Dichlorotetra-μ-Isobutyratodirhenium(III): Enhancement of Cisplatin Action and RBC-stabilizing Properties

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Abstract. *Background: Previous investigations showed antitumor properties of dirhenium carboxylate introduced to tumor-bearing animals at high doses. The development of liposomal forms of rhenium substances and the activity of dichlorotetra-μ-isobutyratodirhenium(III) (Re1) in stabilizing red blood cells (RBC) shown in experiments in vitro and in vivo enabled the use of this substance in the present study. The aim of the work was to investigate the antitumor properties of Re1 in liposomal form alone and together with cisplatin, and to analyze whether Re1 can support RBC in the model of tumor growth. Materials and Methods: Introduction of a single dose of cisplatin and liposomal forms of Re1 according to a scheme of antioxidant therapy was tested in a rat model of specific Guerink (T-8) carcinoma. The dynamics of tumor growth, weights of isolated tumors, RBC morphology and hemoglobin levels were measured. Results: The cluster rhenium compound, Re1, with carboxylic ligands had its own anticancer properties and enhanced cisplatin action on tumor growth. Introduction of the rhenium substance led to an increase in quantities of normal RBC forms in blood of tumor-bearing animals. Possible mechanisms of enhancement of cisplatin efficiency by Re1 according to its structural peculiarities are discussed. Conclusion: A novel antitumor system including the use of a cluster rhenium compound and cisplatin is presented. Enhancement of cisplatin action and antitumor properties of rhenium compound initially took place due to the properties of quadruple metal-to-metal bond between two atoms of rhenium.*

Recent findings in the application of heavy metal compounds in medicine are very significant (1) and demonstrate wider fields of practical use of some new elements. The anticancer effects of platinum, vanadium and gallium substances have been studied, however, the role of some other metal substances, such as rhenium, is practically unknown. A dirhenium cluster compound with propionate ligands was found to have varying degrees of effectiveness against sarcoma S-180, leukemia P-388 and melanoma B-16, with particularly good results against B-16 (2). However, these investigators found the compound to be quite susceptible to decomposition in aqueous solutions. In addition, the complex required very high doses to achieve maximum efficiency. It was also subject to compound instability. It decomposed readily into insoluble rhenium oxides, therefore requiring a considerable amount to be injected in order to have any significant quantity reaching tumor sites. In our work we developed methods of liposomal preparations of rhenium substances that eliminate the above disadvantages (decreased stability, problems with solubility) and enable the use of these compounds in various biochemical experiments (3, 4). In our further investigations, it was shown that some rhenium substances interacted with artificial and natural membranes, and red blood cells (RBC) and some of them were stabilizers of RBC membranes against acidic hemolysis in experiments *in vitro* (5). Dichlorotetra-μ-isobutyratodirhenium(III) (Re1) was a unique stabilizer of RBC against acidic hemolysis in wide range of tested concentrations. During further experiments *in vivo* in models of chemically induced hemolytic anemia, Re1 showed strong antianemic effects comparable with that of tocopherol and was even more effective (6). It was also proposed that the synergistic effect of some metals could play a significant role in antitumor therapy (7).

Taking into consideration possible anticancer, antiradical, RBC-supporting properties of Re1, and the developed method of liposomal forms of rhenium substances, the aim of the present work was to investigate the antitumor properties of Re1 alone and together with cisplatin, to find possible synergism and to analyze whether Re1 can support RBC in the model of tumor growth.

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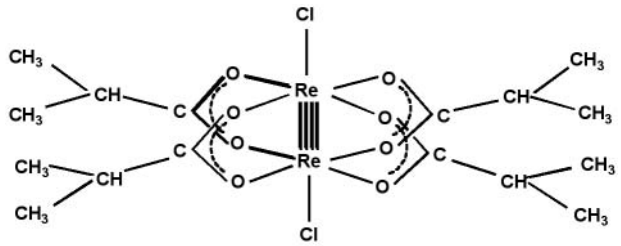

Figure 1. *The structure of dichlorotetra-μ-isobutyratodirhenium(III) (Re1).*

Table I. *Extreme volumes of tumors in vivo of three stages of development.*

Group of animals	Stage of tumor development		
	I (10-13 days)	II (13-17 days)	III (17-21 days)
No. 1: control	10-50	30-70	60-100
No. 2: cisplatin	4-5	15-30	5-35
No. 3: Re1	5-10	15-25	60-82
No. 4: Re1+ cisplatin	4-5	3-5	0-2

Results expressed as % of the volume of tumors of control group on day 21.

