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Influence of *cis*-$[Re_2GABA_2Cl_4]Cl_2$ on the Antioxidant Defense System Parameters of Normal Human Blood

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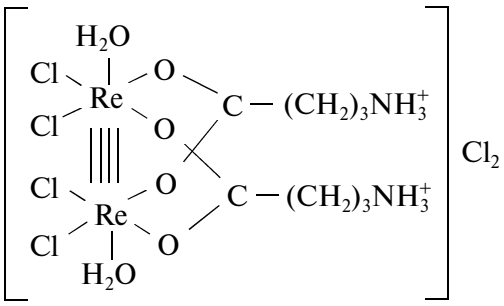
Abstract—The effect of the rhenium complex *cis*-$[Re_2GABA_2Cl_4]Cl_2$ on the antioxidant parameters of normal human blood *in vitro* have been studied. The results suggest that the complex influences various enzymes in the cascade of reactions utilizing active oxygen metabolites. However, the manifestation of this activity varies over the studied concentration range of the complex in the preincubation medium (10^{-12}-10^{-4} M), so the effects appear to be concentration-dependent. The largest differences in antioxidant parameters in comparison with control were observed for the concentrations 10^{-8}, 10^{-5}, and 10^{-4} M. Thus, correlations between the peroxidation level, superoxide dismutase (SOD) activity, antioxidant factor (F), and indexes of resistance of erythrocytes for hemolysis (TR) were found.

Key words: *cis*-$[Re_2GABA_2Cl_4]Cl_2$ complex, antioxidant parameters, human blood

The biological activity of γ-aminobutyric acid (GABA) includes not only its function as a neurotransmitter. GABA also has humoral effects, e.g., it affects blood pressure [1, 2]. It is widely used in pharmacology [3], and together with the low toxicity of the metal rhenium [4] and the significant lability of perrhenic compounds, this stimulates the study of complex binuclear clusters of Re_2^{6+} with GABA. The synthesis, structure, and chemical properties of these compounds have been studied recently under the direction of Professor A. V. Shtemenko at the Department of Inorganic Chemistry, Ukraine State University of Chemical Technology, Dniepropetrovsk [5]. Three types of such clusters have been synthesized: $(AH)_2[Re_2Cl_8]\cdot 2H_2O$ (type I), where an amino acid (A = GABA or other amino acids) is the inner sphere cation, and *cis*-$[Re_2A_2Cl_4]Cl_2$ (type II) and *trans*-$[Re_2A_2Cl_4]Cl_2$ (type III), where the amino acid ligand is coordinated to Re_2^{6+} by carboxylic bridges in *cis*- and *trans*-configurations, respectively, with respect to the Re–Re bond. The type II compounds are the most resistant to hydrolysis in aqueous solutions. Considering these and other chemical properties, it was of interest to study the biological activity of *cis*-diaquatetrachlorodi-*m*-carboxylatodirhenium (*cis*-$[Re_2GABA_2Cl_4]Cl_2$) (III):



Many metal complexes with amino acids (and with other biologically active substances) as the ligands have been used as pharmaceuticals with a wide spectrum of actions [6]. Their biological effects on cell systems are due to one of three mechanisms related to the chemical nature [7]: 1) activity based on a ligand where the role of the metal consists of induction of a suitable shape for transport; 2) the metal ion itself shows activity and the complex is necessary for the transport of the metal ion through the cell membrane; 3) the active species is a complex ion that can interact with the vital centers of cells.

Many complexes have been studied using biochemical and pharmacological tests in efforts to find new, more active and less toxic biologically active compounds. One

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of the most sensitive systems responding to very minor changes in the body is the multicomponent antioxidant–prooxidant system that effects the structural and functional integrity of membranes, the activity of membrane-bound enzymes, cell receptors, and ion channels [8], and as a consequence the integrity of tissues and organs. Therefore, the goal of the present work was to investigate the effect of the *cis*-$[Re_2GABA_2Cl_4]Cl_2$ complex on the functional state of the antioxidant system of blood.

MATERIALS AND METHODS

Blood samples from 23 apparently healthy donors (age range 25-36 years) with type II(A) Rh+ blood were used. After 1 h of preliminary incubation of the blood at 37°C with the rhenium complex (final concentrations 10^{-12}-10^{-4} M from stock solution in physiological solution), erythrocytes were separated from plasma and washed three times with physiological solution using centrifugation at 1500 rpm during 10 min.

Antioxidant state was estimated by studying in hemolysates the activity of superoxide dismutase (SOD), a basic antioxidant defense enzyme catalyzing superoxide anion-radical dismutation, and catalase [9], an equally important antioxidant enzyme preventing the accumulation of H_2O_2.

The peroxidation level was determined by accumulation of malonic dialdehyde (MDA) in plasma and erythrocytes [10]. Another parameter used is the antioxidant state factor (F), which is obtained as the product of the values of catalase and superoxide dismutase activities divided by the MDA level of blood [9]. After preincubation with physiological solution without rhenium, the control blood parameters were obtained.

