REVIEW

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RHENIUM–PLATINUM ANTITUMOR SYSTEMS

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This review provides an overlook of design (in short), antitumor and other biological activity of quadruple-bonded cluster dirhenium(III) compounds and their synergism with cisplatin. In particular, we describe the work of the rhenium-platinum antitumor system (introduction of rhenium and platinum compounds). Among known metal-based anticancer drugs and drug candidates dirhenium(III) compounds differ profoundly due to their strong antiradical and antioxidant properties determined by quadruple bond unsaturation. Such advantages of metal complexes as more expressed redox chemical propertie should be exploited for creating more efficient anticancer drugs. Combination of drugs leads to synergistic effects and/or to lowering toxicity of platinides and is very promising in cancer chemotherapy. The review covers the following items: design of quadruple bonded dirhenium(III) clusters, their spectral and antiradical properties (in short); interaction of the dirhenium(III) compounds with lipids and formation of liposomes; interaction of the dirhenium(III) compounds with erythrocytes and their antihemolytic activity in the models of hemolytic anemia; anticancer activity of dirhenium clusters and work of the rhenium-platinum antitumor system; antianemic and antioxidant properties of the dirhenium(III) compounds in the model of tumor growth; interaction of the dirhenium(III) compounds with nucleobases and DNA. Some modern trends in the field of bioinorganic and medicinal chemistry are also considered regarding their connection to the rhenium-platinum system efficiency: use of combinational therapy and nanomaterials; involvement of some biologically active ligands and redox-activation strategy, etc.

K e y w o r d s: rhenium, platinum, antitumor activity, antioxidant, antihemolytic, hepato-, nephrostabilizing activity.

After discovery and wide use of cisplatin there is a growing interest in transition-metal-containing drugs. Various strategies have been applied for the design of novel drugs with an improved toxicological profile that have been reviewed in detail in [1-7]. It is commonly accepted that many advantages that metal complexes have in the comparison with organic molecules, especially their versatile redox chemistry, should be used for creating more efficient anticancer drugs. An important paradigm for the development of new antitumor pharmaceuticals is represented by dinuclear carboxylate complexes of rhodium, ruthenium and rhenium with so-called ‘chinese lantern’ structure [8-10]. It was postulated that such species could bind to DNA, inhibit DNA replication and protein synthesis [11, 12] in a manner similar to cisplatin [4, 13, 14]. Among this group, the dirhenium(III) compounds may be recognized as especially promising candidates for clinical development due to their very low toxicity [15]. This issue is especially important considering severe limitations for clinical use of some cytostatics as cisplatin originating in its neuro-, hemato-, hepato- and nephrotoxicity [16-18]. Cisplatin, as a systemic anti-proliferative agent, preferentially kills dividing cells, primarily by attacking their DNA at

some level (synthesis, replication or processing) and binds to non-DNA targets. It is not truly selective for cancer cells and damages also proliferating normal cells such as those in the bone marrow and gut epithelium.

A lot of studies have explored the potential of platinum-based combination therapy [19] that means to combine one, two or more known non-platinum anticancer drugs with a platinum compound, for example [20-25]. Such a combination leads to synergistic effects and/or to lowering toxicity of platinum-based drugs and is very promising in cancer chemotherapy. Our work is an example of successful use of two anticancer agents with different mechanism of action in tumor suppression.

We summarize here recent activity and our modest experience in the field of chemistry and biochemistry of rhenium clusters. Herein, we highlight anticancer and modulation properties of these compounds and try to present future trends in their application.

Design of quadruple bonded dirhenium(III) complexes, their spectral, antiradical properties and formation of liposomes (in short)

Till the second half of the 20th century a possibility to realize δ-bonding between two metal atoms was only a prognosis of chemists-theoreticians. Quadruple bond ($\sigma^2\pi^4\delta^2$) may be formed only between two atoms of transition elements, i.e. valence level of which contain d-electrons. Existing of such a bond was suggested first by Kotelnikova and Koz'min [26] and confirmed by Cotton [27] half a century ago in the $[Re_2Cl_8]^{2-}$ anion. Structure of this ion in the complexes with amino acids was later confirmed by us.

Cluster formation stabilized unusual for rhenium state of oxidation +3. Dinuclear fragment Re_2^{6+} with the quadruple metal-metal bond plays role of a single central atom of complex formation with an overall coordination number 10. This makes it possible to choose ligands environment increasing the stock of new compounds with certain properties.

The history of this discovery includes scientific competition between two research groups headed by Drs. Kotelnikova and Koz'min (Institute of General and Inorganic Chemistry, Academy of Science of the USSR, Moscow) and by Prof. Cotton (Massachusetts Institute of Technology, Cambridge, USA). Each side describes this discovery in a different way [28, 29], paying the most attention to the structural aspect, but the main important result of mutual work was the creation of a new branch of chemical knowledge – chemistry of multiple bonded cluster compounds of transition metals.

In spite of a significant progress in theoretical investigations of quadruple metal-metal bond [30] the stock of the quadruple bonded compounds was limited by imperfection of synthetic methods and approaches during following 10 years. This problem was unzipped in the beginning of 80th by elaboration by us of new and universal synthetic methods of dinuclear cluster compounds of rhenium(III). Depending on some conditions of the synthetic procedure it was possible to obtain dirhenium(III) derivatives: octahalogenides; dihalogenotetra-μ-carboxylates; trihalohenotri-μ-carboxylates; *cis*-tetrahalogenodi-μ-carboxylates; *trans*-tetrahalogenodi-μ-carboxylates [31-34]. Some structural types of the dirhenium(III) compounds are presented in Fig. 1.

The main structural unit – dinuclear fragment Re_2^{6+} in this family is a center of complex formation. This review focuses on halogeno-μ-carboxylates of dirhenium(III) as they have been shown to be interesting for biochemical trials.

Dinuclear rhenium(III) compounds with metal-metal bond belong to the class of d^4-d^4 dimers that have electronic configuration of $\sigma^2\pi^4\delta^2$ in the ground state according to quantum chemical calculations, the order of the metal-metal bond is 4 and in the case of an electron capture it is 3.5.

Quadruple bond (delta-, δ-bond) is unique, absent in biologically occuring molecules and may be formed only by atoms of transition metals that contain d-electrons. The fourth component of the Re-Re quadruple bond has much less energy of $\delta\rightarrow\delta^*$ electron transition than $\pi\rightarrow\pi^*$ electron transition, that is a reason of absorption in the long-waved visible area in electronic absorbtion spectra (EAS) and of antiradical, antioxidant properties of the quadruple-bonded dirhenium(III) compounds. These compounds have more unsaturation than double-bonded known antioxidants, containing π-bonds, thus *a priory* may compete in being the mightiest antioxidants.

EAS of dinuclear Re_2^{6+} carboxylates were described in [34, 35]. The analysis of energy position and intensity of the most long-waved band in EAS solutions of rhenium compounds let to assign it to $\delta\rightarrow\delta^*$ electron transition. Gradual substitution of Cl ligands around Re_2^{6+} center by carboxylic ligands

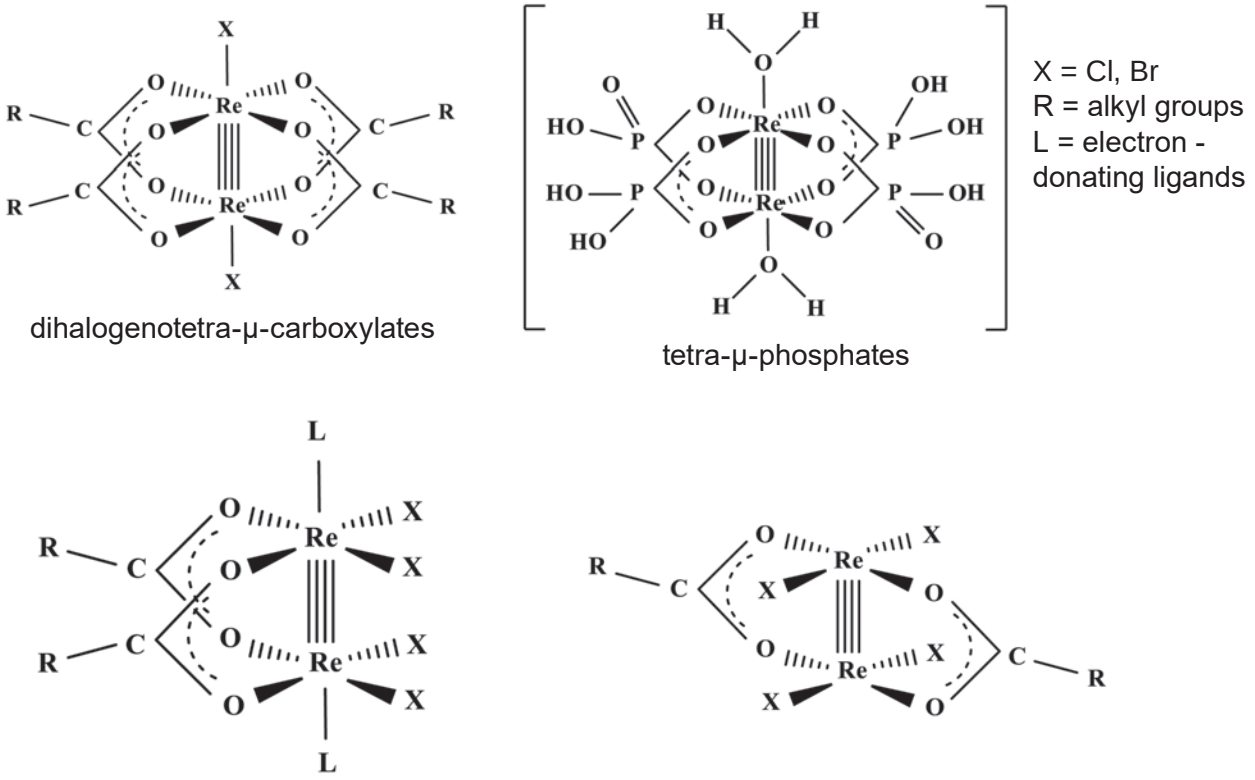


Fig. 1. Some structural types of cluster dirhenium(III) compounds

was accompanied by hypsochromic shift of this band and expected decreasing of its intensity: $Re_2Cl_8^{2-}$ – 14 700 cm^{-1} (ε ~ 1200), *cis*-$Re_2Cl_4(RCOO)_4L_2$ – 15 625 cm^{-1} (ε ~ 500), *trans*-$Re_2Cl_4(RCOO)_4$ – 16130 cm^{-1} (ε ~ 700), $Re_2Cl_3(RCOO)_3$ – 18 000 cm^{-1} (ε ~ 300) and $Re_2(RCOO)_4Cl_2$ – 20 000 cm^{-1} (ε ~ 200). These data let us use EAS as a reliable method of identification of dinuclear cluster rhenium(III) compounds and made it possible to investigate some mechanisms of their interactions with biological molecules.