Materials and Methods

Materials. Cisplatin was synthesized and purified according to (8) and the cluster rhenium compound dichlorotetra-μ-isobutyratodirhenium(III) $[Re_2(i\text{-}C_3H_7CO_2)_4Cl_2]$ (Re1) was synthesized according to the procedure described in (9, 10). Liposomes were prepared from lecytine (Reagent, Ukraine) (11) and analyzed according to Shtemenko *et al.* (3, 4). Cells of Guerin's carcinoma (T-8) were supplied from the R.E. Kavetskiy Institute of Experimental Pathology, Oncology and Radiology, NAS of Ukraine (Kiev, Ukraine).

Animal models. Wistar rats weighing 100-120 g obtained from the vivarium of Dnepropetrovsk Agricultural University were inoculated with tumor carcinoma Guerink (T-8) cells. Tumor transplantation was performed by subcutaneous injection of 20% Guerin's carcinoma cell suspension in the thigh area. *Experiment A.* Animals were divided as follows: Control group No. 1, injection of DMSO in quantities and under conditions as in the groups No. 2 and No. 3; Group No. 2, Re1 injection at a dose of 25 mg/kg in a suspension of DMSO; Group No. 3, Re1 injection at a dose of 200 mg/kg in a suspension of DMSO. Injections were made on the 7th day after tumor cell transplantation, then every day intraperitoneally. *Experiment B.* Control group No. 1 of tumor-bearing animals was not subjected to any treatment. A single intraperitoneal administration of cisplatin at a dose of 8 mg/kg was made on the 9th day after tumor inoculation according to (12) in groups No. 2 and No. 4. An intraperitoneal administration of Re1 in liposomal form at a dose of 7 μM/kg according to the scheme of antioxidant therapy (13) started on the 3rd day after inoculation of tumor cells and was repeated every 2 days until day 21 in groups No. 3 and No. 4. The number of animals in each group was 15.

Measurements. Volumes of tumors were estimated *in vivo* every day in all experiments and groups from day 7 by measuring three diameters according to the formula L x H x W / 2 (14). On day 21, animals were sacrificed under chloroform narcosis according to the rules of the Ethics Committee and the tumors were isolated and weighed. Concentrations of hemoglobin (Hb), quantity and morphology of RBC were measured according to commonly accepted methods. Wilcoxon nonparametric tests were used to compare the tumor volumes according to the absence of treatment and each group of treatment, or between 2 treated groups.

Results

The structure of the rhenium compound is shown in Figure 1.

Guerin's carcinoma (T-8) is a rat-specific tumor that is widely used in the investigation of the influence on tumor growth of different chemotherapeutic agents (15, 16). Growth of tumors in control groups of experiments A and B during 21 days was very rapid and tumors comprised approximately 1/3 of the animals weight on the last day of the experiment. In control groups of both experiments, 25-30% of animals died during 18-21 days after transplantation of tumor, in line with previously described characteristics of this type of tumor (15, 16).

Experiment A. Treatments with Re1 at high doses caused 20-30% inhibition of tumor growth that was independent of the concentration of the introduced substance. No deaths were observed in groups No. 2 and No. 3 and no visible changes in the liver, spleen, kidneys, skin, lung or brain were found. But brown precipitate – products of Re1 decomposition – was found in the peritoneal cavity, being more intensive in animals of group No. 3 where the quantity of introduced Re1 was especially large.

Experiment B. Introduction of cisplatin was very effective leading to significant reduction of tumor growth at all three stages of progression, as compared with the control groups (Table I, groups No. 1 and No. 2).

