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CISPLATIN AND PROGESTERONE SEPARATE AND JOINT ACTION ON RAT THYMUS NUCLEAR PHOSPHOLIPIDS COMPOSITION AND CONTENT

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The antitumor agent cisplatin and progesterone separate and joint action on content of total phospholipids and their individual fractions in nuclei from rat thymus cells were investigated. When used separately, cisplatin and progesterone exhibit its own distinctive properties. Cisplatin reduces, and progesterone, on the contrary, increases the content of total phospholipids. When used together, the effects of these drugs are summed up. Thus total phospholipid content of rat thymus cell nuclei increases by 17% after the joint injection of cisplatin and progesterone. These changes have different effects on quantities of individual phospholipid fractions. It is assumed that the effects of cisplatin and progesterone, both in their separate and joint action, can be mediated by quantitative changes in internuclear lipids that in turn can regulate the main functions of the cell nuclei.

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***Keywords*:** cisplatin, progesterone, rat thymus, nuclear phospholipids.

Introduction. It is well known that nuclear lipids in dose depended manner are capable regulating many essential cellular processes such as DNA replication, transcription and gene expression [1–3]. Furthermore nuclear phospholipids are involved in remodeling of chromatin and epigenetic regulation of gene expression. Alterations of lipid quantity can change the functional and physiological state of the cell [2, 4, 5]. It is possible that quantitative changes in nuclear phospholipids are involved in the molecular mechanisms of action of drugs such as cisplatin and steroid hormones.

Cisplatin or cis-diaminedichloroplatinum (II) is a well-known chemotherapeutic drug, which has been used for treatment of numerous human cancers. Cisplatin induces cytotoxicity by alterations of transcription, DNA replication processes, via induction of all pathways of apoptosis [6–8]. DNA is considered as the primary target for cisplatin [6, 7, 9]. Cisplatin exerts its anti-tumor activity by covalent binding to DNA-forming adducts and therefore by triggering apoptosis [6, 7]. The type of Pt-DNA adducts formed may exert a significant influence on the

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biological activity of the drug. It was found that the genotoxic effect of cisplatin results from the formation of monoadducts, while the formation of double adducts – inside or between strands – results in the cytotoxicity properties of the compound [6, 7]. Nevertheless, the resulting adducts lead to a disturbance of the spatial structure of DNA, which results in inhibition of nucleic acid replication and transcription [6, 7, 9]. However nowadays it has been established, that the anticancer activity of cisplatin is not merely limited to its ability to cross-link DNA and inhibit mitosis that in turn, lead to apoptosis. Thus, the induction of oxidative stress is an "unconventional" mechanism of the cytotoxicity action of cisplatin [6, 9–11]. In addition cisplatin also exhibits important immunomodulatory effects. These effects may be quite important for combating tumors [12].

However, despite its high efficacy, cisplatin exhibits undesirable side effects such as severe kidney problems, allergic reactions decrease immunity to infections andother [6, 13].

Recently was shown, that combination therapies of cisplatin with steroid hormones can reduce toxicity of antitumor drug. It is known that steroid hormones, due to their anti-inflammatory properties, alleviate various toxicities caused by cisplatin [14, 15].

In the light of the foregoing, it is of particular interest to study the separate and combined effects of cisplatin and progesterone on the content of phospholipids in preparations of thymus cells nuclei in female rats.

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 *h*. The group 3 was treated with progesterone (300 *mcg*/*kg*, injected peritoneal). Exposition time for progesterone was 4 *h*. Animals from the group 4 were received the same single dose of progesterone within 20 *h* after the cisplatin injection (4 *h* before decapitation). All animals were killed by decapitation through appropriate timeafter the inhalation anesthesia with chloroform. Then, animals were sacrificed, and the thymus tissue was extracted from each group of animals and used for isolation of nuclei by the method of [16]. Phospholipids of thymus nuclei were extracted by [17]. The fractionation of phospholipids was performed by micro thin layer chromatography (microTLC) using 6×9 cm^2 plates with silicagel L and chloroform–methanol–water in ratio 65:25:4 as a dividing mixture [18]. 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°C for 15 *min* [19]. The quantitative estimation of separated and specific died phospholipids was carried out by special computer software Fujifilm Science Lab 2001 Image Gauge V4.0, which was designed for densitometry. Obtained results were treated by statistics.

All results were expressed as $M \pm m$ from 4 independent experiments. Statistical differences in the results between groups were evaluated by the Student's *t*-test.

Results and Discussion. This article uses the results of studying the effect of cisplatin on the alterations in quantity of total phospholipids and their individual

fractions after 24 *h* of exposure [20]. Cisplatin *in vivo* action reliably decreases the total amounts of phospholipids in nuclear fraction preparations from thymus cells by 20%. On the contrary, the progesterone separate injection leads to increase in total amount of phospholipids in studied preparations by 25% (Tab. 1, Fig. 1).

