

CISPLATIN *IN VIVO* INFLUENCE ON LIPID CONTENT
OF CHROMATIN ON RAT BRAIN CELLS

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The *in vivo* influence of antitumor drug cisplatin on content of phospholipids and neutral lipids in chromatin fraction on rat brain cells was investigated. It was shown, that the drug action leads to decrease in total phospholipids and neutral lipids content about 24 and 20% correspondingly. In spite of these significant changes of total lipids, the alterations in relative quantities and percentage of individual phospholipids as well as neutral lipids in chromatin were negligible. These results indicate that cisplatin excites universal changes in lipid metabolism in chromatin appreciably reducing the absolute quantities of all individual phospholipid and neutral lipid fractions available in chromatin preparations. The significance of these quantitative changes in development of cisplatin antitumor effects was discussed.

Keywords: cisplatin, chromatin, phospholipids, neutral lipids.

Introduction. Cisplatin (cis-diaminedichloroplatinum II) is an effective antitumor drug, which is widely used in chemotherapeutic practice. Cisplatin reveals antineoplastic, cytotoxic and immunomodulator actions, and can induce all the known apoptotic pathways of the cell [1–4]. The efficiency of cisplatin action has a dose-dependent nature. Unfortunately the using of this drug is greatly limited because of its severe side effects [5, 6]. Peripheral neurotoxicity is one of the widespread side effects, which develops in approximately 30–50% of patients receiving cisplatin [5, 6]. It has been established that cisplatin can overpass the hematoencephalic barrier and be accumulated in neural cells after the first injection [6]. The mechanism of neurotoxicity of the antineoplastic agents is unclear, but now it is generally accepted that cisplatin causes oxidative stress due to the generation of reactive oxygen species, which interact with DNA, lipids and proteins [6]. These interactions lead to lipid peroxidation, DNA molecule damages and eventually cell death [5, 6].

The real existence of phospholipids as well as neutral lipids in chromatin fraction is well-known [7–10] and the lipids demonstrate the ability to get into touch with different components of chromatin (with histones, non-histone proteins, DNA) [11]. At the same time, DNA is considered as the primary target for cisplatin, so it is impossible to exclude the participation of chromatin bound lipids in realization of cisplatin antitumor effects. It was also shown that the quantity of nuclear lipids (including the chromatin bound ones) reveals the dependence on nuclei functional activity [7, 8]. Furthermore it was shown that lipids of chromatin in dose-dependent manner can regulate replication, transcription, recombination and repair process [9–12].

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Nowadays a hypothesis that lipids may constitute a significant informational layer in gene regulation space conceptually as “epigenetic code” or lipids contribute epigenetic control exists [11]. So, the knowledge about cisplatin sensitivity of chromatin bound lipids (phospholipids and neutral lipids) may contribute to better understanding antitumor action effects of cisplatin.

In this paper the alterations in content of rat brain cells chromatin lipids after the cisplatin *in vivo* action were described.

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 1 kg animal weight. Rats were decapitated after 24 h of cisplatin injection. Rat brain nuclei were isolated by the method of Blobel and Potter [13]. The chromatin fraction from rat brain cells nuclei was isolated by the method of Umansky [14]. Lipid extraction was carried out by Bligh and Dyer [15]. The fractionation of phospholipids was carried out by micro thin layer chromatography (microTLC) using 6×9 cm² plates with L-silicagel, and chloroform–methanol–H₂O in ratio 65 : 5 : 4 as developing mixture. The same plates and diethyl ester–petroleum ester–formic acid in ratio 40 : 10 : 1 as a dividing mixture were used for the fractionation of chromatin neutral lipids by microTLC method.

After the chromatography the plates with fractionated lipids were dried up at 20°C. Then the plates with fractionated phospholipids were treated by solution of 15.6% CuSO₄ in 8% phosphoric acid and the plates with fractionated neutral lipids were treated by 10% H₂SO₄. The elaborated plates were heated at 180°C for 15 min.

The quantitative estimation of separated and specific dyed phospholipids and neutral lipids was carried out by special computer program FUGIFILM Science Lab. 2001 Image Gauge V 4.0, which was destined by densitometry. The obtained data were treated by statistics.

Results and Discussion. Total phospholipids and neutral lipids content (in µg/g of tissue) in chromatin preparation of rat brain cells in baseline and after *in vivo* treatment of cisplatin was investigated. The results of these studies were presented in Tab. 1.

