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

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The content of total neutral lipid and its individual fractions in rat liver and thymus nuclear matrix 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 matrix preparations by 24% and 15% respectively. Six fractions of neutral lipids were revealed in both liver and thymus nuclear matrix preparations. The relative content (the share) of all neutral lipid fractions is not changed reliably after the *in vivo* action of cisplatin in preparations of both liver and thymus nuclear matrix. At the same time the absolute quantities almost of all fractions in liver nuclear matrix are reliably changed while the absolute quantities only of free cholesterol and cholesterol esters changed reliably in thymus nuclear matrix preparation. These data demonstrate the high-powered action of cisplatin on nuclear lipid metabolic pathways.

Cisplatin – nuclear matrix – neutral lipids – cholesterol

Հետազոտվել է առնետի լյարդի և ուրցագեղձի բջիջների կորիզային մատրիքսի չեզոք լիպիդների բաղադրությունը հակաուռուցքային միացության՝ ցիսպլատինի *in vivo* ազդեցության տակ: Ցույց է տրված, որ ցիսպլատինն իջեցնում է չեզոք լիպիդների ընդհանուր քանակը 24%-ով լյարդի և 15%-ով ուրցագեղձի կորիզային մատրիքսի պատրաստուկներում: Չեզոք լիպիդների վեց ֆրակցիաներ են հայտնաբերվել ինչպես լյարդի, այնպես էլ ուրցագեղձի կորիզային մատրիքսում: Չեզոք լիպիդների բոլոր ֆրակցիաների հարաբերական բաժնեմասը կամ նրանց տոկոսային բաղադրությունը հավաստիորեն չեն փոփոխվում հետազոտված պատրաստուկներում: Միաժամանակ, լյարդի կորիզային մատրիքսի պատրաստուկներում գրեթե բոլոր ֆրակցիաների բացարձակ քանակները հավաստիորեն փոփոխվում են, այն դեպքում երբ ուրցագեղձի կորիզային մատրիքսում հավաստիորեն փոփոխվում են միայն խոլեստերինի և խոլեստերինի եթերների քանակները: Ստացված տվյալները վկայում են բջջակորիզի լիպիդների մատաբոլիզմի վրա ցիսպլատինի զգալի ազդեցության մասին:

Ցիսպլատին – կորիզային մատրիքս – չեզոք լիպիդներ – խոլեստերին

Изучено *in vivo* воздействие противоопухолевого препарата цисплатина на состав нейтральных липидов ядерного матрикса клеток печени и тимуса крыс. Показано, что цисплатин приводит к снижению количества тотальных нейтральных липидов в препаратах ядерного матрикса печени (на 24%) и тимуса (на 15%) крыс. Шесть фракций нейтральных липидов было выявлено в препаратах ядерного матрикса как печени, так и тимуса крыс. *In vivo* воздействие цисплатина не приводит к достоверному снижению относительного количества и процентного содержания выявленных фракций в обоих препаратах ядерного матрикса. Вместе с тем показано, что в препаратах ядерного матрикса печени абсолютное количество почти всех фракций нейтральных липидов достоверно снижается, в то время как в препаратах ядерного матрикса клеток тимуса крыс достоверно снижается только абсолютное количество холестерина и эфиров холестерина. Полученные данные свидетельствует о заметном воздействии цисплатина на внутриядерный метаболизм липидов

Цисплатин – ядерный матрикс – нейтральные липиды – холестерин

The nuclear matrix is a three-dimensional structure which is composed predominantly of non-histone proteins and small amounts of DNA, RNA, phospholipids and neutral lipids [10]. This nuclear skeleton-like structure plays important role in nucleus, such as in initiation of synthesis and replication of DNA and in synthesis, processing and transport of RNA [2,12]. Though lipids are the minor components of nuclear matrix they originally participate in principal nuclear events [3]. In this sense, the investigations of nuclear lipid metabolism are very essential. Lipid metabolic changes may be developed under the *in vivo* action of various exogenous agents. Cisplatin (cis-diammine-dichlorplatinum) is among them. It is well-known exogenous agent the main target of which is cell nucleus and which is clinically used as adjuvant therapy of cancers aiming inducing tumor cells death [8]. In this paper the changes in total neutral lipid content of nuclear matrix preparations from rat liver and thymus cells as well as the relative alterations in individual neutral lipids and absolute changes in 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 [7] and nuclear fraction of thymus – by the method of Allfrey et al [4]. Nuclear matrix preparations were isolated from purified nuclei by the method of Berezney and Coffey [5]. Lipid extraction was carried out by Bligh and Dayer [6]. The fractioning 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 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. Cisplatin injection to rats after 24 hours led to decrease in neutral lipids total amount both in rat liver (by 24% diminution of content) and thymus (by 15% diminution of content) nuclear matrix preparations (fig.1).

