# *In vivo* Action of Cisplatin on Phospholipid Content in Rat Liver and Thymus Chromatin

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## ABSTRACT

The content of total phospholipid 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 phosphilipid content in both rat liver and rat thymus chromatin a 25% and 36% correspondingly. In spite of these significant changes of total phospholipids content the alteration of individual phospholipids percentage in chromatin preparation was negligible. The main difference concerns the relative quantity of choline-contained phospholipids sphingomyelin and phosphatidylcholine. In rat liver chromatin preparations the relative quantity of sphingomyelin decreases and that of phosphatidylcholine increases after the cisplatin action while, on the contrary, in thymus chromatin the relative quantity of sphingomyelin increases and the relative quantity of phosphatidylcholine decreases. Cisplatin leads to the decrease of the absolute quantity of four from five fractions of individual phospholipids both in rat liver and rat thymus chromatin.

**Key words**: *cisplatin, phospholipids, chromatin, phophatidylcholin, sphingomyelin*

## INTRODUCTION

The presence of phospholipids as a component of chromatin is now well demonstrated. Moreover, many enzymes of lipid metabolism such as sphingomyelinase, sphingomyeline-synthase and phosphatidylcholine-depended phospholipase C, have been described and characterized [1, 2]. The chromatin phospholipids, comprising 10% of that present in the nucleus, may play important role in main nuclear functions – replication and transcription [1,3]. They may also have significance in various oncological processes such as formation of tumor or development of preventive means and medicinal remedy. On the other hand cisplatin (cis-diamminedichlorplatinum) is well known effective antitumor agent which is clinically used as adjuvant therapy of cancers aiming to induce tumor cells death [4,5]. 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. At the same time it is well known that DNA forms complexes with phospholipids: phosphatidylcholins, phospatidylethanolamines and sphingomyelins and also with cardiolipin [1,2,6]. DNA-bound lipid complexes have a specific composition which changes depending on the activity of the genome and the phase of the cell cycle. Knowledge about cisplatin sensitivity of this minor component of chromatin – chromatin phospholipids might contribute to a better understanding the cisplatin antitumor action mechanisms which will be favorable to harmless course of chemotherapy. Here the changes of total phospholipid content of chromatin preparations from rat liver and thymus cells as well as the relative alterations of individual phospholipids after the *in vivo* action of cisplatin were described.

## MATERIAL 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 [7] and nuclear fraction of thymus – by the method of Allfrey et al [8]. Chromatin was isolated by the method of Umansky et al [9]. Phospholipid extraction was carried out by Bligh and Dayer [10]. The fractionation of phospholipids were 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 as a dividing mixture. After the chromatography the plates were dried up at $20^OC$ and were treated by 15.6% $CuSO_4$ in 8% phosphoric acid. Then the elaborated plates were heated at $180^OC$ 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

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 [5]. 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 [5, 11]. 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 phospholipids content in chromatin preparations after the *in vivo* action of cisplatin may be the result of such disturbance of these interactions. Total phospholipid 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 phospholipids quantity in rat thymus chromatin is much more than that in liver chromatin and the percentage of changes of total phospholipids 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.