Mortality remained at the same level in the group No. 2 – 20-30% – nevertheless sizes of tumors were much lower in treated animals. Introduction of Re1 alone at a dosage according to the scheme of antioxidant therapy inhibited tumor growth during the first two periods of the observation but it was not so effective at the last stage (Table I, group No. 3). No deaths were observed in this group.

A particularly significant decrease in the measured tumor volumes was found in group No. 4, where cisplatin and Re1 were introduced together.

In this group deaths were not observed during the 21 days of the experiment and reduction of the tumor growth was

Table II. *Weights of isolated tumors (m) and the concentration of Hb (Hb) in blood of animals on day 21 after tumor cells inoculation.*

Group	M (g)	Hb (g/l)
No. 1: control	44.87±25.19	89.86±10.36
No. 2: Re1	36.88±15.60	102.02±14.12
No. 3: cisplatin	9.88±9.90	129.49±12.43
No. 4: cisplatin + Re1	0.28±0.30	153.61±15.34

Table III. *Morphological forms of RBC in blood of animals on day 21 after tumor cells inoculation.*

Group	Discocytes %	Echinocytes %	Damaged RBC %
Normal values	65.00±6.12	23.30±3.12	11.70±2.16
No. 1: control	8.47±1.88	52.57±3.66	58.96±4.54
No. 2: Re1	46.33±4.15	39.47±2.16	12.23±3.04
No. 3: cisplatin	46.36±3.18	24.99±3.84	28.65±3.98
No. 4: cisplatin + Re1	63.87±5.54	29.36±3.68	10.25±2.54

more effective in comparison with cisplatin alone, even at the last stages of tumor development.

Weights of isolated tumors were a little lower in the group treated with Re1 (Table II, group No. 2) as compared with the control group No. 1.

Small isolated tumors were found in both groups No. 3 and No. 4, especially under the effect of both cisplatin and Re1 (group No. 4). In this group, most of the experimental animals had no tumors at all and this kind of chemotherapy can be considered extremely effective.

When comparing Hb concentrations in groups No. 1 and No. 2, where tumors were of large size, and the groups No. 3 and No. 4, where significant reduction of tumors was achieved, an increase in Hb concentration in the blood of experimental animals under the influence of Re1 treatment should be noted. It can be explained as a result of the efficiency of tumor growth inhibition as in the RBC-supporting properties of Re1 mentioned above.

Additional confirmation of this fact can be seen by analyzing the data of RBC morphology presented in Table III.

Development of malignancy led to morphological shifts to damaged forms of RBC and reversible forms – echinocytes. Introduction of Re1 supported production of quantities of discocytes and echinocytes at a rather high level, nevertheless, it did not prevent tumor growth. In the experiment with cisplatin, introduction of the rhenium compound led to a practically normal morphological picture of RBC, and moreover, to reduction of damaged cell quantities. These data correlate well with data of animal mortality in the experimental groups and with Hb levels.

Discussion

Before discussing the results obtained, we find it reasonable to briefly consider some data about structure of the rhenium compound Re1 and its properties. Cluster rhenium compounds with organic ligands contain two atoms of Re and have a unique quadruple bond between two atoms of metal (R-Re), Figure 1 (9). The multiple Re-Re bond contains *d*-electrons typical of some transition metals. The structure of these substances demonstrates their possible role as a free radical trap, as $\delta \rightarrow \delta^*$ transition is the lowest in energy and uptake of a 'spare' electron by upper δ^* does not essentially influence the stability of the bond (its order becomes 3.5) or the geometry of the whole molecule. During *in vitro* experiments in reaction with an artificial stable radical – diphenylpicrylhydrazile – it was shown that the velocity of distinguishing the radical by some dirhenium substances was very high and close to that of natural antioxidants (17). The reaction velocity constant for different structural types of binuclear rhenium carboxylates was different confirming the fact that ligands and their type of arrangement around the cluster unit also took part in the scavenging of the radical. In the case of Re1, four branched alkyl groups symmetrically oriented around the quadruple fragment play a significant role in the formation of the inductive effect and the distribution of electronic density.

