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CISPLATIN AND ESTRADIOL SEPARATE AND JOINT ACTION ON PHOSPHOLIPIDS CONTENT OF CHROMATIN PREPARATION FROM RAT KIDNEY CELLS

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The current study presents the results of in vivo separate and joint action of chemotherapeutic drug cisplatin and sex hormone estradiol on content of total phospholipids and their individual fractions in chromatin preparation from female rat kidney cells. It was shown that the absolute quantities of all phospholipids individual fractions were decreased reliably after the in vivo action of cisplatin. Individual fractions of phospholipids of rat kidney cells chromatin preparations exhibit different sensitivity to estradiol alone treatment. The changes obtained in case of combination of cisplatin therapies with sex hormone estradiol have a positive effect and may be helpful for reducing the cisplatin toxicity.

Cisplatin – estradiol – chromatin from rat kidney cells – phospholipids

Տվյալ աշխատանքում ներկայացված են քիմիոթերապևտիկ դեղամիջոց ցիսպլատինի և սեռական հորմոն էստրադիոլի առանձին ու համատեղ *in vivo* ազդեցության ներքո էգ առնետների երիկամային բջիջներից ստացված քրոմատինի պատրաստուկներում առկա ֆոսֆոլիպիդների ընդհանուր պարունակության և դրանց առանձին ֆրակցիաների քանակական փոփոխությունների վերաբերող տվյալները: Պարզվել է, որ ֆոսֆոլիպիդների բոլոր առանձին ֆրակցիաների բացարձակ քանակները հավաստի կրճատվում են ցիսպլատինի *in vivo* ազդեցության արդյունքում: Էստրադիոլի առանձին կիրառման նկատմամբ առնետի երիկամային բջիջներից ստացված քրոմատինի ֆոսֆոլիպիդները տարբերակված զգայունություն են ցուցաբերում: Ցիսպլատինի և սեռական հորմոն էստրադիոլի համատեղ կիրառման դեպքում գրանցված փոփոխությունները դրական բնույթի են և կարող են օգտակար լինել ցիսպլատինի տոքսիկության նվազեցման համար:

Ցիսպլատին – էստրադիոլ – առնետի երիկամի բջիջներից ստացված քրոմատին – ֆոսֆոլիպիդներ

В настоящем исследовании представлены результаты содержания общих фосфолипидов и их индивидуальных фракций в препаратах хроматина клеток почек крыс самок при отдельном и совместном *in vivo* применении цисплатина и полового гормона эстрадиола. Показано, что при отдельном *in vivo* применении цисплатина достоверно уменьшается абсолютное количество всех отдельных фосфолипидных фракций. Индивидвуальные фракции фосфолипидов препаратов хроматина клеток почек крыс проявляют разную чувствительность к отдельному применению эстрадиола. Изменения, обнаруженные при совместном применении цисплатина и полового гормона эстрадиола, имеют положительный характер и могут быть полезны для снижения токсичности цисплатина.

Цисплатин – эстрадиол – хроматин клеток почек крыс – фосфолипиды

Cisplatin has been cited as being among the most used cytotoxic anticancer medication due to its broader efficacy in the treatment of various types of cancers [3, 20]. Cisplatin used to treat patients with bladder, ovarian, head and neck, lung, testicular, cervical, esophageal, breast and brain cancers [3].

The drug is also characterized by various toxic side effects including nephrotoxicity, cardiotoxicity, hepatotoxicity, neurotoxicity and nausea [9, 18]. Strong evidence from research has demonstrated higher efficacy of combination of chemotherapies of cisplatin together with other drugs in reducing toxic effects [11, 12].

Cisplatin, referred to by chemical name as cis-diamminedichloroplatinum (II), is anticancer and DNA destroying agent that is square planar platinum (II) complex and contains 2 ligands of chloride in a cis configuration orientation [3].

The mechanism of action of cisplatin is mediated by the interaction of this drug with DNA in order to form DNA adducts. While this interaction is the foundation for efficacy of cisplatin in the treatment of cancer, these platinum compounds, interaction is the route cause for cytotoxic effect of cisplatin [3]. Cisplatin treatment has been linked to various toxic side effects including nephrotoxicity, cardiotoxicity, hepatotoxicity, neurotoxicity and nausea [15] . Nephrotoxicity is major undesirable side effect caused by cisplatin treatment. High doses of cisplatin are linked to nephrotoxicity. It is therefore important to note that nephrotoxicity is a dose limiting side effect [3, 9, 18].

