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CONTENT OF NEUTRAL LIPIDS IN RAT LIVER AND THYMUS CHROMATIN UNDER THE IN VIVO ACTION OF CISPLATIN

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The content of total neutral lipid and its individual fractions in rat liver and thymus chromatin was studied. The in vivo action of antitumor agent cisplatin leads to decrease of total neutral lipid content in both rat liver and rat thymus chromatin a 25% and 36% correspondingly. In spite of these significant changes of total neutral lipids content the alteration of individual neutral lipids percentage in chromatin preparation was negligible. The main difference is due to relative quantities of triglycerides and cholesterol content in liver chromatin preparations (on 3,5-3,8%), while in thymus chromatin the most altered lipid fraction was cholesterol (near 5%). At the same time cisplatin leads to reliable decrease in the absolute quantities of all four identified fractions of individual neutral lipids in both liver and thymus chromatin preparations. This demonstrates the high-powered action of cisplatin on nuclear lipid metabolic pathways.

Cisplatin – chromatin - neutral lipids – cholesterol

Հետազոտվել է առնետի լյարդի և ուրցագեղձի քրոմատինի չեզոք լիպիդների բաղադրությունը հակաուռուցքային միացություն ցիսպլատինի *in vivo* ազդեցության տակ: Ցույց է տրված, որ ցիսպլատինն իջեցնում է չեզոք լիպիդների ընդհանուր քանակը 25%-ով լյարդի և 36%-ով ուրցագեղձի քրոմատինի պատրաստուկներում: Չնայած այս զգալի տեղաշարժերին, չեզոք լիպիդների առանձին ֆրակցիաների տոկոսային բաղադրությունն էապես չի փոխվում: Առնետի լյարդի քրոմատինում հայտնաբերված չեզոք լիպիդների չորս ֆրակցիաներից հավաստի փոփոխության է ենթարկվել եռգլիցերիդների (3,8%-ով) և խոլեստերինի (3,5%-ով) տոկոսային բաղադրությունը, իսկ ուրցագեղձի քրոմատինում՝ միայն խոլեստերինի բաժնեմասը (մոտ 5%-ով): Միաժամանակ ցույց է տրվել, որ և լյարդի, և ուրցագեղձի քրոմատինի պատրաստուկներում չեզոք լիպիդների բոլոր առանձին ֆրակցիաների բացարձակ քանակները հավաստիորեն նվազել են: Ստացված արդյունքները վկայում են բջջակորիզի լիպիդների մետաբոլիկ գործընթացների վրա ցիսպլատինի հզոր ազդեցության մասին:

Ցիսպլատին – քրոմատին – չեզոք լիպիդներ – խոլեստերին

Изучено *in vivo* воздействие противоопухолевового препарата цисплатина на состав нейтральных липидов хроматина печени и тимуса крыс. Показано, что цисплатин приводит к снижению количества тотальных нейтральных липидов в препаратах хроматина печени (на 25%) и хроматина тимуса (на 36%) крыс. На фоне такого значительного снижения количества тотальных нейтральных липидов изменение процентного содержания отдельных фракций нейтраль- ных липидов незначительно. Достоверные изменения наблюдаются в процентном содержании триглицеридов (на 3,8%) и холестерина (на 3,5%) в препаратах хроматина печени, а в препаратах хроматина тимуса достоверно снижается только доля холестерина (примерно на 5%). Показано также, что абсолютные количества всех выявленных четырех фракций нейтральных липидов как в препаратах хроматина печени,

жаются после воздействия цисплатина. Полученные результаты свидетельствуют о мощном воздействии цисплатина на внутриядерные процессы метаболизма липидов.

Цисплатин – хроматин – нейтральные липиды – холестерин

The presence of lipids, phospholipids and neutral lipids, as a component of chromatin is now well demonstrated [1, 2, 8, 11]. It was shown that phospholipids predominantly were bound to histones while various neutral lipids were predominantly associated tp different components of chromatin. Thus, most tryglycerides were bound to histones and some of them were associated to non-histone proteins and DNA; most free cholesterol was DNA-bound and a small amount was associated to histones and non-histone proteins. So, DNA is enriched in cholesterol and its esters, free fatty acids and triglycerides; non-histone proteins are enriched in triglycerides and free cholesterol; histones are enriched in triglycerides [8, 12]. These data confirm the role of chromatin neutral lipids in main functions of nuclei – replication and transcription and therefore, any alteration in neutral lipids fractions quantity may be of importance.