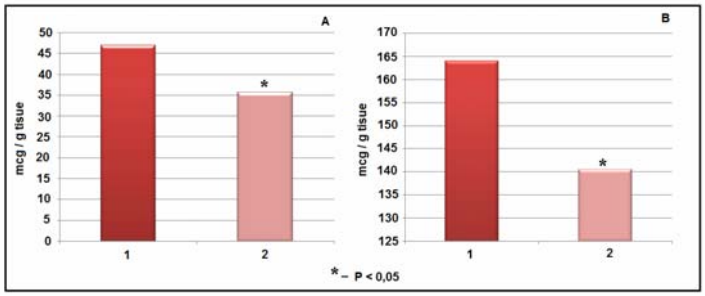

Figure 1. Neutral lipid content (in micrograms per grams of tissue) in nuclear matrix preparations of rat liver (A) and thymus (B) cells before (1) and after (2) the *in vivo* treatment of cisplatin.

The fractioning of neutral lipids disclosed six individual fractions both in liver and thymus nuclear matrix preparations (tabl. 1 and 2). Unlike phospholipids, the composition of neutral lipids of the nuclear matrix has been reported only in a few studies [1, 11, 12]. The relative content (the share) of all neutral lipid fractions is not reliably changed after the in vivo action of cisplatin both in preparations of liver and thymus nuclear matrix (tabl. 1 and 2).

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

#	Neutral lipids	Baseline		Cisplatin	
		Quantity in mcg	%	Quantity in mcg	%
1	Cholesterol	14.50±0.60	29.0	15.00±0.37	30.0
2	Cholesterol Esters	4.25±0.20	8.5	4.00±0.24	8.0
3	Free Fatty Acids	12.00±0.65	24.0	11.50±0.47	23.0
4	Monoglycerides	5,25±0.32	10.5	6.00±0.35	12.8
5	Diglycerides	5,00±0.16	10.0	5.50±0.38	10.2
6	Triglycerides	9.00±0.80	18.0	8.00±0.48	16.0
	Total	50	100	50	100

*p<0,05

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

#	Neutral lipids	Baseline		Cisplatin	
		Quantity in mcg	%	Quantity in mcg	%
1	Cholesterol	21.60±0.72	43.2	9.00±0.50	38.0
2	Cholesterol Esters	5.00±0.49	10.0	4.80±0.23	9.6
3	Free Fatty Acids	7.70±0.50	15.4	8.50±0.15	17.0
4	Monoglycerides	7.40±0.59	14.8	8.35±0.14	16.7
5	Diglycerides	5.50±0.50	11.0	6.30±0.24	12.6
6	Triglycerides	2.80±0.30	5.6	3.05±0.30	6.1
	Total	50	100	50	100

*p<0,05

Taking into consideration that *in vivo* administration of cisplatin leads to reliable decrease in total neutral lipids content in both rat liver and rat thymus nuclear matrix preparations by 24% and 15% respectively (fig.1) the necessity arises to determine the changes in absolute quantities of individual neutral lipids after cisplatin action. The absolute quantities of almost all fractions of neutral lipids in liver nuclear matrix preparations were decreased reliably (tabl. 3 and 4).

Tabl. 3. The quantities (in micrograms per gram of tissue) of individual neutral lipids fractions in nuclear matrix preparations of rat thymus and liver 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 Matrix		Thymus Nuclear Matrix	
		Baseline	Cisplatin	Baseline	Cisplatin
1	CH	13.63±0.49	*10.71±0.18	70 .80±2.60	*53.20±0.91
2	CHE	4.00±0.14	*2.86±0.12	16.40±0.58	*13.48±0.26
3	FFA	11.28±0.40	*8.21±0.35	25.26±0.89	23.80±0.41
4	MG	4.94±0.18	4.58±0.18	24.32±0.75	23.38±0.40
5	DG	4.70±0.17	*3.63±0.16	18.04±0.63	17.64±0.30
6	TG	8.45±0.30	*5.71±0.24	9.18±0.32	8.50±0.12
	Total	47.00±1.68	*35,70±1.50	164.00±5.77	*140.00±2.40

*p<0,05

These results are consonant with our previous data obtained in studies of changes in neutral lipids content in rat liver and thymus chromatin after the cisplatin in vivo action. In those studies the quantities in all four neutral lipid fractions in liver chromatin preparations were also decreased [9].

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

#	Neutral lipids	Liver Nuclear Matrix	Thymus Nuclear Matrix
1	Cholesterol	- 21.4%	- 24.9%
2	Cholesterol esters	- 28.5%	- 17.8%
3	Free Fatty Acids	- 27.2%	- 5.8%
4	Monoglycerides	- 7.3%	- 3.9%
5	Diglycerides	- 22.3%	- 2.2%
6	Triglycerides	- 32.4%	- 7.4%
	T o t a l	- 24.0%	- 14.6%

Taking into consideration that cisplatin treatment decreased the total amount in neutral lipids in thymus nuclear matrix by less than 15%, the reliable diminution in absolute quantities was expressed only in free cholesterol and cholesterol esters fractions (Tables 3 and 4). Thus, the obtained data demonstrate the noticeable action of cisplatin on nuclear lipid metabolic pathways.

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