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ALTERATION OF LIPID CONTENT IN RAT KIDNEY NUCLEAR MEMBRANE PREPARATIONS AFTER THE IN VIVO ACTION OF CISPLATIN

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ABSTRACT

The content of total phospholipid and neutral lipid content and their individual fractions in rat kidney nuclear membranes was studied. The *in vivo* action of antitumor agent cisplatin lead to decrease of total phospholipid and neutral lipid content in rat kidney nuclear membrane preparations a 27.1% and 26.3% correspondingly. Seven fractions of individual phospholipids and six fractions of neutral lipids were revealed in nuclear membrane preparations. The quantities of all individual fractions of both phospholipids and neutral lipids were reliable changed under the cisplatin action. The diminution of content was observed in all fractions of phospholipids besides the content in phosphatidylinositol which quantity was even increased. Of the same kind results were demonstrated in case of neutral lipids: the reliable diminution was observed in all fractions except the content of cholesterol esters which quantity was also increased a little.

Key words: *cisplatin, phospholipids, neutral lipids, kidney nuclear membrane.*

INTRODUCTION

It is well known that cisplatin (cis-diaminedichloroplatinum II) is widely used as an effective chemotherapeutic agent for treatment of various malignancies via induction of cytotoxicity [1]. The latter was accompanied by alterations of transcription, DNA replication processes, via induction of all pathways of apoptosis [1,2]. The main problem for its application is the fact that cisplatin damages tumor cells as well as normal ones and the effectiveness of it is dose-depended. But its use in higher concentrations is limited because of several side effects, such as nephrotoxicity, neurotoxicity and others [3-5]. The kidney cells are able to accumulate the higher effective concentration of cisplatin, than any other organ. Depending on exposure time and concentration of cisplatin the accumulation preferentially causes either apoptosis or necrosis. As in case of cisplatin induced neurotoxicity, nephrotoxicity is due to the production of reactive oxygen species, which interact with DNA, lipids and proteins [4,5]. These interactions lead to lipid peroxidation, DNA molecule damages and eventually renal cell injury and death [3,4]. It is logical, that all these processes may proceed also in normal cells, so the study of lipid metabolism and lipid content changes in kidney nuclei after the cisplatin in vivo action will be of certain interest.

Our previous results showed the reliable changes in phospholipids and neutral lipids quantities in rat kidney intranuclear structures – in chromatin and nuclear matrix caused by cisplatin *in vivo* action [6,7]. It was supposed that those changes may have a comprehensive influence which on the whole must promote the antineoplastic effects of the chemotherapeutic agent. Taking into consideration that the large majority of nuclear lipids is available in nuclear membranes it was occurred to study the lipid quantity and quality changes in nuclear membrane preparations. Cisplatin may have an effect on lipid metabolism of nuclear membranes, on functioning of signal transduction pathways via the quantitative and qualitative alterations in their lipid content.

In this paper the alterations of quantities of total phospholipids and neutral lipids as well as changes of their individual fractions content in nuclear membrane preparations from rat kidney cells after the cisplatin *in vivo* action were investigated.

MATERIAL AND METHODS

The experiments were carried out on albino rats (120-150g weight). Cisplatin was injected peritoneal in concentration of 5 mg per 1000g animal weight. Rats were decapitated after 24 hours of cisplatin injection. Rat kidney nuclei was isolated by the method of Blobel and Potter [8]. Nuclear membrane preparations were isolated from purified nuclei by the method of Berezney et al [9]. Lipid extraction was carried out by Bligh and Dayer [10]. The fractioning of both phospholipids and 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 chloroform – methanol - water in ratio 65:25:4 (in case of phospholipids) and diethyl ester - petroleum ester – formic acid in ratio 40:10:1 (in case of neutral lipids) as dividing mixtures. After the chromatography the plates were dried up at $20^{O}C$ and were treated by 15.6% $CuSO_4$ in 8% phosphoric acid (in case of phospholipids) and by 10% H_2SO_4 (in case of neutral lipids). Then the elaborated plates were heated at $180^{O}C$ 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 *in vivo* action reliably decreases the total amounts of both phospholipids and neutral lipids in nuclear membrane preparations from rat kidney cells by 27.1% and 26.7% correspondingly (Table 1, Fig.1). It is characteristic that of the same kind changes were also demonstrated in kidney intranuclear structures - in chromatin and nuclear matrix preparations [6,7], which demonstrate that antitumor agent leads to appreciable repression of whole lipid metabolism in nuclei of rat kidney cells.

