•Փորձարարական և տեսական հոդվածներ• Экспериментальные и теоретические статьи•
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CISPLATIN ACTION ON PHOSPHOLIPID COMPOSITION IN NUCLEAR FRACTION OF RAT LIVER CELLS

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The in vivo action of antitumor agent cisplatin on total phospholipid content in nuclear fractions of some tissues of rats was studied. The total phospholipid content in rat brain, thymus and kidney nuclear fractions remains unchanged while that in rat liver cells is significantly decreased. The content of six (from seven) fractions of individual phospholipids discovered in nuclei of rat liver cells was markedly decreased after the cisplatin action. These changes were accompanied with increase in phosphatidylcholine content. The quantities of negatively charged phospholipids such as phosphatidylserine, phosphatidic acid and cardiolipin were decreased more than of neutral phospholipids. At the same time the in vivo treatment by cisplatin leads to significant changes of percentage of separate fractions of phospholipids: the percentages of phosphatidylserine, phosphatidic acid, cardiolipin and sphingomyelin were decreased while that of phosphatidylcholine was increased and the percentages of phosphatidylinositol and phosphatidylethanolamine remained unchanged. These remarkable changes of content among the all individual fractions of phospholipids in nuclei of rat liver cells show the profound affect of cisplatin on nuclear lipid metabolism and indicate the necessity of further studies of those changes separately in nuclear membrane fraction and in intranuclear structures, including chromatin and nuclear matrix.

Cisplatin – phospholipids – liver cell nuclei

Ուսումնասիրվել է ցիսպլատին հակաուռուցքային միացության in vivo ազդեցությունը առնետի որոշ հյուսվածքների բջջակորիզների ֆոսֆոլիպիդների պարունակության վրա: Ցույց է տրվել, որ առնետի գլխուղեղի, ուրցագեղձի և երիկամների բջիջների կորիզային ֆրակցիաների գումարային ֆոսֆոլիպիդների պարունակությունը ցիսպլատինի ազդեցության տակ մնում է անփոփոխ, մինչդեռ լյարդի բջիջների կորիզներում ի հայտ է գալիս պարունակության զգալի նվազում: Լյարդի բջջակորիզների ֆրակցիայում հայտնաբերված յոթ առանձին ֆոսֆոլիպիդներից վեցի քանակն էապես նվազում է: Այս փոփոխություններն ուղեկցվում են ֆոսֆատիդիլխոլինի քանակի ավելացմամբ: Գումարային բացասական լիցք ունեցող ֆոսֆատիդիլսերինի, ֆոսֆատիդաթթվի և կարդիոլիպինի քանակն ավելի է նվազում, քան չեզոք ֆոսֆոլիպիդների պարունակությունը: Միաժամանակ, ցիսպլատինի in vivo ազդեցությունը հանգեցնում է նաև բջջակորիզի առանձին ֆոսֆոլիպիդների տոկոսային պարունակության փոփոխման՝ ֆոսֆատիդիլսերինի, ֆոսֆատիդաթթվի, կարդիոլիպինի և սֆինգոմիելինի բաժնեմասի նվազումն ուղեկցվում է ֆոսֆատիդիլխոլինի տոկոսային պարունակության աճով և ֆոսֆատիդիլինոզիտոլի և ֆոսֆատիդիլէթանոլամինի

բաժնեմասի անփոփոխ մնալով: Առանձին ֆոսֆոլիպիդների պարունակության այս խորը փոփոխությունները վկայում են լյարդի բջջակորիզներում լիպիդային մետաբոլիզմի վրա ցիսպլատինի ունեցած զգալի ազդեցության մասին և նախանշում են առաջիկայում այդ փոփոխությունների առանձին հետազոտման անհրաժեշտությունը կորիզաթաղանթներում, ինչպես նաև ներ-կորիզային կառուցվածքներում՝ քրոմատինում և կորիզային մատրիքսում:

Ցիսպլատին - ֆոսֆոլիպիդներ - լյարդի բջիջների կորիզներ

Изучено in vivo влияние противоопухолевого соединения цисплатина на содержание фосфолипидов в ядрах клеток некоторых тканей крыс. Показано, что содержание фосфолипидов в ядрах клеток головного мозга, тимуса и почек не меняется, в то время как в ядрах клеток печени наблюдается его заметное снижение, притом содержание шести из семи отдельных фракций фосфолипидов снижается. Одновременно наблюдается повышение содержания фосфатидилхолина. Содержание негативно заряженных фосфатидилсерина, фосфатидной кислоты и кардиолипина снижается больше, чем содержание нейтральных фосфолипидов. Вместе с тем, in vivo влияние цисплатина приводит к изменениям процентного содержания отдельных фракций фосфолипидов: уменьшение доли фосфатидилсерина, фосфатидной кислоты, кардиолипина и сфингомиелина сопровождается заметным повышением доли фосфатидилхолина, а процентное содержание фосфатидилинозитола и фосфатидилэтаноламина достоверно не меняется. Выявленные глубокие сдвиги в содержании отдельных фракций фосфолипидов свидетельствуют о значительном воздействии цисплатина на липидный метаболизм ядер и указывают на необходимость дальнейших исследований этих сдвигов во фракциях ядерных мембран и внутриядерных структур – хроматина и ядерного матрикса.