Table 1

Total phospholipids content (in mcg/g of tissue) in nuclear preparations of rat thymus cells in baseline, after the cisplatin and progesterone separate and joint in vivo action (p < 0.05, # [20])*

Variants	Baseline[#]	Cisplatin[#]	Progesterone	Cisplatin + Pr
mcg/g of tissue	4000.00 ± 90.00	3200.00 ± 80.00[*]	5000.00 ± 85.00[*]	4680.00 ± 58.00[*]

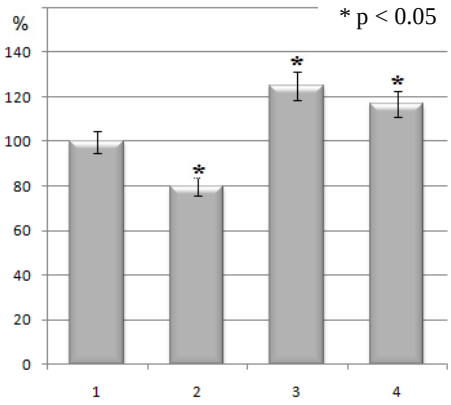


Fig. 1. Changes of total phospholipids content in nuclear preparation of rat thymus cells after the cisplatin and progesterone separate and joint treatment:
1 – baseline (without treatment);
2 – after the cisplatin separate action [20];
3 – after the progesterone separate action;
4 – after joint action of cisplatin and progesterone.

It is known that all metabolic processes are activated in cancer cells [6, 7, 9]. The function of antitumor drugs is to suppress or block all pathways that promote the growth and development of tumor cells.Cisplatin is no exception, inhibits lipid metabolism by inhibiting enzymes [6, 7, 21]. On the contrary, steroid hormones can stimulate metabolic processes [15]. Our results indicate that when used separately, cisplatin and progesterone exhibit characteristic properties. However, the overall result is obtained in case of joint injection of these drugs (Tab. 1, Fig. 1).

The fractionation by microTLC of total phospholipids from nuclear preparations of rat thymus cells in baseline seven individual fractions was identified. The composition of phospholipids does not change either in case of separate or in case of combined use of cisplatin and a steroid. A computer program was used to quantify fractionated phospholipids from nuclear fraction of thymus.

The results of the relative content (in percentage) of all seven phospholipid individual fractions of four studied groups are presented in the Tab. 2.

It should be noted that the major fractions in all studied groups were phosphatidylcholine and phosphatidylethanolamine. The summary content of these two phospholipids is 57.2% in baseline. In third place is phosphatidicacid, the relative percentage of which is 14.4% in baseline (Tab. 2). The remaining 4 individual phospholipids are represented in approximately equal amount. Both separate and combined use of these drugs does not cause significant changes in the relative percentage content of phospholipids (Tab. 2). It is characteristic that the relative

content of individual fractions of phospholipids does not undergo significant changes after the cisplatin and progesterone separate action as well as in case of its combined treatment.

Table 2

The relative content (in percentage) of individual fractions of phospholipids in nuclear preparations of rat thymus cells in baseline and after the cisplatin and progesterone separate and joint action (# [20])

Phospholipids	Baseline#	Cisplatin#	Progesterone	Pr. + CP
Phosphatidylserine	6.00 ±0.40	7.30±0.25	5.60±0.13	5.50±0.19
Sphingomyelin	8.60±0.50	10.90±0.37	9.70±0.12	7.30± 0.25
Phosphatidylinositol	7.00±0.24	7.00±0.30	6.30±0.33	7.20±0.33
Phosphatidylcholine	34.60±0.60	30.10±1.20	43.00±0.62	43.00±0.33
Phosphatidylethanolamine	22.60±0.58	22.00±0.80	18.00±0.33	20.00±0.33
Cardiolipin	6.80±0.20	7.00±0.15	6.40±0.282	6.00±0.22
Phosphatidic acid	14.40±0.80	15.70±0.24	11.00±0.33	11.00±0.19
Total content	100	100	100	100

It is obvious that the obtained changes in percentage content do not represent the reality of alteration in real quantity 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 thymus tissue) in nuclear fraction preparations before and after the treatments were determined (Tab. 3).