Antiradical properties of dirhenium(III) cluster compounds were shown first in the model of chain radical reaction – oxidation of benzyl alcohol by oxygen [36], then by studying reactions of dirhenium(III) compounds with some stable radicals [37, 38]. It was shown that the radical chain was interrupted by two reactions of quadruple bond and the oxidation process was stopped. The reaction of dirhenium(III) carboxylates with stable radicals was more effective than that of some known antioxidants and showed the dependence of the kinetics on the structure of the rhenium complex: interaction of a radical with tetracarboxylates took 30-35 days; with tricarboxylates – several days; *cis*-tetrahalogenodi-μ-carboxylates – a day and *trans*-dicarboxylates – several seconds. Thus, in our hands we keep the traps for radicals with different abilities to react. As the peroxide oxidation of lipids (POL) in cells is a radical chain reaction, the idea emerged about possibility for dirhenium compounds to break POL *in vivo.*

Due to their reactive nature, most of the drugs are rapidly inactivated by binding to proteins or other molecules upon entering the organism and never reaching the tumor in an active form that is considered a major cause of much dose-limiting toxicity [39]. One approach to try and to circumvent these drawbacks is to encapsulate the drug in liposomes. An advantage of liposomes also is that the encapsulated drug is protected from (rapid) degradation and excretion, and it eliminates the binding to neutralizing targets (for example, glutathione in the case of cisplatin). Liposomes of cisplatin can be cross-linked in a way to exhibit favorable pharmacokinetics, i.e., increased serum half-life and improved targeting

tissues or cells of interest [39]. Liposomal cisplatin was shown to be more effective and less toxic to non-cancer cells in liposomal forms in comparison with solutions. Also, long-circulating liposomes are considered to overcome drug resistance [40-43] and in general present a promising delivery system for cisplatin-based cancer treatment.

Most of dirhenium(III) compounds are low-soluble and not stable in water solutions and just liposomal forms of cluster rhenium compounds allow introducing them successfully to biological experiments. Further investigations of the structure and properties of cluster rhenium compounds with different organic ligands including phosphate groups made it possible to discuss the mechanism of interaction of the compounds with membrane lipids (phospholipids) inside liposomes.

In the spectra of $Re_2(i\text{-}C_3H_7COO)_4Cl_2$ in chloroform solution there is a band in the area of 20 000 cm^{-1} that is relevant to $\delta\rightarrow\delta^*$ electron transition of dirhenium tetracarboxylates as it was previously described.

In the phosphatidyl choline mixture a new absorption band in the area of 15 600–14 000 cm^{-1} appeared that increased and shifted with time closer to 14 000 cm^{-1}. At the same time the absorption in the area of 20 000 cm^{-1} (characteristic of dirhenium(III) tetra-μ-carboxylates) decreased. Similar shifts were found in the spectra of other representatives of this structural type – $Re_2(C_3H_7COO)_4Cl_2$, $Re_2(PhCOO)_4Cl_2$, $Re_2(AdCOO)_4Cl_2$ in their chloroform solution with phosphatidyl choline [35, 44, 45]. This fact reflected the process of gradual substitution of carboxylic ligands on phosphate groups of phosphatidyl choline around Re_2^{6+}-centre. The coordination is formed as a monodentate coordination of phosphatidyl choline to equatorial positions of the Re_2^{6+}-centre with destruction of the conjugated Re-carboxylic cycles. Hence, the energy of δ-bond splitting is decreased during interactions of dirhenium carboxylates with phosphatydyl choline.

Liposomes from phosphatidylcholine and other lipids loaded with dirhenium(III) compounds are easily prepared by thin-film method [44, 45], have average size 100-150 nm. They are stable during 8-10 days demonstrating the protective functions of the lipid coating against hydrolysis.

The quadruple bond in liposomes was stored during this period, that was confirmed by EAS.

Recently we have developed an efficient strategy for co-encapsulation of both dirhenium and platinum based drugs into 100–105 nm scale liposomes [44]. The obtained liposomes with two drugs exhibit different shape and spectral characteristics from those incorporating only one drug. Such “nanobins” can be used in anticancer trials, that allows changing surface lipid component in order to obtain more stable vesicles loaded with targeted components with different ratio.

To sum it up, liposomes loaded with rhenium compounds cluster center Re_2^{6+} were not destructed during long period, but there were some changes in ligand environment. These changes took place due to the above described substitution of carboxylate or chlorido ligands by phosphate ones of phosphatidyl choline, which the coating is built of. In the case of dirhenium(III) compounds which contain quadruple bond, encapsulation to lipids led not only to the known advantages such as protection from hydrolysis, long-living, etc., but also to additional activating of the main biologically active unit – the quadruple bond. Elaboration of combined liposomal nanotechnology led to encapsulation of the rhenium-platinum system with definite ratio in one liposome.

Interaction of dirhenium(III) cluster compounds with erythrocytes and their antihemolytic activity in the models of hemolytic anemia

As hemolysis and senescence of human red blood cells (RBC) is an intensive radical process [46] we checked if antiradical properties of binuclear cluster rhenium(III) compounds would be executed in this model. Hemolytic erythrogramm (dependence of kinetics of Hb outcome on cells with time under influence of hemolytics – NaCl, HCl, etc.) of normal human RBC consists of groups of cells with different resistance to hemolysis (Fig. 2, *A*).

Incubation of erythrocytes *in vitro* with cluster rhenium compound $Re_2(i\text{-}C_3H_7COO)_4Cl_2$ led to formation of a group of cells with higher resistance to acidic hemolysis (Fig. 2, *B*). Maximum of hemolysis time shifted to 6 minutes. It was shown that $Re_2(i\text{-}C_3H_7COO)_4Cl_2$, was a unique stabilizer of RBC against acidic hemolysis in the wide range of tested concentrations [47].

Some dirhenium(III) compounds, containing adamantanecarboxylic ligands, were not active in that model at all, i.e., did not influence the stability of normal RBC against acidic hemolysis. But they had a stabilizing effect only in old RBC with lower initial resistance to hemolysis (Fig. 2, *C*). Unloaded

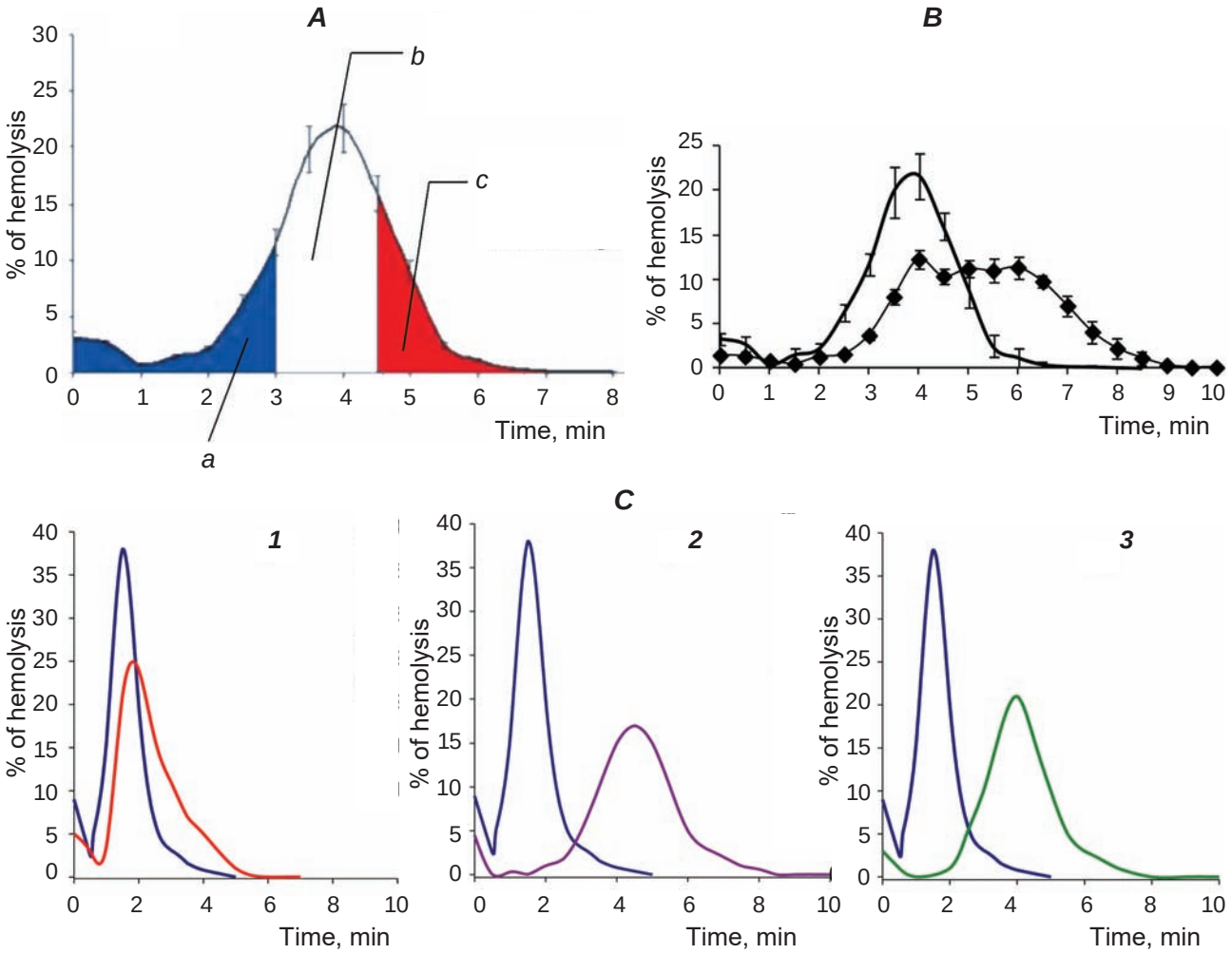


Fig. 2. Hemolytic erythrogramms. ***A*** *– Human normal RBC:* ***a*** *– sector of erythrogramm (24%) that corresponds to old RBC with low stability;* ***b*** *– sector of erythrogramm (50%) that corresponds to adult RBC with average stability;* ***c*** *– sector of erythrogramm (26%) that corresponds to young RBC with strong stability.* ***B*** *– Human normal RBC (solid line) and after incubation with* $Re_2(i\text{-}C_3H_7COO)_4Cl_2$ *(line with cubes).* ***C*** *– Old rats RBC incubated with:* ***1*** *– unloaded liposomes,* ***2*** *– liposomes loaded cis-*$Re_2(AdCOO)_2Cl_4(CH_3CN)_2$, ***3*** *– liposomes loaded cis-*$Re_2(AdCOO)_2Br_4(CH_3CN)_2$

liposomes had some tiny stabilizing effect, shifting the maximum of hemolysis from 1.0 to 1.9 min with no visible changes for the whole time of hemolysis. It is known that empty liposomes had therapeutic effect [48] due to binding to the cell surface and endocytotic uptake of phosphatidyl choline by cell membranes that have protective properties. Interaction of RBC with liposomes loaded with rhenium cluster compounds led to the shift of the erythrogramms to right side and increased significantly the parameters of the hemolytic process that showed the stabilizing properties of the rhenium compounds. This RBC model with the senescing RBC appeared to be very sensitive to the structures of the compounds, where the only difference was in halogens Cl and Br located in axial positions of the quadruple bond. Thus, the stabilizing effect depended on both the structure of the rhenium substance and properties of the RBC membranes.

Binuclear cluster fragment Re_2^{6+} actively reacted with artificial radicals *in vitro*, however the rate of such interaction strongly depended on the ligand environment of the cluster Re_2^{6+}. Besides, the reaction rate decreased with an increase of induction effects of alkyl groups in the ligands. This dependence did not coincide with that obtained by the investigations of artificial radicals shown herein and is more complicated, as the latter includes wider and more

multifunctional interactions in living cells. Presented data showed positive future prospects for Re_2^{6+}-substances applications as therapeutic agents due to their low toxicity and antiradical properties that are put into effect by δ-component of quadruple Re–Re bond and revealed in living RBC.