Table 1

Total phospholipids and neutral lipids content (in mcg/g of tissue) in chromatin preparation of rat brain cells in baseline and after *in vivo* treatment of cisplatin (*- $p < 0.05$)

Variants	Phospholipids of chromatin	Neutral lipids of chromatin
Baseline	184.00 ± 6.70	120.00 ± 2.30
Cisplatin	140.00 ± 3.65*	96.00 ± 3.97*

Our results confirm that total phospholipids content as well as neutral lipids content decreased after the cisplatin action. Cisplatin treatment led to decrease in phospholipids total amount by 24% and the total neutral lipids content decreased by 20% as compared with baseline.

These results demonstrated that cisplatin revealed the universal influence on lipid metabolism of rat brain nuclei. This is in consonant with the data of other investigators who maintain the antitumor drug sensitivity of whole lipid metabolism as well as of its different phases [16].

Five individual fractions of phospholipids were revealed in rat brain chromatin preparations in this case: sphingomyelin, phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine and cardiolipin. The relative percentage content of these fractions is presented in Tab. 2. Phosphatidylethanolamine and phosphatidylcholine are the major components, which quantity together is nearly 58% of chromatin total

phospholipids while the amount of cardiolipin, sphingomyelin and phosphatidylinositol composes approximately 19, 11, 12% of total phospholipids correspondingly (Tab. 2). The fractionation of chromatin neutral lipids from rat brain cells by microTLC disclosed 4 individual fractions (Tab. 3). Cholesterol and free fatty acids together composed more than 72% of total amount of neutral lipids in rat brain chromatin preparations, while the relative content of cholesterol esters and triglycerides are nearly 11 and 16% of total neutral lipids correspondingly (Tab. 3).

Table 2

The relative content (in percentage) of individual phospholipid fractions in chromatin preparations of rat brain cells before and after the cisplatin action

Phospholipids	Baseline, %	Cisplatin, %
Sphingomyelin	12.40 ± 0.60	13.00 ± 0.35
Phosphatidylinositol	11.42 ± 0.64	11.67 ± 0.42
Phosphatidylcholine	33.30 ± 1.08	33.68 ± 0.64
Phosphatidylethanolamine	24.34 ± 1.00	21.00 ± 0.84
Cardiolipin	18.54 ± 1.00	20.65 ± 0.32
Total content	100	100

Table 3

The relative content (in percentage) of individual neutral lipid fractions in chromatin preparations of rat brain cells before and after the cisplatin action

Neutral lipids	Baseline, %	Cisplatin, %
Cholesterol	39.73 ± 1.12	36.80 ± 0.73
Cholesterol esters	11.10 ± 0.34	10.80 ± 0.43
Free fatty acids	32.87 ± 1.13	36.20 ± 0.75
Triglycerides	16.30 ± 0.72	16.20 ± 0.45
Total content	100	100

The chromatin preparations of rat brain cells in baseline and after the *in vivo* action of cisplatin revealed the same individual phospholipids and neutral lipids composition. It is characteristic that the relative content of these individual fractions was not exposed to considerable alterations after the antitumor drug action (Tabs. 2, 3). Taking into consideration the appreciable decrease of total phospholipids and neutral lipids content after the cisplatin *in vivo* action respectively by 24 and 20%, the necessity arises to determine the changes in absolute quantities of all individual lipid fractions. The results of calculation were presented in Tabs. 4, 5.

Table 4

The quantities (in mcg/g of tissue, %) of individual phospholipid fractions in chromatin preparations of rat brain cells before and after the cisplatin action (– p<0.05)*

Phospholipids	Baseline		Cisplatin
Sphingomyelin	22.82 ± 1.10	100	*18.20 ± 0.50
Phosphatidylinositol	21.00 ± 1.20	100	*16.35 ± 0.60
Phosphatidylcholine	61.38 ± 1.90	100	*47.15 ± 0.90
Phosphatidylethanolamine	44.80 ± 1.85	100	*29.40 ± 0.84
Cardiolipin	34.00 ± 1.62	100	*28.90 ± 0.32
Total content	184.00 ± 6.70	100	*140.00 ± 3.65

The quantities of all individual phospholipid and neutral lipid fractions were decreased reliably after the *in vivo* action of cisplatin. Individual fractions exhibit different sensitivity to cisplatin treatment (Tabs. 4, 5). Thus, the quantities of three phospholipid fractions (sphingomyelin, phosphatidylinositol and phosphatidylcholine) were decreased by 20–23%, which is consonant to alteration of chromatin total phospholipids, while the diminution of phosphatidylethanolamine was significantly more (by 34.5%) and that of cardiolipin oppositely less (15.0%). In case of neutral lipids the most sensitivity to cisplatin action was manifested in cholesterol fraction (diminution more than 26.0%) while the quantity of free fatty acids was decreased only by 11.3% (Tab. 4).