Цисплатин – фосфолипиды – ядра клеток печени

It is well known that the platinum drugs represent a unique and important class of antitumor agents. Among them the cisplatin (*cis*-diamminedichloroplatinum (II)) is widely used for the treatment of many malignancies, including testicular, ovarian, bladder, cervical, small-cell and non-small-cell lung cancers [6,7,9]. Although DNA was considered as the primary target of cisplatin [8,10], the cisplatin action at the cellular level still remains unknown. At the same time, it was showed that cisplatin damages, indiscriminately, both cancerous and normal tissue [7], and these global disorders are concerned with many components of nuclei, especially with lipids of nuclear membrane and intranuclear genetic structures, including chromatin and nuclear matrix [6,7]. Nuclei have a very active lipid metabolism which seems to play an important role in the transduction of signals to the genome [1,2, 14]. Positively charged cisplatin *in vitro* interacts with lipids, especially with negatively charged phospholipids [12]. These interactions are significant and should be considered in *in vivo* experiments.

This paper describes the alteration of total phospholipid content of nuclear fractions of rat liver, brain, thymus and kidney cells, as well as the changes among the individual fractions of phospholipids in nuclei of rat liver cells after the in vivo action of cisplatin.

Materials and methods. The experiments were carried out on albino rats (120-150 g weight). Cisplatin was injected peritoneally in concentration of 5 mg per 1000g animal weight. Rats were decapitated after 24 hrs of cisplatin injection. Rat liver, brain, and kidney nuclei were isolated by the method of Blobel and Potter [5].The nuclear fraction from thymus was isolated by the method of Allfrey V.G. et. al [3].

Phospholipid extraction was carried out by Bligh and Dayer [4]. The fractionation of phospholipids were carried out by micro thin layer chromatography using L silicagel, 6x9 sm^2 plates with the thickness of layer equal to 5-7 mcm, using chloroform-methanol-water in ratio 65:25:4 as a dividing mixture. After the chromatography the plates were dried up at 20^0 and were treated by 15,6 % CuSO in 8 % phosphoric acid. Then, the elaborated plates were heated at 189^0 for 15 min. The quantitative estimation of separated and specific dyed phospholipids was carried out by special computer program FUGIFILM Science Lab.2001 Image Gauge V4,0, which was destined for densitometry. Obtained data were undergone statistical analysis.

Results and Discussion. The phospholipid content in nuclei of rat liver, brain, thymus and kidney cells in baseline and after the in vivo treatment by cisplatin was presented in Table 1.

Table 1. Cisplatin in vivo action on total phospholipids content (in mkg/g of tissue) in nuclei of some rat tissues

Version of experiments	Liver	Brain	Thymus	Kidney
Baseline	369,0□16,6	71,0□3,4	800,0□41,3	175,0□8,8
Cisplatin treatment	*333,0□7,7	68,0□3,5	738,5□43,5	180,0□10,6

*P<0,05

The quantity of total phospholipids in nuclear fractions of different tissues varied significantly. The maximum content of total phospholipids was observed in nuclear fraction of thymus (800,0 mcg per gram of tissue), but the reliable changes of total phospholipids content after the *in vivo* action of cisplatin was revealed only in nuclear fraction of rat liver cells (decrease of phospholipids content nearly by 10%) (Table 1.). So, the further investigations were carried out on liver cells.

Seven individual fractions of phospholipids were revealed in nuclei of liver cells (Fig.1). Phosphatidylcholine (PhCh) and phosphatidylethanolamine (PhE) were the major components to form more than 65% of total phospholipids amount, while the percentage of the other five fractions varied within 4-10%. (Table 2).

The results of our study confirm, that phospholipids of rat liver nuclei exhibit diversity in sensitivity to cisplatin treatment. The quantities of phosphatidylserine, (PhS) sphingomyelin (SM), cardiolipin (C) and phosphatidic acid (PhA) were decreased while the content of phosphatidylinositol (PhI) and phosphatidylethanolamine (PhE) remained unchanged. Decrease of sphingomyelin content (~33%) was accompanied by increase in phosphatidylcholine quantity (~9%). (Table 3 and Fig.2).

At the same time the *in vivo* treatment by cisplatin leads to significant changes of percentage of separate fractions of phospholipids in nuclei: the percentages of phosphatidylserine, phosphatidic acid, cardiolipin and sphingomyelin were decreased while that of phosphatidylcholine was increased and the percentages of phosphatidylinositol and phosphatidylethanolamine remained unchanged (Table 2).