Chemically induced anemia (caused by introduction of lead acetate or phenylhidrazine, etc.) led to formation of non-stable population of erythrocytes in comparison with control on the first stage of anemia development.

Introduction of the rhenium compound to experimental rabbits led to a decrease of the non-stable population and increase of stable population of RBC [35]. In the second phase of anemia the formation of very stable population of RBC was noticed. High fragility of RBC and low hematocrit are the signs of hemolytic anemia [46,49]. The ability of RBC to deform is a very important requirement for these cells to navigate narrow capillaries *in vivo.* They reversibly transform from discocytes echynocites and then irreversible destructions start – the process of hemolysis or haemoglobin outcome. The decrease in deformability or membrane defects may play a significant role in hemolysis, caused by different factors. In our experiments quantity of discocites sharply decreased under introduction of phenylhidrazine while quantity of destructed RBC increased. Introductions of tocopherol and a rhenium compound shifted the picture of red blood to the normal state. Introductions of the rhenium compounds in liposomal forms were especially effective. In these experiments we demonstrated the antihemolytic activity of dirhenium(III) cluster compounds *in vivo,* that were not only the result of RBC membrane-stabilizing properties of the compounds, but involved more complex influence on the system of red blood of experimental animals.

Anticancer activity of dirhenium clusters and work of the rhenium-platinum antitumor system

In 1983 the dirhenium cluster compound – $Re_2(EtCOO)_2Br_4(H_2O)_2$ – was proved to have varying degrees of effectiveness against sarcoma S-180, leukemia P-388, and melanoma B-16, with particularly good results against B-16 by Eastland and co-workers [50]. This compound was found to be quite susceptible to decomposition in aqueous solutions. In addition, the complex required very high doses to achieve maximum efficiency also being the result of the compound instability. It is readily decomposed into insoluble rhenium oxide requiring therefore its considerable amount to be injected in order to have a significant quantity to reach tumor sites. Since the discovery of antitumor activity of $Re_2(EtCOO)_2Br_4(H_2O)_2$ this field remained actually unexplored.

Our investigations of anticancer activity of dirhenium cluster compounds started in 2000 with experiments on Guerin's carcinoma (T8). T8 is a rat's specific solid tumor that is widely used in the trials of different chemotherapeutic agents and is sensitive to cisplatin [51, 52]. Growth of transplanted cells in control groups of xenografts during 21 days was very rapid, tumors occupied approximately 1/3 of the animal weight on the last day of the experiment.

The period of 21 days after T8 cells transplantation is considered crucial for survival, the massive deaths of the control animals are usually started, that is in line with previously described aggressive characteristic of this type of tumor. The description of our experiments is also presented, details you may find in [44, 49, 53-56].

We started from the first types of experiments (I) – introductions of rhenium clusters in solutions in large doses. High-dose rhenium therapy caused 20-30% inhibition of tumor growth that was independent of the quantity of introduced substances. No deaths were observed in groups, where rhenium compounds were introduced and no visible changes in the liver, spleen, kidneys, skin, lung or brain were defined. But brown precipitate – products of the cluster rhenium compounds decomposition – was found in the peritoneal cavity, being more intensive in animals of the group, where the quantity of introduced compounds was especially large. That coincided with the results obtained by Eastland's group. The procedure used in experiments of type I cannot be considered as reasonable, since the most of the preparation decomposed and turned into insoluble rhenium dioxide. This fact can also explain the absence of a dose effect. Rhenium cluster compounds clearly demonstrated impossibility to be introduced in solutions and in high acute dose.

As far as liposome forms of dirhenium compounds were elaborated, we used the procedure II – introduction of only rhenium compound at a dosage according to the scheme of antioxidant therapy. The inhibition of tumor growth during the first two periods of the observation was reached, but it was not so effective at the last stage (Fig. 3, Table).

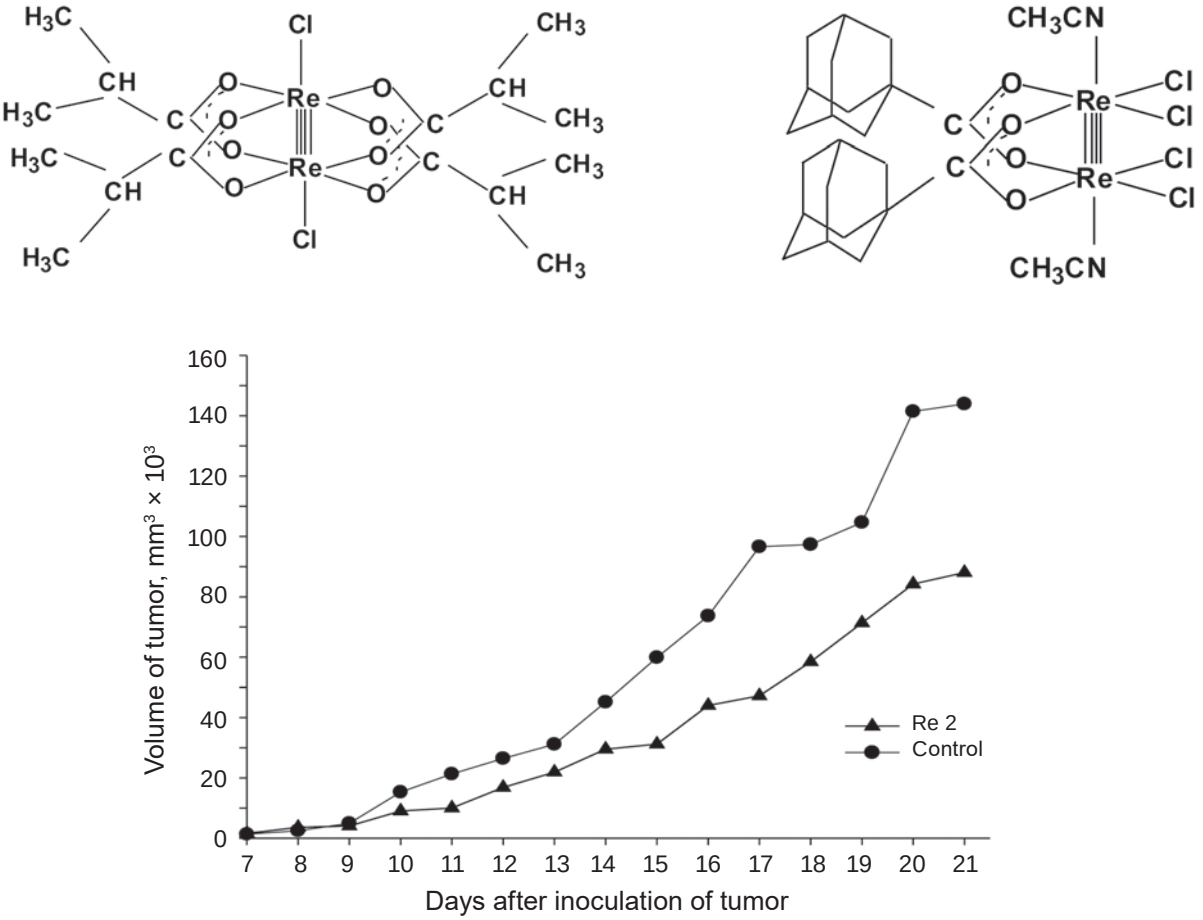


Fig. 3. Volumes of tumors in experiments with Re1 – $Re_2(i\text{-}C_3H_7COO)4Cl_2$, dynamics of tumor growth in a control group and in a group with Re2 – cis-$Re_2(AdCOO)_2Cl_4(CH_3CN)_2$ in liposomes treatment

Volumes of tumors in vivo on three stages of development

Group of animals	Stage of tumor development		
	I (10-13 days)	II (13-17 days)	III (17-21 days)
Control	10–50	30–70	60–100
Cisplatin	4–5	15–30	5–35
[Re1]lip	5–10	15–25	60–82
Cisplatin + [Re1]lip	4–5	3–5	0–2

The dynamics of tumor growth, shown on Fig. 3, is typical of the most of tested rhenium compounds, introduced according to procedure II. Inhibition of the tumor growth under introduction of rhenium compounds according to method of introduction II reached usually 30-45%.

Cluster rhenium compounds, solely introduced in liposome form did not have so strong inhibitive mechanism of interaction with cancer cells as cisplatin, thus this mechanism differs from that of cisplatin. It was shown [57] that different categories of chemically induced and consecutively developing tumors – hyperplasia, adenoma and carcinoma – featured various sensitivity to different agents on some tumor growth stages. It means that each stage of tumor progression has special signal transduction system and special response to different chemotherapeutic agents.

This consideration may partly explain synergistic effect of the rhenium cluster with cisplatin: introduction of cisplatin solution on the 9th day and ten times introduction of the rhenium compound in liposomes with final ratio of platinum and rhenium ratio 1 : 4 (method of introduction III) – the rhenium–platinum antitumor system. A particularly significant decrease in the measured tumor

volumes was found in the groups, where cisplatin and Re1 were introduced together (Fig. 3, Table, Group [Re1]lip + cisPt). In these groups deaths were not observed during 21 days of the experiment and reduction of the tumor growth was more effective in comparison with cisplatin alone, even at the last stages of tumor development. Most of the experimental animals had no tumors at all and this kind of chemotherapy can be considered extremely effective.

It suggests that differing mechanisms of tumor inhibition on different stages of progression by rhenium and platinum compounds resulted in the inhibition of the tumor at the last stage of development. A lot of dirhenium(III) compounds were tested in this experiment (Fig. 4) [44, 53-56], that allowed us to present the rhenium-platinum antitumor system.

The efficacy of the rhenium-platinum antitumor system with the use of some representatives of rhenium cluster compounds of such types as tetracarboxylates, *cis*-dicarboxylates, *trans*-dicarboxyletes of dirhenium(III) complexes was essential, the tumor inhibition reached 95-100%. Our first conclusion about the antitumor system efficiency independence of the ligands nature in the molecule of dirhenium clusters was changed after investigation of the homologues of alkyldicarboxylates (Fig. 4, *B*) [56], where we found the correlation between the length of alkyl chain in carboxylic groups and ability to inhibit the growth of tumor. Really, the dependence in antitumor efficiency grew in the range methyl < ethyl < propyl < butyl < penthyl substituent R in the structure of dicarboxylates of dirhenium, i.e. with hydrophobicity of the alkyl chain.

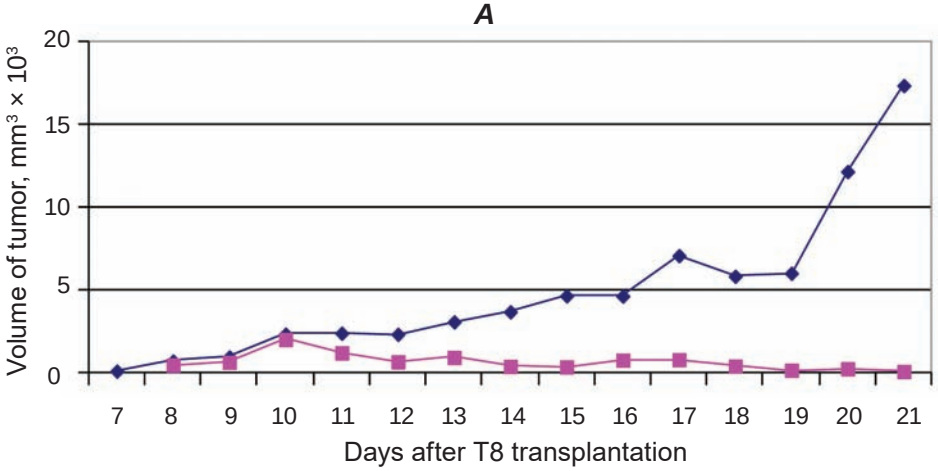
Then a new dirhenium(III) dicarboxylate complex *cis*-$[Re_2(GABA)_2Cl_5(H_2O)_2]Cl$ was shown to possess appreciable antitumor activity being introduced alone, which was higher than those of the previously investigated alkylcarboxylates (up to 60%) (Fig. 4, *C*) [54]. This may find further applications for the development of new antitumor dirhenium(III) species with active ligands. Studies for the synthesis of active dirhenium(III) compounds involving zwitterionic aminocarboxylate ligands, curcuminoid, indolylacetic, etc. ligands are in progress. Our new findings show that both the hydrophobicity (and other possible functions) of ligands and the ligand charge play a significant role in DNA-interactions and in the antitumor activity of the dirhenium complexes.