On the other hand the cisplatin (cis-diamminedichlorplatinum) is well known effective antitumor agent which is clinically used as adjuvant therapy of cancers aiming to induce tumor cells death [6, 7, 9]. It is also well known that cisplatin has a number of possible targets in cells but the major target for it is DNA – the main component of chromatin. So, the knowledge about cisplatin sensitivity of chromatin-bound neutral lipids might contribute to a better understanding the cisplatin antitumor action mechanisms, which will be favorable to harmless course of chemotherapy. In this paper the changes of total neutral lipid content of chromatin 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 [5] and nuclear fraction of thymus – by the method of Allfrey et al [3]. Chromatin was isolated by the method of Umansky et al [13]. Lipid extraction was carried out by Bligh and Dayer [4]. The fractionation of neutral lipids was carried out by micro thin layer chromatography (micro TLC) using L silicagel, 6x9 sm2 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 $20^{O}C$ and were treated by 10% H_2SO_4. Then, the elaborated plates were heated at $180^{O}C$ 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. It is well known that cisplatin, like some other platinum complexes, induces the tumor cell death by various mechanisms, by interactions with various cell targets [7]. Cisplatin's interaction with DNA, with nuclear proteins, with protein components of signal transduction systems are among them. In fact, only a small amount of cellular platinum (<1%) is bound to nuclear DNA, but it is enough to damage DNA and to induce the cell death [6, 7]. Cisplatin binds to DNA leading to the formation of inter- and intrastrand cross-links. These cross-links may hamper the DNA-lipid interactions. Changes of neutral lipids content in chromatin preparations after the in vivo ac-tion of cisplatin may be the result of such disturbance of these interactions. Total neutral lipid content (in mcg/g of tissue) in chromatin preparations of rat liver and thymus cells in baseline and after *in vivo* treatment of cisplatin was presented in fig.1. The lipids quantity in rat thymus chromatin is much more than that in liver chromatin and the percentage of changes of total neutral lipids content after the in vivo action of cisplatin is

distinct: a 36% decrease in rat thymus chromatin and 25% decrease in rat liver chromatin (fig.1). In all probability this is the consequence of differences in thymus and liver chromatin superstructure. It may be the result of the ability of DNA in thymus chromatin to form much more inter- and intrastrand cross-links with cisplatin.

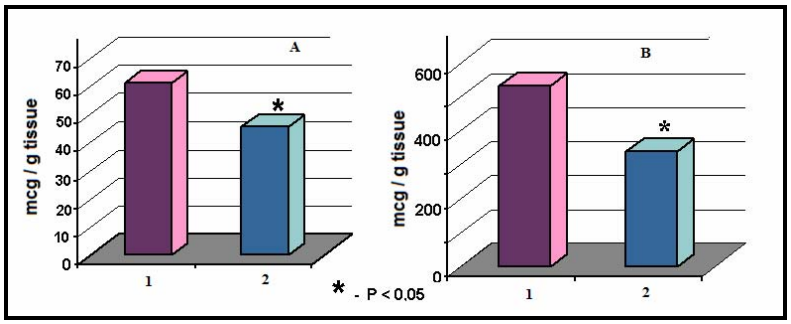

Fig.1. Neutral lipid content (in micrograms per grams of tissue) in chromatin preparations of rat liver (A) and thymus (B) cells before (1) and after (2) the in vivo treatment of cisplatin.

The relative quantities and percentage of separate fractions of neutral lipids in chromatin preparations from liver and thymus cells after administration of cisplatin were demonstrated in tab.1 and 2. Four fractions of separate neutral lipids were revealed in both chromatin preparations. Free cholesterol and free fatty acids together composed over than 60% of total neutral lipids in liver chromatin preparations while the percentage of triglycerides was the most diminutive (6.6%, tab. 1). At the same time the percentage of cholesterol esters, free fatty acids and triglycerides was equal each other in thymus chromatin preparations while the percentage of cholesterol was the most great (37%, tab. 2). *In vivo* action of cisplatin led to reliable changes only of free cholesterol percentage in both chromatin preparations. Its percentage decreased in liver chromatin and on the contrary increased in chromatin of rat thymus (tab. 1 and 2). The alterations of relative quantities of rest fractions of neutral lipids were negligible and not reliable.

