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Abstract. It has been established that by 15 days after birth, the rat in the myocardium of the complex left ventricle and interventricular septum (LV + ISH) complete the proliferation and polyploidy of CMC. The number of CMC workers in the myocardium (LV + ISH) stabilizes at the level of 1,52 ...1,56 x 10^7. In the myocardial parenchyma of newborn rat three populations of muscle cells are unequal in number and function: 1nuclear d-CMC, 1nuclear s-CMC and 2nuclear-CMCs. At t ≤ 15 days, the number of populations of 1nd- and 1ns-CMC decreases to "0" as result of the transition 1nd-CMC →1ns-CMC → 2n-CMC. In the process of postnatal development of rat pups, in the myocardial parenchyma (LV + ISH) a continuous increase in the sizes of 1nd-, 1ns-, 2n-CMCs and an increase in the volume of mitochondrioma in 1nd-, 1ns-, 2n-CMC is determined. The postnatal development of mitochondrioma in CMC is carried out through the implementation of the biological law "division ↔ merger". In the time interval (n/p – 15), the division of MX and the increase in the number of these organelles in CMC are determined. The frequency of MX divisions in 1nd-CMC is 111 mx/day; in 1ns-CMC – 173 mx/day, in the 2n- CMC – 285 mx/day. The frequency of MX divisions in 2n-CMC equal to 267 mx/day, is determined in the time interval (30 – 45) days of postnatal development of rats. In the time interval (15 – 25) days in the 2n-CMC, an intensive fusion of the MX occurs with a frequency of 420 mx/day. The fusion of MX leads to an increase in the volume of organelles and a 2-fold increase in the content of MX DNA molecules in MX. The doubled amount of MX-DNA in MX promotes: intensification of biosynthesis and accumulation of MX-proteins in the MX matrix; an increase in the size of the surface area of cristae which acquire a convoluted and spiral shape; an increase in the volume of MA, the development of physiological hypertrophy of the MX in CMC. During postnatal ontogenesis in populations of CMC of the myocardium complex (LV + ISH), MX sizes increase from a minimum of 0.08 μm^3 in 1nd-CMC of newborn rat to a maximum of 0.9 μm^3 in 2n-CMC of 30- day- old rat.

Key words: cardiomyogenesis, populations of cardiomyocytes, mitochondrial division and fusion, mitochondrial apparatus.

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INTERACTION OF RHENIUM(III) CLUSTER COMPOUNDS WITH OLIGONUCLEOTIDES OF DIFFERENT COMPOSITION

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Introduction. In continuation of our study on the interaction of dirhenium(III) cluster compounds with nucleic acids [1-3], we present a paper devoted to the study on the interaction of three structural types dirhenium(III) complex compounds [4] and cisplatin with adenine-thymine (AT)-rich and guanine-cytosine (GC)-rich (conditionally) oligonucleotides, consisting of twenty mononucleotides. The need for such a study lies in the previously demonstrated efficacy of the Rhenium-Platinum antineoplastic system, which administration to tumor-bearing rats resulted in the almost complete disappearance of the neoplasm [5] and on Jurkat cells [6]. Neighboring nucleic acid base pairs, dGpG (65%) and dApG (25%), are known to be the primary targets of cisplatin and predominantly cause its anticancer activity [7-9].

The purpose of the study was to compare the binding activity of cisplatin and dirhenium(III) complex compounds with oligonucleotides to possibly explain the synergistic or additive effect of these compounds on the cancer cell DNA. It is also an important task to establish a correlation between the structure of rhenium coordination compounds and their ability to interact with certain DNA sequences, which may elucidate the fields of controlled novel anticancer drugs synthesis.