Kidney plays an important role as the main route of cisplatin excretion. Literature data has suggested that kidney has tendency of accumulating cisplatin to higher levels compared to any other organ in the body including the liver [8,14]. However, when concentration of cisplatin within the blood is lower than those in the kidney, it is an indication of toxicity [3]. It is also believed that the mechanism of cisplatin-induced nephrotoxicity is the same as the tumor cytotoxicity. Both mechanisms involve the formation of highly reactive equated platinum species that cross-link DNA and is highly dependent on the availability and the concentration of ambient chloride concentrations [1].

Literature data have demonstrated that when other compounds are combined with cisplatin chemotherapy, there is reduction of undesirable side effects [15] . At the same time it was shown that sex steroids are able to diminish some negative side effects of cisplatin [11] .

At present it is well known that nuclear lipids are vital for cell life. The nuclear morphology and function of intranuclear lipids are dependent on the exact organization of the nuclear membrane, nuclear matrix and chromatin. Approximately 70–80% of polar lipids and CHO–CHO esters are present in the nuclear membrane; the remaining 20–30 % resides inside the nucleus, associated with the nuclear matrix, nucleolus and chromatin [2]. There are two pools of chromatin-bound lipids, namely loosely bound lipids and tightly bound lipids. The tightly bound lipids are important for the direct regulation of gene expression and RNA transcription [12, 13]. In some diseases, lipids are also key molecules in nuclear transport independently of their role in chromatin activity [2]. It seems impossible to exclude the significance of nuclear lipids including chromatin bound ones quantitative alterations for implementation of cisplatin antitumor effects as well as its involvment in molecular mechanisms of steroid hormones action.

This article will focus on cisplatin; one of the most commonly used chemotherapeutic drugs to date and female sex hormone estradiol separate and joint action on content of total phospholipids and their individual fractions in chromatin preparation from female rat kidney cells.

Materials and methods. The investigation was performed on adult female albino rats (120-150 g weight). The animals were kept in 20-22^{0}C and 12 hours dark/light conditions with free

access to water and food (animals were fed with commercial rat feed) in the animal house of faculty of biology of Yerevan State University.

Experiences were fulfilled according to the "International Recommendations on Carrying out of Biomedical Researches with use of Animals" (CIOMS, 1985), to the "Human Rights and Biomedicine the Oviedo Convention" (CE, 1997), to the European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes (CE, 2005) and approved by the National Center of Bioethics (Armenia).

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 after the anesthesia with chloroform. Then, animals were sacrificed, and the kidney was extracted from each group of animals and used for isolation of nuclear fraction and chromatin.

Nuclear fraction from kidney of each group of animals were isolated according to Blobel and Potter [6].

The preparations of pure nuclei (all 4 variants) were used for the isolation of chromatin [17]. Phospholipids of chromatin fraction from rat kidney cells were extracted by Bligh and Dayer [5]. The fractionation of phospholipids was performed by micro thin layer chromatography (microTLC) using 6x9 sm2 plates with silicagel L and chloroform – methanol – water in ratio 65:25:4 as a dividing mixture [16].

After the chromatography the plates with fractionated phospholipids were dried up at room temperature and were treated by 15.6 % CuSO4 in 8% phosphoric acid. Then the elaborated plates were heated at 180^0C for 15 minutes [4].

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 and expressed as M±m (m Standard error of the mean SEM) from 6 independent experiments. Statistical differences in the results between groups were evaluated by the Student's t-test.

Results and Discussion. The total quantities of phospholipids from rat kidney cells chromatin preparations in baseline and after the cisplatin and estradiol separate and joint *in vivo* action are provided in tab. 1.

The results confirm that cisplatin and estradiol were demonstrated opposite effects on total quantity of chromatin phospholipids. The total amount of phospholipids from rat kidney cells chromatin preparations decreased by 25% after the cisplatin *in vivo* action. On the contrary, the estradiol separate injection leads to increase in total amount of phospholipids in studied preparations by 18% (tab. 1).

