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DETERMINATION OF THE ANTICANCER PROPERTIES OF *CIS*- AND *TRANS*-DIADAMANTHYLCARBOXYLATES OF DIRHENIUM(III)

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The aim of the study. *The aim of the work was to investigate in vivo anticancer activity of cis- and trans-diadamanthylcarboxylates of dirhenium(III) alone and together with cisplatin in form of nanobins.*
Materials and methods. *Model of tumor growth, Guerin's carcinoma; intraperitoneal administration of cisplatin, dirhenium(III) compounds in liposomes and of binary liposomes, containing both cytostatics; volumes and final weights of tumors were measured.*
Results. *In vivo antitumor properties of two dirhenium(III) dicarboxylates with 1-adamantanecarboxylic acid moieties as ligands with cis- (**I**) and trans- (**II**) orientation of the carboxylic groups around a cluster fragment alone and together with cisplatin were presented; an attempt to understand differences in a possible mechanism of anticancer activity of the substances were undertaken. Antiradical and DNA-binding properties of **I** and **II** were the matter of consideration.*
Conclusions. *Cis- and trans- compounds of dirhenium **I** and **II** had close antitumor activity in vivo with a little bit superiority of the cis- analog. Mechanisms of anticancer activity of **I** and **II** are different and may also include monofunctional adduct formation and subsequent interstrand cross-linking for the **II** substance, formation of protein-DNA cross-links, etc.*
Keywords: *dirhenium(III) cluster compounds, adamantanecarboxylic acid, cisplatin, model of tumor growth, Calf Thymus DNA, antiradical activity*

1. Introduction

Anticancer activity *in vivo* of *cis*-dicarboxylates of dirhenium(III) including *cis*-diadamantate alone and together with cisplatin was first presented in 2008 [1]. Binuclear clusters of dirhenium(III) with an unique quadruple metal–metal bond have their own anticancer activity and being introduced together with cisplatin in molar ratio 1: 4, that was called by us the "rhenium-platinum antitumor system" (Re-Pt system), is an example of the successful combinational therapy, leading to interruption of the tumor growth, see review [1]. Together with anticancer activity, dirhenium(III) compounds possessed antiradical, antihemolytic, antioxidant, nephro- and hepatoprotective properties that reduced or even almost eradicated the toxic properties of cisplatin.

2. Literature review

It is known, that transplatin in contrast to cisplatin has no cytotoxic activity due to the inability of the *trans*-isomer to form 1,2-GpG intrastrand crosslinks, because of the 180° angle between its two semi-labile chloride ligands, but the substitution of the ammine ligand(s) in transdiamminedichloroplatinum(II) with bulky, planar N-donor ligands affords trans-platinum(II) complexes with high in vitro cytotoxicity, equivalent to their corresponding *cis*-isomers and cisplatin [2]. Structure – reactivity relationship investigations of the dirhenium(III) complexes with different ligands and their orientation around the cluster Re_2^{6+} fragment showed that *trans*- diisobutirates and *trans*-dipivalates had the same anticancer activity *in vivo* as their *cis*-analogs [3], but differed in biochemical behavior, i.e. in antioxidant properties. Involvement of biologically active ligands to the coordination sphere of the complex-formation metal core was shown as a productive strategy in creating of new metal-organic anticancer compounds for platinum [2], ruthenium, osmium [4] and for rhenium substances [1]. For example, a Phase I clinical trial has been carried out with a derivative of satraplatin, in which the cyclohexylamine is replaced with adamantylamine [5]. The adamantane moiety is widely applied in design and synthesis of new drug delivery systems and in surface recognition studies, it is considered as a "lipophylic bullet" in pharmacology and is a part of a range of potent medicines [6, 7]. Recently we have elaborated the method of preparation of nanoliposomes, containing Re-Pt system inside the vesicle.

So called "nanobins" were shown to have higher cytotoxicity against cancer cells, comparing to separately introduced components [8].

3. The aim and objectives of the study

The aim of the work was to investigate *in vivo* anticancer activity of *cis*- and *trans*-diadamanthylcarboxylates of dirhenium(III) alone and together with cisplatin in form of nanobins.