Object and methods. The following compounds served the material under study: cisplatin (Sigma-Aldrich, USA), (cisPt), cis-$Pt(NH_3)_2Cl_2$ (1); dichlorotetra-μ-isobutyratodirhenium(III), ($Re_{tetra-isob}$), $Re_2(i\text{-}C_3H_7COO)_4Cl_2$ (2); bis-dimethylsulfoxide-cis-tetrachlorodi-μ-isobutyratodirhenium(III), ($Re_{cis-isob}$), cis–$Re_2(i\text{-}C_3H_7COO)_2Cl_4(ДМСО)_2$ (3); trans-tetrachlorodi-μ-isobutyratodirhenium(III), ($Re_{trans-isob}$), trans–$Re_2(i\text{-}C_3H_7COO)_2Cl_4$ (4); bis- dimethylsulfoxide-cis-tetrachlorodi-μ-pivalatodirhenium(III), ($Re_{cis-piv}$), cis–$Re_2((CH_3)_3CCOO)_2Cl_4(ДМСО)_2$ (5); trans-tetrachlorodi-μ-pivalatodirhenium(III), ($Re_{trans-piv}$), trans–$Re_2((CH_3)_3CCOO)_2Cl_4$ (6); bis-dimethylsulfoxide-cis-tetrachlorodi-μ-adamantylcarboxylatodirhenium (III), ($Re_{cis-adam}$), cis–$Re_2(C_{10}H_{15}COO)_2Cl_4(ДМСО)_2$ (7); trans- tetrachlorodi-μ-adamantylcarboxylatodirhenium(III), ($Re_{trans-adam}$), trans–$Re_2(C_{10}H_{15}COO)_2Cl_4$ (8); cis-hexachlorodi-μ-3-aminopropanoatodirhenium(III), ($Re_{cis-β-Ala}$), cis-$[Re_2\{β\text{-}AlaH\}_2Cl_6]$ (9); cis-aquapentachlorodi-μ-4-aminobutanoatodirhenium(III) chloride, ($Re_{cis-GABA}$), cis-$[Re_2(GABA)_2Cl_5(H_2O)]Cl$ (10); potassium diaquotetra-μ-hydrogenphosphatodirhenate(III), ($Re_{tetra-phosp}$), $K_2[Re_2(HPO_4)_4(H_2O)_2]$ (11).

Compounds 2 and 11 refer to dirhenium(III) tetracarboxylates and tetraphosphates, respectively; substances 3, 5, 7, 9, 10 refer to cis-dicarboxylates, and 4, 6, 8 – to trans-dicarboxylates of dirhenium (III). Rhenium complex compounds were synthesized at the SHEI «Ukrainian State University of Chemical Technology» according to [3,4,10].

Two types of oligonucleotides were used for this study: DNA (GC = 30%) (length being 20 nucleotides, Mr = 6069 g/mol) sequence 5'-3' «ATTCCTTATCTCTAAGGAAT» and DNA (GC = 10%) (Sigma, length – 20 nucleotides, Mr = 6068 g/mol) sequence 5'-3' «ATTAATTATCTCTAATTAAT», obtained from Sigma-Aldrich, USA; propidium iodide (PI) produced by Sigma-Aldrich, USA was used.

All experiments were performed in 10 mM sodium phosphate buffer with pH = 7.0. Equimolar concentrations of the both nucleotides were prepared, heated to 95°C and gradually cooled to 20°C after 10 minutes (4-6 minutes). 1 μM of oligonucleotide is equivalent to 38 μM of negatively charged phosphate groups, since the 20-membered oligonucleotide has 19 phosphate groups.

The method of competitive complexing with propidium iodide (PI) was carried out according to [11] with some modifications. Solutions of PI and oligonucleotide (three PI molecules per one molecule of oligonucleotide) were mixed in 10 mM phosphate buffer and kept for 10 minutes at 20°C. 1 ml of 5 μM 1-11 compounds solution was added to this mixture and maintained for another 10 minutes. All experiments were performed using the PHERAstar FSX tablet fluorescence reader (BMG LABTECH, Germany). Fluorescence intensity was measured at 600 nm with the excitation wave length of 480 nm. The binding intensity was calculated by the formula:

$$(1 - \frac{F_{DNA–PI–substances\ 1\text{-}11}}{F_{DNA–PI}}) \cdot 100\%,$$

$F_{DNA–PI–substances\ 1\text{-}11}$ – fluorescence intensity of the DNA–PI complex with substances 1-11; $F_{DNA–PI}$ – fluorescence intensity of the DNA–PI complex.

Mixtures of PI and 1-11 substances without DNA (blank experiment) were fluorescently inactive under the same ratios and conditions.

Results of the study and their discussion. The difference in the structure of the selected oligonucleotides is the difference in two sections – 4 and 5, where the first oligonucleotide contains AT, and the second – GC complementary pairs. Therefore, these DNA samples are convenient methods for clarifying the problem of potential drugs binding to specific nucleotide pairs.

PI – is a cationic dye which is widely used in nucleic acid studies. The PI fluorescence efficiency grows with intercalation between nucleic base pairs and is an indicator of the nucleotide chain presence. PI may be displaced by a substance that binds to DNA with a greater affinity, which reflects in the fluorescence intensity decrease (fluorescence quenching). That is why the fluorescence intensity quenching is a value that indicates the intensity of interaction between a substance and DNA.

The **table** shows binding activity of the studied compounds.

Based on the data obtained, it can be noted that the binding values of most compounds under study are greater than that of cisplatin. Thus, substances 2-10 bind to 10% GC – by 1.78-4.15 times, and to 30% GC – by 1.13-2 times more intensively than cisplatin.

