Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

•Փորձարարական և տեսական հոդվածներ •Экспериментальные и теоретические статьи• •Experimental and theoretical articles•

Biolog. Journal of Armenia, 1 (68), 2016

CHANGES IN SPHINGOMYELIN AND PHOSPHATIDYLCHOLINE CONTENT IN RAT LIVER NUCLEAR STRUCTURES DURING THE *IN VIVO* ACTION OF CISPLATIN

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The content of sphingomyelin and phosphatidylcholine in rat liver nuclear membranes, nuclear matrix and chromatin was studied during the *in vivo* action of cisplatin. It was shown that the action of antitumor agent lead to decrease of content of both sphingolipids in all studied nuclear structures. At the same time the sphingomyelin: phospatidylcholine ratio was also significantly (nearly twice) decreased.

Cisplatin – sphingomyelin – phosphatidylcholine – nuclear membrane – nuclear matrix – chromatin

Հետազոտվել է առնետի լյարդի կորիզաթաղանթի, կորիզային մատրիքսի և քրոմատինի սֆինգոմիելինի և ֆոսֆատիդիլխոլինի բաղադրությունը հակառուռուցքային միացություն ցիսպլատինի *in vivo* ազդեցության դեպքում: Ցույց է տրված, որ ցիսպլատինի ազդեցությունը բերում է ուսումնասիրված կորիզային կառույցներում նշված սֆինգոլիպիդների բաղադրության զգալի նվազման: Միաժամանակ զգալիորեն (մոտ երկու անգամ) փոքրանում է նաև սֆինգոմիելին: ֆոսֆատիդիլխոլին քանակական հարաբերությունը:

Ցիսպլատին – սֆինգոմիելին – ֆոսֆատիդիլխոլին – կորիզաթաղանթ – կորիզային մատրիքս – քրոմատին

Изучено *in vivo* воздействие противоопухолевого препарата цисплатина на содержание сфинголипида и фосфатидилхолина в препаратах ядерных мембран, ядерного матрикса и хроматина клеток печени крыс. Показано, что цисплатин приводит к заметному снижению содержания обоих сфинголипидов в трех изученных ядерных структурах. Одновременно значительно (примерно вдвое) уменьшается также количественное соотношение сфингомиелин : фосфатидилхолин.

Цисплатин – сфингомиелин – фосфатидилхолин – ядерная мембрана – ядерный матрикс – хроматин

It is well known that the sphingolipids are very active biological compounds and important components of various membranes which are participated in many processes such as cell proliferation, differentation, intracellular signal tranduction and cell death [1, 5, 15, 19]. They also occupy the principal position among the apoptotic regulators [15, 19], taking into account that the sphingomyeline metabolites such as ceramide and sphingosine

are proapoptotic poisons [2, 3, 15]. The phosphatidylcholine-sphingomyelin metabolism cross-stalk inside the nucleus was also well investigated and the alteration of phosphatidylcholine : sphingomyelin ratio during the cell cycle was clearly demonstrated [3].

These above-mentioned facts confirm the importance of investigation of sphingolipids content alterations in various nuclear structures (nuclear membranes, nuclear matrix and chromatin) caused by *in vivo* action of antitumour factor cisplatin in rat liver cells.

Materials and methods. The experiments were carried out on albino rats (120-150 g 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 preparations were isolated by the method of Blobel and Potter [10]. Nuclear membranes were isolated by the method of Berezney et al [8], nuclear matrix preparations were isolated from purified nuclei by the method of Berezney and Coffey [7] and chromatin was isolated by the method of Umansky et al [20]. Phospholipid extraction was carried out by Bligh and Dayer [9]. The fractionation of phospholipids were carried out by micro thin layer chromatography (micro TLC) using L silicagel, 6 x 9 sm^2 plates with the thickness of layer 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 20^0C and were treated by 15.6 % $CuSO_4$ in 8 % phosphoric acid. 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 high metabolic activity in nuclei is preserved by the presence of numerous different enzymes including those that regulate the lipid metabolism. Many of these enzymes are targets for cisplatin action so as this chemotherapeutic agent is able to act on various pathways of lipid metabolism in nuclei [18]. The decrease of total phospholipid quantity under the *in vivo* action of cisplatin in rat liver nuclear membranes, nuclear matrix and chromatin was demonstrated in our previous studies [12, 13, 14]. Those results demonstrated that cisplatin *in vivo* action provoked quantity changes almost in all individual phospholipids in rat liver nuclear fractions. Taking into consideration the above-mentioned literature data we concentrated our attention especially on alterations in sphingolipids content in nuclear membrane, nuclear matrix and chromatin fractions of liver cells (tabl.1).

Table 1. The content of phosphatidylcholine and sphingomyelin (in micrograms per gram of tissue) and the sphingomyelin : phosphatidylcholine ratio in rat liver nuclear membrane, nuclear matrix and chromatin fractions before and after the cisplatin *in vivo* action.

Nuclear fractions	Sphingomyelin content		Phosphatidylcholine content		The sphingomyelin: phosphatidylcholine ratio	
	Baseline	Cisplatin	Baseline	Cisplatin	Baseline	Cisplatin
Nuclear membrane fraction	217,50±9,23	*111,00±5,13	500,25±21,22	466,20±21,55	0,435	0,238
Nuclear matrix fraction	14,11± 0,42	*7,00±0,20	14,78±0,44	*12,00±0,34	0,955	0,583
Chromatin fraction	13,72±0,36	*7,64±0,25	29,40±0,78	*25,60±0,83	0,467	0,298

*$p < 0.01$

These results showed the different measure of decrease in sphingomyelin and phospatidylcholine amount in all studied nuclear fractions caused by cisplatin treatment. The sphingomyelin content considerably decreased (nearly twice in all studied nuclear fractions), while in case of phosphatidylcholine the diminution was less than 20 % (tabl. 1). It means that cisplatin *in vivo* treatment resulted a new status of nuclear lipid metabolism. This new metabolic state may be the consequence of some processes which concern the sensitivity of some nuclear enzymes such as sphingomyelinase or sphingomyelin-synthase to cisplatin. Changes of activity of these enzymes may lead to increase the tranfer of phosphorylcholine group from sphingomyelin to phosphatidylcholine to a certain extent. At the same time it is well-known that the major metabolic product of sphingomyelin is ceramide, which is generated by nuclear sphingomyelinase and triggers apoptosis and other metabolic changes [3, 4, 6, 16]. Finely, the significant decrease of sphingomyelin : phosphatidylcholine ratio (tabl. 1) may affect on fluidity of nuclear membrane which in its turn may lead to appreciable metabolic exchange between nuclei and cytoplasm.

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Received on 13.10.2015