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CISPLATIN AND ESTRADIOL SEPARATE AND JOINT *IN VIVO* ACTION ON RAT LIVER NUCLEAR PHOSPHOLIPIDS CONTENT

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

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

The alterations in quantities of total phospholipids and changes in their individual fractions content in nuclear preparations from rat liver cells after the estradiol separate and combined with cisplatin *in vivo* action were investigated. It was shown that in contrast to cisplatin separate action, which leads to decrease the total amount of rat liver cells nuclear phospholipids, estradiol separate treatment increased phospholipids absolute quantity by 62 %. In the case of combined use of cisplatin and estradiol, the total quantity of nuclear phospholipids increased by about 13 %. These changes had different impact on the content of individual fractions of phospholipids. Among these changes the alterations obtained in case of cisplatin and estradiol combined treatment deserved greater attention since they most likely have a positive effect and may be helpful for reducing the cisplatin toxicity and eliminating its undesirable spillovers.

Cisplatin – estradiol – rat liver – phospholipids

Հետազոտվել են առնետի լյարդի կորիզային ֆոսֆոլիպիդների ընդհանուր պարունակությունը և դրանց առանձին ֆրակցիաների բացարձակ քանակական փոփոխությունները էստրադիոլի առանձին և ցիսպլատինի հետ համատեղ *in vivo* ազդեցությունից հետո: Պարզվել է, որ ի տարբերություն ցիսպլատինի ազդեցության, որը հանգեցնում էր առնետի լյարդի կորիզային ֆոսֆոլիպիդների ընդհանուր քանակի կրճատմանը, էստրադիոլի առանձին ազդեցության արդյունքում գրանցվում է ֆոսֆոլիպիդների բացարձակ պարունակության աճ 62 %-ով: Ցիսպլատինի և էստրադիոլի համատեղ կիրառման դեպքում ֆոսֆոլիպիդների ընդհանուր քանակն աճում է ընդամենը 13 %-ով: Գրանցված փոփոխությունները տարբեր կերպ են անդրադառնում առանձին ֆրակցիաների բացարձակ քանակների վրա: Առավել մեծ ուշադրության են արժանի ցիսպլատինի և էստրադիոլի համատեղ ազդեցության ժամանակ ստացված փոփոխությունները, որոնք, ամենայն հավանականությամբ, կարող են կարևոր լինել ցիսպլատինի տոքսիկության և անցանկալի կողմնային ազդեցությունների նվազեցման առումով:

Ցիսպլատին – էստրադիոլ – առնետի լյարդ – ֆոսֆոլիպիդներ

Исследовалось содержание общих фосфолипидов и их индивидуальных фракций в препаратах ядер клеток печени крыс при *in vivo* отдельном воздействии эстрадиола и при комбинированном с цисплатином его применении. Показано, что в отличие от воздействия цисплатина, которое приводит к сокращению тотального содержания ядерых липидов, в результате отдельного применения эстрадиола регистрируется повышение общего количества ядерных фосфолипидов на 62 %. При совместном применении эстрадиола и цисплатина общее количество ядерных фосфолипидов повышается всего на 13 %. Эти изменения по-разному влияют на содержание индивидуальных фракций ядерных фосфолипидов. Среди выявленных изменений большего внимания заслуживают сдвиги, обнаруживаемые при сов-

местном воздействии цисплатина и эстрадиола, поскольку они, по всей вероятности, могут быть связаны со снижением токсичности цисплатина и смягчением нежелательных побочных эффектов, вызванных им.

Цисплатин – эстрадиол – печень крысы – фосфолипиды

It is well known that the platinum drug cisplatin (*cis*-diaminedichloroplatinum (II)) is widely used for the treatment of many malignancies. Cisplatin is clinically used as adjuvant therapy of cancers aiming to induce tumor cells death [6, 7]. However, it should be noted that cisplatin damages tumor cells and normal ones. Cisplatin induces citotoxicity by alterations of transcription, DNA replication processes, via induction of all pathways of apoptosis [6, 7]. The effectiveness of cisplatin is dose-dependent, although its use in higher concentrations is limited by several side effects, such as nephrotoxicity, neurotoxicity, ototoxicity and others. These side effects might lead to intoxication of the whole body [11, 14, 16]. It was revealed that the joint use of steroid hormone (estradiol/progesterone) and cisplatin resulted to reduced cisplatin-induced toxicities [5, 9, 10, 15].

