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Dirhenium(III) complex with beta-alanine ligand: anticancer, antioxidant and DNA-binding properties

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

Earlier we have shown that dirhenium(III) dicarboxylate complex with γ-aminobutyric acid possessed higher antitumor activity, than those of the previously investigated alkylcarboxylates, also may act as a modulator of cisplatin mechanism of action and as a stabilizer of red blood cells in tumor-bearing organisms. Thus, the task of the work was to investigate anticancer activity of the complex *cis*-$[Re_2(\beta\text{-Ala})_2Cl_6]$ (**I**) in the model of tumor growth *in vivo* and to realize if the amino acid residue influences the DNA-binding activity of the amino acid derivatives of the cluster rhenium(III) compounds. Antitumor properties of the complex **I** were studied in the model of tumor growth with the use of Wistar rats inoculated by tumor carcinoma Guerink cells. The introduction of the compound alone in free and liposomal forms inhibited the tumor growth by 36 % and 45 % correspondingly, that is more than for dirhenium(III) clusters with alkyl ligands. The combined introduction of **I** and cisplatin had a significant impact on the tumor growth and showed the disappearance of the tumors in most of the animals. No considerable differences were found between introduction of liposomal and free form of **I.** The electronic absorption spectra of Calf Thymus DNA (CT-DNA) exhibit hyperchromism in the presence of increasing amounts of **I.** The DNA band at ~ 260 nm arises from the π-π* transitions of the nucleic acid bases and 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. The binding constant K_b(**I**) = 2.43×10^3 M^{-1} to CT-DNA was obtained that was lower than the values reported for the classical DNA intercalators and compares well with the magnitude of the binding constants for other complexes of dirhenium(III); titration of CT-DNA with cisPt and hydrogen peroxide also leads to a hypochromic effect, weak at low concentrations and more significant at high concentrations of **I**; the DNA binding constants increased in several times when using H_2O_2 or cisplatin that confirms a mechanism for redox activation of interaction of **I** with DNA in a cancer cell. The obtained results demonstrate the possibility of application of the amino acid derivatives of dirhenium(III) clusters in antitumor therapy.

Key words: *dirhenium(III) cluster with amino acid ligand, cisplatin, model of tumor growth, Calf Thymus DNA, binding constants.*

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Introduction

Binuclear clusters of rhenium(III) are the classical complexes with an unique quadruple metal–metal bond (Cotton, Walton, 2005; Golichenko, Shtemenko, 2006; Shtemenko et al., 2013; Shtemenko, Shtemenko, 2017). In our previous work (Shtemenko et al., 2009) it was shown that dirhenium(III) dicarboxylate complex with γ-aminobutyric acid possessed higher antitumor activity, than those of the previously investigated alkylcarboxylates (Shtemenko et al., 2007), also may act as a modulator of cisplatin mechanism of action and as a stabilizer of red blood cells in tumor-bearing organisms. Such results were promising for the using of amino acid complexes of dirhenium(III) as effective antitumor agents. Previously (Golichenko et al., 2015) we have developed methods of synthesis and proved the structure of the complex compound of dirhenium(III) with β-alanine.

Titled compound **I** almost unlimitedly dissolves in water as well as similar GABA dirhenium(III) complex compound. High solubility of such substances in water, in contrast to alkylcarboxylates, greatly facilitates their use in physiological conditions. Also, **I** was shown to have cytotoxic and proapoptotic activity against Jurcat cells (Polokhina et al., 2020). The task of the work was to investigate anticancer activity of **I** in the model of tumor growth *in vivo* and to realize if the amino acid residue influences the DNA-binding activity of the amino acid derivatives of the cluster rhenium(III) compounds.