Systematic studies of structure-activity relationships among dirhodium complexes have provided insight into the molecular characteristics that control their activity [58-63]. In particular, a study performed on a series of dirhodium carboxylate derivatives that exhibit cytostatic activity against the Ehrlich ascites tumor, leukemia L1210, and sarcoma 180 cells, revealed that the activity of this series increases with the lipophilicity of the bridging carboxylate alkyl groups but that further lengthening of the carboxylate group beyond the pentanoate reduces their therapeutic efficacy [60]. Taking this into account as well as the fact that dirhenium isobutyrate analogs were anticancer active ones, we studied in detail properties of the Re-Pt system with rhenium complex with pivalic acid as ligands, namely *cis*–$Re_2[(CH_3)_3CCOO]_2Cl_4{\cdot}2DMSO$ [55].

The inhibition of the tumor growth after introduction of cisplatin in solution (group T8+cisPt) was rather effective in this model and the inhibition of the tumor growth reached up to 84%. The mortality of the experimental animals was very high in both T8 and T8+cisPt groups (up to 35%), which demonstrates the carcinoma aggressiveness and cisplatin toxicity. The introduction of the dirhenium compound alone led to reduction of the tumor size by 57.45% and no rat deaths were recorded in this group. The antitumor effect of $Re_2[(CH_3)_3CCOO]_2Cl_4{\cdot}2DMSO$ (57%) is greater than the effect found for other dirhenium compounds, such as $Re_2[(CH_3)_2COO]_4Cl_2$ with shorter carboxylate chains (by 28–30%) [56] or compared to the effect of $[Re_2(GABA)_2Cl_5(H_2O)]Cl{\cdot}2H_2O$ (by 60%) [51] with the same model. Previously, we proposed that the cationic complex $Re_2(GABA)_2Cl_5(H_2O)]Cl{\cdot}2H_2O$ interacts with DNA more effectively than the neutral alkylcarboxylates [56] by taking into account that the positive charge may facilitate DNA binding due to electrostatic interactions. Our new findings in this report show that both the hydrophobicity of the alkyl ligands and the ligand charge play a significant role in antitumor activity of the dirhenium complexes.

A very significant effect was observed for the group, where cisplatin and $Re_2[(CH_3)_3CCO-O]_2Cl_4{\cdot}2DMSO$ were introduced together. Remarkably, in this case, no deaths were registered for the entire 21-day period of the experiment, while the reduction of the tumor growth was more effective than that of T8+cisPt group and many of the experimental animals had no tumors at all. Therefore, it is obvious that this type of combined chemotherapy with $Re_2[(CH_3)_3CCOO]_2Cl_4{\cdot}2DMSO$ is very effective and comparable to the cases of previously investi-

A



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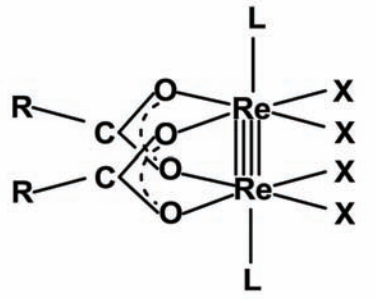

L = H_2O; X = Cl;
R = CH_3, C_2H_5, C_3H_7, C_4H_9

Weight of residual tumors in experiments with *cis*-dicarboxylates dirhenium(III) and cisplatin (*M* ± *m*, *n* = 8-12)

Group	Weight, g
T8	63.30 ± 15.20
cisPt	24.00 ± 4.90
$Re_{acetate}$ + cisPt	13.80 ± 2.40
$Re_{propionate}$ + cisPt	1.50 ± 0.30
$Re_{butirate}$ + cisPt	0.28 ± 0.10
$Re_{pivalate}$ + cisPt	0.06 ± 0.01

C

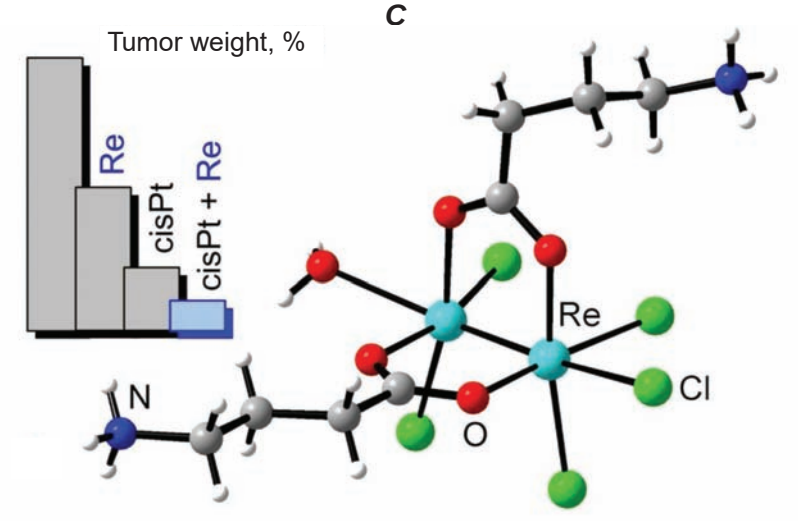

Fig. 4. Antitumor properties of the rhenium-platinum system with different cluster rhenium compounds in comparison with cisplatin. ***A*** *– Dynamics of tumor growth under influence of cisplatin (blue),* $Re_2(i\text{-}C_3H_7COO)_4Cl_2$ *and cisplatin (rose);* ***B*** *– Weight of residual tumors in experiments with cisplatin and rhenium(III) dicarboxylates.* ***C*** *– X-ray structure of cis-*$[Re_2(GABA)_2Cl_5(H_2O)_2]Cl$ *and weights of residual tumors*

gated compounds. Additionally, no significant structure-efficacy relationship is observed in the case of combined treatments as recorded in treatments with single dirhenium compounds. But the best results were obtained, when both compounds were put in one liposome in the ratio 1 : 4 [44]. The tumor was reduced from the first days of the experiment and no harmful effects were noticed for the liver, kidneys and RBC morphology. These results demonstrated efficacy of the encapsulated Re-Pt system into lipid coating and showed the way to enhance the antitumor properties of the system (and other preparations) by this procedure.

In a thorough theoretical discussion of chemoprevention, Lippman and Hong [64] stated that our understanding of neoplastic evolution considerably improved and led to revolution in drug development – a turn from toxic drugs to molecular targeting. Identifying multiple molecular targets for effective combinations of preventive agents is a major focus of chemoprevention or chemotherapeutic study. Many studies were carried out to evaluate the combinations of antitumor drugs, for example interferon resulted in synergistic interactions with antitumor ruthenium complexes [65]. New 5-fluoracil analogues and folate antagonist, the inhibitor of topoisomerase I irinotecan and the third-generation platinum compound oxaliplatin [66-68] developed, based on distinct mechanism of cytotoxicity and resistance, as well as effective combination patterns show themselves to be very promising.

The known affinity of sulfur for platinum complexes has resulted in the investigation of so-called "protecting agents" with a view to correcting side-effects of platinum therapy, without reducing its antitumor activity too much (for example, nucleophilic sulfur compounds, such as sodium thiosulfate (STS), biotin, glutathion, sodium 2-mercaptoethanesulfonate (mesna) and its oxidized S-S-bridged dimer (dimessna, BNP-7787)). The protective effect of these compounds is prevention or reversal of Pt-S adducts in proteins. It has been shown that protein-bound cisplatin cannot be released by STS, although STS is able to break the Pt-thioether bond in methionine model systems. Possible Re1 functioning as "protecting agent" has not been studied, but there is a more feasible side of its mechanism of action. Possible mechanisms and numerous examples of cisplatin action modulation are discussed in detail in the review of Fuertes et al [19, 68], where biochemical modulation is formulated as a manipulation of cellular biochemical pathways by chemical agents to produce selective enhancement of the efficiency of antitumor drug. Biochemical modulation of mechanism of action platinum-based compounds is considered as a rather efficient and promising strategy in cancer treatment, even in comparison with new metal-based drugs. This definition – biochemical modulation – includes possible mechanisms of complex action of several substances mentioned above. Among the most important factors for understanding possible enhancement mechanisms of the rhenium compounds we should underline the following: (i) enhancement of cisplatin accumulation as it was shown for dipyridamole, amphotericine B and cyclosporine. An increase in cell membrane permeability is one of the known properties of these substances that lead to the enhancement [69]. Our previous works showed the unusual ability of some rhenium substances to increase conductivity of artificial lipid membrane [70, 71] and the formation of membrane pores which provoke K^+ efflux; ii) platinum detoxification by glutathione. Having strong reducing potential, showed herein, cluster rhenium compounds may interfere with the glutathione system both at the substrate level and at the enzyme level of enzymes as was demonstrated with example of L-SR-buthionine sulfoximine (L-BSO), an inhibitor of γ-glutamyl-cysteine synthetase; iii) intracellular ATP-level regulation, which determines apoptotic death as was shown for a complex of substances (MAP-regime); cluster rhenium substances may change the bioenergetic cellular index as they are active antioxidants; iv) interactions with ceramide-sphingosine-sphingosine-1-phospate rheostate that determined balance between survival and apoptosis as rhenium compounds were shown to interact with phosphate groups in liposomes.

The occurrence of drug resistance is one of the main challenges for cancer chemotherapy [72-76]. Tumor resistance to anticancer drugs has multiple and complex mechanisms. From the contemporary knowledge a heterogeneous population of tumor cells contains inherently chemoresistant (intrinsic resistant, cancer stem cells or cancer initiating cells) that drive tumorogenesis; and other cells, initially responsive, but acquired mutations and became resistant after drug application (secondary resistant or acquired resistance). Resistant to cisplatin Guerin carcinoma (T8*) – is a very convenient model for investigation of efficacy of new drugs for overcoming cisplatin resistance [76]. In our experiments cisplatin

reduced T8* effectively (only by 30-35%). Application of the Re-Pt system led practically to elimination of the resistant tumor. Resistant cancer cells display a rich repertoire of self-defense biochemical reactions. But among plenty of methods proposed to overcome drug resistance, the following two most promising ones were named: a combination of drugs targeting alternative pathways simultaneously and employment of immunotherapy in the form of monoclonal antibodies and vaccines that can augment immune response against cancer [72]. Also, some metal complexes were proposed for therapy and diagnosis of drug resistance [77]. This allows us to suppose, that combination of two anticancer metal-containing drugs used by us with different mechanism of action may be useful to overcome drug resistance.