The most important observation, in our opinion, is the difference in the binding activity of cisplatin and rhenium(III) cluster compounds to GC- and AT-rich oligonucleotides, which is shown in the **table** and is clearly demonstrated in the **figure.**

Table – Binding activity values of the studied compounds in%

Compound No	Compound designation	Binding activity of the compound, %	
		GC10%	**GC30%**
1	cisPt	17.62±0.88	33.85±1.69
2	$Re_{tetra\text{-}isob}$	47.02±2.35	57.98±2.89
3	$Re_{cis\text{-}isob}$	31.31±1.56	39.88±1.99
4	$Re_{trans\text{-}isob}$	54.88±2.74	56.42±2.82
5	$Re_{cis\text{-}piv}$	47.03±2.35	48.83±2.44
6	$Re_{trans\text{-}piv}$	45.25±2.26	53.32±2.66
7	$Re_{cis\text{-}adam}$	47.02±2.35	53.31±2.66
8	$Re_{trans\text{-}adam}$	70.6±3.52	68.48±3.42
9	$Re_{cis\text{-}\beta\text{-}Ala}$	41.2±2.06	38.33±1.91
10	$Re_{cis\text{-}GABA}$	73.19± 3.18	68.94± 3.00
11	$Re_{tetra\text{-}phosp}$	19.67±0.98	17.33±0.86

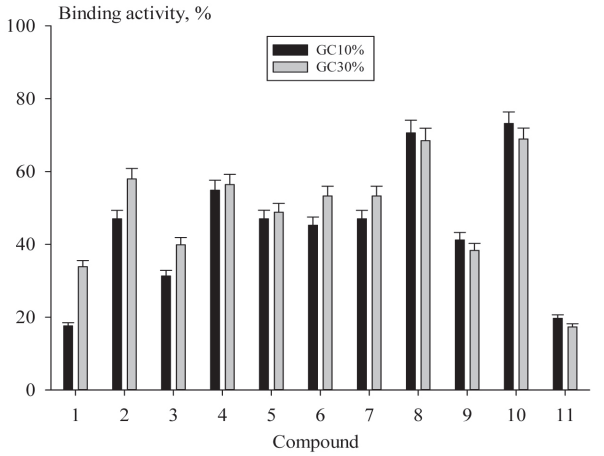

Figure – Binding activity of cisplatin and rhenium (III) cluster compounds with oligonucleotides.

Thus, cisplatin binds almost to 30% GC nucleotide practically twice more intensely than to 10% GC nucleotide, which corresponds to previously obtained data. It is known that enzymatic hydrolysis in the product of the interaction between cisplatin and calf thymus DNA, followed by chromatographic-mass spectrometric determination of the obtained products structure, proved that the main product was 1,2 – an intrachain adduct of cis-[$Pt(NH_3)_2${d(GpG)}] (cis-GG) (up to 50%); 23-28% belonged to the cis-AG adduct [12].

Further studies have shown that cisplatin binds in a bidentate manner to the adjacent purine bases via N7, preferably to two adjacent guanines, regardless of the DNA type, double-stranded or single-stranded (plasmid, oligonucleotide) [13]. Among the studied nucleotides we have found that in the GC-rich oligonucleotide there

were 2 guanine residues, one pair of peripheral binding and a cis-GG adduct formed.

Rhenium compounds are characterized by a smaller difference in binding to the studied oligonucleotides. Thus, for compounds 2, 3 and 6 this difference is 10.96, 8.57 and 7.97%, respectively, and for other compounds this difference does not exceed 6%. Therefore, it is possible to note the difference in the specificity of binding to adenine and guanine nucleic bases.

Higher binding activity of 30% GC, similar to cisplatin, was found for tetracarboxylate type 2 compound; other rhenium(III) complex compounds bind more strongly to 10% GC than to 30% GC (3, 5, 6), or bind to both types of oligonucleotides with a statistically unreliable difference (4, 9) or even equally.

Such observations suggest different mechanisms for binding of cisplatin and rhenium(III) cluster compounds and may explain the synergistic or additive activity of the Rhenium-Platinum combination effect.

The highest activity (up to 80%) in the interaction with oligonucleotides was displayed by compounds 8 and 10, which ligands are adamantyl ligand and γ-aminobutyric acid. The lowest activity is in compound 11 with phosphate ligands, which are negatively charged in the neutral medium.

We explain such facts by electrostatic interactions between ligands in the composition of rhenium compounds and phosphate groups in the nucleotide composition, as well as by hydrophobic interactions that can be performed through the contact of adamantyl residues and nucleic base E-rings.

When comparing the binding activity of compounds 3 and 4, 5 and 6, 7 and 8, which are cis- and trans- isomerides, it should be noted that the trans- isomerides interact somewhat more actively with both types of oligonucleotides. This may be explained by the higher reactivity of the corresponding trans-isomers [4].