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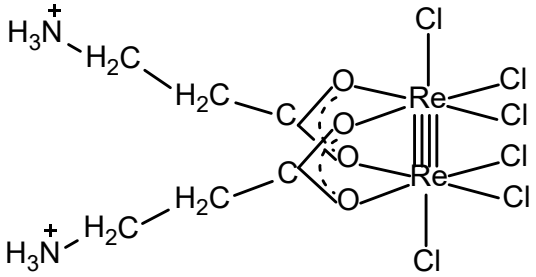


Structure of *cis*-[$Re_2(\beta\text{-Ala})_2Cl_6$] (I)

Objects and methods of research

Materials

Cisplatin (cisPt) and **I** were synthesized at Ukrainian State University of Chemical Technology at the Department of Inorganic Chemistry (Golichenko et al., 2015).

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). Calf thymus DNA (CT-DNA) with molecular weight 328 Da, $\varepsilon_{260}=0.6600\cdot10^4 M^{-1}\cdot cm^{-1}$ was purchased from Serva (FRG). All chemical reagents were of analytical grade.

Animal model studies

The animal model was described previously (Shtemenko et al., 2007, 2009; Li et al., 2015). Tumor transplantation was performed by subcutaneous injection of 20 % Guerin's carcinoma (T8) cell suspension in the thigh area. Control group of tumor-bearing animals was not subjected to any treatment.

A single intraperitoneal administration of cisPt at a dose of 8 mg/kg was made on the ninth day after tumor inoculation and intraperitoneal administration of preparations in liposome forms in dose of 7 μM/kg of the rhenium compound **I** or rhenium-platinum (4:1) systems started on the third day after inoculation of tumor cells and was repeated every 2 days until the day 21: group [**I**]lip+cisPt.

Introduction of co-encapsulated drugs started on the third day after inoculation of tumor cells and was repeated every 2 days until day 21: group [**I**+cisPt]lip. The number of animals in each group was 8. All manipulations with animals have been 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.

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.

CT-DNA binding constant measurements

Absorption measurements were performed on a Hewlett-Packard diode array spectrophotometer (HP 8453) according to (Shtemenko et al., 2013) with some modifications: the buffered calf thymus CT-DNA solutions that were used exhibited a ratio of 1.8 : 1 for the absorptions at 260 and 280 nm, indicating that the DNA was sufficiently free from proteins. Binding titration experiments were performed using a fixed concentration of CT-DNA (0.12 μM) with increasing concentrations of metal complex (0 to 200 μM) in 5 mM Tris–HCl buffer, pH 7.5, and 20 mM NaCl. Complex-DNA solutions were incubated for 5 min before the absorption spectra were recorded. The DNA binding constant, Kb (M^{-1}), was obtained by fitting the titration data according to the equation (1).

$$1/(A-A_0)=1/A_0 + 1/[K_b \times A_0 \times C_{(complex)}] \quad (1)$$

After plotting $1/(A–A_0)$ vs 1/C(complex) and recording the slope and intercept of the resulting curve, where A_0 and A are the absorption values before and after adding the complex, respectively and C (complex, mol/L) is the concentration of the added complex.

Results and discussion

Animal studies

*Anticancer activity of **I** and **I**+cisPt in vivo*

The **I** exhibits average activity against tumor cell growth, being introduced as in free, as in liposomal forms (Fig. 1).