In another model – an investigation of the protective properties of cluster rhenium compounds against RBC hemolysis (5) – Re1 revealed itself as a strong stabilizer of the erythrocytic membrane against acidic hemolysis. The stability of RBC to acidic hemolysis was very sensitive to the structure and concentration of tested compounds and responded to any change in their structure (*cis*-, *trans*-isomers, any other substitutes). During this work, plenty of clusters were tested and an interesting observation was made: chlorine complexes acted as stabilizers and bromide ones as hemolytics, indicating that atoms of chlorine represented a significant part of the molecule taking part in the protective process. In experiments *in vivo* in models of chemically induced anemia in rats (18) and in rabbits (6), introduction of Re1 led to extended life of experimental animals and it augmented the level of hemoglobin, morphological forms and stability of RBC at all stages of anemia development. As hemolysis of erythrocytes was shown to be generated by reactive oxygen species of radical nature (19), we explain these stabilizing properties of Re1, first of all, with the ability of the quadruple bond to react with radicals. Antioxidant properties of ruthenium compounds (substances with anticancer activity and blockers

of mitochondrial uniport system for calcium), their ability to decrease free radical production and protection of heart contractile function were also more explained by ruthenium ion action than by its complexation (20). At the same time, this work highlighted the fact that complexation played its role in the membrane crossing. Investigations of cluster rhenium compounds with ligands different in nature and type of orientation around the dirhenium cluster fragment Re_2^{6+} in the process of interaction with phosphatidylcholine in solution or in liposomes (4) clearly showed the dependence of the interaction velocity on ligands. Support of the hematological state of an organism may not result from RBC membrane stabilizing effects of Re1 alone but could involve more complex interactions with the bone-marrow and RBC production (12).

As a quadruple bond is not normally available in biological molecules, it is easily detectable in biological material by electronic absorption spectra (EAS) due to the $\delta \rightarrow \delta^*$ transition (20,000-14,000 cm^{-1}). Absorption in this area bears witness not only to the existence of a quadruple bond but can also characterize substitution around Re_2^{6+}. For example, a solution of Re1 in chloroform had an absorption maximum at 20,000 cm^{-1}. After the addition of phosphatidylcholine, a new band appeared at 14,500-14,000 cm^{-1}, which is characteristic for phosphate groups coordinated around a dirhenium fragment (4), the intensity of which increased with time. Coordination may take place *via* nucleophilic substitution of chlorine (one or both) or carboxylic groups (one, several, *cis-, trans-*) or a combination. All appropriate derivatives are now available and their structure is confirmed (21). These data demonstrate a coordination of phosphate groups of phosphatidylcholines around the dirhenium fragment that is very important for interactions with living cells and possible further activation or deactivation of some signal cellular pathways. Moreover, these data may explain possible interaction of Re1 with substances such as proteins, hydrocarbons.

Recently, we showed that Re1 may interact with blood proteins (22) and some enzymes *in vitro*, for example, with acidic phosphatase, amino acid aminotranspherases and glucosooxidase (results not published), which change their activity.

We can summarize that Re1 has several reaction centers that may be responsible for its important properties due to its structure: a) rhenium, a metal with low toxicity (23) as a main component of the molecule; b) quadruple metal-to-metal bond that is responsible for antiradical and antioxidant properties; c) chlorine and carboxylic ligands capable of interacting with polar molecules of living cells; d) branched alkyl groups of tetraisobutyrate and the rather symmetrical structure capable of hydrophobic interactions.

Our results, shown in this paper in Experiment A, demonstrate that at high doses, Re1 can inhibit tumor growth and prolong the life of experimental animals. These results correlate with those obtained for dirhenium propionate (2). The procedure used in Experiment A cannot be considered as reasonable as most of the preparation decomposed and turned into insoluble rhenium dioxide. This fact can also explain the absence of a dose effect. But even in these experiments, Re1 demonstrated low toxicity, antioxidant and antihemolytic activity.

