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CHANGES IN POLYPHOSPHOINOSITIDES CONTENT IN NUCLEAR MEMBRANES OF RAT LIVER AND THYMUS CELLS UNDER THE ACTION OF CISPLATIN

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The content of polyphosphoinositides in nuclear membranes of rat liver and thymus cells before and after the in vivo action of cisplatin has been studied. The percentage of monophosphoinositides was the highest (41-42% of total polyphosphoinositides) in both rat liver and thymus nuclear membrane fractions while the percentage of triphosphoinositides was the smallist: near 26%. Cisplatin treatment reduced the quantity of monophosphoinositides for nearly 30% in liver and more than 10% in thymus nuclear membrane fractions. The diminution of monophosphoinositides was accompanied by the increase in diphosphoinositides and triphosphoinositides content in both rat liver and thymus nuclear membranes. The obtained results testify that along with suppression nuclear lipid metabolism by in vivo action of cisplatin which was expressed by decreasing the quantities of almost all phospholipid fractions in nuclear membranes (including the fraction of monophosphoinositides) the triphosphoinositides/ monophosphoinositides ratio was significantly increased. This indicates that antitumor agent cisplatin may have an effect upon functioning the phosphoinositide metabolic pathway in rat liver and thymus nuclei.

Nuclear membranes – polyphosphoinositides – cisplatin

Հետազոտվել է առնետների լյարդի և ուրցագեղձի բջիջների կորիզաթաղանթի պոլիֆոսֆոինոզիտիդների բաղադրությունը ցիսպլատինի in vivo ազդեցության դեպքում: Ցույց է տրվել, որ ստուգիչ տարբերակներում մոնոֆոսֆոինոզիտիդների տոկոսային բաղադրությունը ամենաբարձրն է (41-42 %), իսկ տրիֆոսֆոինոզիտիդներինը՝ նվազագույնը (մոտ 26 %) նշված հյուսվածքների կորիզաթաղանթի պատրաստուկներում: Ցիսպլատինի ներարկումը բերում է մոնոֆոսֆոինոզիտիդների քանակության մոտ 30 %-ով նվազման լյարդի և ավելի քան 10 %-ով նվազման՝ ուրցագեղձի կորիզաթաղանթների պատրաստուկներում: Մոնոֆոսֆոինոզիտիդների թվաքանակի նվազումը ուղեկցվում է տրիֆոսֆոինոզիտիդների թվաքանակի մեծացմամբ ինչպես լյարդի, այնպես էլ ուրցագեղձի կորիզաթաղանթներում: Ստացված արդյունքները վկայում են այն մասին, որ կորիզաթաղանթների լիպիդների մետաբոլիզմի ճնշման ֆոնի վրա, որը պայմանավորված է ցիսպլատինի in vivo ազդեցությամբ և ուղեկցվում է ֆոսֆոլիպիդների գրեթե բոլոր ֆրակցիաների թվաքանակի նվազմամբ, տրիֆոսֆոինոզիտիդ/մոնոֆոսֆոինոզիտիդ փոխհարաբերությունը զգալիորեն աճում է: Սա նշանակում է, որ հակաուռուցքային դեղամիջոց ցիսպլատինը կարող է ներազդել առնետների լյարդի և ուրցագեղձի բջջակորիզներում ընթացող ֆոսֆոինոզիտիդային մետաբոլիկ ուղու գործունեության վրա:

Կորիզաթաղանթներ – պոլիֆոսֆոինոզիտիդներ – ցիսպլատին

Изучен состав полифосфоинозитидов ядерных мембран клеток печени и тимуса крыс после in vivo воздействия цисплатина. Показано, что в контрольных вариантах в препаратах ядерных мембран указанных тканей содержание монофосфоинозитидов наибольшее (41-42%), а содержание трифосфоинозитидов – наименьшее (примерно 26 %).

Введение цисплатина приводит к снижению количества монофосфоинозитидов в препаратах ядерных мембран клеток печени (на 30%) и клеток тимуса (на 10%) крыс. Снижение количества монофосфоинозитидов сопровождается повышением содержания трифосфоинозитидов в ядерных мембранах обеих тканей. Полученные результаты свидетельствуют о том, что на фоне подавления липидного метаболизма в ядерных мембранах, что обусловлено in vivo воздействием цисплатина и сопровождается снижением количества почти всех фракций фосфолипидов, соотношение трифосфоинозитид/монофосфоинозитид заметно повышается. Это означает, что антиопухолевое вещество цисплатин может воздействовать на функционирование фосфоинозитидного метаболического пути в ядрах клеток печени и тимуса крыс.