Investigations of morphology of residual tumors showed unusually large quantities of gigantic cells in the tumor tissues (Fig. 5, *A*), isolated from tumor-bearing animals where rhenium-platinum system was applied, together with usually observed chemically caused pathomorphological changes as fibrosis, necrotic, apoptotic, chimeric mitotic cels, etc.

Gigantic cells are common for chemically induced morphological changes in cancer cells but there is not final recognition about their destiny – are they 'terminal' or it is some kind of 'delay' in cancer progression [78] that is far beyond our issue matters. But formation of large quantities of giants in the residual tissue of T8 in experiments where rhenium-platinum antitumor system was applied in comparison with experiments, where cisplatin or rhenium substances were solely introduced allowed us to suggest that combination of two metal based compounds switched some additional signal transduction pathways important for cancer cells survival or death.

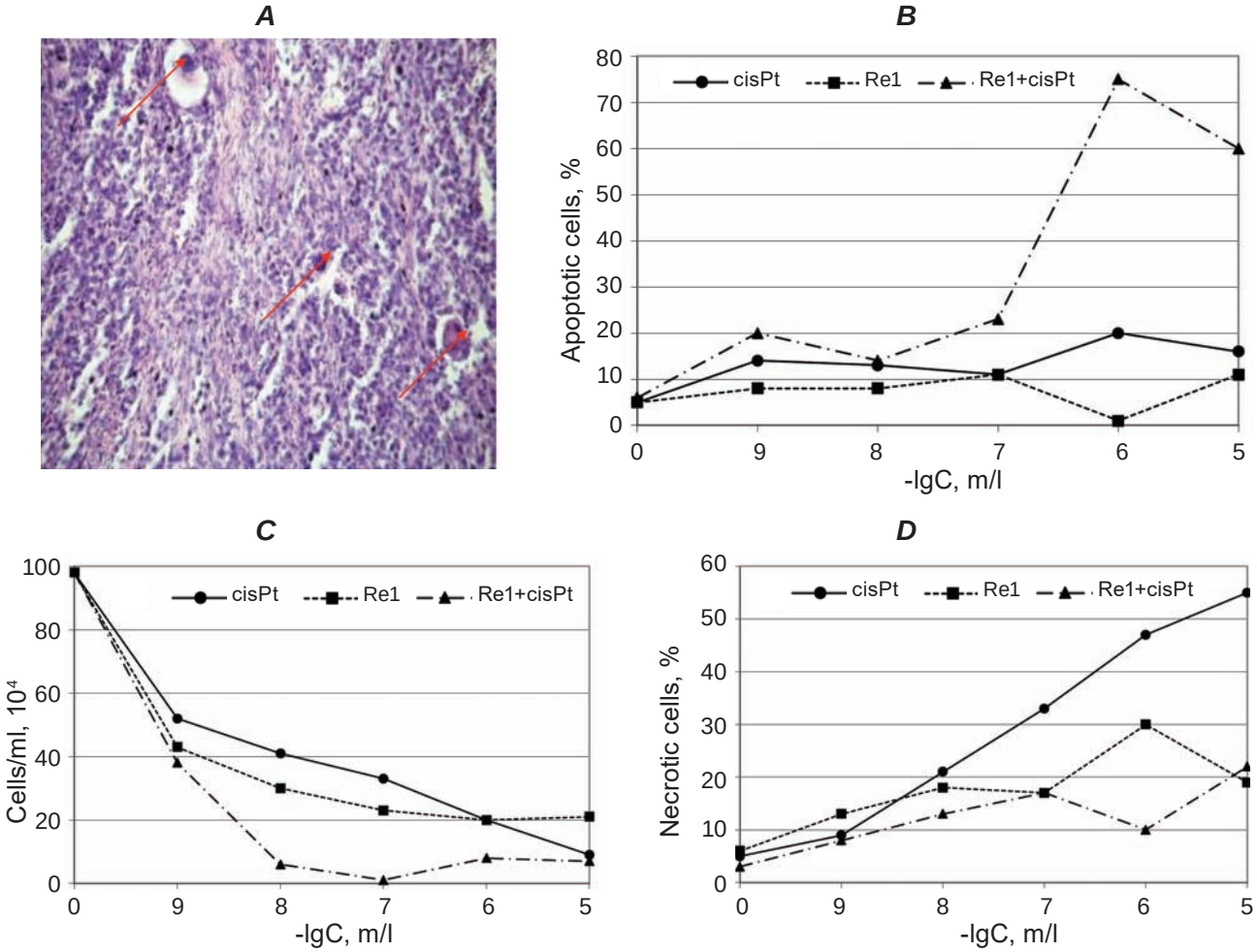

Fig. 5. ***A*** *– Gigantic cells in residual T8 tissue.* ***B***, ***C*** *and* ***D*** *– Influence of rhenium–platinum antitumor system, where Re1 –* $Re_2(i\text{-}C_3H_7COO)_4Cl_2$ *and its components on the growth of leukemic cells Jurkat*

Results of the experiments with human leukemic cells (Fig. 5, *B*, *C*, *D*) supported this observation as influence of the system was more effective (*B*) and more pro-apoptotic (*C*, *D*) to this kind of cancer cells in comparison with the influence of cisplatin and rhenium compound.

Further our investigations of anticancer properties of the rhenium-platinum antitumor system were aimed to involve new dirhenium compounds with biologically active ligands and to elaborate nanotechnology with the use of combined liposomes [44, 55]. The antitumor effect of some newly synthesized dirhenium compounds with biologically active ligands having been introduced alone, was greater than the effect found for other dirhenium compounds, such as *cis*-$[Re_2(GABA)_2Cl_5(H_2O)]Cl{\cdot}2H_2O$ in the same model [not published results]. Previously, we supposed that this cationic complex interacted with DNA more effectively than the neutral alkylcarboxylates, owing to the positive charge that may facilitate DNA binding due to electrostatic interactions.

Antianemic and antioxidant properties of the rhenium(III) compounds in the model of tumor growth

Anemia is a common complication of malignancies [79-83]. Cancer can give rise to anemia by various routes, the mechanisms behind cancer-related anemia's are very complicated, remain debatable [62, 63] and only some aspects are discussed herein. A negative impact of anemia on the outcome of cancer patients treated with chemo-, radiotherapy is well-known, with a reduction of treatment efficacy by anemia-induced tumor hypoxia being a popular explanation. Bone marrow is a very active tissue, and cancer treatment can sometimes be very hard on normal bone marrow, causing anemia. Drug treatment is most likely to cause anemia in this way, particularly with drugs such as platinum-based drugs. New lines of evidence suggest that abnormalities in the production of erythropoietin (EPO) in kidney tissue are involved. The hypoproliferative state in anemia of cancer appears to be related to either decreased EPO production by injured kidney or impaired bone marrow response to EPO [84-86]. The use of EPO subsequently expanded to include the correction of drug-induced anemia (such as with chemotherapy drugs) and other types of cancer-related anemia. Under influence of cisplatin deep destructions in the system of RBC of tumor-bearing animals occurred: resistance of RBC greatly decreased (Fig. 6, *A*), maximum of hemolysis (1.5 min) shifted to left side in comparison with intact animals (3.5 min).

Under cisplatin introductions quantity of discocytes sharply decreased and quantity of destructed RBC increased (Fig. 6, *C*), that underlined the harmful influence of the cisplatin therapy on the production of RBC.

Use of rhenium(III) carboxylates of tetracarboxylates and *cis*-dicarboxylates types led to increase in RBC resistance (Fig. 6, *B*), hematocrit, quantities of discocytes and to decrease in quantities of destructed RBC (Fig. 6, *C*). Similar RBC-supporting functions of rhenium(III) carboxylates of such types of compounds were shown to manifest in the model of tumor growth without cisplatin [55] and in the models of chemically induced hemolytic anemias [35]. Interestingly, that one of representatives of the type of *trans*-dicarboxylates – *trans*-$Re_2(C_3H_7COO)_2Cl_4$ did not influence so positively the RBC properties, introduced as a component of the rhenium-platinum antitumor system, nevertheless it did show anticancer activity close to *cis*-$[Re_2(C_3H_7COO)_2Cl_4(H_2O)_2$ (Fig. 6, *C*). This fact requires more detailed further investigations.

Thus, the use of the rhenium-platinum antitumor system eliminated the toxic influence of malignancy and cisplatin on RBC state. Such properties of the rhenium compounds should not be explained only by RBC membrane stabilizing properties as it was possible in experiments *in vitro*, and to our mind involve bone marrow processing. This statement was shown in our further experiments with bone marrow investigations [87]. Bone marrow structure and cell numbers of cisplatin-treated rats showed a deep depression of erythroid germ, decrease in the number of juvenile, mature forms of erythroid cells and polychromatophilic normoblasts, that is followed by decrease in the RBC production to the bloodstream. Introduction of the rhenium-platinum system led to up to 3-fold increase in erythroblasts, basophilic and polychromatophilic normoblasts compared to group T8+cisPt. Moreover, the appearance of single polychromatofilic normoblasts with two nucleus and single megakaryocytic cells was found under the influence of the rhenium compounds introduction.

These investigations demonstrated that dirhenium(III) compounds as a part of the Re-Pt system had anti-anemic properties, influenced positively morphological and biochemical parameters of blood and erythroid germ of the bone marrow, effectively

