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 (70), 2018

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

E.S. GEVORGYAN, ZH.V. YAVROYAN, N.R. HAKOBYAN, A.G. HOVHANNISYAN, E.G. SARGSYAN

Yerevan State University, Department of Biophysics, gevorgyan_emil@yahoo.com

The content of total phospholipids and neutral lipids as well as their individual fractions in nuclear matrix preparations from rat brain cells after *in vivo* action of the antitumor drug cisplatin was investigated. It was revealed that cisplatin administration reduced the total quantity of nuclear matrix phospholipids and neutral lipids by about 28% and 25% respectively. 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 lipids metabolism in nuclear matrix preparations to antitumor drug cisplatin action. It was supposed that the cisplatin antitumor action can be performed also via quantitative changes of internuclear lipids, which are able to regulate the principal functions of cell nuclei.

Cisplatin – brain – nuclear matrix – phospholipids – neutral lipids

Առնետի գլխուղեղի բջիջների կորիզային մատրիքսում ուսումնասիրվել է ընդհանուր ֆոսֆոլիպիդների և չեզոք լիպիդների, ինչպես նաև դրանց առանձին ֆրակցիաների պարունակությունը հակաուռուցքային դեղամիջոց ցիսպլատինի *in vivo* ազդեցության ժամանակ: Ցույց է տրվել, որ կենդանիներին ցիսպլատինի ներարկումը բերում է կորիզային մատրիքսի ֆոսֆոլիպիդների և չեզոք լիպիդների ընդհանուր քանակի նվազման՝ համապատասխանաբար մոտ 28 % և 25 %-ով: Ընդհանուր լիպիդների քանակի այդ նվազումն ուղեկցվում է ֆոսֆոլիպիդների և չեզոք լիպիդների առանձին ֆրակցիաների քանակական փոփոխություններով: Ստացված արդյունքները վկայում են հակաուռուցքային դեղամիջոց ցիսպլատինի նկատմամբ կորիզային մատրիքսի լիպիդների մետաբոլիզմի ունեցած բարձր զգայունության մասին: Ենթադրվում է, որ ցիսպլատինի հակաուռուցքային ազդեցությունը կարող է իրականացվել նաև ներկորիզային լիպիդների քանակական փոփոխությունների ուղիով, որոնք կարող են ներգործել բջջակորիզի հիմնական կարգավորիչ գործառույթների վրա:

Ցիսպլատին – գլխուղեղ – կորիզային մատրիքս –ֆոսֆոլիպիդներ – չեզոք լիպիդներ

Исследовалось содержание общих фосфолипидов и нейтральных липидов, а также их индивидуальных фракций в препаратах ядерного матрикса из клеток головного мозга крыс при *in vivo* воздействии противоопухолевого препарата цисплатина. Установлено, что при обработке цисплатином сокращается общее количество фосфолипидов и нейтральных липидов ядерного матрикса соответственно на 28 % и 25 %. Убывание количества тотальных липидов сопровождается изменениями в содержании индивидуальных фракций фосфолипидов и нейтральных липидов. Данные указывают на высокую чувствительность метаболизма липидов ядерного матрикса к действию противоопухолевого препарата цисплатина. Предполагается, что противоопухолевое действие цисплатина осуществляется посредством количественных изменений липидов ядерного матрикса, ответственных за регуляцию основных функций клеточного ядра.

Цисплатин – головной мозг – ядерный матрикс – фосфолипиды – нейтральные липиды

Previous results showed the reliable changes in phospholipids and neutral lipids quantities in nuclear structures, namely nuclear membranes, chromatin and nuclear matrix of rat different tissues after the administration of widely used antitumor agent cisplatin. Those changes were demonstrated in rat liver and thymus cells as well as in rat kidney and brain cells [4-8]. In this paper we complete the series of articles on the same topic bringing the results of studies about the alterations of quantities of total phospholipids and neutral lipids as well as about the changes of their individual fractions content in nuclear matrix preparations from rat brain cells after the cisplatin *in vivo* action.

Materials and methods. The experiments were carried out on albino rats (120-150g weight). Cisplatin was injected peritoneal in concentration of 5 mg per 1000g animal weight. Rats were decapitated after 24 hours of cisplatin injection. Rat brain nuclei were isolated by the method of Blobel and Potter [3]. Nuclear matrix preparations were isolated from purified nuclei by the method of Berezney and Coffey [1]. Lipid extraction was carried out by Bligh and Dayer [2]. The fractionating of both phospholipids and neutral lipids was carried out by micro thin layer chromatography (micro TLC) using L silicagel, 6x9 sm^2 plates, 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 dividing mixtures. After the chromatography the plates were dried up at 20^0C 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 180^0C 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. It is well known that the nuclear matrix is a salt-extracted biochemical intranuclear fraction of the nucleus which composed predominantly of non-histone proteins and small amounts of DNA, RNA, phospholipids and neutral lipids [12]. Its participation in basic functions of nucleus is also well known [12-14], so some changes in its composition may influence on its functioning. Lipid quantitative changes provoked by cisplatin may be bound up with antitumor effects of drug. At the same time the higher concentrations of cisplatin may also promote the drug negative toxic effects [9-11]. So, cisplatin *in vivo* action on intranuclear lipids quantity may be of some importance.

