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IN VIVO ACTION OF CISPLATIN ON LIPID CONTENT IN NUCLEAR MEMBRANES FROM RAT BRAIN CELLS

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The *in vivo* action of antitumor drug cisplatin on content of phospholipids and neutral lipids in nuclear membrane fraction from rat brain cells has been investigated. It was shown, that the drug action leads to decrease in total phospholipids and neutral lipids content by about 27.5% and 25% respectively. In spite of these significant changes of total lipids the alterations in relative percentage content of individual phospholipids as well as neutral lipids in nuclear membrane preparations were negligible. These results indicate that cisplatin excites universal changes in lipid metabolism in nuclear membranes appreciably reducing the absolute quantities almost of all individual phospholipid and neutral lipid fractions available in nuclear membranes preparations. The significance of these quantitative changes in development of cisplatin antitumor effects was discussed.

Cisplatin – nuclear membrane – phospholipids – neutral lipids

Հետազոտվել է ֆոսֆոլիպիդների և չեզոք լիպիդների քանակը առնետի ուղեղի բջիջներից ստացված կորիզային թաղանթի ֆրակցիայում հակաուռուցքային դեղամիջոց ցիսպլատինի *in vivo* ազդեցությունից հետո: Ցույց է տրվել, որ ցիսպլատինի ազդեցությունը նվազեցնում է ընդհանուր ֆոսֆոլիպիդների և չեզոք լիպիդների քանակը, համապատասխանաբար 27,5 % և 25 %-ով: Չնայած ընդհանուր լիպիդների այդ զգալի փոփոխություններին, առանձին ֆոսֆոլիպիդների, ինչպես նաև չեզոք լիպիդների հարաբերական տոկոսային պարունակության փոփոխությունները կորիզային թաղանթի պատրաստուկներում չնչին են: Նշված արդյունքները ցույց են տալիս, որ ցիսպլատինը կորիզային թաղանթում առաջացնում է նմանատիպ փոփոխություններ, զգալիորեն նվազեցնելով համարյա բոլոր առանձին ֆոսֆոլիպիդների և չեզոք լիպիդների ֆրակցիաների բացարձակ քանակները: Քննարկվում է այդ քանակական փոփոխությունների նշանակությունը ցիսպլատինի հակաուռուցքային ազդեցությունների դրսևորման հարցում:

Ցիսպլատին – կորիզային թաղանթ – ֆոսֆոլիպիդներ – չեզոք լիպիդներ

Исследовалось *in vivo* воздействие противоопухолевого препарата цисплатина на содержание фосфолипидов и нейтральных липидов во фракциях препаратов ядерной мембраны из клеток мозга крыс. Показано, что воздействие препарата приводит к уменьшению содержания тотальных фосфолипидов и нейтральных липидов соответственно на 27,5 % и 25 %. Несмотря на эти значительные изменения тотальных липидов, изменения в относительном процентном содержании отдельных фосфолипидов, также как и нейтральных липидов в препаратах ядерной мембраны, незначительны. Данные результаты показывают, что цисплатин приводит к однотипным изменениям в метаболизме липидов, значительно уменьшая абсолютные значения почти во всех фракциях отдельных фосфолипидов и нейтральных липидов в препаратах ядерной мембраны. Обсуждается значение данных количественных изменений в проявлении противоопухолевых эффектов цисплатина.

Цисплатин – ядерная мембрана – фосфолипиды – нейтральные липиды

It is well known that cisplatin (cis-diaminedichloroplatinum II) is an effective antitumor drug which is widely used in chemotherapeutic practice and reveals antineoplastic, cytotoxic, immune-modulator actions and induction of apoptotic pathways of the cell [7, 9, 11]. Cisplatin can overpass the hematoencephalic barrier and accumulate in neural cells after the first injection. The efficiency of this drug is dose-depended though its usage in higher concentrations is contraindicated because of diverse negative side effects, such as nephrotoxicity, ototoxicity, neurotoxicity and others [8, 12, 13, 20]. Peripheral neurotoxicity is one of the widespread side effects which develop in approximately 30-50 % of patients receiving cisplatin [3, 13]. The mechanism of neurotoxicity caused by cisplatin administration is unclear though it is obvious that the drug generates reactive oxygen species which interact with DNA, lipids and proteins. These interactions lead to lipid peroxidation, DNA molecule damages and eventually cell death [3, 13]. Taking into consideration that the cell nuclei is the main target for cisplatin action one may suppose the involvement of metabolic changes of nuclear lipids in manifestation of neurotoxicity. It is obvious that the large majority of nuclear lipids is available in nuclear membranes which plays significant role in nuclear functions of vital importance [1, 2]. Cisplatin may have an effect on lipid metabolism of nuclear membranes, even if on functioning of signal transduction pathways via the quantitative and qualitative alterations in their lipid content. So, the knowledge about the alterations in content of nuclear membrane lipids (phospholipids and neutral lipids) after *in vivo* action of cisplatin may contribute to better understanding antitumor action effects of this drug.