In general, the process of PI displacement with rhenium compounds can have the following mechanism: when a rhenium compound interacts with DNA, its unwinding occurs due to the formation of a covalent bond [1-3]. In this case, the stacking interactions between the nucleic bases are disrupted and PI, as an intercalator, also loses the ability to interact with the nucleotide planes, which leads to its release. Therefore, the obtained results confirm the previously obtained data on the active interaction of rhenium compounds with nucleic acids.

Conclusions

1. Using the method of competitive propidium iodide complex formation it has been first shown that rhenium(III) complex compounds of various structural types are capable of interacting with oligonucleotides with intensity exceeding the cisplatin binding intensity depending on the ligands structure and reaches 73.2%.

2. The mechanism of rhenium(III) complex compounds binding to oligonucleotides is shown to be different from that of cisplatin in terms of interaction specificity with adenine and guanine nucleic bases, which may explain the synergistic or additive antineoplastic effect of rhenium compounds and cisplatin administration.

Prospects of further research. The data obtained suggest that rhenium(III) complex compounds are potent anticancer substances. We believe that further studies of these compounds efficacy on human cancer cell cultures in experimental carcinogenesis models are promising. We also consider it necessary to further elucidate the mechanism of Rhenium compounds binding to oligonucleotides of various compositions, for example, to non-canonical DNA found in promotion sites of oncogenes.

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ВЗАЄМОДІЯ КЛАСТЕРНИХ СПОЛУК РЕНІЮ(III) З ОЛІГОНУКЛЕОТИДАМИ РІЗНОГО СКЛАДУ

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Резюме. У зв'язку з отриманими раніше даними про ефективність протипухлинної системи Реній-Платина проведено дослідження взаємодії комплексних сполук ренію(III) трьох структурних типів з 20-членними олігонуклеотидами, які відрізняються за вмістом AT- і GC-нуклеотидних комплементарних пар, методом конкурентного комплексоутворення с пропідій йодидом. Показано, що ренієві сполуки зв'язуються з олігонуклеотидами обох типів інтенсивніше, ніж цисплатин; процес зв'язування залежить від структури і просторової орієнтації лігандів навколо кластерного диренієвого фрагменту. Одержані результати можуть бути основою комбінаційної протиракової терапії, заснованої на одночасному введенні комплексних сполук ренію(III) і цисплатину.

Ключові слова: кластерні сполуки ренію(III), олігонуклеотиди, AT- і GC- комплементарних пари олігонуклеотидів, пропідій іодид.

ВЗАИМОДЕЙСТВИЕ КЛАСТЕРНЫХ СОЕДИНЕНИЙ РЕНИЯ(III) С ОЛИГОНУКЛЕОТИДАМИ РАЗНОГО СОСТАВА

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Резюме. В соответствии с полученными ранее данными об эффективности противоопухолевой системы рений-платина, проведено исследование взаимодействия кластерных соединений рения(III) трёх структурных типов с 20-членными олигонуклеотидами, которые отличаются содержанием AT- и GC- нуклеотидных комплементарных пар, методом конкурентного комплексообразования с пропидий иодидом. Показано, что соединения рения связываются с олигонуклеотидами обоих типов интенсивнее, чем цисплатин; процесс связывания зависит от структуры и ориентации лигандов вокруг дирениевого кластерного фрагмента. Полученные результаты могут быть основой комбинационной антираковой терапии, включающей одновременное введение комплексного соединения рения и цисплатина.

Ключевые слова: кластерные соединения рения(III), олигонуклеотиды, AT- і GC- комплементарные пары олигонуклеотидов, пропидий иодид.

INTERACTION OF RHENIUM(III) CLUSTER COMPOUNDS WITH OLIGONUCLEOTIDES OF DIFFERENT COMPOSITION

Polokhina K. V., Golichenko O. A., Shtemenko O. V., Shtemenko N. I.

Abstract. According to previously obtained data about efficacy of the antineoplastic rhenium-platinum system, the study was performed on three structural types rhenium(III) cluster compounds interaction with 20-membered oligonucleotides that differed in content of AT- and GC- complementary base pairs, using the method of competitive complex formation with propidium iodide. It is shown that rhenium compounds are bound with oligonucleotides of both types more intensively than cisplatin; the binding process depending on the structure and ligands orientation around the cluster dirhenium fragment. The results obtained may be the base of the combination anticancer therapy including simultaneous administrations of a complex rhenium compound and cisplatin.

Key words: rhenium(III) cluster compounds, oligonucleotides, AT- and GC-complementary pairs of oligonucleotides, propidium iodide.

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