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CISPLATIN *IN VIVO* ACTION ON CONTENT OF NEUTRAL LIPIDS IN RAT LIVER AND THYMUS NUCLEAR MEMBRANES

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The content of total neutral lipid and its individual fractions in rat liver and thymus nuclear membrane preparations was studied. The in vivo action of antitumor agent cisplatin leads to decrease of total neutral lipid content in both liver and thymus nuclear membrane preparations a 24% and 20% correspondingly. Six fractions of neutral lipids were revealed in both liver and thymus nuclear membrane preparations. The cisplatin action leads to decrease of relative content and percentage of free cholesterol while the relative content and percentage of other fractions do not reliably changed. At the same time the absolute quantities of all fractions are reliably changed which demonstrates the high-powered action of cisplatin on nuclear lipid metabolic pathways. The absolute quantities of all fractions reliable decreased under the cisplatin action in both liver and thymus nuclear membrane preparations.

Cisplatin – nuclear membranes – neutral lipids – cholesterol

Հետազոտվել է առնետի լյարդի և ուրցագեղձի կորիզաթաղանթների չեզոք լիպիդների բաղադրությունը հակաուռուցքային միացություն ցիսպլատինի *in vivo* ազդեցության տակ: Ցույց է տրված, որ ցիսպլատինն իջեցնում է չեզոք լիպիդների ընդհանուր քանակը 24%-ով լյարդի և 20%-ով ուրցագեղձի կորիզաթաղանթների պատրաստուկներում: Չեզոք լիպիդների վեց ֆրակցիաներ են հայտնաբերվել ինչպես լյարդի, այնպես էլ ուրցագեղձի կորիզաթաղանթներում: Ցիսպլատինի ազդեցությունը բերում է պատրաստուկներում խոլեստերինի հարաբերական քանակի և տոկոսային բաղադրության հավաստի նվազման այն դեպքում, երբ մնացած ֆրակցիաների հարաբերական քանակը և տոկոսային բաղադրությունը հավաստիորեն չի փոփոխվում: Միաժամանակ, բոլոր ֆրակցիաների բացարձակ քանակները հավաստիորեն փոփոխվում են, ինչը վկայում է բջջակորիզի լիպիդների մետաբոլիզմի վրա ցիսպլատինի զգալի ներգործության մասին: Բոլոր ֆրակցիաների բացարձակ քանակները ինչպես լյարդի, այնպես էլ ուրցագեղձի կորիզաթաղանթներում հավաստիորեն նվազում են:

Ցիսպլատին – կորիզաթաղանթներ – չեզոք լիպիդներ – խոլեստերին

Изучено *in vivo* воздействие противоопухолевого препарата цисплатина на состав нейтральных липидов печени и тимуса крыс. Показано, что цисплатин приводит к снижению количества тотальных нейтральных липидов в препаратах ядерных мембран печени (на 24%) и тимуса (на 20%) крыс. Шесть фракций нейтральных липидов было выявлено в препаратах ядерных мембран как печени, так и тимуса крыс. *In vivo* воздействие цисплатина приводит к достоверному снижению относительного количества и процентного содержания свободного холестерина, в то время как относительное количество и процентное содержание остальных фракций достоверно не изменяется. Одновременно показано, что абсолютное количество всех фракций нейтральных липидов достоверно снижается как в препаратах ядерных мембран печени, так и в препаратах ядерных мембран тимуса крыс, что свидетельствует о значительном воздействии цисплатина на внутриядерные процессы метаболизма липидов.

Цисплатин – ядерные мембраны – нейтральные липиды – холестерин

The nuclear envelope is a unique dynamic structure, which serves not only as a physical barrier between the nucleus and the cytoplasm, but also as a regulator of many cellular events. It is evident that the main components of inner and outer nuclear membranes, including the phospholipid and neutral lipid components, may play important role in these various events [1,2,7]. Changes in nuclear lipid metabolism, which may be realized under the *in vivo* action of different endogenous and exogenous regulators, will lead to sudden alterations in functioning of various nuclear events: such as macromolecular exchanges between the cytosole and nucleoplasm or signal transduction nuclear pathways [11,13]. Cisplatin (cis-diamminedichlorplatinum) is among those well known exogenous agents which is clinically used as adjuvant therapy of cancers aiming to induce tumor cells death [8, 12]. This compound has a number of possible targets in cells but the major target for it is nuclei: nuclear membranes and a number of intranuclear genetic structures, including chromosomes, chromatin and nuclear matrix. In this paper the changes of total neutral lipid content of nuclear membranes preparations from rat liver and thymus cells as well as the relative alterations of individual neutral lipids, and absolute changes their quantities after the *in vivo* action of cisplatin were described.