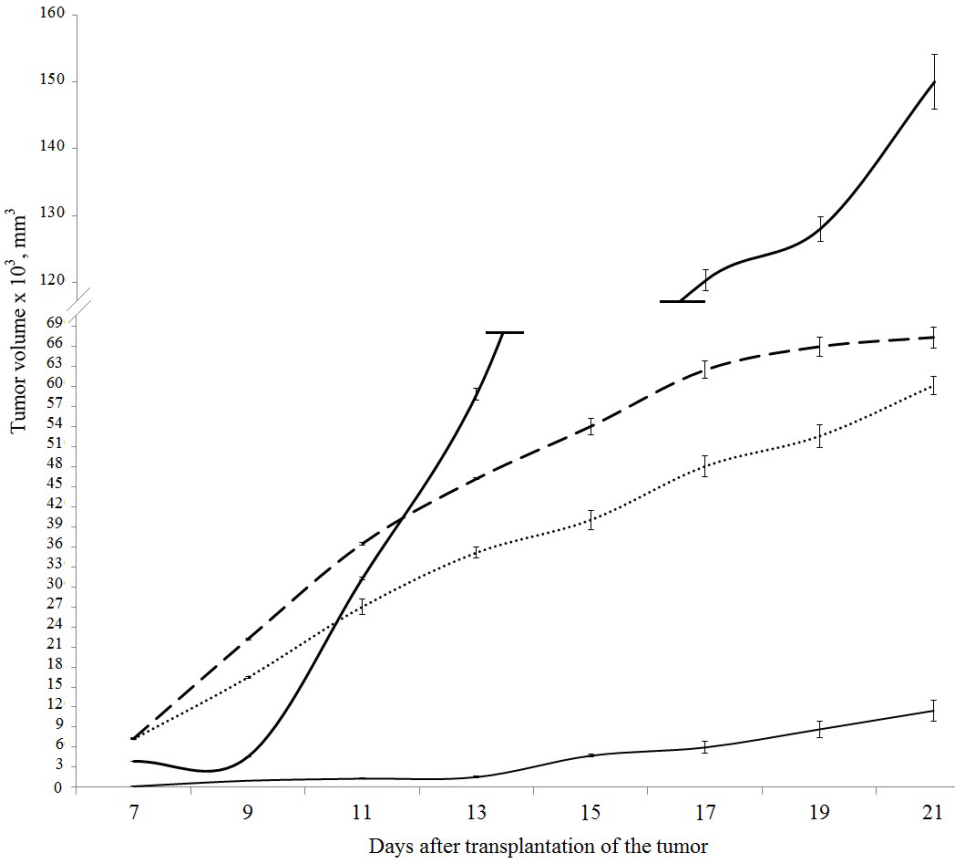


Fig. 1. Dynamics of the control tumor growth (▬ T8) and under influence of introductions of cisplatin (— T8+cisPt); I in nanoliposomes (····· T8+[I]nl); and in water solutions (– – – T8+[I]sl)

Introduction of the encapsulated dirhenium compound **I** alone led to reduction of the tumor weights by 45 % and of free **I** – by 36 % correspondingly, that is much lower in comparison to cisPt (Table 1).

Table 1.

Weights of the residual tumors and values of the reduction of the tumor (*M*±*m*, *n*=6)

Groups	Weight of tumors, g	Reduction of tumor, %
T8	63.27 ± 15.19	-
T8+cisPt	10.40 ± 1.26 #	83.56 ± 1.991
T8+[**I**]sl	40.31 ± 10.34##	36.29 ± 16.343
T8+[**I**]nl	34.35 ± 5.21##	45.71 ± 8.285
T8+cisPt+[**I**]sl	13.87 ± 0.69	78.08 ± 1.091
T8+cisPt+[**I**]nl	2.72 ± 0.14 ##	95.70 ± 0.221

 # p<0.05 versus T8; ## p<0.05 versus T8+cisPt

The antitumor effect of free **I** is close to the effect of encapsulated form of **I** that is unusual for the dirhenium(III) clusters due to their instability in water and appropriate requirements of the liposomal technology application. It is a very valuable result giving possibility to use stable in water dirhenium compounds in anticancer trials.

It is interesting to note, that the substance (cis–$Re_2(CH_3CH_2CCOO)_2Cl_4$) – a propionate derivative, which differs from I only by absence of amino groups, had very low activity in the same model, being introduced as in free, as in liposomal (16.61 %) forms (Leus et al., 2012).