Cisplatin *in vivo* action reliably decreases the total amounts of both phospholipids and neutral lipids in nuclear matrix preparations from rat brain cells by 27.5% and 25%

Table 1. Total phospholipids and neutral lipids content (in mcg/g of tissue) in nuclear matrix preparations of rat brain cells in baseline and after *in vivo* treatment of cisplatin

Variants	Phospholipids in nuclear matrix from rat brain cells, mcg/g of tissue	Neutral lipids in nuclear matrix from rat brain cells, mcg/g of tissue
Variants	Phospholipids in nuclear matrix from rat brain cells, mcg/g of tissue	Neutral lipids in nuclear matrix from rat brain cells, mcg/g of tissue
Baseline	83.00±2.60	56.00±1.90
Cisplatin	*60.00±2.20	*42.00±2.23

*-$p < 0.05$

Taking into consideration that of the same kind changes were also demonstrated in brain nuclear membrane and chromatin preparations one may conclude that antitumor agent leads to appreciable repression of whole lipid metabolism in nuclei of rat brain cells.

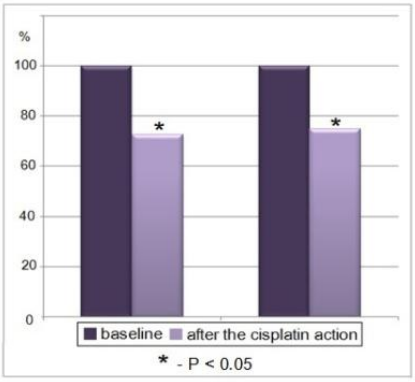

Fig. 1. Changes in total phospholipids (left diagrams) and neutral lipids content (right diagrams) in nuclear matrix preparations of rat brain cells in baseline and after *in vivo* treatment of cisplatin

The diminution of total lipids quantity was the consequence of diversified changes in relative content of both individual phospholipids and different neutral lipid fractions in nuclear matrix preparations of rat brain cells. In spite of trifling amount of total lipids separated in nuclear matrix preparations six individual fractions of phospholipids and three fractions of neutral lipids were revealed which was sufficient unexpected. The percentage of some of them was appreciably changed after the cisplatin action. Thus, the most significant changes in relative content among phospholipids fractions were observed in case of Sphingomyelin and Cardiolipin (an addition by 5.7% and a diminution by 6.8% respectively) (tab. 2).

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

N	Phospholipids	Baseline	Cisplatin
		%	%
1	Sphingomyelin	15.40±0,53	21.12±0.88
2	Phosphatidylinositol	11.00±0,52	11.52±0.39
3	Phosphatidylcholine	27.70±0.11	30.33±0.73
4	Phosphatidylethanolamine	24.00±0.61	22.72±0.97
5	Cardiolipin	18.70±0.50	11.94±0.54
6	Phosphatidic acid	3.20±0.27	2.37±0.22
Total content		100	100

In case of neutral lipids the significant addition in relative content was determined in fraction of Total Glycerides (nearly by 13%) while in two other fractions the diminution in relative content was observed (tab. 3). These diversified changes in relative content of different phospholipid and neutral lipid fractions testify the possibility of alteration in their absolute content.

Taking into consideration these perceptible changes in relative content of individual phospholipid and neutral lipid fractions in rat brain nuclear matrix preparations one may presume the diversified changes in their absolute quantities. In fact, the following tables (tab. 4 and tab. 5) demonstrate such changes in absolute quantities of separate lipid fractions.

Table 3. The relative content (in percentage) of different neutral lipid fractions in nuclear matrix preparation of rat brain cells in baseline and after the *in vivo* treatment of cisplatin

N	Neutral lipids	Baseline	Cisplatin
		%	%
1	Total Glycerides (mono-, di-, tri-)	25.60±2.20	38.42±2.24
2	Cholesterols and Cholesterols esters	29.80±2.14	24.08±1.85
3	Free fatty acids	44.60±2.11	37.50±1.23
Total content		100	100

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

N	Phospholipids	Baseline	Cisplatin
1	Sphingomyelin	12.78±0.44	12.67±0.53
2	Phosphatidylinositol	9.13±0.43	*6.90±0,23
3	Phosphatidylcholine	23.00±0.60	*18.20±0,44
4	Phosphatidylethanolamine	19.92±0.51	*13.65±0,58
5	Cardiolipin	15.52±0.40	*7.16±0,32
6	Phosphatidic acid	2.65±0.23	*1.42±0.13
Total content		83.00±2.60	*60.00±2.20

*-p < 0.05

Table 5. The absolute quantities (in micrograms per gram of tissue) of different neutral lipid fractions in nuclear matrix preparation of rat brain cells before and after the cisplatin action