Materials and methods. The experiments were carried out on albino rats (120-150g weight). Cisplatin was injected peritoneal in concentration of 5 mg per 1000 g animal weight. Rats were decapitated after 24 hours of cisplatin injection. Rat liver nuclei were isolated by the method of Blober and Potter [6] and nuclear fraction of thymus – by the method of Allfrey et al [3]. Nuclear membranes were isolated by the method of Berezney et al [4]. Lipid extraction was carried out by Bligh and Dayer [5]. The fractionation of neutral lipids was carried out by micro thin layer chromatography (micro TLC) using L silicagel, 6x9 sm^2 plates with the thickness of layer 5-7 mcm, using diethyl ester – petroleum ester – formic acid in ratio 40:10:1 as a dividing mixture. After the chromatography the plates were dried up at 20^0C and were treated by 10% H_2SO_4. 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 FUGIFILM Science Lab 2001 Image Gauge V 4.0, which was destined for densitometry. Obtained results were treated by statistics.

Results and Discussion. Total neutral lipid content (in mcg/g of tissue) in nuclear membrane preparations of rat liver and thymus cells in baseline and after *in vivo* treatment of cisplatin was presented in fig.1. Cisplatin treatment reliable decreased the total neutral lipids content in both nuclear membrane preparations: a 24% decrease in rat liver nuclear membranes and 20% decrease in rat thymus nuclear membranes (fig.1).

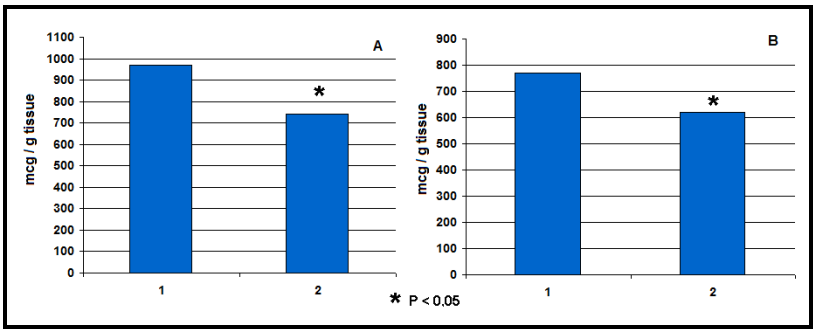

Fig. 1. Neutral lipid content (in micrograms per grams of tissue) in nuclear membrane preparations of rat liver (A) and thymus (B) cells before (1) and after (2) the *in vivo* treatment of cisplatin.

The relative quantities and percentage of individual fractions of neutral lipids in nuclear membrane preparations from liver and thymus cells after administration of cisplatin were demonstrated in tab. 1 and 2. Six fractions of separate neutral lipids were revealed in both nuclear membrane preparations. Over 78 % (in liver nuclear membranes) and near 76 % (in thymus nuclear membranes) of total neutral lipids were represented by free cholesterol, free fatty acids and cholesterol esters, while the percentage of three other fractions (monoglycerides, diglycerides and triglycerides) was only 21-24% (tab. 1, tab. 2).

Tab. 1. The relative content (in micrograms) and percentage of individual neutral lipid fractions in nuclear membrane preparations of rat liver cells before and after the cisplatin action

#	Neutral lipids	B a s e l i n e		C i s p l a t i n	
		Quantity in mcg.	%	Quantity in mcg.	%
1	Cholesterol	18.50±1.50	37.0	*15.40±1.35	30.8
2	Cholesterol Esters	8.15±1.10	16.3	9.05±1.32	18.1
3	Free Fatty Acids	12.40±0.60	24.8	14.20±0.83	28.4
4	Monoglycerides	4,80±0.48	9.6	4.90±0.45	9.8
5	Diglycerides	3,40±0.65	6.8	3.65±0.31	7.3
6	Triglycerides	2.75±0.46	5.5	2.80±0.15	5.6
	T o t a l	50	100	50	100

*p<0.05

In vivo action of cisplatin led to reliable changes only of free cholesterol percentage in both nuclear membrane preparations. Its percentage decreased in both liver and thymus nuclear membranes (tab. 1 and 2). The alterations of relative quantities of rest fractions of neutral lipids were negligible and not reliable.

