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THE EFFECT OF CISPLATIN AND HYDROCORTISONE CO-ADMINISTRATION ON RAT LIVER CHROMATIN CONDENSATION

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Cisplatin (*cis*-diammine-dichloro-platinum) is a broad-spectrum anticancer drug widely used for treatment of malignancies. The drug interacts with DNA resulting to intra- and inter-strand cross-links which contribute to cytotoxicity. To prevent nausea, vomiting and inflammation in clinical practice the cisplatin most commonly is co-administrated with hydrocortisone to cancer patients. It is well known that glucocorticoids exert biological action via interaction of hormone-receptor complexes with genomic DNA. To address the question whether co-treatment with cisplatin and hydrocortisone could modulate cisplatin-DNA interaction, the internucleosomal fragmentation of rat liver DNA after treatment of rats with cisplatin and hydrocortisone was investigated. The results of this study indicate that hydrocortisone–cisplatin interaction could prevent loosening of chromatin in internucleosomal chromatin regions induced by cisplatin in 48 hours and might alter the drug cytotoxicity.

Hydrocortisone – cisplatin – DNase 1 – DNA fragmentation

Ցիսպլատինը (*ցիս*-դիամին-դիքլոր-պլատին) ամենատարածված հակաուռուցքային դեղամիջոցներից մեկն է: Փոխազդելով ԴՆԹ-ի մոլեկուլի հետ, դեղամիջոցը առաջացնում է ներ- և միջշղթայական կապեր, որոնք պայմանավորում են ցիսպլատինի ցիտոտոքսիկությունը: Հիվանդների մոտ սրտխառնոցը, փսխումը և բորբոքային զարգացումները կանխելու համար քեմոթերապիայի ընթացքում ցիսպլատինի հետ համատեղ ներարկվում է հիդրոկորտիզոն: Հայտնի է, որ գլյուկոկորտիկոիդների կենսաբանական ազդեցությունը միջնորդվում է հորմոն-ռեցեպտորային համալիրների և գենոմային ԴՆԹ-ի փոխազդեցությամբ: Ցիսպլատինի և հիդրոկորտիզոնի համատեղ կիրառման հնարավոր ազդեցությունը ցիսպլատին-ԴՆԹ-ածանցյալների վրա ուսումնասիրելու համար տվյալ աշխատանքում հետազոտվել է առնետների լյարդի բջջակորիզների ԴՆԹ-ի ինտերնուկլեոսոմային ֆրագմենտավորումը ցիսպլատինի և հիդրոկորտիզոնի համատեղ ներարկումից հետո: Ցույց է տրվել, որ հիդրոկորտիզոնի և ցիսպլատինի համատեղ ազդեցությունը կարող է կանխել ցիսպլատինի ներգործությամբ պայմանավորված քրոմատինի ինտերնուկլեո-սոմային հատվածների ապակոնդենսավորումը, դրանով իսկ ազդելով դեղամիջոցի ցիտոտոքսի-կության վրա:

Հիդրոկորտիզոն – ցիսպլատին – ԴՆազ 1 –ԴՆԹ-ի ինտերնուկլեոսոմային ֆրագմենտավորում

Цисплатин (цис-диамино-дихлорплатин) является противоопухолевым препаратом широкого действия, применяемым при лечении злокачественных новообразований. Препарат взаимодействует с ДНК, образуя внутри- и межцепочечные связи, что обуславливает его цитотоксичность. Для предотвращения тошноты, рвоты и развития воспалительных процессов в клинике практикуется совместное с цисплатином введение раковым больным гидрокортизона. Установлено, что биологическое действие глюкокортикоидов обусловлено взаимодействием гормон-рецепторных комплексов с геномной ДНК. С целью выяснения эффекта совместного применения цисплатина и гидрокортизона на характер связывания циспла-

тина с молекулой ДНК в настоящей работе исследован характер интернуклеосомального фрагментирования ДНК ядер клеток печени крыс при единовременном введении цисплатина и гидрокортизона. Показано, что совместное воздействие гидрокортизона и цисплатина может предотвратить вызванную цисплатином деконденсацию интернуклеосомальных участков хроматина, таким образом действуя на его цитотоксичность.