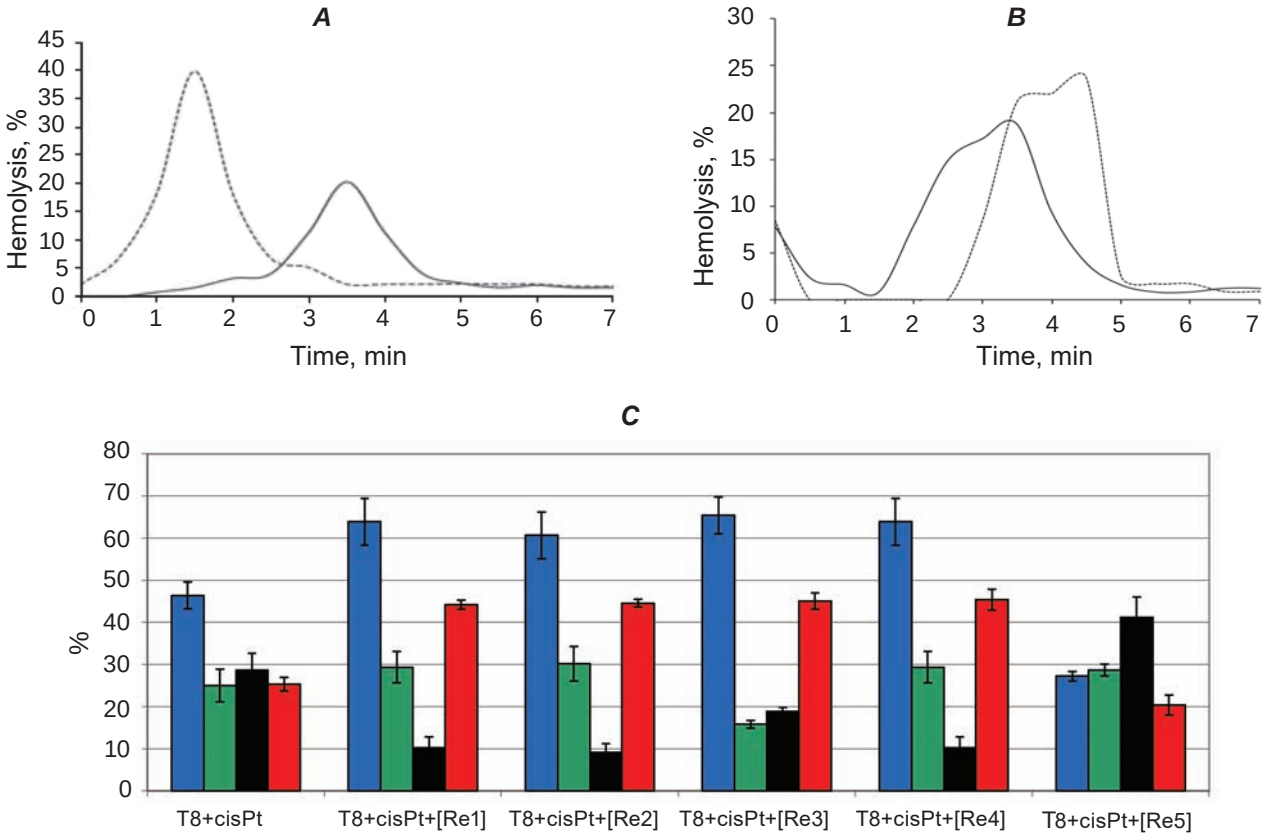

Fig. 6. Hemolytic erythrograms of rats RBC. ***A*** *– Intact animals (solid), group T8 + cisPt (dash).* ***B*** *– Groups of tumor-bearing rats with introduction of rhenium–platinum system where the rhenium components were: cis-$Re_2(C_2H_5COO)_2Cl_4(H_2O)_2$ (solid) and cis-$Re_2(C_3H_7COO)_2Cl_4(H_2O)_2$ (dash).* ***C*** *– Hematocrit (red), quantity of RBC morphological forms (discocytes – blue, echinocytes – green, destructed forms – black) in blood of rats under influence of rhenium–platinum antitumor system, where Re1 – $Re_2(i\text{-}C_3H_7COO)_4Cl_2$; Re2 – cis-$Re_2(AdCOO)_2Cl_4(CH_3CN)_2$; Re3 – cis-$[Re_2(GABA)_2Cl_5(H_2O)_2]Cl$; Re4 – cis-$Re_2(C_3H_7COO)_2Cl_4(H_2O)_2$, trans-$Re_2(C_3H_7COO)_2Cl_4$*

increased hemoglobin levels, hematocrit, state of red blood cells and decreased content of pathological forms of RBC together with antineoplastic properties in the model of tumor growth.

As pathogenesis of the cancer anaemia involves combination of a shortened erythrocyte survival in the circulation with the failure of the bone marrow to increase red cell production, attempts have been made to find enhancement of platinum-based drugs with recombinant human erythopoietin [85]. However, erythropoetin corrected anemia but did not improve cancer control or survival of patients. Thus, we may conclude, that rhenium compounds have their own anticancer properties and, furthermore, antihemolytic ability and both can be independently executed in the model of tumor growth.

Development of tumors and T8 carcinoma followed by a free radical burst usual for malignancy development [88-90] led to accumulation of malonic dialdehyde (MDA) in plasma of tumor-bearing rats (Fig. 7, *A*, *B*, group T8) as a result of intensive approximately 4-fold peroxide oxidation of lipids (POL) in comparison to control.

Introduction of cisplatin led to approximately half lower POL intensity due to the slowing down tumor growth [91]. Introduction of dirhenium(III) **Re1**–**Re4** compounds with lower anticancer activity in comparison to cisplatin, nevertheless, led to lower MDA accumulation than in blood of T8-bearing animals. In the case of the rhenium-platinum system application of some rhenium compounds (Re1, Re4) decreased the intensity of POL practically to the level of intact animals. Thus, these rhenium compounds revealed their antioxidant properties in the model of tumor growth *in vivo.* This is not true again for the dirhenium(III) compound Re5 with *trans*-

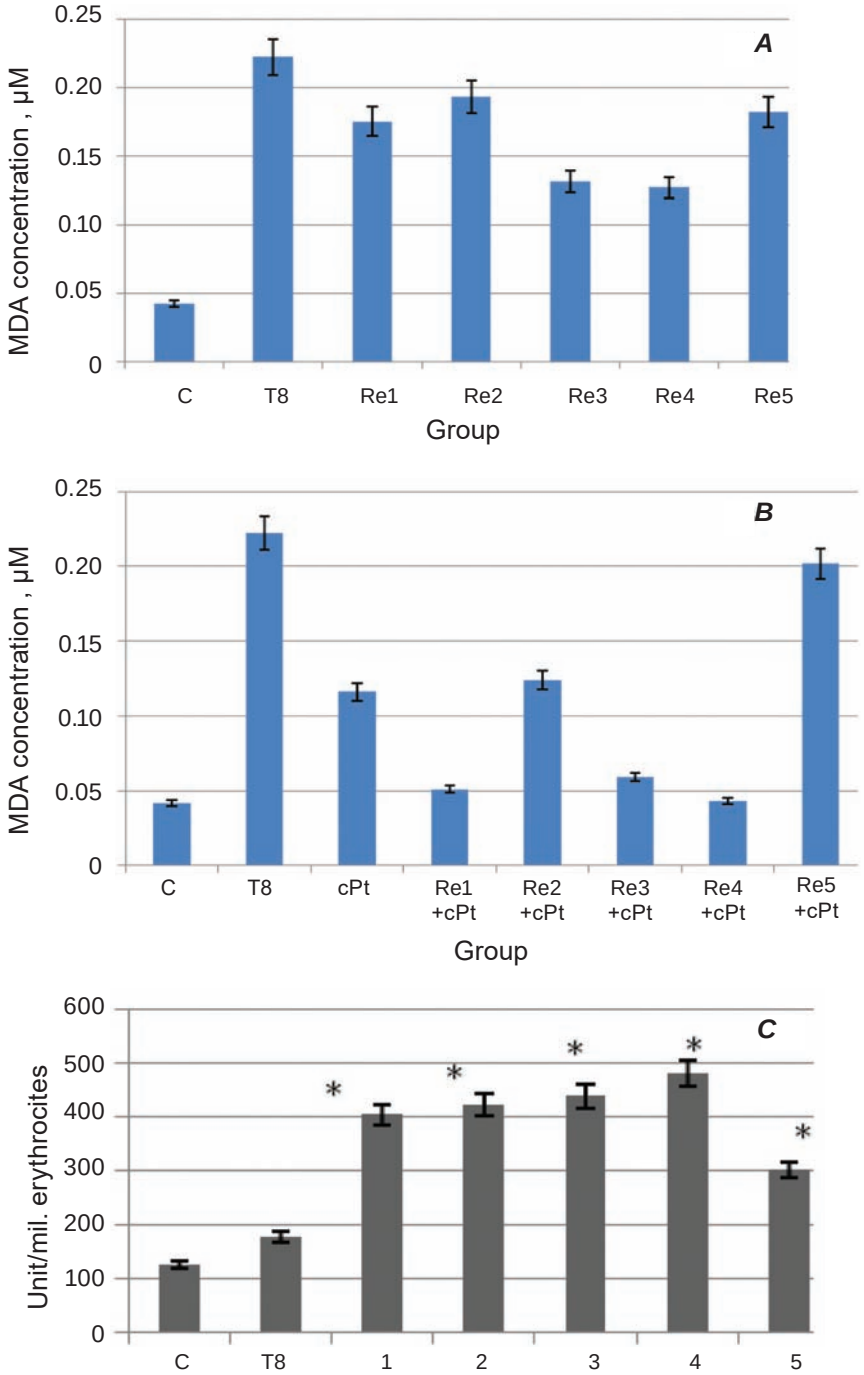

*Fig. 7. Level of MDA in plasma of control (**C**) rats and tumor-bearing rats under influence of dirhenium(III) cluster compounds (**A**) and rhenium–platinum antitumor system (**B**), where Re1 – $Re_2(i\text{-}C_3H_7COO)_4Cl_2$; Re2 – cis-$Re_2(AdCOO)_2Cl_4(CH_3CN)_2$; Re3 – cis-$[Re_2(GABA)_2Cl_5(H_2O)_2]Cl$; Re4 – cis-$Re_2(C_3H_7COO)_2Cl_4(H_2O)_2$, Re5 – trans-$Re_2(C_3H_7COO)_2Cl_4$. (**C**) Activity of SOD in RBC of tumor-bearing rats under introductions of the rhenium cis-dicarboxylates with alkyl ligands: 1 – methyl; 2 – propyl; 3 – isobutyl; 4 – pivalyl; 5 – trans-$Re_2(C_3H_7COO)_2Cl_4$*

orientation of carboxylic groups around cluster fragment (Fig. 7, *A*, *B*, Re5).

These abilities to decrease POL intensity depended on the structure of dirhenium(III) compounds and were shown to be realized in other tissues of tumor-bearing xenografts and in other models with depleted redox state. Antioxidant properties of some dirhenium(III) compounds were higher than

those of well-known antioxidants, for example, alpha-tocopherol in the model of chemically induced anemia [35]. If the introductions of the known antioxidants led to a decrease in the intensity of POL up to 1.5-2-fold, the introduction of dirhenium(III) complexes reached the 4-5 and more times efficacy. The mechanism of action of the known antioxidants is based on the interaction of radicals with their conjugated π-bonds (for example vitamins A, E) with formation of a stable unit, which interrupts the radical chain reaction [92]. As it was shown above, the quadruple bonded cluster rhenium fragment easily bounds electrons of radicals on the energetically low δ*-untibonding orbital in three-stage reaction. Some metal-organic compounds with antioxidant properties are known [93], but their antioxidant properties are realized due to the existence of the π-unsaturated ligands, for example, of polyphenol or flavonoid nature. Quadruply bonded dirhenium compounds thus represent a new class of highly effective δ-antioxidants that are, given their nontoxicity, are very promising medicines.

The antioxidant and anticancer properties of dirhenium dicarboxylates of *cis*- and *trans*-configuration with different length of organic ligands in the model of tumor growth were studied and their different ability to activate superoxidedismutase (SOD) together with different antioxidant properties and similar antitumor effect were studied [91]. Dirhenium(III) *cis*-dicarboxylates were characterized by higher degree of activation of erythrocyte SOD in comparison to *trans*-isomer (Fig. 7, *C*).

The dependence between the structure of dirhenium (III) dicarboxylates and their ability to activate SOD was the reason to investigate the process of their interaction with proteins. We investigated differences in the interactions between binuclear cluster rhenium(III) compounds of *cis*- and *trans*-configuration with native bovine serum albumin (BSA) and human serum albumin (HSA) by methods of electronic absorbtion spectroscopy, tryptophan fluorescence and circular dichroism spectroscopy [94]. It was shown that in the process of interaction of both compounds with proteins the formation of the different complexes took place via His moieties with preservation of quadruple Re-Re bond. *Trans*-isomer interacted with molecular environment of Trp-214 (HSA) and Trp-212 (BSA) in hydrophobic pocket of the IIA subdomain of homological proteins. For the *cis*-isomer more complex mechanism took place that included not only the sub-domain IIA, but also at least one more site of binding of the rhenium substance in the subdomain IB of the protein. Different changes in the secondary structure of the homological proteins were shown in the complexes with configuration isomers. Similar investigations were accomplished with native bovine erythrocyte SOD with the changing of the method of Trp-fluorescence on Tyr-fluorescence [95]. Binding of both complexes to His moieties and changing of the secondary structures of the enzyme did not influence its active center. Even more, for *cis*-dicarboxylate the SOD-like activity was demonstrated to be on the first minutes of the xantine-oxidase reaction, in contrast to *trans*-dicarboxylate. The studied features of the interaction between rhenium compounds and SOD *in vitro* explained only partly the strong activation of SOD in the experiments *in viv*o, shown above.