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 brain nuclei were isolated by the method of Blobel and Potter [6]. Nuclear membrane preparations were isolated from purified nuclei by the method of Berezney et al [4]. Lipid extraction was carried out by Bligh and Dayer [5]. 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 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. Cisplatin *in vivo* action reliably decreases the total amounts of both phospholipids and neutral lipids in nuclear membrane preparations from rat brain cells by 27.5 % and 25.0 % correspondingly (tab. 1, fig.1). It is characteristic that of the same kind changes were also demonstrated by us in brain chromatin preparation [14] as well as in nuclear membranes and intranuclear structures of other tissues [14-18]. All these results demonstrate that this antitumor agent leads to appreciable repression of whole lipid metabolism in rat cell nuclei.

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

Variants	Phospholipids in nuclear membrane from rat brain cells (mcg/g of tissue)	Neutral lipids in nuclear membrane from rat brain cells (mcg/g of tissue)
Baseline	462.00±9.20	300.00±3.15
Cisplatin	*335.00±7.63	*225.00±3.50

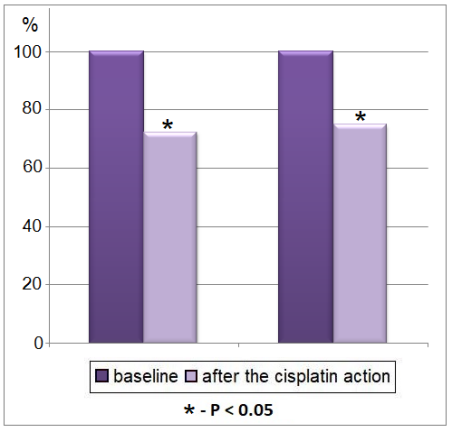

Fig. 1. Changes in percent of total phospholipids (left diagrams) and neutral lipids (right diagrams) content in nuclear membrane preparations of rat brain cells in baseline and after *in vivo* treatment of cisplatin

The fractionation of nuclear membrane phospholipids revealed seven individual fractions in baseline and after the cisplatin action. Phosphatidylcholine and phosphatidylethanolamine are the major fractions among them, their portion jointly was near 49 %, while the percentage content other five fractions jointly is a little more than half 51 % (tab. 2). Cisplatin treatment essentially changed the relative content of individual phospholipid fractions: the content of five fractions was increased a little while the content of two of them (phosphatidic acid and cardiolipin) was reliable decreased (tab. 2).

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

N	Phospholipids	Baseline	Cisplatin
		%	%
1	Phosphatidylserine	10,10±0,59	12,25±0,24
2	Sphingomyelin	13,82±0,70	14,55±0,34
3	Phosphatidylinositol	8,74±0,26	12,05±0,26
4	Phosphatidylcholine	27,50±0,84	28,00±0,57
5	Phosphatidylethanolamine	21,20±1,50	22,00±0,52
6	Cardiolipin	10,44±0,80	5,75±0,24
7	Phosphatidic acid	8,20±0,78	5,40±0,26
	Total content	**100**	**100**

The fractionation of neutral lipids demonstrated the presence of six individual fractions in baseline as well as after the cisplatin action (tab. 3). As in case of phospholipids, cisplatin treatment lead to diversified changes in relative content of individual neutral lipid fractions. The relative content of two of them (cholesterol and triglycerides) was increased in percentage, for the other two (cholesterol esters and free fatty acids) – was decreased while the relative content of monoglycerides and diglycerides was not reliably changed (tab. 3).