Table 1. Total phospholipids and neutral lipids content (in mcg/g of tissue) in nuclear membrane preparations of rat kidney cells in baseline and after *in vivo* treatment of cisplatin. (*-P < 0.05)

Variants	Phospholipids in nuclear membrane from rat kidney cells (mcg/g of tissue)	Neutral lipids in nuclear membrane from rat kidney cells (mcg/g of tissue)
Baseline	550.00±6.70	390.00±4.22
Cisplatin	*401.00±3.65	*286.00±2.14

The fractionation of nuclear membrane phospholipids revealed seven individual fractions in baseline as well as after the cisplatin action. Phosphatidylcholine and phosphatidylethanolamine are the major fractions among them, their portion jointly was more than 50%, while the share of each fraction of phosphatidic acid, cardiolipin, phosphatidylinositol and phosphatidylserine was less than 10% (Table 2). Cisplatin treatment essentially changed the relative content of individual phospholipid fractions: four fractions decreased their percentage content while three of them enhanced it (Table 2).

The fractionation of neutral lipids demonstrated the presence of six individual fractions in baseline as well as after the cisplatin action (Table 3). As in case of phospholipids, cisplatin treatment lead to diversified changes of relative content of individual neutral lipid fractions. Half of them increased their percentage content, the others – decreased it. The most perceptible alteration was observed in percentage content of cholesterol esters (more than 7%) (Table 3).

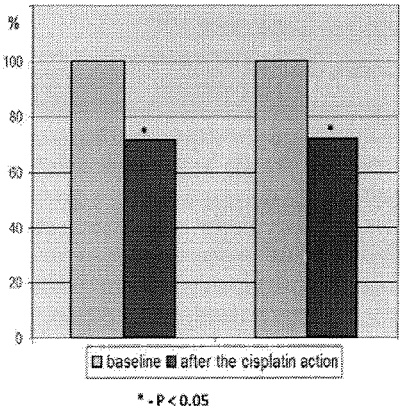

Figure1. Changes in percent of total phospholipids (left diagrams) and neutral lipids content (right diagrams) in nuclear membrane preparations of rat kidney cells in baseline and after *in vivo* treatment of cisplatin.

Table 2. The relative content (in percentage) of individual phospholipid fractions in nuclear membrane preparations of rat kidney cells before and after the cisplatin action.

N	Phospholipids	Baseline	Cisplatin
		%	%
1	Phosphatidylserine	9,76±0,25	10,40±0,22
2	Sphingomyelin	15,00±0,35	13,60±0,36
3	Phosphatidylinositol	8,60±0,26	14,00±0,62
4	Phosphatidylcholine	33,50±0,35	27,00±0,48
5	Phosphatidylethanolamine	20,00±0,49	24,00±0,42
6	Cardiolipin	7,60±0,25	7,00±0,54
7	Phosphatidic acid	5,54±0,24	4,00±0,26
Total content		100	100

Table 3. The relative content (in percentage) of individual neutral lipid fractions in nuclear membrane preparations of rat kidney cells before and after the cisplatin action.

N	Neutral lipids	Baseline	Cisplatin
		%	%
1	Monoglycerides	13.40±1.03	14,00±0.20
2	Diglycerides	10.70±1.68	11,90±0.82
3	Cholesterol	25.30±1.70	23,60±1,12
4	Cholesterol esters	12.80±1,19	20,00±0,98
5	Free fatty acids	25,40±1,54	21,00±0,73
6	Triglycerides	12.40±0,48	9,50±0,28
Total content		100	100

Changes in percentage content do not represent the reality of alteration in content of both phospholipid and neutral lipid individual fractions after the cisplatin action. In order to elucidate this the absolute quantities in micrograms per gram of tissue of individual lipids in nuclear membrane preparations before and after the cisplatin action were determined.
The absolute quantities of six phospholipid individual fractions were decreased reliably while the phosphatidylinositol amount was increased after the *in vivo* action of cisplatin (Table 4). The most diminution of content among phospholipid fractions was observed in case of phosphatidic acid, phosphatidylcholine, sphingomyelin, and cardiolipin by 47.5%, 41.2%, 33.3% and 32.8% correspondingly, which was more than the decrease of total phospholipid content (27.1%). The decreases of phosphatidylserine and phosphatidylethanolamine content (by 21.8%, and 12.5% correspondingly) were less than those for nuclear matrix total phospholipid while the quantity of phosphatidylinositol was increased by 18.7% (Table 4).