SOD-like activity, shown later for other rhenium compounds and their ability to decompose hydrogen peroxide like catalase (CAT, approximately 30-40% in comparison to native enzyme CAT, unpublished results) made certain impact on their ability to diminish oxidative stress and opened one more direction of application of the rhenium cluster compounds as SOD and CAT-mimetics. For more effective antioxidant intervention the use of metal-organic compounds with SOD- and CAT-like activity on the base of manganese [96] and cerium [97] were shown.

How antioxidants may be anticancer agents: antioxidants have been suggested to inhibit NF-κB activation by scavenging reactive oxygen intermediates that act as signaling molecules to activate the NF-κB pathway and by directly inhibiting IKK kinase activity by modifying critical Cys residues in the IKK kinase activation loop [98, 99]. For example, chemical modification of the curcumin molecule led to more potent inhibitors curcuminoids of NF-κB activity than curcumin, while exhibiting both anti-inflammatory and anticancer activities [100, 101].

Influence of the rhenium cluster compounds on the state of the liver and kidneys requires special survey, as to our knowledge, there are no liver and kidneys protectors of metal-organic origin. In short: as nontoxic antioxidants, dirhenium(III) compounds influenced positively the state of the liver and kidney and prevented cisplatin-induced nephro- and hepatotoxicity [102, 103]. Although the mechanisms underlying the side-effects induced by cisplatin in the liver and kidneys tissues are not understood clearly, it was attributed to the combination of multiways, such as

generation of reactive oxygen species, which could interfere the antioxidant defense system and result in oxidative damage in different tissue and reaction with thiols in proteins and glutathione, which could cause cell dysfunctions [103]. Several antioxidants were tested in the animal model to find optimum combinations to prevent hepato-toxicity of cisplatin [104-107], and the conclusion was made about possible success which could be found in combinations of antioxidants. Dirhenium(III) compounds were found by us as unique antioxidants in decreasing intensity of POL (MDA level was found to be depleted to normal levels) in the liver and kidney tissues in experiments not only on tumor-bearing rats and use of cisplatin but also in the models of acute chemical intoxication [67-69]. Histopathology of the tissues, level of diagnostic enzymes, experiments on isolated hepatocytes and other models of kidney and liver injuries confirmed protective abilities of rhenium substances. Comparative studies of hepato- and nephro-protective properties of the compounds showed better results for dirhenium(III) carboxilates ligands derived from adamantanic acid. A tentative scheme of influence of cluster rhenium compound with quadripol bonds on erythropoiesis through regulation of synthesis of erythropoietin in kidneys was proposed [87].

Interaction of the rhenium(III) compounds with DNA and nucleobases

Interaction with DNA is still considered to be crucial in our choice among anticancer agents, nevertheless we know that any substance binds to a lot of non-nucleic acids targets being introduced to an organism. Our recent findings showed that dirhenium(III) complexes bind to nucleobases and supercoiled natural DNA [55, 108].

To probe the binding of DNA to *cis*–$Re_2[(CH_3)_3CCOO]_2Cl_4{\cdot}2DMSO$, the electronic absorption spectra, obtained by titrating calf timus DNA (CT-DNA) with solutions of the compound, were performed and are depicted in Fig. 8, *A*.

It is notable that the electronic absorption spectra traces of CT-DNA exhibit pronounced hyperchromism in the presence of increasing amounts of the rhenium compound. The DNA band at ~260 nm arises from the π-π* transitions of the nucleic acid bases. Changes in the intensity and slight wavelength shifts of this characteristic band reflect the corresponding structural modifications of the DNA, which include changes in stacking, disruption of the hydrogen bonds between complementary strands, covalent binding of the DNA bases, intercalation of aromatic rings and others. For example, hypochromism and red-shifting of the band at 260 nm are associated with intercalative binding of the complex between the DNA base pairs. The extent of hypochromism is commonly consistent with the strength of the intercalative interaction. On the other hand, hyperchromism of the absorption band at 260 nm involves non-intercalative binding and usually results from disruption of the DNA double helical structure. The hyperchromism observed for the CT DNA, induced by the addition of *cis*–$Re_2[(CH_3)_3CCOO]_2Cl_4{\cdot}2DMSO$, implies that its binding mode is not intercalative (intercalation is not possible for this compound), but most likely, it involves unwinding of the DNA with possible covalent interactions between the dirhenium(III) complex and the nucleic acid bases (the extent of DNA unwinding has been correlated with the formation of monofunctional or bifunctional covalent adducts by cisplatin [32]). Moreover, the new absorption band that appears at ~330 nm at higher complex concentrations, indicates the formation of a new DNA- *cis*–$Re_2[(CH_3)_3CCOO]_2Cl_4{\cdot}2DMSO$ adduct, which also supports covalent binding of the rhenium compound to CT-DNA. The covalent binding mode of the CT-DNA to the rhenium compound is also corroborated by the fact that coordination of the model nucleobase 9-ethyladenine to the dirhenium core via sites N6/N1 is known.

The calculated value of binding constant was: K_b 2.2×10^3 M^{-1}. As expected, the determined K_b value is lower than the values reported for the classical DNA intercalator ethidium bromide (1.4×10^6 M^{-1}) and for other complexes bearing intercalating groups; this K_b value indicates that the compound binds to DNA with a lower affinity than the classical intercalators but it is compared well with the magnitude of the binding constants for other non-intercalating complexes.

The interactions of transition metal complexes with DNA have been investigated by supercoiled plasmid DNA cleavage experiments, which in some cases, are associated with redox-active or photoactivated metal complexes. Supercoiled DNA cleavage entails relaxation of the supercoiled circular pUC18 DNA into the nicked circular and linear forms. When circular plasmid DNA is subjected to electrophoresis, the fastest migration is observed for the supercoiled form of DNA (Form I). If one strand is

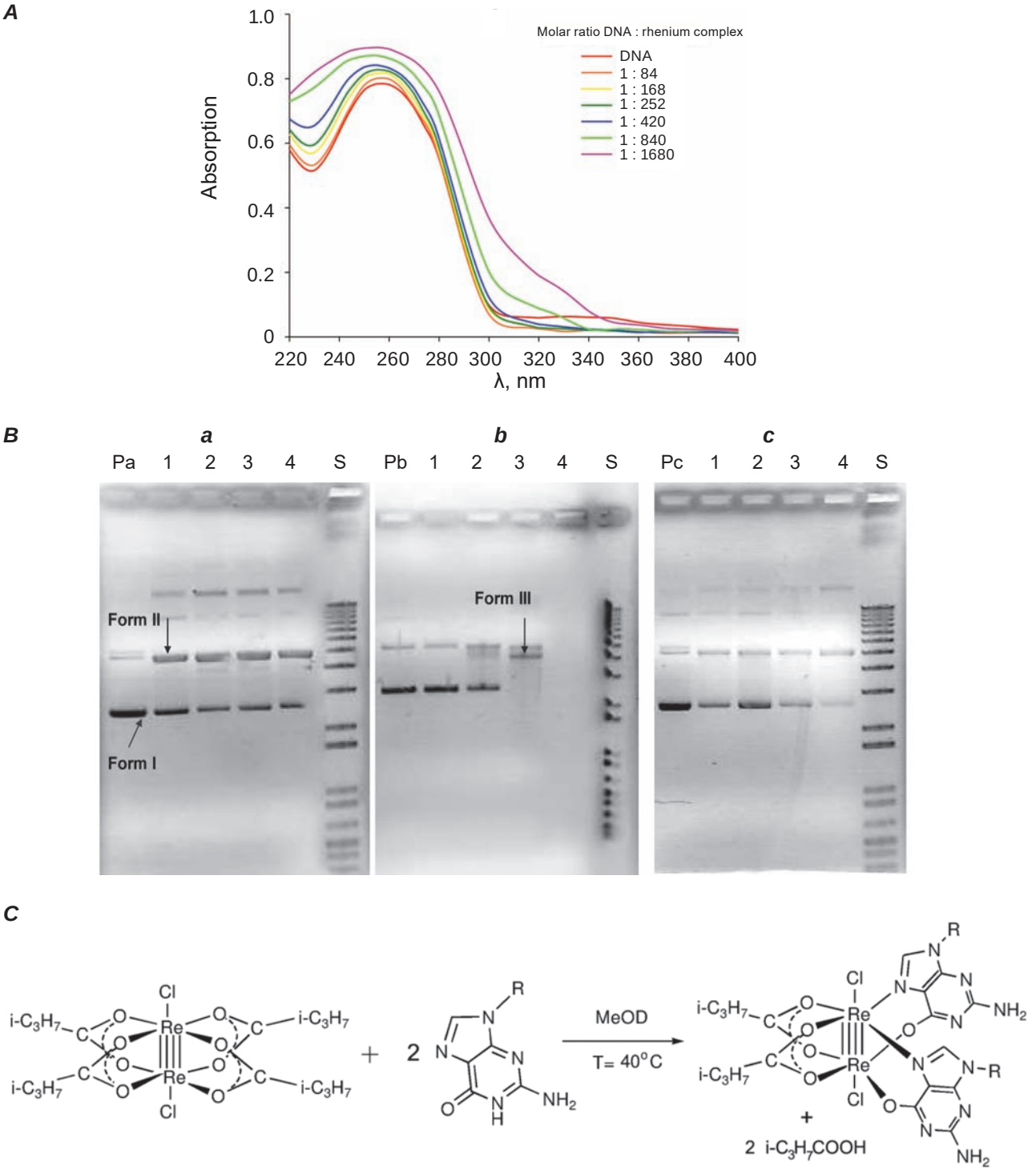

Fig. 8. ***A*** *– Electronic absorption spectra of CT-DNA upon addition of cis–$Re_2[(CH_3)_3CCOO]_2Cl_4$·2DMSO in different molar ratio;* ***B*** *– Electrophoregrams applying to the interaction of pUC18 plasmid with increasing concentration of cis–$Re_2[(CH_3)_3CCOO]_2Cl_4$·2DMSO (a); in the presence of hydrogen peroxide (b); in the presence of mercaptoethanol (c). Lanes P – untreated plasmid; 1 – 10 μM, 2 – 20 μM, 3 – 40 μM, 4 – 80 μM, S – standart.* ***C*** *– Reaction between $Re_2(i\text{-}C_3H_7COO)_4Cl_2$ (**I**) and 9-R-GuaH, R = Me, Et*

cleaved, the supercoiled form relaxes and produces a slower moving open circular form called DNA (Form II). If both strands are cleaved, a linear form of DNA (Form III) is generated, which migrates at a position between Forms I and II in the electrophoresis gel.