Гидрокортизон – цисплатин – ДНаза 1 – интернуклеосомальная фрагментация ДНК

Cisplatin [*cis*-diammine-dichloro-platinum] is one of the most commonly used antitumor drugs and is recognized as highly effective cytotoxic agent, which forms DNA-adducts due to chemical modification of nuclear DNA. Cisplatin cumulating in normal tissues and organs in the course of treatment is responsible for severe adverse effects emerging as secondary leukemia, thymomas and other pathologies of hematopoietic and immune systems even considerable time after the drug treatment. Thus, curative effect of cisplatin is attenuated due to dose-limiting toxicity and to growing resistance of the cancer cells to the drug. To prevent inflammation, cisplatin-induced allergic reaction, nausea and vomiting, the hydrocortisone is commonly co-administered with cisplatin. Due to pro-apoptotic properties the hydrocortisone elicit immunosuppressive supportive effect to tumour therapy in lymphoid cells. However, the data regarding the impact of GCs treatment on cytotoxic effect of cisplatin treatment in solid tumour cells are contradictive. Cell life or die decisions are multifactorial phenomena, which depend on activation or suppression of broad spectrum of different genetic programs. These programs could be triggered by modulation in the pattern of chromatin condensation in cells that undergo cytotoxic insults. However, little is known regarding the possible effect of co-treatment with cisplatin and hydrocortisone on chromatin structure. Considering liver as the main organ responsible for detoxification of pharmacologic agents, the present study is aimed at investigating the influence of co-treament of rats with cisplatin and hydrocortisone on chromatin condensation in liver nuclei.

It is recognized that DNA internucleosomal cleavage and DNase 1 accessibility reflect structural features of chromatin [3]. Based on this, in present study it is assayed whether co-administration of cisplatin and hydrocortisone is capable to affect the accessibility of liver chromatin to DNase 1 and artificially activated endogenous Ca^{2+}/Mg^{2+}endonuclease.

Materials and methods. *Animals* Animals were treated according to regulations of Committee for Bioethics of Yerevan State University.

Albino inbred 6 week old male rats were used throughout all experiments. The animals were standardized by weight (to 100g). Cisplatin and other reagents were purchased from Sigma-Aldrich. Vehicle (saline) and cisplatin (10mg/kg weight) and hydrocortisone (5mg/kg) were injected intra-peritoneal. Hydrocortisone was injected repeatedly in 24 hours. Animals were sacrificed under light ether anesthesia by decapitation after 48 h treatment with cisplatin and livers were collected.

Liver nuclei isolation Liver nuclei were isolated according to Hewish and Burgoyne [2]. Sucrose solutions utilized throughout the nuclei isolation procedure were buffered with 20 mM Tris containing 15 mM NaCl, 60 mM KCl, 0,15mM spermine and 0.5mM spermidine, pH 7,4.

DNA fragmentation assay 100 µl aliquote samples of nuclear suspension normalized to 1000 µg/ml DNA were transferred to the Eppendorf tubes and 60 mM $MgCl_2$ and 10mM $CaCl_2$ were added to yield final concentrations of 6mM $MgCl_2$ and 1mM $CaCl_2$ in aliquoted probes. The ions were added to activate endogenous Mg^{+2} - and Ca^{+2} /Mg^{+2} -dependent nuclear endonucleases, which initiated internucleosomal DNA cleavage [9]. DNA isolation was performed according to standard protocol [4].

Nuclear DNA was subjected to electrophoresis in 1,8 % agarose gels (8v/cm). DNA was visualized by ethidium bromide staining and DNA fragmentation was assessed after gel densitometry using Fuji Film Image Gauge ver.4.0 program for determination of relative content of DNA fragments.

DNase I assay 1000 μl aliquots were removed from nuclear suspensions and placed in test tubes. The tubes were heated to 37^0C and 100 μl DNase I stock solutions were added (to final concentrations of 10 Kunit/ml). DNase I stock solution was prepared in 25 mM Tris, pH 7,5). No DNase 1 was added to blank sample. At the end of incubation the tubes were chilled in ice and perchloric acid was added to final concentration 5%. The tubes were chilled for additional 10 minutes and undigested material was pelleted by centrifugation 6000 g 10 min. The amount of acid-soluble DNA released by Dnase I digestion was assayed in supernatant. The percentage of digestion was calculated as (A_{260} x 100/ $A_{260 \text{ the blank sample}}$).