N	Neutral lipids	Baseline	Cisplatin
1	Total Glycerides (mono-, di-, ri-)	14.31±0.48	16.14±0.52
2	Cholesterols and Cholesterol esters	16.69±0.59	*10.11 ±1.72
3	Free fatty acids	25.00±1.18	*15.75±0.52
Total content		56.00±1.90	*42.00±2.23

*-p < 0.05

The diminution of content among individual phospholipids was observed in case of five fractions with the exception of sphingomyelins. The most decrease in quantity was observed in case of cardiolipins (by 53.9%), phosphatidic acids (by 46.4%) and phosphatidylethanolamines (by 31.5%). These diminutions of content were more than the decrease of total phospholipids while the decreases of two other fractions (phosphatidylinositols and phosphatidylcholines) were less than the content of nuclear matrix total phospholipids (tab. 4). In case of neutral lipids cisplatin treatment led to certain increase in total glycerides content while the absolute quantity of cholesterols together with their esters was decreased by 40% and that of free fatty acids – by 37% (tab. 5).

These results are consistent with the previous data concern the cisplatin action on lipid content in nuclei and in its different structures (nuclear membrane, chromatin and nuclear matrix) from rat liver, thymus, kidney and brain cells though the content diminution of various phospholipids and neutral lipids fractions of course was sufficient different [4-8]. In all probability this may be explained by the specificity of lipid metabolism in nuclei of various tissues. Changes in content of different phospholipids and neutral lipids fractions caused by cisplatin *in vivo* action may play a definite role in

functioning of a number of pathways in nuclei (such as phosphoinositide or other regulatory pathways) where these lipids are participated. It seems that these alterations in nuclear lipid metabolic pathways should be considered particular negative side effects during the basic antitumor effect of cisplatin.

REFERENCES

1. *Berezney R., Coffey D.S.* The nuclear protein matrix: Isolation, structure and functions Adv. Enzyme Regul., *14*, 63-100, 1976.
2. *Bligh E.G., Dyer W.Y.* A rapid method of total lipid extraction and purification. "Can. J. Biochem. Physiol.", *37*, 911-917, 1959.
3. *Blobel G., Potter V.R.*, Nuclei from rat liver: Isolation method that combines purity with high yield. "Science", *154*, 76-79, 1966.
4. Gevorgyan E. S., Yavroyan Zh. V., Hovhannisyan A. G., Hakobyan N.R. Action of Cisplatin on Phospholipid Content in Rat Liver and Thymus Chromatin. "Electronic Journal of Natural Sciences", *19*, 2, 3-6., 2012.
5. *Gevorgyan E.S., Hovhannisyan A.G., Yavroyan Zh.V., Hakobyan N.R.* Content of Neutral Lipids in Rat Liver and Thymus Chromatin under the in vivo Action of Cisplatin "Biological Journal of Armenia", *64*, 3, 97-101, 2012.
6. *Gevorgyan E.S.,Yavroyan Zh.V.,Hovhannisyan A.G.,Hakobyan N.R.,Sargsyan E.G.* Cisplatin in vivo action on lipid content in chromatin from rat kidney cells. "Biological Journal of Armenia", *3*, 68, 12-18, 2016.
7. *Gevorgyan E.S., Yavroyan Zh.V., Hakobyan N.R., Hovhannisyan A.G.* Cisplatin *in vivo* influence of lipid content of chromatin on rat brain cells. "Proceedings of the Yerevan State University", *51*, 1, 21-26, 2017.
8. *Gevorgyan E.S., Yavroyan Zh.V., Hakobyan N.R., Hovhannisyan A.G., Sargsyan E.G. In vivo* action of cisplatin on lipid content in nuclear membranes from rat brain cells. Biolog. Journal of Armenia, *70*, 1, 16-21, 2018.
9. *Hanigan M. H., Devarajan P.* Cisplatin nephrotoxicity: molecular mechanisms. "Cancer Therapy", 1, 47-61, 2003.
10. *Hashem R.M., Safwar G.M., Rashed L.A., Bakry S.* Biochemical findings on cisplatin-induced oxidative neurotoxicity in rats. "International journal of Advanced Research", *3*, Issue 10, 1222-1234, 2015.
11. *Miller R.P., Tadagavadi R.K., Ramesh G., Reeves W.B.* Mechanisms of cisplatin Nephrotoxicity. "Toxins", 2, 2490-2518, 2010.
12. *Samuel Sh.K., Spencer V.A., Bajno L., Sun J.-M., Holt L.T., Oesterreich S., Davie J.R.* In situ cross-linking by cisplatin of nuclear matrix-bound transcription factors to nuclear DNA of human breast cancer cells. "Cancer Research", *58*, 3004-3008, 1998.
13. *Struchkov V.A., Strazhevskaya N.B.* Structural and functional aspects of nuclear lipids in normal and tumor cells. "Biochemistry" (Moscow), *65*, 5, 620-643, 2000.
14. *Wilson R.H.C., Coverley D.* Relationship between DNA replication and the nuclear matrix. "Genes to Cells", 18, 17-31, 2013.

Received on 19.04.2018