To achieve the aim, the following objectives were set:

1. Determination of the antitumor properties of two dirhenium(III) cis- and trans-dicarboxylates with 1-

adamantanecarboxylic acid in vivo alone and together with cisplatin;

2. Establish a possible mechanism of anticancer activity of the substances and an attempt to understand differences in action of **I** and **II**;

3. Consideration of the antiradical and DNA-binding properties of **I** and **II** in the context with results obtained.

4. Materials and methods

Cisplatin (cisPt) was purchased from Polymed (PolyMed Therapeutics, Inc., Houston, TX). Dirhenium(III) dicarboxylates bis-dimethylsulfoxide-cis-tetrachlorodi-μ-1-adamantylcarboxylatodirhenium(III) **I** and trans-tetrachlorodi-μ-1-adamantyl carboxylatodirhenium(III) **II** with formulas cis-$Re_2(C_{10}H_{15}COO)_2Cl_4{\cdot}2DMSO$ **(I)** and trans-$Re_2(C_{10}H_{15}COO)_2Cl_4$ **(II)** were synthesized in the Ukrainian State University of Chemical Technology at the Department of Inorganic Chemistry [9]. Cells of Guerin's carcinoma (T8) were received from the R. E. Kavetskiy Institute of Experimental Pathology, Oncology and Radiology, National Academy of Science of Ukraine (Kiev, Ukraine). All chemical reagents were of analytical grade.

Preparation of liposomes. Liposomes, containing **I** or **II,** and **I** or **II** with cisplatin in molar ratio 4 : 1 (nanobins), were prepared by the thin-film method using the reagent L-a-Phosphatidylcholine (Egg, Chicken), where the main component was 1-Palmitoyl-2-oleoylphosphatidylcholine, (POPC), MW 760.08 g/mol in CHCl3 (Avanti, Polar Lipids,

Inc., Alabaster, AL) as a lipid component according to [10].

Animal model. The animal model was described previously [3]. Tumor transplantation was performed by subcutaneous injection of 20 % Guerin's carcinoma (T8) cell suspension in the thigh area. Control tumor-bearing animals were not subjected to any treatment, group T8. A single intraperitoneal administration of cisPt at a dose of 8 mg/kg was made on the ninth day after tumor inoculation, group T8+cisPt; intraperitoneal administration of liposomes in a dose of 7μM/kg of the rhenium compounds **I** and **II** or rhenium-platinum (4:1) system – groups (T8+[**I**]nl); (T8+[**II**]nl); (T8+[**I**+ cisPt]nl); (T8+[**II** +cisPt]nl) started on the third day after inoculation of tumor cells and was repeated every 2 days until the day 21. The number of animals in each group was 8.

On day 21, the animals were sacrificed under chloroform narcosis according to the rules of the Ethics Committee and the tumor cells were isolated and weighed. Wilcoxon nonparametric tests were used to compare the parameters, obtained from the group without treatment and each group of treatment, or between two treated groups.

Measurements Volumes of tumors were estimated in vivo daily for all experiments and groups from day 7 by measuring three diameters according to the formula L × H × W/2. On day 21, the animals were sacrificed by chloroform narcosis according to the rules of Ethic Committee and the tumors were isolated and weighed. Wilcoxon nonparametric tests were used to compare the tumor volumes between the groups that received treatment and the control groups.

All manipulations, involving the animals, were carried out under narcosis in accordance with the EU Directive 2010/63/EU for animal experiments and Permission of the Ministry of Education and Science of Ukraine.

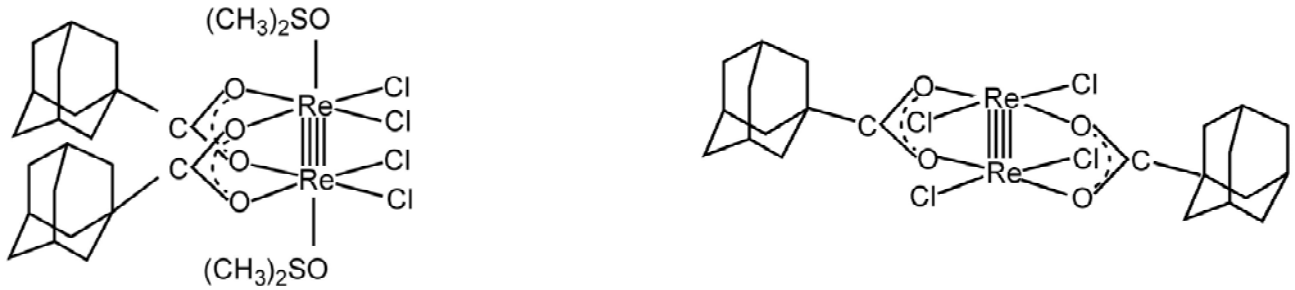
5. Results

The considered compounds, which structures are presented on Fig. 1, are not isomers because the cis-complex compound contains DMSO as an axial ligand.

Introductions of **I** and **II** in liposomes led to the significant effects of tumor inhibition (Fig. 2).

The dynamics of the tumor growth differs under the influence of *cis*- and *trans*- compounds: if the volume of tumors in experiments with **II** stopped to grow and the volumes became practically the same till the end of the experiment, under the influence of **I** there is a significant decrease in volumes sizes beginning from the 15 days after inoculation of cancer cells that resulted in a little bit better activity of **I** in comparison to **II**. Notably to underline, that both substances showed practically the same efficacy.