Tab. 2. The relative content (in micrograms) and percentage of individual neutral lipid fractions in nuclear membrane preparations of rat thymus cells before and after the cisplatin action

#	Neutral lipids	B a s e l i n e		C i s p l a t i n	
		Quantity in mcg.	%	Quantity in mcg	%
1	Cholesterol	19.25±1.16	38.5	17.10±1.20	34.2
2	Cholesterol Esters	7.40±1.04	14.8	7.90±0.67	15.8
3	Free Fatty Acids	11.35±1.13	22.7	12.73±0.47	25.5
4	Monoglycerides	5.00±0.40	10.0	5.10±0.25	10.2
5	Diglycerides	4.50±0.45	9.0	4.75±0.23	9.5
6	Triglycerides	2.50±0.44	5.0	2.42±0.15	4.8
	T o t a l	50	100	50	100

*p<0.05

These relative quantities among the individual fractions of neutral lipids after the cisplatin action were resulted when we took equal amounts of lipids (50 mcg) both in baseline and cisplatin-treated probes. Taking into consideration that in vivo administration of cisplatin leads to reliable decrease of total neutral lipids content in both rat liver and rat thymus nuclear membrane preparations a 24% and 20% correspondingly (fig.1) the necessity arises to determine the changes of absolute quantities of individual neutral lipids after cisplatin action. The absolute quantities of all fractions of neutral lipids in liver and thymus nuclear membrane preparations were decreased reliably (tab. 3).

Cisplatin in vivo action leads to decrease of all neutral lipid fractions in both liver and thymus nuclear membrane preparations (tab. 4). The most significant reduction was revealed in free cholesterol fraction (36.5% in liver and 28.5% in thymus nuclear membrane preparations) and the less decrease was detected in free fatty acids fraction (12.6% in liver and 9.6% in thymus nuclear membrane preparastions). The quantities of other neutral lipid fractions decreased in various extent.

Tab. 3. The quantities (in micrograms per gram of tissue) of individual neutral lipids fractions in nuclear membrane preparations of rat liver and thymus cells before and after the cisplatin action (CH – cholesterol; CHE – cholesterol esters; FFA – free fatty acids; MG – monoglycerides, DG – diglycerides, TG – triglycerides).

#	Neutral lipids	Liver Nuclear Membrane		Thymus Nuclear Membrane	
		Baseline	Cisplatin	Baseline	Cisplatin
1	CH	58.90±14.35	*227.92±10.90	296.45±19.33	*212.04±8.16
2	CHE	158.11±6.68	*133.94±9.03	113.96±5.42	*97.96±2.40
3	FFA	40.56±11.00	*210.16±8.85	74.79±10.79	*158.10±3.55
4	MG	93.12±3.94	*72.52±1.77	77.00±5.12	*63.24±2.07
5	DG	65.96±3.61	*54.02±1.66	69.30±5.52	*58.90±1.93
6	TG	53.35±1.44	*41.44±3.19	38.50±4.02	*29.76± 2.19
	T o t a l	970.00±41.0	*740.00±35.40	770.00±50.20	*620,00±20.30

*p<0.05

This appreciable diminution of neutral lipids quantities under the antitumor agent cisplatin action is very important as during malignant transformation the ratio of neutral lipids to phospholipids increases dramatically and new fractions of neutral lipids appear [14].

Tab. 4. The alteration (in percent) of individual phospholipid quantities in liver and thymus nuclear membrane preparations under the cisplatin *in vivo* action

#	Neutral lipids	Liver Nuclear Membrane	Thymus Nuclear Membrane
1	Cholesterol	- 36.5%	- 28.5%
2	Cholesterol esters	- 15.3%	- 14.0%
3	Free Fatty Acids	- 12.6%	- 9.6%
4	Monoglycerides	- 22.1%	- 17.9%
5	Diglycerides	- 18.1%	- 15.0%
6	Triglycerides	- 22.3%	- 22.7%
	T o t a l	- 23.7%	- 19.5%

*p<0.05

These results are consonant with our previous data obtained in studies of changes of neutral lipids content in rat liver and thymus chromatin after the cisplatin *in vivo* action. In those studies the quantities of all four neutral lipid fractions in both chromatin preparations were also decreased [9]. Similar results were obtained also in studies of phospholipids content in rat liver and thymus chromatin preparations under the cisplatin *in vivo* treatment. The quantities of all five phospholipid fractions revealed in chromatin preparations as well as the total phospholipid content were also reliable decreased [10]. So all these results demonstrate the deep and multiform transformation of lipid metabolism in nuclei caused by cisplatin injection.

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