If to compare influence of the liposomal forms introductions of other different dirhenium(III) clusters on the tumor growth, it is clear that the effect of **I** is greater than the effect found for dirhenium(III) compounds with alkyl ligands, such as dirhenium(III) complexes with pivalic (cis–$Re_2((CH_3)_3CCOO)_2Cl_4$), isobutiric ($Re_2(i\text{-}C_3H_7COO)_4Cl_2$) or adamanthylcarbonic (cis-[$Re_2(C_{10}H_{15}COO)_2Cl_4$]) ligands, that had reached 28–30 % (Leus et al., 2012) but lower than the effect of the homologues substance $[Re_2(GABA)_2Cl_5(H_2O)]Cl\cdot 2H_2O$ (by 60 %) in the same model (Shtemenko et al., 2009). These results once more confirm our previous conclusions about growth of antitumour activity in the range of dirhenium(III) substances with the increase of the ligand chain and about positive influence of the introduction of the amino acid moiety to the ligand arrangement.

Our earlier studies have shown that combination therapy using cisPt and dirhenium(III) clusters in reduction of tumors in tumor-bearing animals was very effective (Shtemenko et al., 2009, 2013, 2015; Shtemenko, Shtemenko, 2017). Thus, it was not a surprising thing, that a substation effect was observed in groups T8+[**I**]sl and T8+[**I**]nl+cisPt, where cisplatin and I were introduced together (Fig. 2, Table 1).

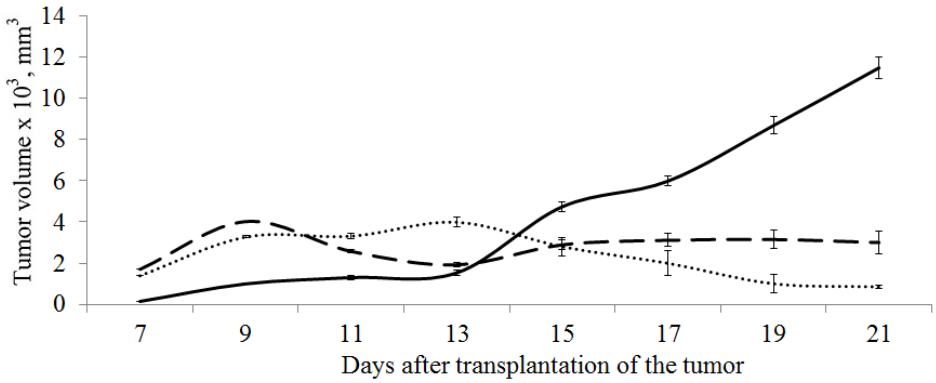


Fig. 2. Dynamics of the tumor growth under influence of introduction of cisPt (— T8+cisPt); (····· T8+cisPt+[I]nl); (- - - T8+[I]sl)

The reduction of the tumor growth was more effective than that in T8+cisPt group and the most of the experimental animals had no tumors at all. Practically no considerable differences were found between introduction of liposomal and free form of **I**, once more demonstrating the possibility of application of the amino acid derivatives of dirhenium(III) clusters in antitumor therapy.

DNA-binding activity

Investigation of DNA-binding activity is one of the most important procedure for the choice of a potentially anticancer drug (Martınez-Lillo et al., 2011; Majer et al., 2015; Ismail et al., 2019). To probe the binding of DNA to **I**, the electronic absorption spectra, obtained by titrating CT-DNA with solutions of **I**, were performed according to reported procedures (Shtemenko et al., 2013; Polokhina et al., 2016; Paramonova et al., 2016) and are depicted in Fig. 3.

The electronic absorption spectra CT-DNA exhibit hyperchromism in the presence of increasing amounts of **I.** The DNA band at ~ 260 nm arises from the π-π* transitions of the nucleic acid bases and 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.

By plotting $1/(A–A_0)$ vs 1/C according to eq. 1, the value K_b**I** = 2.43×10^3 M^{-1} for the binding constant of the DNA complex to **I** was obtained. The determined K_b value for **I** is lower than the values reported for the classical DNA intercalators; this K_b value (2.43×10^3 M^{-1}) indicates that **I** binds to DNA with a lower affinity than the classical intercalators but it compares well with the magnitude of the binding constants for other non-intercalating complexes of dirhenium(III) (Polokhina et al., 2016; Paramonova et al., 2016), which is in the range $3.4 \times 10^2 – 2{,}7 \times 10^3$ M^{-1}.