Table 5

The quantities (in mcg/g of tissue, %) of individual neutral lipid fractions in chromatin preparations of rat brain cells before and after the cisplatin action (* – $p < 0.05$)

Neutral lipids	Baseline		Cisplatin	
	Cholesterol	47.68 ± 1.34	100	*35.20 ± 0.70
Cholesterol esters	13.32 ± 0.41	100	*10.30 ± 0.43	
Free fatty acids	39.44 ± 1.36	100	*35.00 ± 0.75	
Triglycerides	19.56 ± 0.86	100	*15.50 ± 0.40	
Total content	120.00 ± 2.28	100	*96.00 ± 3.97	

These lipid quantity alterations in chromatin of brain cells are consequences of deep and multiform transformation of nuclear lipids metabolism caused by antitumor drug cisplatin. It was known for a long time that cell nuclei are sites for active metabolism of lipids, because lipids exchanged different enzymes, that supported the necessary level of individual phospholipids or neutral lipids fraction [7, 8, 10, 12, 17]. Cisplatin interacting with nuclei may damage the native structure of nuclear components including lipid metabolic enzymes, changing their activities and may excite lipids quantity alterations. It was above-mentioned that the most significant reduction of quantity was detected in phosphatidylethanolamine fraction and less o in cardiolipin fraction.

It is clear that the diminution of any individual lipid quantity may effect on some nuclear functions. For example, it is known, that phosphatidylethanolamine promotes chromatin decondensation, induces transition of chromatin from solenoid to nucleosome conformation and activates RNA polymerase [18], therefore, the decrease of its content after the cisplatin action may alter the function of chromatin. Cardiolipin as the major phospholipid fraction in chromatin is tightly bound with DNA and alteration of its quantity may effect on regulation of activities of DNA topoisomerase, RNA polymerase, DNA replication. The diminution of DNA-bound cardiolipin content may also alter the condensation of chromatin [18]. All these effects confirm the supposition that alteration of cardiolipin quantity in chromatin of brain cells can effect on basic functions of nuclei: replication and transcription.

The considerable decrease in quantities of both choline-contained phospholipids in rat brain chromatin (Tab. 4) is not corresponding with the results of our previous similar studies, devoted to cisplatin action on lipid content in liver and thymus chromatin, where we found the multidirectional alterations of these choline-contained phospholipids quantity [19, 20]. The difference in cisplatin sensitivity to these phospholipids (perhaps to all lipids) can be associated with specificity of lipid metabolism in these tissues. At the same time, it is well known that the metabolism of nuclear lipids is regulated the cytoplasm [8]. So, cisplatin may act upon this regulation too, and the influence of chemotherapeutic agent may have various manifestations in different tissues.

The remarkable diminution of phosphatidylinositol content (by 21%) as well as that of sphingomyelin (by 22%) demonstrates the disturbance of functioning of nuclear phosphoinositol and sphingomyelin cycles by cisplatin action, which was also showed in our previous studies in case of rat liver and thymus chromatin [19, 20].

The decrease in absolute content of all neutral lipid fractions also demonstrates the strong influence of cisplatin on lipid metabolism in rat brain chromatin (Tab. 5). Nowadays it is well-known that there exist two pools of DNA-bound lipids: loosely bound and tightly bound ones [21]. In all probability cisplatin decreases the quantities of both loosely and tightly bound lipids as in case of rat liver and thymus chromatin [19, 20]. As it is well-known that some neutral lipids: glycerides, cholesterol and their esters (together with cardiolipin), play the key role in supramolecular organization of chromatin [21], such significant alteration of their content really will be able to damage it.

Taking into consideration that DNA-bound lipid pools of malignant cells significantly differ from those of normal cells both by phospholipid composition and by the presence of some additional neutral lipid fractions, one may indicate that lipids could play an important role in cancer cells [8]. So, DNA-bound lipids may be hypersensitive target sites for anticancer agents as cisplatin. These results allow one to conclude that cisplatin *in vivo* influence on lipid content in rat brain chromatin has a comprehensive character, concerns various sides of nuclear lipid metabolism.

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