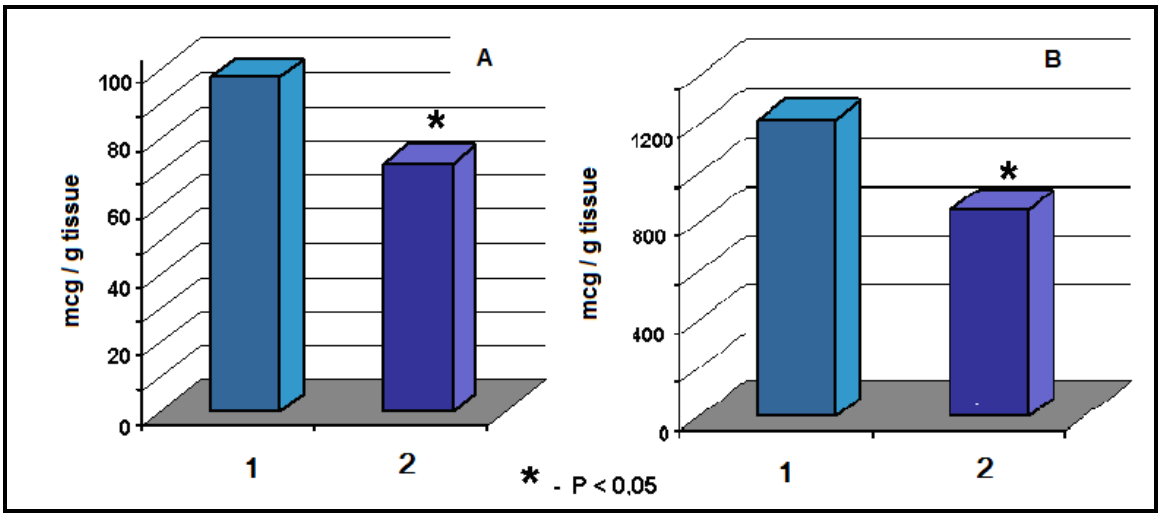


Fig.1. Phospholipid content (in micrograms per grams of tissue) in chromatin preparations of rat liver (A) and thymus (B) cells before (1) and after (2) the in vivo treatment of cisplatin.

Five fractions of individual phospholipids were revealed in both chromatin preparations. In liver chromatin preparations phosphatidylcholine and phosphatidylethano-lamine are major fractions and their percentage is near 60% of total chromatin phospholipids while in thymus chromatin preparations along with them the percentage of cardiolipin is also rather high. The relative quantities and percentage of individual phospholipid fractions in chromatin preparations from liver and thymus cells after administration of cisplatin was demonstrated in Tables 1 and 2.

Table 1. The relative content (in micrograms) and percentage of individual phospholipids fractions in chromatin preparations of rat liver cells before and after the cisplatin action.

| # | Phospholipids | Baseline | | Cisplatin | |
|---|---|---|---|---|---|
| | | Quantity in mcg. | % | Quantity in mcg. | % |
| 1 | Sphingomyelin | 7.00±0.10 | 14.0 | * 5.25±0.13 | 10.5 |
| 2 | Phosphatidylinositol | 6.75±0.40 | 13.5 | 6.25±0.20 | 12.5 |
| 3 | Phosphatidylcholine | 15.00±0.50 | 30.0 | *17.59±0.37 | 35.2 |
| 4 | Phosphatidylethanolamine | 14.75±0.56 | 29.5 | 14.31±0.38 | 28.6 |
| 5 | Cardiolipin | 6.50±0.47 | 13.0 | 6.60±0.23 | 13.2 |
| | Total | 50 | 100 | 50 | 100 |

*P<0.05

Table 2. The relative content (in micrograms) and percentage of individual phospholipids fractions in chromatin preparations of rat thymus cells before and after the cisplatin action.

| # | Phospholipids | Baseline | | Cisplatin | |
|---|---|---|---|---|---|
| | | Quantity in mcg. | % | Quantity in mcg. | % |
| 1 | Sphingomyelin | 5.75±0.41 | 11.5 | * 7.30±0.58 | 14.6 |
| 2 | Phosphatidylinositol | 5.60±0.45 | 11.2 | 5.25±0.37 | 10.5 |
| 3 | Phosphatidylcholine | 16.10±0.45 | 32.2 | *13.70±0.73 | 27.4 |
| 4 | Phosphatidylethanolamine | 10.65±0.47 | 21.3 | 11.00±1.26 | 22.0 |
| 5 | Cardiolipin | 11.90±0.72 | 23.8 | 12.75±1.26 | 25.5 |
| | Total | 50 | 100 | 50 | 100 |

*P<0.05

One can see that phospholipid fractions of rat liver and thymus chromatin exhibits diversity in sensitivity to cisplatin *in vivo* action. The relative content of phosphatidylcholine and sphingomyelin was reliably changed in both chromatin preparations while the alterations of relative quantities of rest phospholipids (phosphatidylinositol, phosphatidylethanolamine and cardiolipin) were negligible and not reliable. It is worth noticing that the quantity of sphingomyelin in chromatin preparations of liver cells decreased and phosphatidylcholine content increased after cisplatin action (Table 1) while in thymus chromatin preparations one can see the contrary phenomenon (Table 2).