The washed erythrocytes were subjected to acid hemolysis according to [11]. The total resistance to hemolysis was calculated using the formula [12]:

$$TR = E_1 \cdot t_1 + E_2 \cdot t_2 + ... + E_n \cdot t_n,$$

where TR is total resistance of erythrocytes (% · min); E is the percentage of erythrocytes of a given fragility group hemolyzed per 1 min; t is the time (min) of hemolysis determining the fragility of a given group; 1, 2, ..., n are various erythrocyte groups.

The results were assessed statistically and their reliability was determined using Student's t-criterion.

RESULTS AND DISCUSSION

Acid hemolysis of erythrocytes treated with 10^{-4} and 10^{-8} M of the rhenium complex with GABA was on average 8% ($p < 0.05$) and 12% ($p < 0.01$) reduced (Fig. 1), respectively, in the experiments versus the controls. Thus, the mean time of hemolysis and the time of maximal hemolysis of erythrocytes increased and the share of hemolysis during the hemolytic peak was reduced. In contrast, incubation of erythrocytes with 10^{-5} M of the complex resulted in increased hemolysis on average by 31% ($p < 0.01$).

Hemolytic effects of substances are often connected with peroxidation processes in erythrocytes, and, in contrast, the induction of the enzymatic antioxidant defense system is connected with increased resistance of erythrocytes to hemolysis [13, 14].

The MDA contents in our experiments in erythrocytes treated with the *cis*-$[Re_2GABA_2Cl_4]Cl_2$ was unchanged compared with controls at all concentrations in the range 10^{-4}-10^{-12} M. Thus, the compound showed neither oxidant nor antioxidant activity (Fig. 2) under our experimental conditions. However, examining the data concerning MDA in the plasma fraction of blood (Fig. 3) showed a negative correlation with susceptibility to acid hemolysis; the MDA level in plasma of blood treated with 10^{-5} M complex increased by 13% ($p < 0.01$), and this was 3% ($p < 0.01$) and 5% ($p < 0.001$) reduced after preincubation with 10^{-7} and 10^{-8} M, respectively.

It should be noted that these data, first, show the importance of determination of MDA content not only in intact blood or in erythrocytes where its level is higher, but also in plasma where the MDA level is significantly lower but is more indicative in certain cases. Second, a feedback correlation "stability to hemolysis–peroxidation degree" was confirmed. Third, the data show a selective influence depending on the concentration of the rhe-

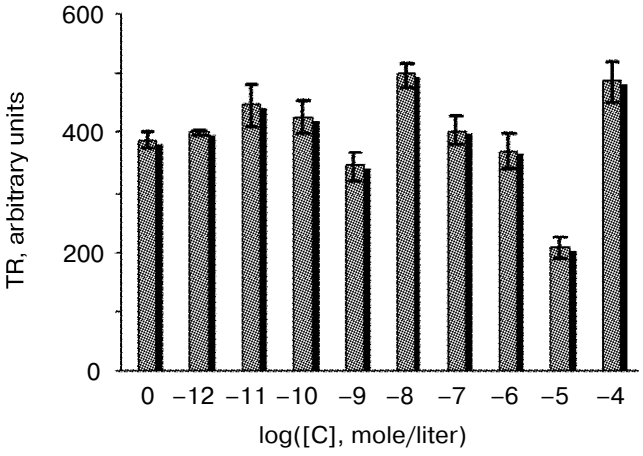

Fig. 1. Dependence of erythrocyte total resistance (TR) for hemolysis on the concentration of the rhenium complex with GABA during preincubation. The results correspond to M ± m, n = 3-15.

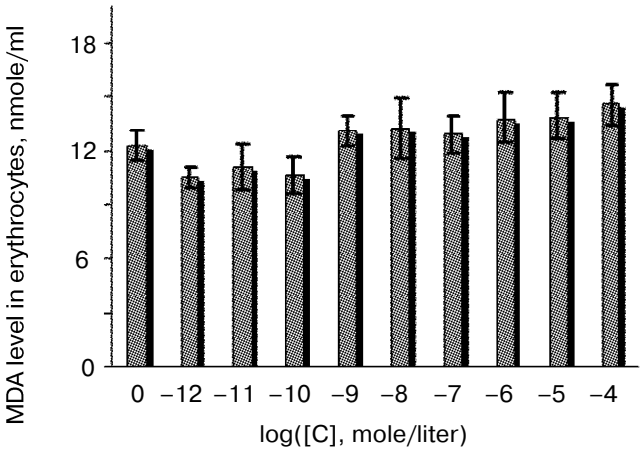

Fig. 2. Dependence of erythrocyte peroxidation level on concentration of rhenium complex with GABA during preincubation. The results correspond to M ± m, n = 3-7.