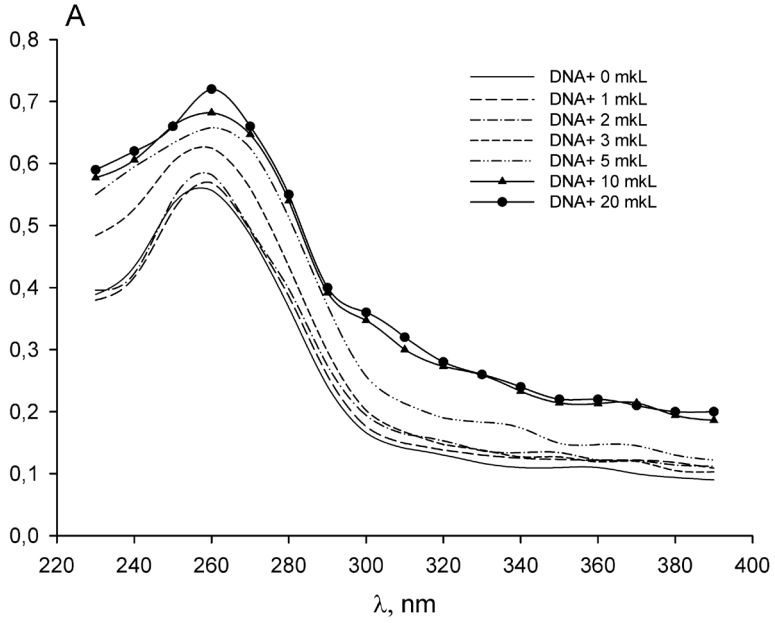
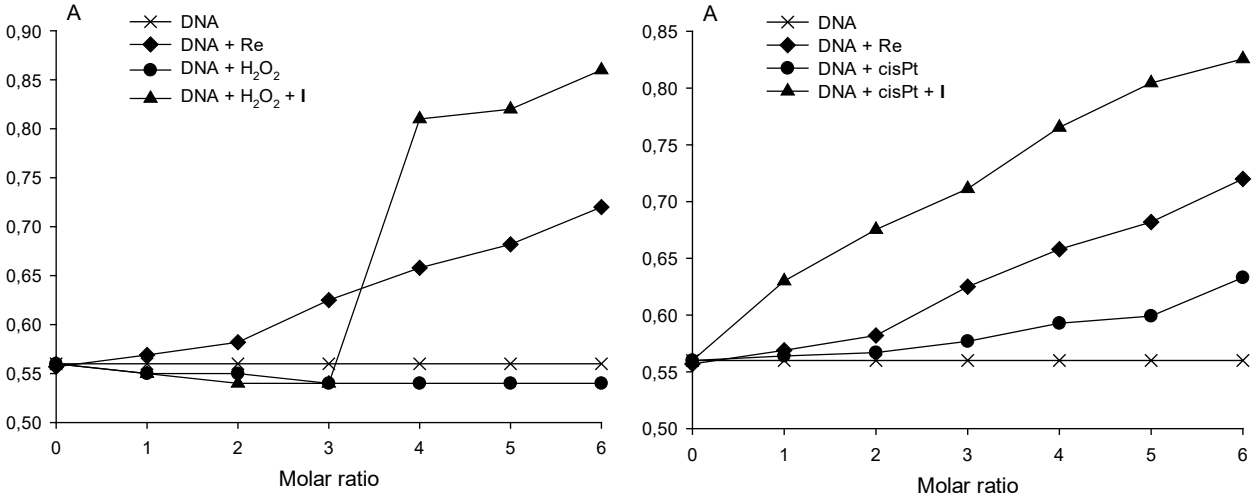