Being introduced in low doses alone according to the schedule of experiment B, Re1 showed antitumor activity at the first and the second stage of tumor growth and no activity at the third stage. This demonstrates that Re1 does not have a strong inhibitive mechanism of interaction with cancer cells, and this mechanism differs from that of cisplatin. During recent investigations (24), different categories of chemically induced and consecutively developing tumors – hyperplasia, adenoma and carcinoma – featured various sensitivities to different agents. The different sensitivity of some tumor growth stages to different chemotherapeutic agents may partly explain the enhancement of cisplatin action by Re1, demonstrated in group No. 4. It suggests that differing mechanisms of inhibition at different stages resulted in an inhibition of the tumor at the last stage of development.

In a thorough theoretical discussion of chemoprevention, Lippman and Hong (25) stated that our understanding of neoplastic evolution considerably improved and led to a 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 combinations of antitumor drugs, for example interferon, have resulted in synergistic interactions with antitumor ruthenium complexes (26); new 5-fluorouracil analogues and folate antagonist, the inhibitor of topoisomerase I irinotecan and the third-generation platinum compound oxaliplatin (27, 28) were developed, based on distinct mechanism of cytotoxicity and resistance, as well as effective combination patterns show themselves to be very promising.

As pathogenesis of the anaemia of cancer involves the 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 platinides with recombinant human erythopoietin (12, 29). However, erythropoetin corrected anemia but did not improve cancer control or survival of patients. Thus, we may conclude, that Re1 has its own anticancer properties and, furthermore, antihemolytic ability and both can be independently executed in the model of tumor growth.

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 (30) (for example, nucleophilic sulfur compounds, such as sodium thiosulfate (STS), biotin, glutathion, sodium 2-mercapto-ethanesulfonate (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 a "protecting agent" has not been studied, but there is a more feasible side of its mechanism of action.

More commonly, the idea of regulation of redox potential in cancer cells is used by many authors (15, 16, 31-33), especially in connection with intrinsic or acquired resistance to chemotherapy. It has been observed that changes in the glutathione levels in blood and the development of various hematotoxicities in the host are inversely related in cisplatin-mediated chemotherapy. 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 party explain some important characteristics of cancer, such as activated proto-oncogenes, genomic instability and drug resistance. Thus, application of an antioxidant such as Re1 could result not only in lowering of oxidative stress by reducing radical production, but may also have regulatory functions.

Possible mechanisms and numerous examples of cisplatin action modulation are discussed in detail in the review by Fuertes *et al.* (34), 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 the mechanism of action of 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 Re1 enhancement mechanisms, we should underline the following: (i) Enhancement of cisplatin accumulation as 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. Our previous works showed the unusual ability of some rhenium substances to increase conductivity of artificial lipid membrane (35) 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 as was demonstrated with the 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; Re1 was shown to interact with phosphate groups.

In the present paper and at this stage of our knowledge about rhenium substances and their mechanisms of interaction with living cells, it is impossible to suggest which biochemical pathway(s) are manipulated by them. It is not known whether rhenium substances reach and interact with DNA. But the enhancement of cisplatin action by rhenium substances is confirmed by our investigations of other cluster rhenium compounds with different organic ligands. This shows that our results are connected primarily with properties of quadruple metal-metal bond.

Conclusion

The novel antitumor system containing a cluster rhenium compound and cisplatin is presented. The structure of dichlorotetra-μ-isobutyratodirhenium(III) determines several reaction centers which may be responsible for the properties exhibited here. Enhancement of cisplatin action and antitumor properties of the rhenium substance was primarily due to the properties of the quadruple metal-metal bond between the two rhenium atoms.

References

1 Reedijk J: Medicinal application of heavy metal compounds. Curr Opin Chem Biol *3*: 236-240, 1999.

2 Eastland GW, Yang G and Thompson T: Studies of rhenium carboxylates as antitumor agents. Part II. Antitumor studies of *bis*(μ-propionato) diaquotetrabromodirhenium (III) in tumor-bearing mice. Meth Find Exptl Clin Pharmacol *5*: 435-438, 1983.

3 Shtemenko AV, Shtemenko NI, Oliynik SA and Zelenuk MA: Lyposome forms of rhenium cluster compounds in models of Haemolytic anemia. *In*: Metal Ions in Biology and Medicine. John Libbey Eurotext, Paris *7*: 558-561, 2002.