Table 3

The absolute content (in mcg/mg of tissue) of individual fractions of phospholipids in nuclear preparations of rat thymus cells in baseline and after the cisplatin and progesterone separate and joint action ($p < 0.05$, # [20])*

Phospholipids	Baseline#	Cisplatin#	Progesterone	Pr. + CP
Phosphatidylserine	240.00±7.91	233.60±25.60	*281.50±6.65	255.50±8.88
Sphingomyelin	344.00±11.67	348.80±32.00	*482.50±5.40	343.50±11.70
Phosphatidylinositol	280.00±6.55	*224.00±11.90	*325.0±14.80	*337.00±15.40
Phosphatidylcholine	1384.00±25.23	*963.20±38.40	*2150.0±31.00	*2012.40±26.16
Phosphatidylethanolamine	904.0±18.60	*704.00±25.60	890.00±16.30	936.00±15.00
Cardiolipin	272.0±3.24	*224.00±12.80	320.00±14.10	280.80 ±13.21
Phosphatidic acid	576.0±2.11	*502.40±7.69	551.00±7.50	*514.80±8.90

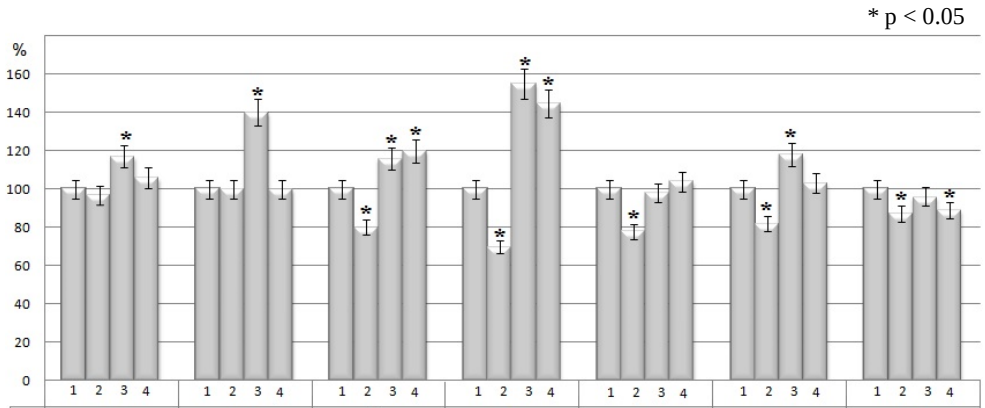
Taking into consideration the significant changes in total nuclear phospholipids content after the cisplatin and progesterone separate and joint action the necessity arises to determine the absolute quantities of lipids individual fractions. The calculation results indicate a significant sensitivity of individual phospholipid fractions to the cisplatin and progesterone separate and joint action.

The reliable decreases of quantity of five from seven individual phospholipids after cisplatin injection were registered. Thus absolute content of phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine, cardiolipin and phosphatidic acid reduced by 20, 30, 22, 18 and 13%, respectively. The absolute quantity of phosphatidylserine and sphingomyelinremains unchanged after the cisplatin separate action (Tab. 3, Fig. 2). A separate injection of progesterone also causes alterations

in the absolute content of five individual phospholipids, the quantities of which increases. Progesterone-sensitive phospholipids include phosphatidylserine, sphingomyelin, phosphatidylinositol, phosphatidylcholine and cardiolipinthe, the absolute quantity of which increases by 17, 40, 16, 55 and 18%, respectively (Fig. 2). In this case absolute quantity of phosphatidylethanolamine and phosphatidic acid remains unchanged (Tab. 3).

The combined use of cisplatin and progesterone affects the absolute content of individual phospholipids of rat thymus nuclei in different ways. Thus, sphingomyelin, the absolute amount of which does not change in case of cisplatin sepatarate action and increase in case of progesterone alone use, does not show sensitivity to joint action of these drugs (Tab. 3, Fig. 2).

As noted earlier, the absolute amount of phosphatidylethanolamineand cardiolipin decreased in case of separate injection of cisplatin and increased with the alone injection of progesterone. With the combined use of these drugs, the baseline level of phosphatidylethanolamineand cardiolipin is restored. The control level of phosphatidylserine is also restored with the joint injection of cisplatin and progesterone. At the same time, there is an increase in the content of phosphatidylinositol by 20%, and the absolute amount of phosphatidic acid reduced by 11% (Tab. 3, Fig. 2). The results obtained reflect the "negotiations" at the molecular level, in which one of the parties wins – cisplatin or progesterone.



	PS	SM	PI	PC	PE	C	P.A.
1	100	100	100	100	100	100	100
2	97	100	80	70	78	82	87
3	117	140	116	155	98	118	96
4	106	100	120	145	104	103	89

Fig. 2. The alteration (in percent) in individual phospholipid quantities in rat thymus cells nuclei preparations after the cisplatin and progesterone separate and joint action: 1 – baseline; 2 – after the cisplatin separate action [20]; 3 – after the progesterone separate action; 4 – after joint action of cisplatin and progesterone; PS –phosphatidylserine; SM – sphingomyelin; PI – phosphatidylinositol; PC – phosphatidylcholine; PE – phosphatidylethanolamine; C – cardiolipin; P.A. – phosphatidic acid.