Table 4. The quantities (in micrograms per gram of tissue) of individual phospholipid fractions in nuclear membrane preparations of rat kidney cells before and after the cisplatin action. (*P < 0,05).

N	Phospholipids	Baseline	Cisplatin
1	Phosphatidylserine	53,68±1,38	*42,00±0,90
2	Sphingomyelin	82,50±1,92	*55,00±1,46
3	Phosphatidylinositol	47.30±1,43	*56,14±1,30
4	Phosphatidylcholine	184.30±2,00	*108,27±1,96
5	Phosphatidylethanolamine	110,00±1,70	*96,24±1,68
6	Cardiolipin	41,80±1,40	*28,07±1,16
7	Phosphatidic acid	30,47±1,38	*16,00±1,04

Changes of choline-contained phospholipids quantity probably testify the sensitivity of some nuclear enzymes such as sphingomyelinase or sphingomyelin-synthase to cisplatin. The significant and reliable decrease of phosphatidylcholine and sphingomyelin quantities confirm the probable influence of cisplatin on activity of these enzymes. These results indicate that cisplatin may affect on phosphatidylcholine/sphingomyelin crosstalk mechanism which exists in nuclei [12]. At the same time these significant changes in sphingomyelin content in nuclear membrane preparations as well as the enhancing of absolute quantity of phosphatidylinositol demonstrate the disturbance of functioning of nuclear sphingomyelin and phosphoinositol regulatory cycles by cisplatin *in vivo* action. The involvement of these phospholipids in signal transduction events in nuclei has been widely described [11-13].
In case of neutral lipids the similar situation was observed: the absolute quantity of five fractions was reliably decreased in a different extent while on the contrary the amount of cholesterol esters was increased (Table 5). These alterations confirm that cisplatin *in vivo* action leads to perceptible redistribution between the mono-, di- and triglycerides as well as between the cholesterol and its esters which was recently demonstrated also in case of kidney nuclear matrix [7].

Table 5. The quantities (in micrograms per gram of tissue) of individual neutral lipid fractions in nuclear membrane preparations of rat kidney cells before and after the cisplatin action. (*P < 0,05).

N	Neutral lipids	Baseline	Cisplatin
1	Monoglycerides	52,30±4,02	*40,00±0,51
2	Diglycerides	41,73±1,30	*34,00±2,10
3	Cholesterol	98,67±6,50	*67,50±3,10
4	Cholesterol esters	50,00±1,15	*57,20±1,75
5	Free fatty acids	99,00±5,30	*60,10±2,43
6	Triglycerides	48,30±1,86	*27,20±0,80

So, the obtained results demonstrate their accordance with our previous data concerning the diminution of phospholipids and neutral lipids content in kidney chromatin and nuclear matrix preparations [6,7]. This indicates the comprehensive action of cisplatin on lipid metabolism in various nuclear structures which may offer some serious prerequisites for alteration the functioning those nuclear processes where these lipids participate, regulate or act [12-14]. Although the cisplatin action is specific in different tissues which is clearly seen in manifestations of various negative side effects including ototoxicity, gastrotoxicity, myelosuppression, allergic reactions [15,16] and nephrotoxicity (as the main negative effect) [7,16], alterations of quantities of nuclear lipids in rat kidney as well as in rat liver, thymus [17,18] and brain [19] cells, on the whole, are similar. In all probability this similarity indicates that cisplatin displays its antitumor effects also via changes of nuclear lipids quantity and these alterations are not directly connected with toxic effects of the drug. So, all these results demonstrate the deep and multiform transformation of lipid metabolism in nuclei caused by cisplatin *in vivo* action.

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