Introductions of the investigated substances together with cisplatin in binary liposomes was very effective and led practically to disappearance of cancer cells in some experimental animals, Fig. 3, Table 1.



I **II**

Fig. 1. Structure of bis-dimethylsulfoxide-cis-tetrachlorodi-μ-1-adamantylcarboxylatodirhenium(III) **I** and trans-tetrachlorodi-μ-1-adamantylcarboxylatodirhenium(III) **II**

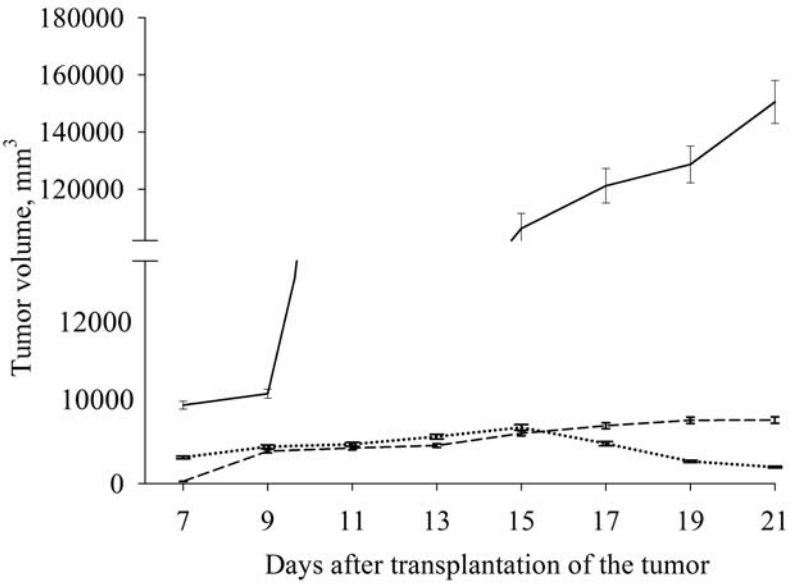


Fig. 2. Dynamics of the tumor growth: control (— T8); under introductions of **I** and **II** in liposomes (⋯T8+[**I**]nl); and (- - - T8+[**II**]nl) accordingly

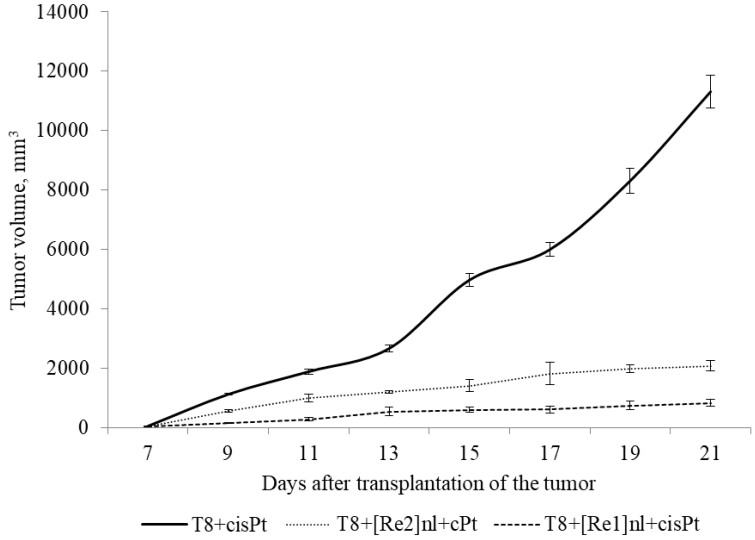


Fig. 3. Dynamics of the tumor growth under introductions of cisplatin (– T8+cisPt); complexes of rhenium with cisplatin in liposomes (⋯T8+[**II** +cisPt]nl); (- - - T8+[**I**+ cisPt]nl)

Table 1

Weights of tumors of experimental animals, g

Group	Control T8	T8 + cisPt	T8+[**I**]nl	T8+[**II**]nl	T8+[**I** +cisPt]nl	T8+[**II** +cisPt]nl
Weight, g	64.36±10.42	14.18±2.36	18.24±3.40	22.46±4.56	0.54±0.12	1.96±0.42

Nevertheless the dynamics of the tumor inhibition was practically the same for experiments with **I** and **II**, *cis*- substance was more effective than *trans*-analog. The average weight of isolated tumors was twice larger and a difference in the size of tumors also was evident on the dynamics curve.