At present it is well known that nuclear lipids are major regulators of many essential cellular processes such as DNA replication, transcription and gene expression [1, 2, 17]. Recent advances demonstrated the involvement of nuclear lipids in remodeling of chromatin and epigenetic regulation of gene expression [12, 13]. It seems impossible to exclude the significance of nuclear lipids quantitative alterations for cisplatin antitumor effects [8].

Our previous results showed the reliable changes in total quantities of nuclear phospholipids and in content of their individual fractions of rat liver cells after the cisplatin *in vivo* action [8]. Regulatory role of nuclear phospholipids via their implications in DNA replication, transcription, chromatin assembly, acetylation and methylation of histones, and in other distinct nuclear processes is well-known. Taking into consideration the cisplatin-depended nuclear phospholipid alterations, it is assumed that the implementation of cisplatin antitumor effects through these changes could not be excluded. On the other hand, it has already shown, that estradiol and progesterone showed a beneficial effect in elimination of cisplatin-induced nephrotoxicity [10]. In view of the above developments it seems interesting to explore the cisplatin and estradiol separate and joint *in vivo* action on rat liver nuclear phospholipids content.

In this paper the alterations of quantities of total phospholipids as well as changes in their individual fractions content in nuclear preparations from rat liver cells after estradiol separate and its joint *in vivo* action with cisplatin were investigated.

Materials and methods. The investigation was performed on adult female albino rats (120-150 g weight). The animals were divided into 4 groups. Group 1 is a control group of animals without treatment. Animals of groups 2 and 4 received single dose of cisplatin (8 mg/kg). Cisplatin was injected peritoneal. Exposition time for cisplatin was 24 hours. Group 3 was treated with estradiol (200 mcg/kg, injected peritoneal). Exposition time for estradiol was 4 hours. Animals from Group 4 within 20 hours after the cisplatin injection (4 hours before decapitation) received the same single dose of estradiol. All animals were killed by decapitation through corresponding time. Before extraction of the liver, perfusion through the portal vein with ice-cold saline was done in order to remove the remaining blood. Rat liver nuclei were isolated by the method of Blobel and Potter [4]. Phospholipid extraction was carried out by Bligh and Dayer [3]. The fractionation of phospholipids was performed by micro thin layer chromatography (micro TLC) using 6x9 sm^2 plates with L silicagel and chloroform-methanol-water in ratio 65:25:4 as a dividing mixture. After the chromatography the plates were dried up at 20^0C and were treated by 15.6% $CuSO_4$ in 8% phosphoric acid. Then the elaborated plates were heated at 180^0C for 15 minutes. 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. The total quantities of rat liver cells nuclear phospholipids in baseline and after the cisplatin and estradiol separate and joint *in vivo* action are provided in tab. 1 (tab.1). The total amount of rat liver cells nuclear phospholipids after treatment with the antitumor drug cisplatin decreased by 10% [8]. On the contrary, estrogen treatment leads to increase of phospholipids content by 62% in rat liver nuclear fraction (tab.1). The combined injection of cisplatin and estradiol increased the quantity of nuclear total phospholipids by 13.5% in comparison to baseline, although in comparison with estradiol received group of animals, it was reduced by 48.5 % (tab.1 and fig.1). The results confirm that cisplatin and estradiol were demonstrated opposite effects on total quantity of nuclear phospholipids. However, in the case of joint action these effects were summed up (tab. 1 and fig.1).

Table 1. Total phospholipids content (in mcg/g of tissue) in nuclear preparations of rat liver cells in baseline and after the cisplatin and estradiol separate and joint *in vivo* action

Variants	Baseline	Cisplatin	Estradiol	Cisplatin+ Estradiol
mcg/g tissue	3700,00±61,50	*3337,00±51,30[1]	*6000,00 ±172,57	*4200 ±145,00