Statistics All results were expressed as M ± S.D. Statistical differences in the results between groups were evaluated by the two-tailed Student's t-test. A probability (p) value of < 0.05 was considered significant.

Results and Discussion. Ca^{+2}/Mg^{+2}-dependent endonuclease is known to efficiently create sharp DNA ladder composed of genomic DNA and is recognized to be useful tool for chromatin research [7]. It was demonstrated that Mg^{+2}-dependent endonucleases are involved in initial DNA cleavage in nuclei where they perform large scale DNA fragmentation (DNA fragments exceeding 1000 b.p. in length), whilst Ca^{+2}/Mg^{+2}-dependent endonuclease is responsible mainly for olygonucleosomal DNA fragmentation (1000-200 b.p. in length) [5,6]. To study whether DNA-binding of hydrocortisone receptors can interfere with DNA-cisplatin adducts chromatin structure-dependent assay was employed, using artificially activated intra-nuclear apoptotic Mg^{+2} and Ca^{+2}/Mg^{+2}-dependent endonucleases which are responsible for DNA internucleosomal fragmentation (laddering) in apoptosis [8].

In present study it was revealed that in 48 hours of cisplatin administration to rats the internucleosomal DNA ladder generated by endogenous $Ca^{2+/}Mg^{2+}$endonuclease in liver chromatin loses its characteristic sharpness and we detect widening of DNA bands visualized by agarose gel electrophoresis (fig. 1).

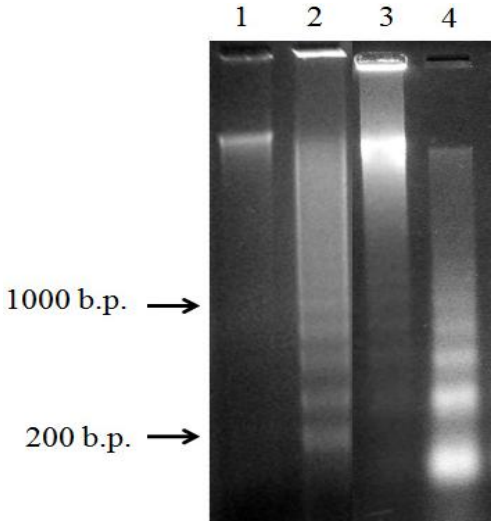

Fig. 1. Rat liver DNA internucleosomal fragmentation in control (lanes 1,2) and after cisplatin administration (lanes 3,4). Lanes 1 and 3- DNA after nuclei isolation, 2 and 4-nuclei incubated with Ca^{+2} (1 mM) and Mg^{+2} (6mM) ions 60 min.

However, changes in DNA ladder configuration after treatment of rats with cisplatin were not accompanied by modulation in DNase 1 accessibility of chromatin (fig. 2).

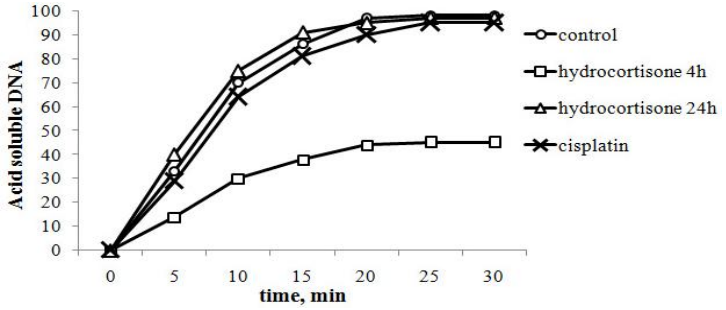

Fig. 2. Kinetics of DNA cleavage in liver chromatin by DNase 1 after treatment of rats with cisplatin and hydrocortisone

These data suggest that after cisplatin injection liver chromatin did not undergo general loosening. We suppose that local unwinding of chromatin areas in vicinity of DNA-cisplatin adducts has no appreciable effect on chromatin condensation in general [1].