Fig. 3. Electronic absorption spectra of CT-DNA upon addition of I

Titration of CT-DNA with cisPt and hydrogen peroxide also leads to a hypochromic effect, weak at low concentrations and more significant at high concentrations of substance (Fig. 4).



a) b)

Fig. 4. The intensity of absorption (A) at 260 nm. The molar ratio of DNA-hydrogen peroxide (a) or cis-Pt (b) and DNA-I: 1 – 1 : 84; 2 – 1 : 168; 3 – 1 : 252; 4 – 1 : 420; 5 – 1 : 840; 6 – 1 : 1680

This suggests for another mechanism of the interaction of cisPt, H_2O_2 in comparison to **I**, which leads to significant chemical modification of the DNA helix and may be the explanation of the efficacy of the combinational therapy.

As it was shown previously, titration of CT-DNA by **I** after hydrogen peroxide and cisPt addition leads to a sharp increase in absorption spectra in the area of absorption of nucleic bases. Binding constant K_b(cis-Pt) = 1.080 × 10^3 M^{-1}, $K_b(H_2O_2)$ = 0, $K_b(H_2O_2$+**I**) = 24.423 × 10^3 M^{-1}, K_b(cis-Pt+**I**) = 7.783 × 10^3 M^{-1}.

The DNA binding constants increases in several times when using H_2O_2 (Fig. 4a) or cis-Pt (Fig. 4b). The obtained data confirm a mechanism for redox activation of interaction of antitumor Rhenium compounds with DNA in a living cell so-called «prodrug strategy» (Zhang, Sadler, 2017), which explains the activity of some drugs, such as **I**, which become active only in a cancer cells, where the redox state is much different from the redox state of the normal cells. Interestingly, that difference between redox-activated **I** and non-redox-activated **I** on the Fig. 4 and differences between K_b**I** vis K_b(H_2O_2+**I**) and K_b(cis-Pt+**I**) is much more greater than the same for other investigated (non-amino acid derivatives) that reaffirms perceptiveness of use of such substances in medicine and defines directions of synthesis of the biologically active compounds.

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Комплекс диренію(III) з бета-аланіновим лігандом: протипухлинні, антиоксидантні та ДНК-зв'язуючі властивості

К.В. Полохіна, С.О. Бабій, О.А. Голіченко, Н.І. Штеменко

Раніше нами було показано, що дикарбоксилатна комплексна сполука диренію(III) з γ-аміномасляною кислотою має більш високу протипухлинну активність, ніж для раніше досліджених алкілкарбоксилатів, а також може діяти як модулятор механізму дії цисплатину і як стабілізатор еритроцитів в організмах–пухлиноносіях. Таким чином, завдання роботи полягало у тому, щоб дослідити протипухлинну активність комплексу *cis*-$[Re_2(\beta\text{-Ala})_2Cl_6]$ (**I**) в моделі росту пухлини *in vivo* і зрозуміти, чи впливає амінокислотний залишок на ДНК-зв'язуючу активність амінокислотних похідних кластерних сполук ренію(III). Протипухлинні властивості комплексу **I** вивчали на моделі росту пухлини з використанням щурів лінії Вістар, інокульованих клітинами пухлини карциноми Герена. Введення тільки однієї сполуки у вільній та ліпосомальній формах інгібувало ріст пухлини на 36 % і 45 % відповідно, що більше, ніж для кластерів диренію(III) з алкільними лігандами. Комбіноване введення **I** і цисплатину значно впливає на розмір пухлини та призводить до зникнення пухлин у більшості тварин. Не було виявлено суттєвих відмінностей між введенням ліпосомальної і вільної форми речовини **I**. У електронних спектрах поглинання ДНК тимусу теляти (СТ-ДНК) спостерігається гіперхромізм у присутності зростаючої кількості **I**. Смуга ДНК при ~260 нм відповідає π-π* переходам основ нуклеїнових кислот. Зміни в інтенсивності і незначний зсув цієї характеристичної смуги відображають відповідні структурні модифікації ДНК, які включають зміни в укладанні, розрив водневих зв'язків між комплементарними ланцюгами, ковалентне зв'язування основ ДНК, інтеркаляцію ароматичних кілець та ін. Була отримана константа зв'язування K_b(**I**) = 2.43×10^3 M^{-1} для СТ-ДНК, значення якої нижче, ніж для класичних інтеркаляторів ДНК, і порівняння з величинами констант зв'язування для інших комплексів диренію(III); титрування СТ-ДНК цисплатином і перекисом водню також призводить до гіпохромного ефекту, слабкого при низьких концентраціях і більш значного при високих концентраціях **I**; константи зв'язування ДНК збільшувались у кілька разів при використанні H_2O_2 або цисплатину, що підтверджує механізм окисно-відновної активації взаємодії **I** з ДНК у раковій клітині. Отримані результати демонструють можливість застосування амінокислотних похідних кластерів диренію(III) в протипухлинній терапії.