1-our previous results – p<0,05*

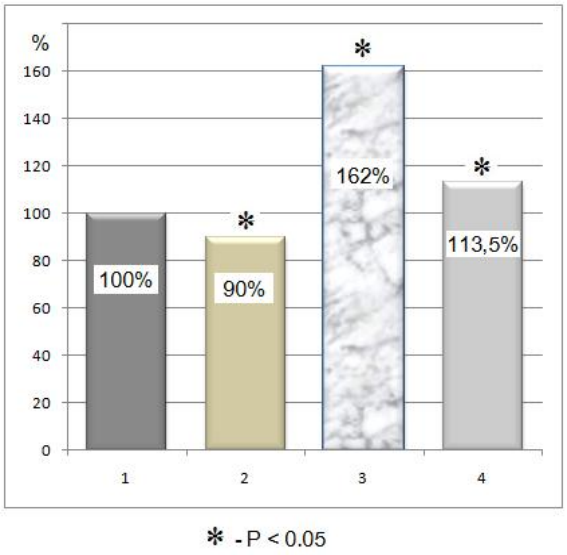

Fig. 1. Changes in total phospholipids content in nuclear preparation of rat liver cells in baseline and after the cisplatin and estradiol separate and joint treatment.
1 – baseline, 2 – after the cisplatin action, 3 – after estradiol action,
4 – after joint action of cisplatin and estradiol

The fractionation of nuclear phospholipids by the microTLC method revealed seven individual phospholipids in baseline and in the case of cisplatin and estradiol separate and joint action. This means that neither cisplatin and estradiol separate nor

combined action cause qualitative changes. Phosphatidylserine, sphingomyelin, phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine, cardiolipin and phosphatidic acid were obtained among the phospholipids of rat liver cells nuclei preparations. Phosphatidylcholine and phosphatidylethanolamine were the major components to form more than 65% of total phospholipids amount in baseline, while the percentage of the other five fractions varied within the range of 4-10%. (tab. 2).

Table 2. The relative content (in percentage) of individual fractions of phospholipids in nuclear preparations of rat liver cells in baseline and after the cisplatin and estradiol separate and joint action.

N	Phospholipids	Baseline	Cisplatin	Estradiol	Cisplatin+ Estradiol
1	Phosphatidylserine	5.80±0.34	3.90±0.28	3.32±0.22	5.35±0.23
2	Sphingomyelin	7.60±0.33	5.60±0.22	4.58±0.24	7.40±0.1
3	Phosphatidylinositol	10.40±0.24	11.10±0.25	5.40±0.42	9.04±0.21
4	Phosphatidylcholine	39.70±0.40	45.80±0.42	51.24±1.07	40.20±1.23
5	Phosphatidylethanolamine	26.10±0.28	26.00±0.39	26.30±0.43	23.60±0.87
6	Cardiolipin	4.80±0.21	3.90±0.23	3.63±0.27	5.2±0.33
7	Phosphatidic acid	5.60±0.20	3.70±0.15	5.53±0.26	9.17±0.45
Total content		100	100	100	100

It is characteristic that the relative content of these individual fractions was not exposed to considerable alterations after the cisplatin and estradiol separate action as well as in case of its combined treatment. It must be mentioned that more significant changes undergo the relative content of phosphatidylcholine, which increased under the separate action of cisplatin and estradiol. On the other hand, after the joint action of cisplatin and estradiol, the baseline level of phosphatidylcholine relative content was restored (tab. 2).

Taking into consideration the significant changes in total nuclear phospholipids content after the cisplatin and estradiol separate and joint *in vivo* action the necessity arises to determine the changes in absolute quantities of individual fraction of lipids in all variants. Calculation results are presented in tab. 3. Individual fractions of phospholipids of rat nuclei exhibit different sensitivity to cisplatin treatment. The absolute content of phosphatidylinositol and phosphatidylcholine remained unchanged after the cisplatin action, while the quantities of all the other decreased reliably. Absolute content of phosphatidylserine, sphingomyelin, phosphatidylethanolamine, cardiolipin and phosphatidic acid reduced by 41.4%, 35.5%, 15.2%, 33.3% and 41.3% respectively. These obtained alterations in lipid quantity of rat liver nuclei cells are consequences of deep and multiform transformation of nuclear lipids metabolism caused by antitumor drug cisplatin [8]. After the estradiol alone action as well as in case of cisplatin and estradiol joint treatment the absolute quantity of phosphatidylserine and sphingomyelin remained unchanged. Changes were not found in quantity of phosphatidylinositol after the joint action of cisplatin and estradiol, while estradiol alone action decreased the content of this phospholipid by 15%. Among the nuclear phospholipids, the phosphatidylcholine exhibits the greatest susceptibility to estradiol alone action. The absolute quantity of this phospholipid increased by 110% in comparison with baseline as well as with cisplatin separate action. However, it should be noted that cisplatin and estradiol joint action lead to increased quantity of phosphatidylcholine only about 18 % (tab.3 and fig.2).