6. Discussion

Cisplatin is a widely used anticancer drug, which induces apoptosis in cancer cells by covalently modifying the DNA [11]. Its geometric isomer transplatin binds to biological molecules by different mechanisms [12] and has no cytotoxic activity. The difference in antitumor activity between the two isomers is attributed to the inability of the *trans*- isomer to form 1,2-GpG intrastrand crosslinks due the angle between its two labile chloride ligands [2]. This led to the early belief that only platinum complexes with *cis*-leaving groups were endowed with antitumor activity [13]. Further development of synthetic activity has dispelled this notion [14, 15]. Substitution of the ammine ligand(s) in *trans*-diamminedichloroplatinum(II) with bulky ligands affords *trans*-platinum(II) complexes with high *in vitro* cytotoxicity, equivalent to their corresponding *cis*- isomers and cisplatin. *Trans*-platinum(II) complexes of this type exhibit a spectrum of activity that differs significantly from that of any other anticancer agent in the National Cancer Institute

database [16]. Moreover, they could circumvent cisplatin resistance in some types of cancer cell lines [17].

In our experiments we see that *cis*- and *trans*-compounds of dirhenium **I** and **II** had close antitumor activity *in vivo*. Previously we investigated the pairs of *cis*- and *trans*- dicarboxylates of dirhenium(III) with isobutirate and pivalate ligands [18], that had different antitumor properties *in vivo* and reacted differently with DNA *in vitro*. In those experiments *cis*- analog was more active than *trans*-analog, approximately in 2–3 times. Adamanthyl ligand is a bulky steroid-like ligand, existence of which reverses the difference. In the Table 3 some characteristics, obtained by us earlier, are presented.

Table 2

Constants of reaction of **I** and **II** with radicals and DNA

Substance	Reaction with Calf thymus (CT) DNA Constant of binding Kb, M^{-1}		Reaction with radicals Velocity constant k_v , c^{-1}	
	CT DNA	CT DNA + H_2O_2	1,3,5-triphenyl-verdazyle radical (Vd)	1,1-Diphenyl-2-picryl-hydrazyl (DPPH)
I	$2.311 \cdot 10^3$	$2.790 \cdot 10^3$	$6.72 \cdot 10^{-4}$	$4.71 \cdot 10^{-3}$
II	$2.144 \cdot 10^3$	$5.510 \cdot 10^3$	$2.14 \cdot 10^{-3}$	$6.90 \cdot 10^{-2}$
References	[19]		[20, 21]	

Constants of binding with DNA of **I** and **II** are close that supports the data, obtained here from antitumor activity *in vivo*. But, spectral investigation of interaction of **I** and **II** with DNA showed additional differences between *cis*- and *trans*- analogs [19]. It was demonstrated, that platinum complexes with *trans*-configurations switches on additional mechanisms in cancer cells media [13]: the presence of bulky planar ligands in transplatinides increased the propensity for monofunctional adduct formation and subsequent interstrand cross-linking; these complexes formed DNA-topoisomerase I cross-links that are capable of triggering DNA strand breaks and apoptosis; such ternary DNA−protein cross-links were not observed, following the treatment with cisplatin, and could explain, in part, the distinctive cellular response, evoked by transplatinum(II) complexes with bulky planar ligands; within the *trans*-platinides it was shown, that the bulky iminoether ligand configuration is a major determinant of activity. Analogically, we may propose, that the mechanisms of anticancer activity of **I** and **II** are different and may also include monofunctional adduct formation and subsequent interstrand cross-linking for the **II** substance, formation of protein-DNA cross-links, etc.

Interestingly, that in the presence of hydrogen peroxide in the DNA-complex medium or in the reactions with artificial radicals the *trans*- complex is much more active (see Table 2). This was explained by us by red-ox activation of the quadruple bond [1] and by better accessibility of the quadruple bond to radical attack in the dirhenium(III) compounds with *trans*-cofigurations of [22].

Study limitations. Unfortunately, it is currently impossible to establish the exact mechanism of the antitumor action of rhenium complexes. Even for the simpler cisplatin molecule, discussions about the mechanism of its biological action continue. At the same time, the data of our work allow us to come closer to understanding the possible mechanism of anticancer activity of rhenium(III) complex compounds with different structures.

Perspectives. As some *trans*-platinides circumvent cisplatin resistance in some types of cancer cell lines, very actual is to follow experiments with **I, II** and resistant to cisplatin cells.

7. Conclusions

1. *Cis*- and *trans*- compounds of dirhenium **I** and **II** had close antitumor activity *in vivo* with a little bit superiority of the *cis*- analog.

2. Mechanisms of anticancer activity of **I** and **II** are different and may also include monofunctional adduct formation and subsequent interstrand cross-linking for the **II** substance, formation of protein-DNA cross-links, etc.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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