Next, it was attempt to determine whether hydrocortisone administration to rats can modulate chromatin condensation in early (4 hours) and late phases (24 hours) of hormone action. Results presented in fig. 3 demonstrate that treatment of rats with hydrocortisone did not alter DNA fragmentation in rat liver nuclei in 4 h.

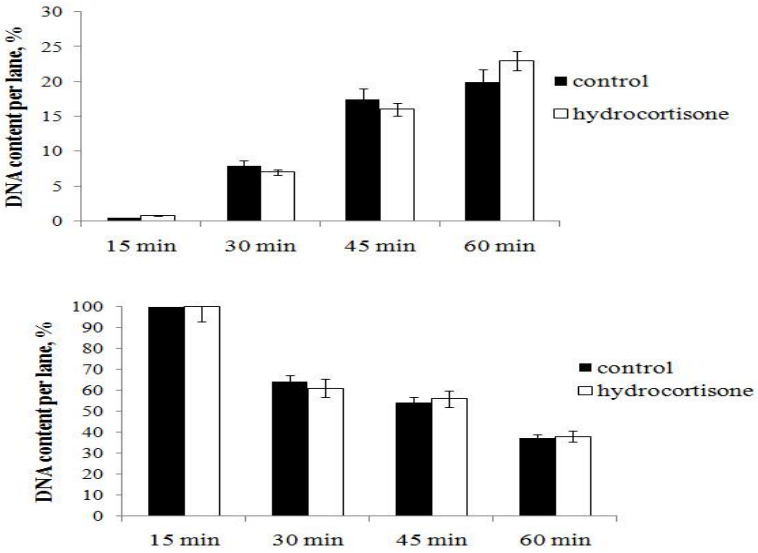

Fig. 3. Intensity of rat liver chromatin DNA internucleosomal fragmentation during different periods of incubation of isolated nuclei in the presence of Ca^{+2} (1 mM) and Mg^{+2} (6 mM) ions in control and after 4 hours of hydrocortisone injection to animals. a-content of 200 b.p. fragments, b- fragments length exceeding 1000 b.p.

However, liver chromatin accessibility to DNase 1 show that it becomes more resistant to DNase 1, which reflects chromatin condensation in 4 hours of hydrocortisone administration to rats. Our data come to show that in 24 hours of treatment with hormone,

susceptibility of chromatin to DNase 1 returns to level demonstrated by liver chromatin of rats not treated with hydrocortisone (control group), while intensity of DNA internucleosomal fragmentation was elevated nearly two-fold (fig. 4,5).

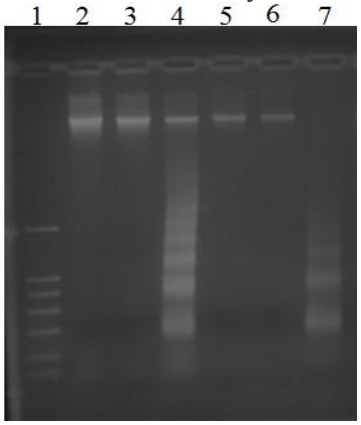

Fig. 4. Rat liver DNA internucleosomal fragmentation in control (lanes 2-4) and after 24 hours of hydrocortisone injection to animals (lanes 5-7), lane 1- DNA ladder sample. Lane 2,5- DNA after nuclei isolation, lanes 3,6- nuclei incubated 60 min with 0,15 mM spermine and 0.5 mM spermidine, lanes 4,7 - nuclei incubated with Ca^{+2} (1 mM) and Mg^{+2} (6mM) ions 60 min

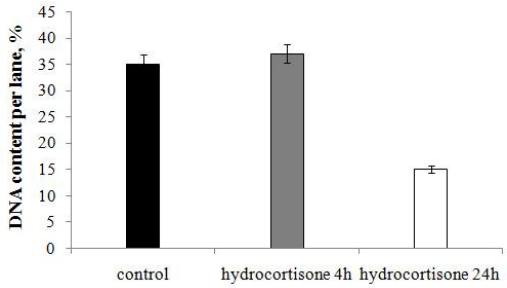

Fig. 5. Intensity of rat liver chromatin DNA internucleosomal fragmentation in control and after 4 and 24 hours of hydrocortisone injection to animals. Fragments length exceeding 1000b.p.