Ключові слова: *кластери диренію(III) з амінокислотними лігандами, цисплатин, модель росту пухлини, ДНК тимусу теляти, константи зв'язування.*

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Комплекс дирения(III) с бета-аланиновым лигандом: противоопухолевые, антиоксидантные и ДНК-связывающие свойства

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Ранее нами было показано, что дикарбоксилатное комплексное соединение дирения(III) с γ-аминомасляной кислотой обладает более высокой противоопухолевой активностью, чем у ранее исследованных алкилкарбоксилатов, а также может действовать как модулятор механизма действия цисплатина и как стабилизатор эритроцитов в организмах – носителях опухоли. Таким образом, задача работы состояла в том, чтобы исследовать противоопухолевую активность комплекса *cis*-$[Re_2(\beta\text{-Ala})_2Cl_6]$ (**I**) в модели роста опухоли *in*

vivo и понять, влияет ли аминокислотный остаток на ДНК-связывающую активность аминокислотных производных кластерных соединений рения(III). Противоопухолевые свойства комплекса **I** изучали на модели роста опухоли с использованием крыс линии Вистар, инокулированных клетками опухоли карциномы Герена. Введение только одного соединения в свободной и липосомальной формах ингибировало рост опухоли на 36 % и 45 % соответственно, что больше, чем для кластеров дирения(III) с алкильными лигандами. Комбинированное введение **I** и цисплатина оказало значительное влияние на размер опухоли и приводило к исчезновению опухолей у большинства животных. Не было обнаружено существенных различий между введением липосомальной и свободной формы **I**. В электронных спектрах поглощения ДНК тимуса телёнка (СТ-ДНК) наблюдается гиперхромизм в присутствии увеличивающихся количеств **I**. Полоса ДНК при ~ 260 нм соответствует π-π* переходам оснований нуклеиновых кислот. Изменения в интенсивности и незначительные сдвиги длин волн этой характеристической полосы отражают соответствующие структурные модификации ДНК, которые включают изменения в укладке, разрыв водородных связей между комплементарными цепями, ковалентное связывание оснований ДНК, интеркаляцию ароматических колец и др. Была получена константа связывания K_b(**I**) = 2.43 × 10^3 M^{-1} для СТ-ДНК, значение которой ниже, чем значения для классических интеркаляторов ДНК, и сравнимо с величинами констант связывания для других комплексов дирения(III); титрование СТ-ДНК цисплатином и перекисью водорода также приводит к гипохромному эффекту, слабому при низких концентрациях и более значительному при высоких концентрациях **I**; константы связывания ДНК увеличивались в несколько раз при использовании H_2O_2 или цисплатина, что подтверждает механизм окислительно-восстановительной активации взаимодействия **I** с ДНК в раковой клетке. Полученные результаты демонстрируют возможность применения аминокислотных производных кластеров дирения (III) в противоопухолевой терапии.

Ключевые слова: *кластеры дирения(III) с аминокислотными лигандами, цисплатин, модель роста опухоли, ДНК тимуса телёнка, константы связывания.*

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