These relative changes among the individual phospholipids fractions after the cisplatin action were demonstrated when we took equal amounts of phospholipids (50 mcg) both in baseline and cisplatin-treated probes. Taking into consideration that *in vivo* administration of cisplatin leads to reliable decrease of total phospholipids 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 phospholipid fractions after cisplatin action. The quantities of four phospholipid 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 (Table 3).

Changes of choline-contained phospholipids quantity testify the sensitivity of some nuclear enzymes such as sphingomyelinase or sphingomyelin-synthase to cisplatin. Changes in sphingomyelin content may be due to the presence of sphingomyelinase and sphingomyelin synthase in the chromatin [1]. The small decrease of phosphatidylcholine quantity (near 14%) and the significant diminution of the other choline-hold lipid sphingomyelin (over than 44%) in liver chromatin preparations as well as the reverse changes of these phospholipids in thymus chromatin preparations (decrease of phosphatidylcholine content over 40% and sphingomyelin quantity near 14%) confirm the probable influence of cisplatin on activity of these enzymes (Table 4.).

Table 3. The quantities (in micrograms per gram of tissue) of individual phospholipids fractions in chromatin preparations of rat liver and thymus cells before and after the cisplatin action (SP-sphingomyelin, PI – phosphatidylinositol, PC – phosphatidyl-choline, PE – phosphatidylethanolamine, CL – cardiolipin).

| # | Phospholipids | Liver Chromatin | | Thymus Chromatin | |
|---|---|---|---|---|---|
| | | Baseline | Cisplatin | Baseline | Cisplatin |
| 1 | SP | 13.72±0.36 | *7.64±0.25 | 139.59±10.70 | 123.66±6.13 |
| 2 | PI | 13.23±0.35 | *9.09±0.35 | 135.95±10.43 | *88.94±4.41 |
| 3 | PC | 29.40±0.78 | 25.60±0.83 | 390.84±29.97 | *232.08±11.51 |
| 4 | PE | 28.91±0.77 | *20.80±0.67 | 258.54±19.83 | *186.34±9.24 |
| 5 | CL | 12.74±0.34 | *9.60±0.31 | 288.88±22.15 | *215.99±10.71 |
| | T o t a l | 98.00±2.60 | *72.73±2.35 | 1213.80±93.08 | *847±42.00 |

*P<0.05

This opposed action of cisplatin in liver and thymus chromatin preparations may be depended on specificity of lipid metabolic events in liver and thymus nuclei. These results also indicate that cisplatin may affect on phosphatidylcholine/sphingomyelin crosstalk mechanism which exists in nuclei [12].

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

| # | Phospholipids | Liver Chromatin | Thymus Chromatin |
|---|---|---|---|
| 1 | Sphingomyelin | - 44.3% | - 11.4% |
| 2 | Phosphatidylinositol | - 31.3% | - 34.6% |
| 3 | Phosphatidylcholine | - 12.9% | - 40.6% |
| 4 | Phosphatidylethanolamine | - 28.1% | - 27.9% |
| 5 | Cardiolipin | - 24.7% | - 25.2% |
| | T o t a l | - 25.8% | - 30.2% |

On the other hand, changes in sphingomyelin content as well as the appreciable lowering of absolute quantity of phosphatidylinositol in both liver and thymus chromatin preparations (31%-35%, Table 4) demonstrate the disturbance of functioning of nuclear phosphoinositol and sphingomyelin

cycles by cisplatin action. The involvement of these phospholipids in signal transduction nuclear events has been widely described [13, 14].

The decrease in cardiolipin quantity in both liver and thymus chromatin (Tables 3, 4) may promote destroying the chromatin structure as it is well known that cardiolipin together with some neutral lipids plays the key role in the supramolecular organization of chromatin [6,15]. Particularly, cardiolipin is the major DNA-bound phospholipid in chromatin and almost all of chromatin cardiolipin is localized in the DNA. This anionic phospholipid is tightly bound to DNA and plays important structural-functional role: it promotes chromatin decondensation, substitutes H1 histone from linker DNA, induces transition of chromatin, activates RNA polymerase [15]. So, the variation of absolute quantities of cardiolipin caused by cisplatin *in vivo* action may damage the functioning of main nuclear processes: transcription and replication.

On the bases of these results we conclude that cisplatin *in vivo* action on phospholipid content in both rat liver and thymus chromatin has a comprehensive character and concerns various sides of lipid metabolism in nuclei.

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