It is obvious that the obtained changes in percentage content do not represent the reality of alteration in real content of both phospholipid and neutral lipid individual fractions after the cisplatin action. In order to clear up this problem the absolute quantities of individual lipids (in micrograms per gram of brain tissue) in nuclear membrane preparations before and after the cisplatin action were determined.

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

N	Neutral lipids	Baseline	Cisplatin
		%	%
1	Monoglycerides	12,00±1,26	11,86±1,00
2	Diglycerides	11,20±0,79	11,50±1,67
3	Cholesterol	25,60±1,81	32,60±1,35
4	Cholesterol esters	16,20±1,00	13,60±0,60
5	Free fatty acids	26,00±0,70	18,66±1,70
6	Triglycerides	9,00±1,30	11,78±1,34
Total content		**100**	**100**

The absolute quantities of six phospholipid individual fractions were decreased reliably while the phosphatidylinositol quantity was not changed after the *in vivo* action of cisplatin (tab. 4). The most diminution of content among phospholipid fractions was observed in case of cardiolipin and phosphatidic acid by 60,0% and 52,2% correspondingly, which was much more than the decrease of total phospholipid content (27,1%). The decreases of choline-hold phospholipids sphingomyelin and phosphatidylcholine as well as phosphatidylethanolamine content (23-27%) were as big as the decrease of total phospholipids content (tab. 4). In all probability the latter results testify that in brain cell nuclear membranes the cisplatin *in vivo* action has no specific affect concerning the metabolic pathways where those three phospholipids have a share. In spite of that the most notable results were demonstrated in case of phosphatidylinositol which share in phospholipid total amount was reliably increased (by 3,31%) (tab. 2). This indicates that cisplatin probably may affect on functioning of phosphoinositide regulatory circle which is known exists in nuclei and which has been widely described [19, 21]. The absence of alteration of absolute quantity of phosphatidylinositol against the background of diminution of other individual phospholipids content after the cisplatin action may be elucidated by cisplatin ability to affect on phosphoinositide regulatory circle via increasing the monophosphoinositide: triphosphoinositide ratio. At the same time the significant diminution of cardiolipin and phosphatidic acid content boldly confirms the cisplatin influence on phospholipid metabolic pathways in nuclei of brain cells (tab. 4).

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

N	Phospholipids	Baseline	Cisplatin
1	Phosphatidylserine	46,70±1,65	*41,04±0,80
2	Sphingomyelin	63,85±2,00	*48,74±1,07
3	Phosphatidylinositol	40,37±1,80	40,36±0,87
4	Phosphatidylcholine	127,00±3,00	*93,80±1,90
5	Phosphatidylethanolamine	98,00±1,63	*73,70±1,32
6	Cardiolipin	48,23±2,95	*19,26±0,80
7	Phosphatidic acid	37,85±2,00	*18,10±0,87

The similar situation was observed in case of neutral lipids: the absolute quantity of four fractions was reliably decreased in a different extent while diminution of the amounts of triglycerides and cholesterol was not reliable. (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 brain nuclear membrane (tab. 5).

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

N	Neutral lipids	Baseline	Cisplatin
1	Monoglycerides	36,00±0,45	*26,70±2,25
2	Diglycerides	33,60±0,27	*25,85±1,94
3	Cholesterol	76,80±2,16	73,35±3,00
4	Cholesterol esters	48,60±3,00	*30,60±1,35
5	Free fatty acids	78,00±2,10	*42,00±1,95
6	Triglycerides	27,00±1,98	26,50±1,74

The obtained results demonstrate their accordance with our previous data concerning the diminution of phospholipids and neutral lipids content in rat brain chromatin [14] as well as in different nuclear structures from rat liver, thymus and kidney cells [14-16, 21]. This indicates the comprehensive action of cisplatin on lipid metabolism in various nuclear structures from different tissues. Although the cisplatin action is specific in different tissues which is clearly seen in manifestations of various negative side effects including nephrotoxicity (as the main negative effect), neurotoxicity, ototoxicity, gastrotoxicity, myelosuppression, allergic reactions etc. The alterations of quantities of nuclear lipids in various rat tissues, on the whole, are similar. In all probability this similarity indicates that cisplatin displays its antitumor effects also via changes of nuclear lipids quantity and these alterations are not directly connected with toxic effects of the drug. So, all these results demonstrate the deep and multiform transformation of lipid metabolism in nuclei caused by cisplatin *in vivo* action.

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