Ядерные мембраны – полифосфоинозитиды – цисплатин

Polyphosphoinositides make up only a small fraction of cellular phospholipids, yet they control almost all aspects of a cell's life and death. These lipids gained tremendous research interest as plasma membrane-signaling molecules when discovered in the 1970s and 1980s. The research in the last 15 years has added a wide range of biological processes regulated by phospatidilinositides, turning these lipids into one of the most universal signaling entities in eukaryotic cells [3, 14]. Phosphoinositide signaling pathways are present also in nuclei. The existence of a nuclear phosphatidilinositol metabolism is widely recognized [4, 12, 16]. Derangements of nuclear phosphoinositide metabolism are responsible for a number of human diseases ranging from rare genetic disorders to the most common ones such as cancer, obesity, and diabetes. The levels of nuclear polyphosphoinositides are changed in response to various stimuli, suggesting that they may serve to regulate specific nuclear functions. These levels may be changed also in response to variuos antitumor agents, to different medicines against cancer diseases. which may appear a valuable information for treatment. Cisplatin (cis-diamminedichlorplatinum) is among these renowed antitumor agents [9]. Its effectiveness seems to be due to the unique properties of cisplatin, which enters cells via multiple pathways and forms multiple different DNA-platinum adducts. Cisplatin kills cancer cells by damaging DNA and inhibiting DNA synthesis [15]. This antitumor agent acts upon cellular self-defense system by activating or silencing a variety of different genes, resulting in dramatic epigenetic and/or genetic alternations. As a result, the development of cisplatin resistance in human cancer cells in vivo and in vitro by necessity stems from bewilderingly complex genetic and epigenetic changes in gene expression and alterations in protein localization [17]. Thus, the cisplatin in vivo action affects manifold metabolic pathways in nuclei including the lipid metabolism. From this point of view the study of changes in polyphosphoinositides content in nuclei and particularly in nuclear membranes in rat liver and thymus cells under the in vivo action of cisplatin appears to be of definite interest.

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 [8] and nuclear fraction of thymus – by the method of Allfrey et al [2]. Nuclear membranes were isolated by the method of Berezney et al [6]. The extraction of nuclear membrane lipids was carried out by Bligh and Dayer [7]. Polyphosphoinositides were extracted by the method of Bergelson et al [1]. The source for isolation of polyphosphoinositides were the residues remained on filter after the phospholipids extraction. 3 ml of chloroform-methanol-HCl mixture (in 2:1:0.01 ratio) was added on these residues. After the shaking from time to time and incubation (20 min) the mixture was centrifuged (5 min, at 3.000 rot/min speed). This extraction procedure was repeated also 2 times. The residues were poured out after the third centrifugation and the liquid supernatants were joined together. This joined extract was washed in turn by 1N HCl, by the mixture of chloroform-methanol- 1N HCl (in 3:48:47 ratio) and by the mixture

of chloroform-methanol- 0.01N HCl (in 3:48:47 ratio). After each washing procedure the extracts were centrifuged (5 min., at 3.000 rot/min speed) and the supernatants were poured out. The final acidic extract of polyphosphoinositides was muddy and methanol was added for liquidating this muddiness. The fractionation of phosphoinositides were carried out by micro thin layer chromatography (micro TLC) using KCK silicagel, 6x9 sm^2 plates with the thickness of layer 5-7 mcm, using chloroform-methanol-4N NH_4OH in ratio 9:7:2 as a dividing mixture. After the chromatography the plates were dried up at 20OC 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. The phosphatidilinositol (monophosphoinositides) 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. Our previous results showed that the total phospholipids quantity in rat liver nuclear membranes preparations was a 25 % more (1450 mcg/g of tissue) than that in thymus nuclear membranes (1160 mcg/g of tissue) and the percentage of changes of total phospholipids content after in vivo action of cisplatin is nearly the same in both tissues nuclear membrane preparetions: a 23 % decrease of phospholipid quantity in nuclear membranes of liver cells and a 20 % decrease in thymus nuclear membranes [10]. These results demonstrated the universal influence of this cytotoxic antitumor agent on lipid metabolism in nuclear membranes of two tissues which sufficiently differ from each other.

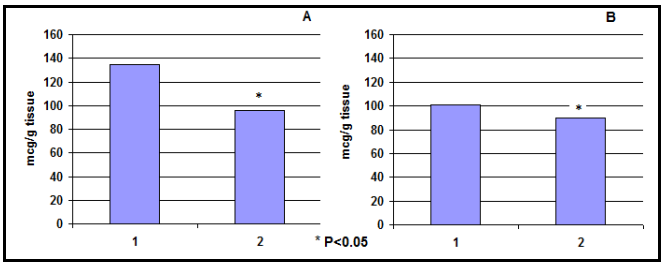

Fig. 1. Phosphatidilinositol (monophosphoinositides) content (in mcg/g of tissue) in rat liver (A) and thymus (B) nuclear membrane preparations before (1) and after (2) *in vivo* action of cisplatin

Polyphosphoinositides separated from rat liver and thymus nuclear membrane preparations were fractionated by micro thin layer chromatography into three phosphoinositide fractions: monophosphoinositides (the same as phosphatidilinositol), diphosphoinositides and triphosphoinositides. The relative quantities and percentage of each of them in nuclear membrane preparations from liver and thymus cells after administration of cisplatin were demonstrated in tab. 1 and 2. One can see that more than 40 % of total phosphoinositides was presented as monophosphoinositides in both liver and thymus nuclear membrane preparations, while the relative percentage of di- and triphosphoinositides was only 32-33 % and 26-27 % successively.

