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CISPLATIN AND ESTRADIOL JOINT ACTION ON PHOSPHOLIPID COMPOSITION OF NUCLEAR FRACTION FROM FEMALE RAT BRAIN CELLS

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The *in vivo* action of antitumor drug cisplatin and sex hormone estradiol on content of total phospholipids and their individual fractions in nuclei from rat brain cells was investigated. It was shown that these drugs manifested an opposite effect of decreasing (in case of cisplatin) and increasing (in case of estradiol) the total phospholipid content while the content of individual phospholipids expressed distinct alterations. The joint action of these two drugs on the whole restored the baseline level of phospholipids content. The obtained results are discussed in terms of antagonistic effects of studied drugs.

Cisplatin – estradiol – nuclei from rat brain cells – phospholipid

Ուսումնասիրվել է հակաքաղցկեղային դեղամիջոց ցիսպլատինի և սեռական հորմոն էստրադիոլի *in vivo* ազդեցությունը առնետի գլխուղեղի բջջակորիզից անջատած ընդհանուր ֆոսֆոլիպիդների և դրանց առանձին ֆրակցիաների բաղադրության վրա: Ցույց է տրված, որ այդ նյութերը հակառակ ազդեցություն են ունենում՝ փոքրացնելով (ցիսպլատինի դեպքում) և մեծացնելով (էստրադիոլի դեպքում) ընդհանուր ֆոսֆոլիպիդների քանակը, մինչդեռ առանձին ֆոսֆոլիպիդների պարունակությունը ենթարկվում է տարաբնույթ փոփոխությունների: Այս երկու նյութերի համատեղ ազդեցությունը ընդհանուր առմամբ վերականգնում է ֆոսֆոլիպիդների բաղադրության ստուգիչի մակարդակը: Ստացված արդյունքները քննարկվում են ուսումնասիրվող նյութերի հակամարտ ազդեցության առումով:

Ցիսպլատին – Էստրադիոլ – առնետի գլխուղեղի բջջակորիզ – ֆոսֆոլիպիդ

Исследовано *in vivo* влияние противоопухолевого препарата цисплатина и полового гормона эстрадиола на содержание общих фосфолипидов и их отдельных фракций в ядрах клеток головного мозга крыс. Показано, что эти вещества имеют противоположное действие. Цисплатин уменьшает содержание, а эстрадиол, наоборот, повышает содержание общих фосфолипидов, а содержание отдельных его фракций изменяется по-разному. Совместное воздействие этих веществ в общем восстанавливает контрольный уровень содержания фосфолипидов. Полученные результаты обсуждаются в аспекте антагонизма действия изучаемых веществ.

Цисплатин – эстрадиол – ядра клеток головного мозга крыс – фосфолипид

More than two decades ago the increase of phospholipid content in some nuclear fractions (nuclear membranes, nuclear matrix and chromatin) of rat brain cells after the *in vivo* action of estradiol has been shown [1, 2]. The augmentation of total phospholipid

content was accompanied by distinct changes of individual phospholipid fractions, particularly by increasing the monophosphoinositides content and decreasing the content of triphosphoinositides or by alteration of ratio of choline-content phospholipids: sphingomyelin and phosphatidylcholine. Thus, estradiol *in vivo* action in concentrations and exposition that lead to activation of biosynthetic processes resulted to the appreciable metabolic alterations in rat brain cells [1, 2].

Simultaneously, our recent data showed that the *in vivo* action of antitumor drug cisplatin decreased the content of phospholipids and neutral lipids in nuclear membrane and chromatin fractions from rat brain cells [6, 7]. These results indicate that cisplatin excites universal changes in lipid metabolism in nuclear membranes and chromatin appreciably reducing the absolute quantities almost of all individual phospholipid and neutral lipid fractions available in preparations.

The opposite effects on phospholipid compositon of estradiol and cisplatin may have a definite interest bearing in mind that steroid hormones in some cases participate in chemotherapy work. Steroids can be used as part of treatment to help in destroying cancer cells and make chemotherapy more effective. They may help in reducing an allergic reaction of patients to certain drugs, in low doses they may be used as anti-sickness drugs to improve the appetite of patients. At the same time the sex steroids have a very important role reducing some negative side effects of cisplatin such as nephrotoxicity or neurotoxicity [8, 12]. So, these two drugs manifesting an opposite effects on nuclear lipid composition in rat brain cells simultaneously are useful in treating many types of cancer, in chemotherapy, expressing an identical effect. This was the main motive which causes us to study the joint action of cisplatin and estradiol on phospholipid composition of nuclear fraction from rat brain cells.