Table 1. The relative content (in micrograms) and percentage of individual neutral lipids fractions in chromatin 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	15.25±0.38	30.5	* 13.50±0.55	27.0
2	Cholesterol Esters	12.65±0.42	25.3	11.50±0.38	23.0
3	Free Fatty Acids	15.50±0.53	31.0	16.50±0.47	33.0
4	Triglycerides	6.60±0.78	13.2	8.50±0.46	17.0
	T o t a l	50	100	50	100

*p<0.05

These relative changes among the separate fractions of neutral lipids after the cisplatin action were demonstrated 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 chromatin a 25% and 36% correspondingly (fig.1) the necessity arises to determine the absolute changes of individual fractions of neutral lipids after cisplatin action. The quantities of all neutral lipid fractions in liver and thymus chromatin preparations decreases reliably

which demonstrates the deep and multiform transformation of lipid metabolism in nuclei and particularly in chromatin caused by cisplatin (tab. 3).

Table 2. The relative content (in micrograms) and percentage of individual neutral lipids fractions in chromatin 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	18.75±0.80	37.5	* 21.20±1.00	42.4
2	Cholesterol Esters	10.00±0.50	20.0	8.50±0.20	17.0
3	Free Fatty Acids	11.20±0.40	22.4	10.30±0.36	20.6
4	Triglycerides	10.05±0.68	20.1	10.00±0.90	20.0
	T o t a l	50	100	50	100

*p<0.05

Table 3. The quantities (in micrograms per gram of tissue) of individual neutral lipids fractions in chromatin preparations of rat liver and thymus cells before and after the cisplatin action
(CH- cholesterol; CHE– cholesterol esters; FFA – free fatty acids; TG – triglycerides).

#	Neutral lipids	Liver Chromatin		Thymus Chromatin	
		Baseline	Cisplatin	Baseline	Cisplatin
1	CH	18,70±0,52	*12,39±0,33	199,87±17,63	*144,16±7,42
2	CHE	15,52±0,43	*10,55±0,29	106,60±9,40	*57,80±3,05
3	FFA	19,01±0,53	*15,14±0,41	119,39±10,57	*70,04±3,63
4	TG	8,10±0,22	*7,80±0,21	107,14±9,45	*68,00±3,50
	T o t a l	61,33	45,88	533,00	340,00

*p<0.05

Taking into consideration that some neutral lipids: diglycerides, cholesterol and its esters (together with cardiolipin) play the key role in the supramolecular organization of chromatin [12] such a significant diminution of cholesterol and its esters quantities in liver chromatin preparations and tryiglycerides, cholesterol and its esters quantities in thymus chromatin preparations (tab. 4) indicate that one of the main in vivo effects of cisplatin concerns the disturbance of supramolecular organization of chromatin.

Nowadays it is well known that there exist two pools of DNA-bound lipids: loosely bound and tightly bound ones. The loosely bound lipids may act as linkers between DNA replicon transcription, whereas the tightly bound lipids may be the specific sites of attachment of DNA loops to the nuclear matrix [12]. The obtained results demonstrate that cisplatin in vivo effect led to decrease of quantities of both loosely and tightly bound lipids (tab. 3). 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 [10,12].

So, bearing in mind that lipids of chromatin, especially glycerides and cholesterol, play a structural role and stabilize the supramolecular organization of chromatin the decreasing of neutral lipids quantities under the cisplatin action will promote the destroying the chromatin structure. Naturally, this will be accompanied by deep functional disturbances of nuclear functions.

Table 4. decrease (in percent) of individual phospholipid quantities in liver and thymus chromatin under the cisplatin in vivo action.

#	Neutral lipids	Liver Chromatin	Thymus Chromatin
1	Cholesterol	- 33.7%	- 27.9%
2	Cholesterol esters	- 32.0%	- 45.8%
3	Free Fatty Acids	- 20.4%	- 41.3%
4	Triglycerides	- 3.7%	- 36.5%
5	T o t a l	- 25.2%	- 36.2%

On the bases of these results we conclude that cisplatin in vivo action on neutral lipid content in both rat liver and thymus chromatin has a comprehensive character, may damage the supramolecular structure of chromatin and disturb the functioning of main nuclear processes: transcription and replication.

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