The results of this study revealed permanent activation of DNA internucleosomal fragmentation in 48 hours hydrocortisone injection (fig. 6), which can reflect the loosening of chromatin in internucleosomal regions of the fiber.

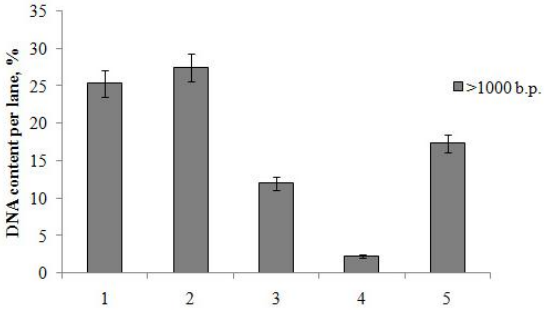

Fig. 6. Intensity of rat liver chromatin DNA internucleosomal fragmentation in 1-control, 2-after treatment of rats with hydrocortisone 4 hours, 3- treatment with hydrocortisone 24 hours, 4- treatment with hydrocortisone 48 hours, 5-co-treatment with hydrocortisone and cisplatin.

DNA internucleosomal fragmentation in liver chromatin of rats which were co-treated with hydrocortisone and cisplatin is less intensive than in chromatin isolated from animals treated only with hydrocortisone. This data indicate that hydrocortisone–cisplatin interaction could prevent loosening of chromatin in internucleosomal chromatin regions induced by cisplatin in 48 hours and might alter the drug cytotoxicity.

REFERENCES

1. *Bellon S.F., Coleman J.H., Lippard S.J.* DNA unwinding produced by site-specific intrastrand cross-links of the antitumor drug cis-diamminedichloroplatinum (II), Biochemistry, *30*, 32, 8026-35, 1991.
2. *Hewish D.R., Burgoyne L.A.* Chromatin Substructure, The Digestion of Chromatin DNA at Regularly Spaced Sites by a Nuclear Deoxyribonuclease, Biochemical and Biophysical Res. Com, *52* , 504-510, 1973.
3. *Hughes F.M., Cidlowski J.* Apoptotic nuclease assays, Methods enzymol, *322*, 47-62, 2000.
4. *Sambrook J., Russel D.* Molecular Cloning, A laboratory manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 3rded, *1*, 6, 4-6, 2001.
5. *Shiokawa D., Tanuma S.* Differential DNases are selectively used in neuronal apoptosis depending on the differentiation state, Cell Death and Differentiation, *11*, 1112-1120, 2004.
6. *Shiokawa D., Shika Y., Tanuma S.I.* Identification of two functional nuclear localization signals in DNase γ and their roles in its apoptotic DNase activity. Biochem. J., *376*, 377-381, 2003.
7. Widlak P., Peng L., Wang X., Garrard W. T. Cleavage Preferences of the Apoptotic Endonuclease DFF40 (Caspase-activated DNase or Nuclease) on Naked DNA and Chromatin Substrates, The Journal Of Biological Chemistry, *275*, *11*, 17, 8226-8232, 2000.
8. Yakovlev A.G., Wang G., Stoica B.A., Boulares H.A., Spoonde A.Y., Yoshihara K., Smulson M.E. A Role of the Ca^{2+}/Mg^{2+}-dependent Endonuclease in Apoptosis and Its Inhibition by Poly(ADP-ribose) Polymerase, The Journal of Biological Chemistry, *275*, 21302-21308, 2000.
9. *Yakovlev A.G., Wang G., Stoica B.A., Simbulan 0., Rosenthal C.M., Yoshihara K., Smulson M.E.* Role of DNASIL3 in Ca^{2+}- and Mg^{2+}-dependent cleavage of DNA into oligonucleosomal and high molecular mass fragments, Nucleic Acids Res., *27*, 1999-2005, 1999.

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