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 [1, 22]. It is necessary to mark that metabolism of nuclear lipids is regulated independently of that of cytoplasm. Furthermore, the modification of nuclear lipid metabolism is involved in cell proliferation or apoptotic processes induced by different stimuli, including drug action [1, 22].

Given the immunomodulatory ability of cisplatin, it can be assumed that nuclear lipids are also involved in these processes in rat thymus cells [12]. Alterations in absolute quantities of phospholipid fractions in rat thymus nuclei caused by cisplatin and progesterone separate action can mediate the own specific effects of these drugs. Quantitative changes in nuclear phospholipids are also likely to mediate the effects of cisplatin and progesterone when they are used together. So, it can be assumed that nuclear lipids are key elements for the normal course of basic nuclear processes and the quantitative changes, that were identified, help to find out how cisplatin and progesterone show their impact when they act separately and together. Actually alterations of absolute quantities of nuclear phospholipids are tools by which specific properties of cisplatin and progesterone are exhibited in case of their separate action and revealed peculiar summation of its opposite effects in case of joint action of these drugs.

The changes obtained in case of cisplatin and progesterone joint treatment most likely have a positive effect and may be helpful for reducing the cisplatin toxicity and eliminating its undesirable spillovers.

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ՑԻՍՊԼԱՏԻՆԻ ԵՎ ՊՐՈԳԵՍՏԵՐՈՆԻ ԱՌԱՆՁԻՆ ԵՎ ՀԱՄԱՏԵՂ ԱԶԴԵՑՈՒԹՅՈՒՆԸ ԱՌՆԵՏԻ ՈՒՐՑԱԳԵՂՁԻ ԿՈՐԻԶԱՅԻՆ ՖՈՍՖՈԼԻՊԻԴՆԵՐԻ ԿԱԶՄԻ ԵՎ ԲԱՂԱԴՐՈՒԹՅԱՆ ՎՐԱ

Հետազոտվել է առնետների ուրցագեղձի բջիջների կորիզային ֆոսֆոլիպիդների ընդհանուր պարունակության և դրանց առանձին ֆրակցիաների բացարձակ քանակների փոփոխությունները ցիսպլատինի և պրոգեստերոնի

առանձին և համատեղ ազդեցությունից հետո: Առանձին կիրառելիս այս դեղերը դրսևորում են իրենց բնորոշ ազդեցությունները: Ցիսպլատինը կրճատում է, իսկ պրոգեստերոնն ընդհակառակը, ավելացնում է ֆոսֆոլիպիդների ընդհանուր պարունակությունը: Համատեղ կիրառելիս, գրանցվում է այս դեղերի գումարային ազդեցությունը: Այսպես՝ առնետի ուրագեղձի բջիջների կորիզային ընդհանուր ֆոսֆոլիպիդների քանակն աճում է 17%-ով ցիսպլատինի և պրոգեստերոնի համատեղ ազդեցությունից հետո: Այս փոփոխությունները տարբեր ազդեցություն են գործում ֆոսֆոլիպիդների առանձին ֆրակցիաների քանակի վրա: Ենթադրվում է, որ առանձին և համատեղ կրառելիս ցիսպլատինը և պրոգեստերոնը առաջացնում են ներկորիզային լիպիդների քանակական փոփոխություններ, որոնք իրենց հերթին կարող են կարգավորել բջջակորիզի կարևոր ֆունկցիաները:

А. Г. ОГАНЕСЯН, Н. Р. АКОПЯН, Ж. В. ЯВРОЯН, Э. С. ГЕВОРКЯН

ОТДЕЛЬНОЕ И СОВМЕСТНОЕ ДЕЙСТВИЕ ПРЕПАРАТОВ ЦИСПЛАТИН И ПРОГЕСТЕРОН НА СОСТАВ И СОДЕРЖАНИЕ ЯДЕРНЫХ ФОСФОЛИПИДОВ КЛЕТОК ТИМУСА КРЫС

Исследовалось содержание общих фосфолипидов и их индивидуальных фракций в препаратах ядер клеток тимуса крыс при отдельном и совместном применении препаратов цисплатин и прогестерон. При отдельном применении эти лекарства проявляют присущие им свойства – цисплатин сокращает, а прогестерон, наоборот, повышает количество общих фосфолипидов. При совместном применении регистрируется суммарный эффект этих лекарств. Так, содержание общих фосфолипидов ядер клеток тимуса крыс возрастает на 17% при совместном применении препаратов цисплатин и прогестерон. Эти изменения по-разному влияют на содержание отдельных фосфолипидных фракций. Предполагается, что данные препараты при отдельном и совместном применении вызывают количественные изменения внутриядерных липидов, которые, в свою очередь, могут регулировать важные функции клеточного ядра.