Materials and methods. The investigation was performed on adult female albino rats (120-150 g weight). The animals were divided into 4 groups. The group 1 was a control group of animals without treatment. Animals of group 2 and group 4 received a single dose of cisplatin (8 mg/kg). Cisplatin was injected peritoneal. Exposition time for cisplatin was 24 hours. The group 3 was treated with estradiol (200 mcg/kg, injected peritoneal). Exposition time for estradiol was 4 hours. Animals from the group 4 were received the same single dose of estradiol within 20 hours after the cisplatin injection (4 hours before decapitation). All animals were killed by decapitation through appropriate time. Then, animals were sacrificed, and the brain tissue was extracted from each group of animals and used for isolation of nuclei by the method of Blobel and Potter [4]. Phospholipids of brain nuclei were extracted by Bligh and Dayer [1]. The fractionation of phospholipids was performed by micro thin layer chromatography (microTLC) using 6x9 sm^2 plates with silicagel L and chloroform – methanol – water in ratio 65:25:4 as a dividing mixture. After the chromatography the plates were dried up at room temperature and were treated by 15.6 % $CuSO_4$ in 8 % phosphoric acid. 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 Fujifilm Science Lab 2001 Image Gauge V42, which was designed for densitometry. Obtained results were treated by statistics.

Results and Discussion. Cisplatin *in vivo* action reliably decreases the total amounts of phospholipids in nuclear fraction preparations from rat brain cells by 26.4 % (tab. 1, fig.1). On the contrary, the estradiol separate injection leads to increase in total amount of phospholipids in studied preparations by 28,6 % (tab. 1, fig.1). However the combined injection of cisplatin and estradiol restored the baseline value of phospholipids amount (tab. 1, fig.1). The results confirm that in case of separate action cisplatin and estradiol exhibit opposite effects on total amount of nuclear phospholipids, whereas in case of their joint action a cumulative effect was revealed.

Table 1.

Total phospholipids content (in mcg/g of tissue) in nuclear preparations of rat brain cells in baseline and after the cisplatin and estradiol separate and joint *in vivo* action.

Variants	phospholipids content mcg/g tissue
Baseline	1400.00±80.00
Cisplatin	*1030.00 ±62.00
Estradiol	*1800.00±42.00
Cisplatin+ Estradiol	1300.00±51.00

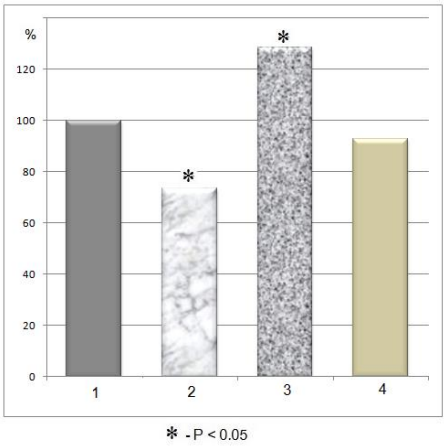

Fig. 1. Changes of total phospholipids content in nuclear preparation of rat brain cells after the cisplatin and estradiol separate and joint treatment.
1 – baseline, 2 – after the cisplatin separate action, 3 – after the estradiol separate action, 4 – after joint action of cisplatin and estradiol

The fractionation of total phospholipids from nuclear preparations of rat brain cells in baseline and after the cisplatin and estradiol separate and joint treatment showed the presence of seven individual phospholipids (fig. 2).

It should be noted that the major fractions in all studied groups were phosphatidylcholine and phosphatidylethanolamine (58.4-63.4 % of total phospholipid content). The relative content (in percentage) of all seven phospholipid individual fractions of four studied groups varied distinctly (tab. 2). Among the major fractions the changes of phosphatidylethanolamine relative content were more than that for phosphatidylcholine (the difference was 4.9 % vs. 3.3 %). The most appreciable changes among the minor phospholipid fractions were observed in case of phosphatidylinositol (the difference was 5.2 %) (tab. 2).

It is obvious that the obtained changes in percentage content do not represent the reality of alteration in real content of phospholipid individual fractions after the drugs action. In order to clear up this problem the absolute quantities of individual phospholipids (in micrograms per gram of brain tissue) in nuclear fraction preparations before and after the drugs injection were determined (tab. 3).

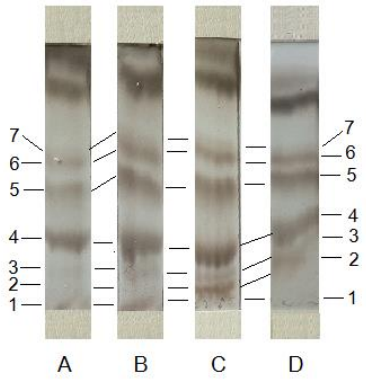

Fig. 2. Chromatograms of fractionation of phospholipids from rats brain nuclear fractions bymicroTLC method.
A – baseline; B – after the cisplatin separate action; C –after the separate action of estradiol;
D – after the cisplatin and estradiol joint action.
1 –phosphatidylserine, 2 – sphingomyelin, 3 – phosphatidylinositol,
4 – phosphatidylcholine, 5 – phosphatidylethanolamine, 6 – cardiolipin,
7 –phosphatidic acid

Table 2.

