylcholine by 34,8 %, 34,0 % and 32,3 % correspondingly, which was more than the decrease of total phospholipid content (25.1 %). The decreases of phosphatidylethanolamine and phosphatidylinositol content (by 9,5 %, and 8,1 % correspondingly) were less than those for nuclear matrix total phospholipid (tab. 4).

Table 4. The quantities (micrograms per gram of tissue) of individual phospholipid fractions in nuclear matrix preparations of rat kidney cells before and after the cisplatin action. (*p < 0.05)

N	Phospholipids	Baseline	Cisplatin
1	Sphingomyelin	15.60±0.25	*10.30±0.18
2	Phosphatidylinositol	8.70±0.31	*8.00±0.17
3	Phosphatidylcholine	30.00±0.43	*20.30±0.43
4	Phosphatidylethanolamine	20.00±0.57	*18.10±0.35
5	Cardiolipin	11.20±0.34	*7.30±0.40
Total content		85.5±1.90	*64.00±1.40

In case of neutral lipids we observe diverse quantitative changes of them. Thus, the diminution of content after the cisplatin action was observed only in four fractions: in free fatty acids (by 50,4 %), in triglycerides (by 40,2 %), in monoglycerides (by 37,3 %) and in cholesterol (by 20,2 %), while in diglycerides and in cholesterol esters negligible and not reliable increase of content was revealed (by 10,0 % and by 4,6 % correspondingly) (tab. 5). These alterations confirm that cisplatin *in vivo* action leads to perceptible redistribution between the mono-, di- and triglycerides as well as between the cholesterol and its esters in kidney nuclear matrix.

Table 5. The quantities (micrograms per gram of tissue) of individual neutral lipids fractions in nuclear matrix preparations of rat kidney cells before and after the cisplatin action (*p < 0.05)

N	**Neutral lipids**	**Baseline**	**Cisplatin**
1	Cholesterol	20.30±0.65	*16.20±0.35
2	Cholesterol esters	9.85±0.30	10.30±0.48
3	Free fatty acids	16.60±0.78	*8.24±0.43
4	Triglycerides	9.70±0.46	*5.80±0.35
5	Diglycerides	5.88±0.20	6.46±0.15
6	Monoglycerides	11.17±0.20	*7.00±0.30
Total content		73,50±1,90	54.00±1.50

Diminution of phospholipids and neutral lipids content in kidney nuclear matrix is consonant with lipid content changes in kidney chromatin showed previously [9]. This indicates the comprehensive action of cisplatin on lipid metabolism in intranuclear structures which may offer some serious prerequisites for alteration the functioning those processes where these lipids participate, regulate or act [5, 13, 18]. 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 [11, 16] and nephrotoxicity (as the main negative effect) [14, 16], the mention-above alterations of quantities of intranuclear lipids in rat kidney as well as in rat liver, thymus [6, 8] and brain [7] cells, on the whole, are similar. Such identity of cisplatin-depended lipids behavior in intranuclear structures from various tissues in all probability indicates that cisplatin displays its antitumor effect also via changes of intranuclear lipids quantity.

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Received on 15.05.2017