4 Shtemenko AV, Zelenuk MA, Shtemenko NI and Verbytska YC: Spectrophotometric investigation of the interaction of rhenium complex compounds with phosphatidylcholine in liposomes. Ukr Biokhim Journal (Ukr) *74*: 38-43, 2002 (in Ukrainian).

5 Shtemenko NI, Piroshkova-Patalakh IV and Shtemenko AV: Screening and testing strategy for biological activity of rhenium cluster compounds. *In*: Metal ions in biology and medicine. John Libbey Eurotext, Paris *6*: 616-18, 2000.

6 Oliynik SA, Shtemenko NI and Shtemenko AV: Antihemolytic activity of cluster rhenium complexes with organic ligands. Dopovidi NANU (Ukr) *6*: 194-198, 2001 (in Ukrainian).

7 Collery P, Shtemenko N, Shtemenko A, Bourleaud M, Etienne JC, Maymard I and Loriquet P: Supplementation by rhenium compounds instead of iron compounds during the treatment by erythropoeitin of anemia in cancer patients. *In*: Metal Ions in Biology and Medicine. John Libbey Eurotext, Paris *8*: 534-537, 2004.

8 Klucnikov NG: Handbook on Inorganic Synthesis. Moscow. Chemistry Ed., pp. 184-186, 1965 (in Russian).

9 Shtemenko AV and Kotel'nikova AS: Formation of binuclear halogenecarboxylates of rhenium with quadruple metal-to-metal bonds: Isvestiya Acadamy of Sciences of USSR. Chemistry (Rus) *11*: 2630-2631, 1980 (in Russian).

10 Shtemenko AV and Bovykin BA: Chemistry of Binuclear Rhenium Clusters. *In*: Rhenium and Rhenium Alloys. TMS publication, Pensylvania, pp. 189-197, 1997.

11 Munechika NC: United States Patent: 5, 662, 931 September 2, 1997.

12 Taylor SK: Is recombinant human erythropoietin (rh-epo) more than just a treatment of anemia in cancer and chemotherapy? Medical Hypothesis *60*: 89-93, 2003.

13 Meerson FZ, Evstigneeva ME and Ustinova EE: Effect of chronic haemolytic anemia on heart contractile function and increase of its resistance to hypoxia. Pat Physiol Exp Therap (Rus) *5*: 25-29, 1983 (in Russian).

14 Seelig HS, Berger MR and Keppler BK: Antineoplastic activity of three ruthenium derivatives against chemically induced colorectal carcinoma in rats. J Cancer Res Clin Oncol *118*: 195-200, 1992.

15 Kakarkin AJ, Penyuk AA, Pushkar MS and Kakarkina LI: Effect of complex containing antioxydants and enterosorbent on nephro- and urotoxicity of cyclophosphamide in rats with Guerin's carcinoma. Exper Oncol (Ukr) *23*: 287-290, 2001 (in Ukrainian).

16 Chekhun VF, Lebed OI, Tryndyak VP, Makovetsky VP, Todor IN and Kulik GI: Structural alterations of plasma membranes of Guerin's carcinoma cells upon the development of resistance to doxorubcine. Exper Oncol (Ukr) *24*: 279-283, 2002 (in Ukrainian).

17 Oliynik SA, Shtemenko NI and Shtemenko AV: Antiradical activity of cluster rhenium complexes with organic ligands. Dopovidi NANU (Ukr) *7*: 176-180, 2001 (in Ukrainian).

18 Shtemenko AV, Shtemenko NI, Olyinik SA and Zelenuk MA: Liposomal forms of rhenium cluster compounds in models of hemolytic anemia. *In*: Metal Ions in Biology and Medicine, John Libbey Eurotext. Paris *7*: 558-561, 2002.

19 Tavazzi B, Amorini AM, Fazzina G, Di Pierro D, Tuttobene M, Giardina B and Lazzarino G: Oxidative stress induces impairment of human erythrocyte energy metabolism through the oxygen radical-mediated direct activation of AMP-deaminase. Biological Chem *276*: 48083-48092, 2001.