Thus, the results show that the antineoplastic drug cisplatin, which is a well known DNA-damaging factor, disorders many metabolic pathways in nuclei including the lipid metabolism. The suppression effect on phospholipid biosynthesis leads to nonspecific, universal decreasing of quantities of some phospholipid fractions.

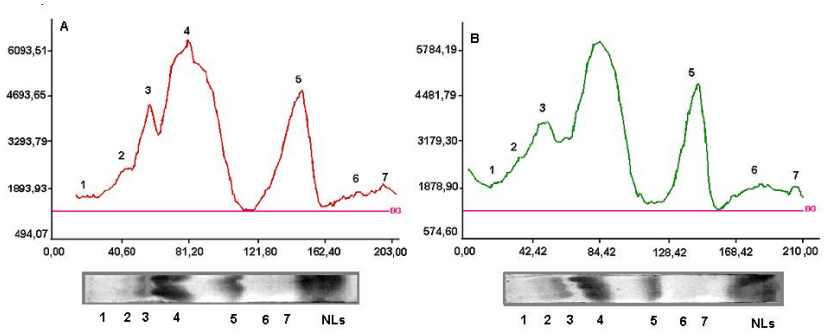

Fig.1. The chromatograms and densitograms of rat liver nuclear phospolipids, Fractionated by microTCL.
A- baseline, B - after the cisplatin *in vivo* treatment.
1- phosphatidylserine, 2-sphingomyelin, 3- phosphatidylinositol, 4-phosphatidylcholine, 5 - phosphatidylethanolamine, 6 - cardiolipin, 7 - phosphatidic acid, NLs- neutral lipids

Table 2. The percentage content of individual phospholipid fractions in nuclei of rat liver cells after the cisplatin in vivo treatment

Version of experiments	PhS	SM	PhI	PhCh	PhE	C	PhA
Baseline	5,8	7,6	10,4	39,7	26,1	4,8	5,6
Cisplatin treatment	3,8	5,6	11,1	45,8	26,1	3,9	3,7

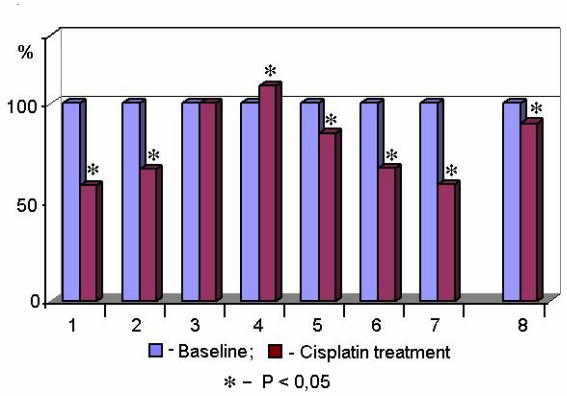

Fig.2. Changes (in %) of quantities of individual phospholipids in nuclei of rat liver cells after the cisplatin *in vivo* treatment. The phospholipid quantities in baseline version is equal to 100%.
1-phosphatidylserine, 2-sphingomyelin, 3- phosphatidylinositol, 4- phosphatidylcholine, 5 – phosphatidylethanolamine, 6 – cardiolipin, 7 - phosphatidic acid, 8 – total phospholipids.

At the same time the maximum decreasing level of negatively charged phospholipids such as phosphatidylserine, phosphatidic acid and cardiolipin may be also the result of interaction of some molecules of positively charged cisplatin with them, as it was demonstrated in *in vitro* experiments by other authors [11-13]. Such possibility should not be excluded and may be considered in *in vivo* experiments [11].

Table 3. The quantities of individual phospholipids fractions (in mkg/g of tissue) in nuclei of rat liver cells after the cisplatin in vivo treatment.

Phospholipids	Baseline	Cisplatin treatment
Phosphatidylserine (PhS)	21,5□1,6	12,6□1,4*
Sphingomyelin (SM)	29,0□ 2,0	18,7□2,0*
Phosphatidylinositol (PhI)	38,3□2,2	37,4□3,3
Phosphatidylcholine (PhCh)	146,5□ 5,3	159,2□1,6*
Phosphatidylethanolamin (PhE)	96,2□2,8	81,6□3,8*
Cardiolipin (C)	18,0□2,5	12,0□0,4*
Phosphatidic acid (PhA)	20,8□1,8	12,2□1,0*

*-P<0,05

Therefore, the decreasing effect caused by cisplatin was more expressive in case of sphingomyelin which may be explained as consequence of possible activation of the process of transferring phosphorylcholine group from sphingomyelin molecule to phosphatidylcholine by antitumor agent. Decrease in the quantity of sphingomyelin may be also the result of cisplatin activation of acidic sphingomyelinase which was demonstrated by other authors [11,12].

These remarkable changes of content of all individual fractions of phospholipids in nuclei of rat liver cells show the profound affect of cisplatin on nuclear lipid metabolism and indicate the necessity of further studies of those changes separately in nuclear membrane fraction and in intranuclear structures, including chromatin and nuclear matrix.

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