Agarose gel electrophoresis studies of plasmid pUC18 were performed in the presence of $Re_2[(CH_3)_3CCOO]_2Cl_4{\cdot}2(CH_3)_2SO$ and the gels obtained, with the DNA cleavage induced by increasing concentrations of the complex, are illustrated in Fig. 8, *B*. The gradual conversion of the supercoiled Form I to a mixture of supercoiled (Form I) and (Form II) DNA takes place and increasing amounts of Form II are produced with higher concentrations of $Re_2[(CH_3)_3CCOO]_2Cl_4{\cdot}2(CH_3)_2SO$ (Fig. 8, *B*, lanes *1-4*, concentration of the complex 10, 20, 40 and 80 μM, respectively). These findings indicate unwinding and the strong DNA-cleaving abilities of the dirhenium complex. Moreover, binding of the plasmid DNA to the rhenium complex results in decreased mobility of Form I (lanes *1-4*), which indicates changes in the DNA conformation caused by kinking of the duplex induced by the metal binding. Similar observations for cisplatin have been attributed primarily to the formation of intrastrand bifunctional 1,2-Pt(GG) and 1,2-Pt(AG) adducts inducing DNA bending at the lesion sites. Accordingly, the reduced mobility of the Form I in the presence of $Re_2[(CH_3)_3CCOO]_2Cl_4{\cdot}2(CH_3)_2SO$ (lanes *1-4*) suggests that the rhenium complex most likely binds covalently to DNA as has been proposed for other metal complexes which exhibit similar DNA mobility decrease in the gel electrophoresis assays. The presence of the slowest moving bands in Fig. 8, *B* a (slower than Form II) indicates formation of high molecular weight adducts that may be explained by the formation of DNA-rhenium compound interstrand-strand adducts similar to cisplatin and dirhodium compounds.

Interestingly, a significantly different electrophoretic behavior of the supercoiled plasmid DNA+rhenium compound is observed in the presence of H_2O_2 (Fig. 8, *B*, lanes *1-4*): under these conditions, increasing the concentration of the complex leads to less Form II as compared to the corresponding lanes in Fig. 8, *B* B (lanes *1-4*); additionally, it leads to a mixture of supercoiled (Form I) and linear DNA (Form III) with decreasing amounts of Form I and increasing amounts of Form III (Fig. 8, *B*, lanes *1-3*). In lane *4* of the electrophoresis gel (Fig. 3, *b*) there are no traces of any form of the plasmid, which is the result of hydrolysis taking place under high concentration of I and hydrogen peroxide. These results show the enhanced nuclease activity of the complex in the presence of H_2O_2. In this vein, it is known that cisplatin induces production of high concentrations of hydroxyl radical and reactive oxygen species (by up to 70%) in cells and tissues.

In the presence of mercaptoethanol (Fig. 8, *B*, B 3c), the cleaving ability of the complex is also enhanced but no Form III and high molecular weight fragments are observed as compared to $Re_2[(CH_3)_3CCOO]_2Cl_4{\cdot}2(CH_3)_2SO$ only (Fig. 8, *B*, 3*a*). Taking into account that in the presence of H_2O_2 and 2-mercaptoethanol (control lanes, Pb and Pc, Fig. 8, *B*, 3*b,c*), no significant amounts of Form II III are observed, it is concluded that DNA strand scission takes place only in the presence of the rhenium complex. These findings indicate that the DNA interactions with $Re_2[(CH_3)_3CCOO]_2Cl_4{\cdot}2(CH_3)_2SO$ are redox-activated.

Experiments *in vitro* showed possibilities of the dirhenium complexes to bind to nucleobases (Fig. 8, *C*) [108].

The DNA binding studies support relatively strong interactions of rhenium compounds with DNA by alteration of the DNA conformation, groove binding, likely formation of covalent interstrand adducts, disruption, kinking and unwinding of the DNA duplex as well as supercoiled DNA cleavage. These effects render natural DNA a plausible target of the dirhenium(III) compounds in living cells and provide insight about their anticancer activity.

As cisplatin is a pro-oxidant, the redox-activating properties of the rhenium compounds may explain the efficacy of the Re-Pt antitumor system.

As a whole, quadruple bond is a unique bond absent among biologically occurring molecules, its chemistry and biochemistry is arising to bring a lot of discoveries being developed. Elaboration of the synthetic methods described herein presents possibility to create a dirhenium(III) quadruple-bonded compound of any class with organic, haloid or phosphate ligands or their mixture to our choice. Quadruple Re–Re bond supports the lower valence state of the metal that may be important in 'prodrug strategy', i.e. entrapping by cancer cells the metal atom may change its valence state to be a good catalyst of the cellular redox processes crucial for survival. Shown ability to coordinate different ligands in diverse manner around Re_2^{6+}-coordination centre pre-

sents a possibility to involve diverse (also specific) ligands to coordination sphere thus to move from 'dirty' drug to more specific targeting. For example, involvement of amino acids in this process, gives a perspective to work with short peptides. By design of dirhenium(III) compounds with ligands of high specific ability to bind to well-defined target in cancer cells we could operate more tolerant to other cells and to exploit both the redox regulation potential of the cluster fragment and its coordination ability as well.

Dependence of the absorption bands position in EAS area of $\delta \rightarrow \delta^*$ transition from the quantity of bidentate ligands around cluster fragments in dirhenium(III) compounds gave an expectancy to watch interactions with lipids even inside a liposome. The process of substitution around cluster rhenium fragment shown in such a way inside a nano – vehicle may make definite impact on the activity of the quadruple bond as presented more functional potential for reaction ability. Encapsulation of cluster rhenium compounds to lipid coating has not only protective but activation significance for the quadruple Re–Re bond. These findings have their own significance for nanobiotechnology. Different approaches are used by us now to elaborate solid lipid nanoparticles, nanoliposomes with mixed composition inside that opens perspective to take control of drug release and to use nanobased combinational therapy.

Antiradical and antioxidant properties of the dirhenium(III) compounds are of great interest as represent a new type of antioxidant activity. Really, antiradical and antioxidant activity of the known natural and synthetic substances is based on their ability to accept and delocalize an electron through the system of conjugated π-bonds. Here we have shown the ability of quadruple bond with δ-component to scavenge an unpaired electron and to diminish oxidative stress, thus we have a range of pharmacophores – antioxidant units in our hands with different antioxidant capacities which may be chosen according to requirements.

Dirhenium(III) compounds have their own anticancer activity that is mainly conditioned by dirhenium cluster fragment but depends on the nature of the ligands; the synergistic effect in application of cisplatin and dirhenium(III) cluster compounds showed eliminated tumor growth and cancer cells with high efficiency; combination of two metal based compounds switched some additional signal transduction pathways crucial for cancer cells survival or death. Only some aspects of the mechanism of anticancer activity of the rhenium-platinum system have been studied.

Identifying multiple molecular targets for effective combinations of preventive agents is a major focus of contemporary chemoprevention or chemotherapeutic study. As cisplatin is a multifunctional molecule, it can bind to a lot of targets in the living cell. Dirhenium(III) compounds are more multifunctional than cisplatin due to their more complicated structure and more multitargeting should be expected. They interact with DNA, may enhance cisplatin accumulation, interfere with the glutathione system, change intracellular ATP-level or manipulate other biochemical pathway(s) of the cancer cells that altogether led to mighty synergistic effect of both compounds.

Dirhenium(III) compounds as antioxidants behaved as antihemolytics, hepato- and nephroprotectors. The idea of regulation of redox potential in cancer cells is central to our mind. Cancer cells can generate large amounts of hydrogen peroxide, which may contribute to their ability to mutate and damage normal tissues, and, moreover, facilitate tumor growth and invasion. It has been suggested that persistent oxidative stress in tumor cells could partly explain some important characteristics of cancer, such as activated proto-oncogenes, genomic instability, drug resistance, etc. Thus, application of antioxidants such as dirhenium(III) compounds can result not only in lowering of oxidative stress by distinguishing the extent of radical process, but also may have regulatory functions.

Overall, the above results strongly indicate that application of nontoxic dirhenium(III) compounds with quadruple bond is an emerging concept in the development of new anticancer therapeutics.

Future perspectives

Rhenium-platinum antitumor system may be effective in a lot of human cancer types and could be a mighty weapon in our war against cancer. Dirhenium(III) compounds with unique quadruple bond represent a perspective platform for development of new anticancer therapeutics as a useful component in cancer combined therapies or as pharmacophore units with antioxidant properties in design of future medicines. Quadruple-bonded rhenium(III) compounds may be used not only in treatment of cancer but also in any diseases with depleted redox

states as antihemolytics, hepato- and nephroprotectors.

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ПРОТИПУХЛИННІ СИСТЕМИ РЕНІЙ–ПЛАТИНА

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Огляд присвячено розгляду процесів виявлення протипухлинних й інших біологічних властивостей кластерних сполук диренію(III) з почверним зв'язком та їх синергізму із цисплатином. Зокрема, описано роботу протипухлинної системи реній–платина, яка створюється за одночасного введення ренієвих і платинових сполук. Серед відомих металвмісних потенційних антиракових ліків диреній(III)-сполуки вирізняються значною антирадикальною і антиоксидантною активністю, обумовленою ненасиченістю почверного зв'язку. Такі переваги металовмісних сполук як широкі редокс-хімічні властивості слід використовувати для створення ефективних протиракових сполук. Сумісне введення декількох препаратів приводить до синергічного ефекту або/також до зниження токсичності платинидів і є перспективним у протираковій терапії. Представлена робота висвітлює такі питання: синтез диреній(III)-сполук із почверним зв'язком (коротко) та їхні спектральні і антирадикальні властивості; взаємодія диренієвих(III) сполук із ліпідами та формування наноліпосом; взаємодія диреній(III)-сполук з еритроцитами та їхня антигемолітична активність у моделях гемолітичної анемії; антиракова активність диренієвих кластерів та робота системи реній–платина; антианемічна і антиоксидантні властивості кластерних сполук ренію в моделі пухлинного росту; взаємодія диреній(III)-сполук із нуклеїновими основами і ДНК. Розглянуто також деякі сучасні тенденції в розвитку біонеорганічної і медичної хімії стосовно ефективності системи реній–платина: використання комбінаційної терапії та наноматеріалів; залучення біологічно активних лігандів, стратегія редоксактивації і т.і.

Ключові слова: реній, платина, протипухлинна активність, антигемолітики, гепато- та нефростабілізуюча активність.

ПРОТИВООПУХОЛЕВЫЕ СИСТЕМЫ РЕНИЙ–ПЛАТИНА

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Обзор посвящен рассмотрению процессов проявления противоопухолевых и других биологических свойств кластерных соединений дирения(III) с четверной связью и их синергизму с цисплатином. В частности, описана работа противоопухолевой системы рений–платина, которая создается при одновременном введении рениевых и платиновых соединений. Среди известных металлосодержащих потенциальных противораковых препаратов дирений(III)-соединения отличаются значительной антирадикальной и антиоксидантной активностью, обусловленной ненасыщенностью четвертичной связи. Такие преимущества металлоорганических соединений как более выраженные редокс-химические свойства следует использовать для создания эффективных противораковых препаратов. Совместное введение нескольких препаратов приводит к синергическому эффекту или/также к снижению токсичности платини-

дов и является перспективным в противораковой терапии. Представленная работа освещает следующие вопросы: синтез дирений(III)-соединений с четвертичной связью (кратко) и их спектральные и антирадикальные свойства; взаимодействие дирений(III)-соединений с липидами и формирование нанолипосом; взаимодействие дирений(III)-соединений с эритроцитами и их антигемолитическая активность в моделях гемолитической анемии; антираковая активность дирениевых кластеров и работа системы рений–платина; антианемическая и антиоксидантные свойства кластерных соединений рения в модели опухолевого роста; взаимодействие дирений(III)-соединений с нуклеиновыми основаниями и ДНК. Рассмотрены также некоторые современные тенденции в развитии бионеорганической химии и медицинской химии в связи с эффективностью системы рений–платина: использование комбинационной терапии и наноматериалов; вовлечение биологически активных лигандов, стратегия редоксактивации и т.д.

Ключовые слова: рений, платина, противоопухолевая активность, антигемолитики, гепато- и нефростабилизующая активность.

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