One can see that more than 40 % of total phosphoinositides was presented as monophosphoinositides in both liver and thymus nuclear membrane preparations, while the relative percentage of di- and triphosphoinositides was only 32-33 % and 26-27 % successively. Cisplatin administration led to appreciable decrease in relative content and percentage of monophosphoinositides in both liver and thymus nuclear membrane. At the same time the relative content and percentage of two other phosphoinositides were increased in both nuclear membrane prepartations (tab. 1 and 2).

Table 1. The relative content (mcg) of separate fractions of phosphoinositides in rat liver nuclear membrane preparations before (1) and after (2) in vivo action of cisplatin

#	Phosphoinositides	Baseline		Cisplatin	
		Quantity, mcg	%	Quantity, mcg	%
1	Monophosphoinositides	21.00±0.85	42.0	* 15.00±0.63	30.0
2	Diphosphoinositides	16.00±0.62	32.0	* 20.00±0.77	40.0
3	Triphosphoinositides	13.00±0.73	26.0	15.00±0.67	30.0
	Total	50	100	50	100

Table 2. The relative content (mcg) of phosphoinositides fractions in rat thymus nuclear membrane preparations before (1) and after (2) in vivo action of cisplatin

#	Phosphoinositides	Baseline		Cisplatin	
		Quantity, mcg	%	Quantity, mcg	%
1	Monophosphoinositides	20.35±0.85	40.7	* 13.00±0.67	26.0
2	Diphosphoinositides	16.43±0.23	32.9	16.50±0.78	33.0
3	Triphosphoinositides	13.22±0.19	26.4	* 20.50±0.30	41.0
	Total	50	100	50	100

The absolute quantities of diphosphoinositides and triphosphoinositides per 1 g of tissues were also reliably increased (tab. 3).

Table 3. Content of mono- , di- and triphosphoinosotides (mcg/g of tissue) in rat liver and thymus nuclear membrane preparations before and after the in vivo action of cisplatin

#	Phosphoinositides	Liver		Thymus	
		Baseline	Cisplatin	Baseline	Cisplatin
1	Monophosphoinositides	134.9±4.46	*95.5±3.08	100.9±2.54	*90.2±1.60
2	Diphosphoinositides	102.8±4.15	*127.3±3.07	81.6±1.52	*114.5±2.76
3	Triphosphoinositides	85.6±2.56	95.5±2.75	65.5±1.42	*142.2±0.95
4	Triphosphoinositides/ Monophosphoinositides	0.64	1.00	0.65	1.58

Thus, these results showed that in vivo action of antitumor agent cisplatin against the background of significant decreasing total phospholipid content (including phosphatidilinositol content) in nuclear membranes in both tissues [10] lead to reliable increase of diphosphoinositides quantities in liver and thymus nuclear membranes and to reliable increase of triphosphoinositides quantities particularly in thymus nuclear membranes (tab. 3).

It is well known that the transformation of monophosphoinositides into diphosphoinositides and subsequently into triphosphoinositides is conducted by definite phosphoinositide kinases. The activation of these kinases may be connected with destruction of certain nuclear structures caused by cisplatin action. Triphosphoinositides/ monophosphoinositides ratio may demonstrate the functional status of nuclear membranes concerning the functioning of several nuclear signaling pathways. These results are in accordance with literature data indicated that cisplatin-induced DNA damage activates phosphatidilinositol 3-kinase/Akt cascade both in vitro and in vivo which has been shown to mediate cell survival via the regulation of numerous proteins [11,13]. It is well known that cisplatin-induced DNA damage activates various signaling pathways to prevent or

Promote cell death [8]. So, cisplatin, being one of the most effective anticancer agents widely used in the treatment of tumors, often manifests contradictory effects preventing the cell death. Although extensive DNA damage can induce cell death by apoptosis, several signaling pathways, including phosphatidilinositol 3-kinase/Akt cascade and others, can regulate cisplatin-induced apoptosis. Since signaling pathways regulate cisplatin sensitivity, one way to improve the efficacy of cisplatin is to use it in combination with agents that target the signaling pathways and contribute to cisplatin resistance [8, 18]. For example, blocking the phosphatidilinositol 3-kinase/Akt cascade with a phosphatidilinositol 3-kinase inhibitor wortmannin increased thr efficacy of cisplatin [13]. These findings are very important especially in practical medicine. The results support the idea that combination therapy with cisplatin and wortmannin would increase the therapeutic efficacy of cisplatin [13]. The increase of triphosphoinositides/ monophosphoinositides ratio after the cisplatin action was observed in both liver and thymus nuclear membrane preparations (tab. 3). It is remarkable that this ratio was higher in case of thymus nuclear membrane preparations which showed that this transmuting process was more effective in thymus nuclear membranes which may be the result of huge differences among metabolic pathways in liver and thymus tissues.

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