20 Leperre A, Millart H, Prevost A, Trenque T, Kantelip JP and Keppler BK: Comparable effects of ruthenium red and *cis* $[Ru(NH_3)_4Cl_2]Cl$ on the isolated ischaemic-reperfused heart. Fundam Clin Pharmacol *9*: 545-553, 1995.

21 Shtemenko AV, Stolyarenko VG and Domasevitch KV: Structure and spectral characteristics of $(NH_4)_2Re_2(HPO_4)_4(H_2O)_2$. Russ Inorg Chem *51*: 1014-1019, 2006 (in Russian).

22 Shtemenko N, Gorelaya M and Alexandrova L: Further investigations of influence of rhenium compounds with blood proteins. *In*: Metal Ions in Biology and Medicine: John Libbey Eurotext, Paris *8*: 243-245, 2004.

23 Oliynik SA, Shtemenko NI and Shtemenko AV: Toxicity of rhenium substances: discussion of the problem. Contemp Prob Toxicol (Ukr) *1*: 11-14, 2001 (in Ukrainian).

24 Estensen RD, Jordan MM, Wiedmann TS, Galbraith AR, Seele VE and Watternberg IW: Effect of chemopreventive agens on separate stages of progression of benzo{*a*}pyrene induced lung tumors in A/J mice. Carcinogenesis *25*: 197-201, 2004.

25 Lippman SM and Hong WK: Cancer prevention by delay. Clin Cancer Res *8*: 314-346, 2002.

26 Kreuser ED, Keppler BK, Berdel WE, Piest A and Thiel E: Synergistic antitumor interactios between newly synthesized ruthenium complexes and cytokines in human colon carcinoma cell lines. Semin Oncology *19*: 73-81, 1992.

27 Grinevic I, Mans DRA, Peters GI and Schwartsmann G: Irinotecan and oxaliplatin: an overview of the novel chemptherapeutic options for the treatment of advanced colorectal cancer. Brasil J Med Biol Res *34*: 1087-1103, 2001.

28 Wan-Long l, Ding-Guo L, Qiang C and Han-Ming L: Clinical and experimental study of oxaliplatin in treating human gastric carcinoma. World J Gastroenterol *10(19)*: 2911-2915, 2003.

29 Henke M, Laszig R, Rube C, Schafer U, Haase K-D, Schacher B, Mose S, Beer K, Burger U, Doigherty C and Frommhaid H: Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: randomised, double-blind, placebo-conrolled trial. Lancet *362*: 1255-1260, 2003.

30 Reedijk J: Why does cisplatin reach guanine-N7 with competing S-donor ligands available in the cell? Chem Rev *99*: 2499-2510, 1999.

31 Prasad SB and Khynriam D: Cisplatin-induced changes in tissue glutathione level and glutathione-related enzymes in Dalton's lymphoma-bearing mice. *In*: Metal Ions in Biology and Medicine. John Libbey Eurotext, Paris *8*: 368-372, 2004.

32 Prasad SB and Kharbangar A: Cisplatin-mediated biochemical changes in mitochondria in tumor-bearing mice. *In*: Metal ions in biology and medicine. John Libbey Eurotext, Paris *8*: 575-579, 2004.

33 Yurchenko OV, Todor IN, Tryndyak VP, Tregubova NA, Kovtonyuk OV, Slyanik GI, Kulik GI and Chekhun VF: Resistance of Guerin's carcinoma cells to cisplatin: biochemical and morphological aspects. Exp Oncol (Ukr) *25*: 64-68, 2003.

34 Fuertes MA, Alonso C and Perez JM: Biochemical modulation of cisplatin mechanisms of action: enchancement of antitumor activity and circumvention of drug resistance. Chem Rev *103(3)*: 645-662, 2003.

35 Bovykin BA, Omelchenko AM, Shtemenko AV and Chasova EV: The unusual ability of a rhenium (Y) complex containing nicotinamide to raise the conductivity of lipid membranes. Biophysics *40*: 525-529, 1995.

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