The relative content (in percentage) of individual fractions of phospholipids in nuclear preparations of rat brain cells in baseline and after the cisplatin and estradiol separate and joint action

N	Phospholipids	Baseline	Cisplatin injection	Estradiol injection	Cisplatin and Estradiol joint injection
1	Phosphatidylserine	6.4±0.7	4.5±0.3	5.2±0.3	5.7±0.1
2	Sphingomyelin	7.8±1.2	8.0±0.4	9.2±0.4	8.0±0.2
3	Phosphatidylinositol	9.5±0.3	7.6±0.3	6.3±0.4	4.3±0.2
4	Phosphatidylcholine	34.9±1.8	33.0±1.1	32.1±0.1	35.4±0.6
5	Phosphatidylethanolamine	23.5±1.5	27.9±0.7	28.4±0.6	28.0±0.6
6	Cardiolipin	11.5±1.8	12.0±0.6	13.6±0.4	13.5±0.3
7	Phosphatidic acid	6.4±0.8	7.0±0.6	5.2±0.3	5.1±0.2
	Total content	100	100	100	100

The results showed the reliable decreases in the content of all individual phospholipids after cisplatin injection. Moreover, the content of phosphatidylserine, phosphatidylinositol and phosphatidylcholine was diminished more than it of total phospholipids (tab. 3). These results are consequences of deep and multiform transformation of nuclear lipid metabolism caused by antitumor drug cisplatin and correspond to our previous data [6, 7].

In contrast to cisplatin action the estradiol treatment leads to diverse changes of individual phospholipids content. The quantity of four phospholipid fractions was significantly increased, the content of phosphatidylserine and phosphatidic acid was increased negligible while the phosphatidylinositol content was even decreased (tab. 3). These results all in all coincide with our previous data and confirm the activation of lipid metabolism by steroid hormone in nuclear fraction of rat brain cells [1].

Table 3.

The absolute quantities (in micrograms per gram of tissue) of individual phospholipid fractions in nuclear preparations of rat brain cells in baseline and after the cisplatin and estradiol separate and joint action.

Phospholipids	Baseline	Cisplatin injection	Estradiol injection	Cisplatin and Estradiol joint injection
Phosphatidylserine	90.00±3.13	*46.20±1.78	92.21±4.42	*73.45±1.82
Sphingomyelin	109.00±3.60	*82.40±2.00	*163.37±8.00	104.00±2.34
Phosphatidylinositol	134.00±2.12	*78.20±1.75	*111.52±6.11	*55.53±3.11
Phosphatidylcholine	487.00±4.26	*340.00±3.30	*566.40±1.87	460.35±7.27
Phosphatidylethanolamine	330.00±4.60	*287.00±2.60	*502.68±9.69	*364.00±4.10
Cardiolipin	160.00±4.00	*123.60±2.47	*240.72±7.31	*176.00±3.37
Phosphatidic acid	90.00±3.30	*72.60±2.53	93.10±4.76	*66.70±3.00

*-$p<0,05$

Cisplatin and estradiol joint action manifested the tendency of restoring the baseline content of all phospholipid fractions but in various measure (tab. 3). Thus, for example, the quantities of sphingomyelin and phosphatidylcholine were fully restored, while the quantities of cardiolipin and phosphatidylethanolamine differed from their baseline content only by 10 %. The quantity differences in case of phosphatidylserine, phosphatidic acid and phosphatidylinositol were much more (18 %, 26 % and 59 % correspondingly). These results demonstrate the antagonistic effects of cisplatin and estradiol concerning the lipid metabolic pathways in nuclear fractions of rat brain cells. It is characteristic that the only phospholipid fraction that decreased its quantity during both cisplatin and estradiol separate and joint action was phosphatidylinositol: the pivotal phospholipid of phosphoinositide signaling pathway. Thus the further study of quantity changes of polyphosphoinositides under the *in vivo* action of mentioned drugs seems to be much remarkable.

The mechanism of joint action of two biological active drugs is rather complex action especially if both cisplatin and estradiol are capable to provoke deep metabolic changes in brain cells. It is well known that lipid metabolism is regulated by multiple signaling pathways and generates a variety of bioactive lipid molecules [11]. Lipid metabolism participates in the regulation of many cellular processes such as cell growth, proliferation, differentiation, apoptosis, chemotherapy response and drug resistance etc. [10]. Therefore, the significance of the joint action of two drugs leading to deep differences in lipid metabolism is difficult to overestimate. At first site, the opposite to cisplatin action effect of estradiol seems to be negative concerning the cisplatin antitumor activity but at the same time it was shown that sex steroids are able to diminish some negative side effects of cisplatin [5, 9]. So, the joint action of cisplatin and steroid hormones must face to thorough investigation in various studies of diverse processes proceed in cell nuclei.

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