Table 3. The absolute quantities (in micrograms per gram of tissue) of individual phospholipid fractions in nuclear preparations of rat liver cells in baseline and after the cisplatin and estradiol separate and joint action

hospholipids	Baseline	Cisplatin	Estradiol	Cisplatin+Estradiol
Phosphatidylserine	215.00±16.00	*126.00±14.00	199.00±13.16	222.00±9.66
Sphingomyelin	290.00±20.00	*187.00±20.00	275.00±14.4	307.00 ±8.83
Phosphatidylinositol	382.00±22.00	374.00±33.00	**324.00±25.2	391.00 ±8.80
Phosphatidylcholine	1465.00±53.00	1592.00±16.00	*3074.00±62.20	*1726.00 ±51.65
Phosphatidylethanolamine	962.00±28.00	*816.00±38.00	*1578.00±25.80	950.00 ±36.53
Cardiolipin	180.00±15.00	*120.00±18.50	*218.00±16.21	*220.00 ±16.00
Phosphatidic acid	208.00±13.00	*122.00±10.00	*332.00±15.60	*384.00 ±19.00

**-p<0.05; **- p < 0.1;*

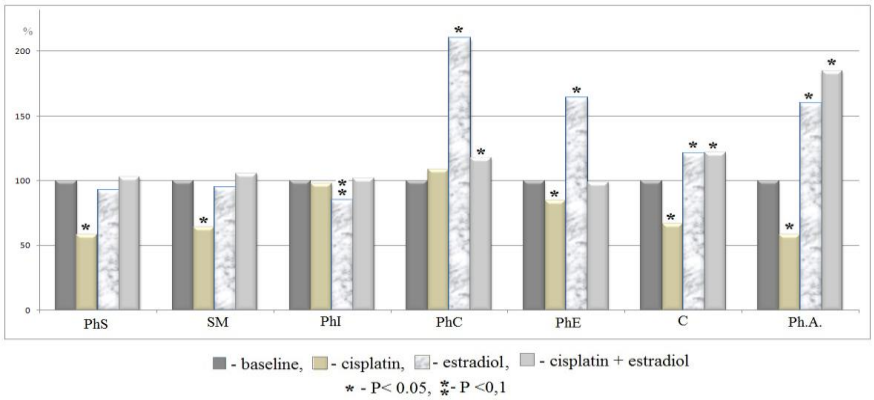

Fig.2. Changes (in %) of absolute quantity of rat liver nuclear phospholipids individual fractions after the cisplatinand estradiol separate and joint action

PhS – phosphatidylserine, SM - sphingomyelin, PhI – phosphatidylinositol,
PhC - phosphatidylcholine, PhE– phosphatidylethanolamine,
C – cardiolipin, Ph.A. – phosphatidic acid

The absolute content of another nuclear phospholipid phosphatidylethanolamine has also been significantly increased by about 64 % after estradiol separate action, whereas the joint action of cisplatin and estradiol lead to recovery reference level. Cardiolipin and phosphatidic acid absolute content has been increased by 21 % and 60 % respectively after the estradiol separate action (tab. 3 and fig. 2). Estradiol and cisplatin combined treatment revealed increase of cardiolipin quantity as well as phosphatidic acid absolute content by 22 % and about 85 % respectively (tab.3 and fig.2).

It is well known, that nuclear lipids are components of the intranuclear structures, such as nuclear membrane, nuclear matrix, nucleolus and chromatin. These lipids are involved in numerous biological processes, including signal transduction and a variety of metabolic pathways [2, 17]. It is worthy to mention that metabolism of nuclear lipids is regulated independently of that of cytoplasm. Furthermore, the modification of lipid metabolism is involved in cell proliferation or apoptotic processes induced by different stimuli, including hormones and drug action. As result of lipids metabolism secondary messengers are produced in the cell nucleus, which play a pivotal role in multiple signaling networks as well as in chromatin organization and transcription control [2, 12, 13, 17].

The quantitative changes in nuclear phospholipids in case of cisplatin alone action should be considered negative side effects. However, the changes obtained in case of cisplatin and estradiol joint treatment most likely have a positive effect and may be helpful for reducing the cisplatin toxicity and eliminating its undesirable spillovers. Thus, it can be concluded that nuclear lipids are key elements for the correct functioning of all nuclear processes. The quantitative changes, that were identified, have served to elucidate how cisplatin and estradiol are show their impact in case of separate and joint action.

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