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

*-p<0,05

Variants	**Phospholipids content mcg/g tissue**
Baseline	288,0±6,35
Cisplatin	*216,0±5,48
Estradiol	*340,0±11,77
Cisplatin+ Estradiol	*254,0±8,54

The combined injection of cisplatin and estradiol decreased the quantity of chromatin total phospholipids by 12 % in comparison to baseline, while in comparison

with estradiol received group of animals, it was reduced by 25 % (tab 1). In comparison with cisplatin separate action increase of chromatin total phospholipids quantity by about 18 % was registrated (tab.1). Consequently in case of joint action of these drugs there is competition, but the effect of estradiol is prevalent.

We've been fractioning lipids to identify individual phospholipid fractions responsible for the total phospholipids quantities alterations in rat kidney cells chromatin preparations. The fractionation of chromatin phospholipids from rat kidney cells by the microTLC method in baseline and after the cisplatin and estradiol separate and joint treatment showed the presence of five individual phospholipids (fig.1).

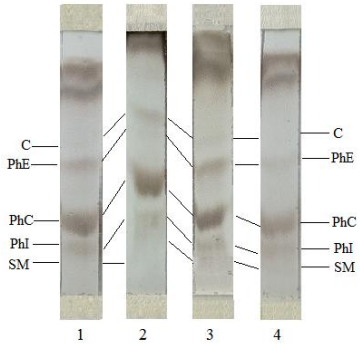

Fig. 1. Chromatograms of fractionation of chromatin phospholipids from rats kidney cells by microTLC method.
1 – baseline, 2 – after the cisplatin separate action, 3 –after the separate action of estradiol, 4 – after the cisplatin and estradiol joint action.
SM – sphingomyelin, PhI – phosphatidylinositol, PhC – phosphatidylcholine, PhE – phosphatidylethanolamine, C – cardiolipin.

Sphingomyelin, phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine and cardiolipin were obtained among the phospholipids of rat kidney cells chromatin prepararations. The present results indicated that neither cisplatin nor estradiol separate nor combined action cause qualitative changes of phospholipids. The relative content of individual phospholipid fractions testified that phosphatidylethanolamine and phosphatidylcholine were the major components and formed about 59 % of total phospholipids amount in baseline, while the sum percent content of the other three fractions is about 41 % (tab.2). It must be mentioned that the relative percentage content of individual phospholipid fractions from rat kidney chromatin preparations undergo to negligible changes after the cisplatin and estradiol separate and joint action (tab.2).

Table 2. The relative content (in percentage) of individual fractions of phospholipids in chromatin preparations of rat kidney cells in baseline and after the cisplatin and estradiol separate and joint action
*-p<0,05

N	Phospholipids	Baseline	*Cisplatin Injection	Estradiol injection	Cisplatin and estradiol joint injection
1	Sphingomyelin	14,50±0,35	15,70±0,60	9,70±0,61	12,56±0,66
2	Phosphatidylinositol	12,00±0,30	12,40±0,38	12,20±0,64	13,50±0,86
3	Phosphatidylcholine	32,50±0,58	30,80±0,44	39,35±0,85	39,60±0,85
4	Phosphatidylethanolamine	26,00±0,40	24,40±0,73	20,15±0,70	16,77±0,90
5	Cardiolipin	15,00±0,28	16,70±0,50	18,60±0,66	17,57±0,60
Total content		100	100	100	100

**Gevorgyan et al; 2016 [10]*

Taking into consideration 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, the necessity arises to determine the changed in absolute quantities of individual fraction of lipids in all studied variants (tab.3 and fig. 2).

The absolute quantities of all individual fractions of phospholipids were decreased reliably after the in vivo action of cisplatin [10] (tab.3). Diminution of phospholipids in kidney chromatin preparations indicates the comprehensive action of cisplatin on lipid metabolism in intranuclear structures which may some serious prerequisites for alteration the functioning those processes where these lipids participate, regulate or act [10, 12, 13].

Individual fractions of phospholipids of rat kidney cells chromatin preparations exhibit different sensitivity to estradiol alone treatment. The quantity of three phospholipid fractions was significantly increased, while the sphingomyelin and phosphatidylethanolamine content was even decreased (tab.3).