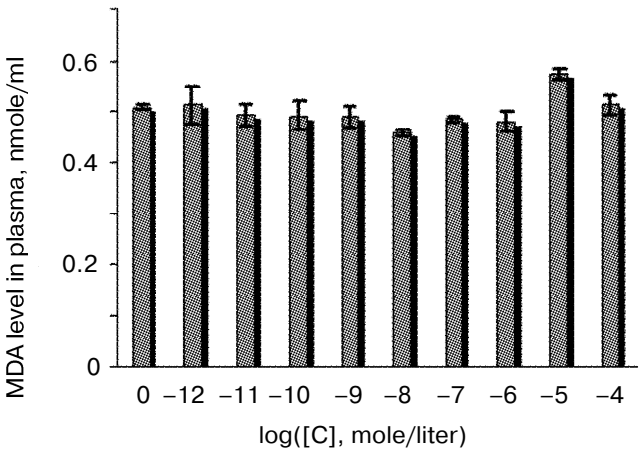

Fig. 3. Dependence of plasma MDA level on concentration of rhenium complex with GABA during preincubation. The results correspond to M ± m, n = 3-7.

nium complex, which acts either as antioxidant and stabilizer of erythrocytes membranes or as a prooxidant and hemolytic depending on concentration.

The activity of catalase for the decomposition of H_2O_2 increased (from 2 to 34% versus control) for various concentrations of the rhenium complex (10^{-4}, 10^{-10}, 10^{-11} M). This suggests activation of the process of protection against peroxidation either due to increased substrate concentration limiting the reaction rate of this enzyme [15] or due to a direct interaction of the complex or its active species with the enzyme active center.

Estimates of changes in SOD activity (table) induced by treatment of blood with the rhenium complex revealed a tendency similar to that for the acid hemolysis.

Thus, on studying the changes in the antioxidant parameters the extreme values of SOD (blood) and MDA (plasma) activity were accompanied by the most pronounced changes in erythrocyte stability to hemolysis at 10^{-4}, 10^{-5}, and 10^{-8} M concentrations of the Re complex. These data also correlate with the values of the antioxidant state factor.

The toxicity of *cis*-$[Re_2GABA_2Cl_4]Cl_2$ was studied at the Ukrainian Scientific Research Institute of Oncology and Radiology (Kiev, Ukraine); the LD_{50} of the free form of the compound was 1200 mg/kg. If this parameter is compared with those observed for active platinum compounds widely used in clinical practice as antitumor drugs [16] and palladium compounds, the platinum metal toxi-

Effect of *cis*-$[Re_2GABA_2Cl_4]Cl_2$ concentration on the levels of MDA, catalase, and SOD in blood (M ± m)

Complex concentration, M	MDA, nM	n	Catalase activity × 10^4, IU/ml	n	SOD activity, IU/ml	n	F, nM
0	12.79 ± 0.86	7	4.30 ± 0.25	9	2913 ± 87	8	979
10^{-4}	15.10 ± 1.12	6	5.75 ± 0.41*	5	3184 ± 277	5	1213
10^{-5}	14.46 ± 1.26	6	5.40 ± 0.62	6	2394 ± 198*	6	894
10^{-6}	14.26 ± 1.37	6	4.40 ± 0.74	6	3124 ± 217	6	964
10^{-7}	13.41 ± 1.00	6	4.50 ± 0.44	6	3039 ± 128	5	1019
10^{-8}	13.65 ± 1.70	5	4.60 ± 0.39	5	3301± 31*	5	1112
10^{-9}	13.58 ± 0.85	5	4.85 ± 0.35	5	2813 ± 78	5	1005
10^{-10}	11.12 ± 1.10	5	4.55 ± 0.41	5	2632 ± 257	5	1077
10^{-11}	11.55 ± 1.32	5	5.15 ± 0.17**	5	2557 ± 239	5	1140
10^{-12}	11.01 ± 0.60	3	5.40 ± 0.60	3	2583 ± 147	3	1267

* $p < 0.05$.
** $p < 0.01$ (significance versus control).

city in cisplatin in the free form and in the less toxic liposome form is found to be 55 and 38 times, respectively, above the toxicity of the studied rhenium compound. And the *cis*-diaminedichloroplatinum complex (cDDP) toxicity exceeds that of the rhenium complex by more than 110-fold [17]. The analogous compounds of palladium are 3-5-fold more toxic than *cis*-$[Re_2GABA_2Cl_4]Cl_2$ [18].

Based on LD_{50} values, the pharmacological dose of 100 mg/kg was calculated [19]. This dose was tested in chemotherapy of transplantable s.c. tumor T-8. The value T/C = 37.37% found suggests good antitumor activity of the Re complex with this tumor model [20]. T/C is a characteristic of the antitumor activity on solid tumors expressed as the ratio of masses of tumors of treated (T) and control (C) animals; the drug is promising if T/C < 45%.

From the results of this study, we conclude that the cluster rhenium compound with GABA has biological activity and its effect is concentration-dependent, i.e., at some concentrations there is induction of antioxidant processes and antihemolytic effect, at other concentrations there is acceleration of hemolysis and activation of peroxidation processes, and in a third case the interaction with the complex does not change the measured parameters versus the control. This suggests multiple mechanisms of interaction of the Re complex with blood.

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