Table 3. The absolute quantities (in micrograms per gram of tissue) of individual phospholipid fractions in chromatin preparations of female rat kidney cells in baseline and after the cisplatin and estradiol separate and joint action
*-p<0,05

N	Phospholipids	Baseline	**Cisplatin Injection	Estradiol injection	Cisplatin and estradiol joint injection
1	Sphingomyelin	41,76±1,00	*33,90±1,30	*33,00±2,07	*32,00±1,68
2	Phosphatidylinositol	34,56±0,86	*26,85±0,82	*41,46±2,18	34,30±2,19
3	Phosphatidylcholine	93,60±1,07	*66,55±1,00	*133,80±2,89	100,50±2,16
4	Phosphatidylethanolamine	74,88±1,15	*52,70±1,58	68,50±2,40	*42,60±2,30
5	Cardiolipin	43,20±0,81	*36,00±1,00	*63,24±2,26	44,60±1,52

**Gevorgyan et al; 2016 [10]*

The absolute quantity of phosphatidylinositol, phosphatidylcholine and cardiolipin differed from their baseline content by 43 %, 46 % and 20 % correspondingly (fig. 2).

Though among the rat kidney cells chromatin preparations phospholipids, the phosphatidylcholine and cardiolipin exhibit great susceptibility to estradiol alone action (tab.3, fig.2), cisplatin and estradiol joint treatment restored the baseline value of phosphatidylinositol, phosphatidylcholine and cardiolipin (tab.3, fig.2). On the same time the absolute quantity of sphingomyelin and phosphatidylethanolamine decreased significantly after the joint action of cisplatin and estradiol correspondingly by 23,3 % and 43% in comparison with baseline (tab.3, fig 2). It is well known that chromatin phospholipids in dose dependent manner are capable of regulating DNA replication, transcription and gene expression [1, 7, 12, 19]. It has been shown the involvement of nuclear lipids in remodeling of chromatin and epigenetic regulation of gene expression [7, 12, 19].

In light of these findings our results obtained on quantitative changes of rat kidney chromatin phospholipids individual fractions take on considerable importance.

The data presented above indicated that in case of separate action cisplatin and estradiol demonstrated its own abilities suppress or stimulate metabolic processes. The quantitative changes in chromatin phospholipids in case of cisplatin alone action should be considered negative side effects. The changes obtained in case of combination of cisplatin therapies with sex hormone estradiol most likely have a positive effect and may be helpful in reduction of undesirable side effects.

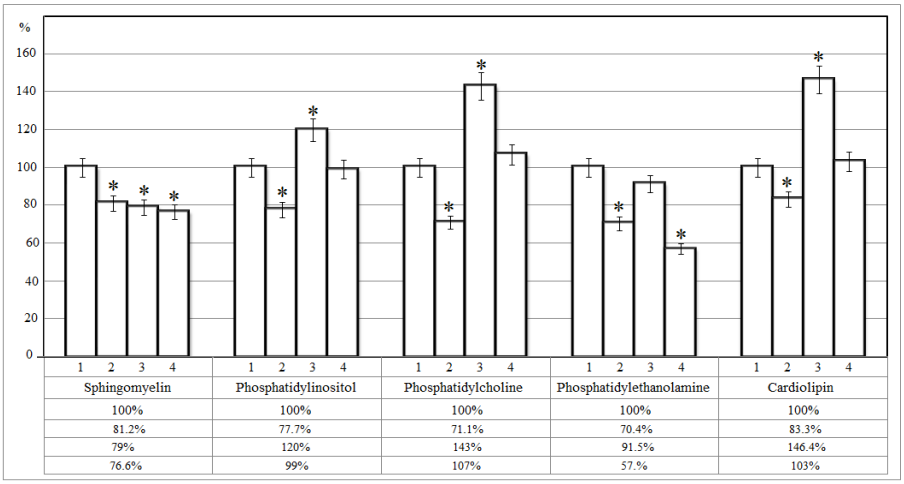

Fig.2. Changes (in %) of absolute quantities of individual phospholipids of chromatin from rat kidney cells. 1 – baseline, 2 – after the cisplatin separate action (Gevorgyan E.S. et al, 2016) [10]), 3 – after the estradiol separate action, 4 – after the cisplatin and estradiol joint action

Thus, it can be concluded that in the cell nucleus, lipids are not ”a minor component” of the intranuclear environment, but are key elements for the correct functioning of nuclear processes [2].

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