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LEAD DIGLYCYRRHIZATE AND ITS EFFECT ON THE OXIDASE ACTIVITY OF NEUTROPHILS

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Lead glycyrrhizate was prepared. Its effect on the oxidase activity of neutrophils was compared with that of glycyrrhizic acid. It has been found that glycyrrhizic acid and lead diglycyrrhizate cause a dose-dependent reduction in the formation of active forms of oxygen by the neutrophils after their activation by phorbolic ester and a chemotoxic peptide. It has been determined that the effect of lead diglycyrrhizate is weaker than that of glycyrrhizic acid.

Key words: glycyrrhizic acid, lead diglycyrrhizate, oxidase activity, neutrophils, active forms of oxygen.

Glycyrrhizic acid (GA) is a natural glycoside that is isolated from *Glycyrriza glabra* L., affects the metabolism of steroidal hormones, and exhibits mineral corticoid-like activity [1]. It has been found that GA possesses antiviral, immunotropic, antiallergic, antitumor, and anti-ulcer activities [2]. It is also widely used as a hepatoprotector [3].

However, heavy metals can be complexed if GA is used as an antidote for lead poisoning [4]. This will evidently facilitate their conversion to mildly toxic compounds. It seemed interesting to synthesize lead diglycyrrhizate ($PbGA_2$) and compare the effects of GA and $PbGA_2$ on the oxidase activity of neutrophils because it is known that the activity of NADPH-oxidase is an important indicator for studying anti- and pro-oxidant properties of compounds.

$PbGA_2$ was prepared in aqueous-alcohol by reacting the potassium salt of glycyrrhizic acid with $Pb(NO_3)_2$.

The resulting compound consists of two molecules of glycyrrhizic acid covalently bound to Pb.

The IR spectrum of $PbGA_2$ contains an absorption band due to vibration of OH at 3450 cm^{-1}, C=O conjugated to a double bond at 1695-1645 cm^{-1}, and COO bound to Pb at 1605-1545 cm^{-1}.

The effects of GA and $PbGA_2$ on the generation of active forms of oxygen (AFO) by neutrophils were compared using two activating agents: phorbol-12-myristate-13-acetate (PMA) (1), which activates proteinkinase C (PKC) by phosphorylating components of the NADPH-oxidase complex [5], and the chemotoxic peptide N-formylmethionylleucylphenylalanine (FMLP) (2), which binds to a specific receptor on the neutrophil membrane [6].

The intensity of spontaneous chemiluminescence did not change after adding $PbGA_2$ to cells containing incubation medium and neutrophils. However, incubation of neutrophils with $PbGA_2$ produced a dose-dependent reduction of AFO production when PMA was used as the activator (Fig. 1a). Thus, AFO production by neutrophils decreased by 7±3% relative to the control after adding $PbGA_2$ (0.1 μM) and by 47±2% at 100 μM.

AFO production by neutrophils incubated beforehand with $PbGA_2$ also decreased if the activator was FMLP, which induces AFO formation in response to activation of the FMLP receptor (Fig. 1b). However, the dependence of the oxidase activity on $PbGA_2$ concentration was slightly different. AFO production decreased by 3±1.5% compared to the control in response to FMLP after incubation of neutrophils with $PbGA_2$ (0.1 μM). As the concentration of $PbGA_2$ increased to 1 μM, the response of neutrophils to FMLP was inhibited by 21±3%. The response decreased by 68±3% after 10-min incubation with 100 μM $PbGA_2$.

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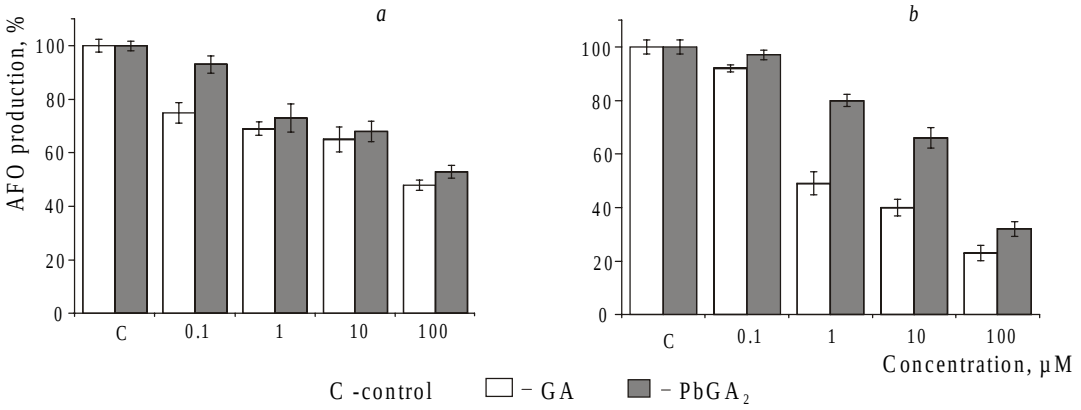

Fig. 1. Effects of GA and $PbGA_2$ in various concentrations on chemiluminescence of neutrophils after activation of AFO by FMA (1 μM) (a) and FMLP (10 μM) (b). Averages for the effect from results of 25 (a) and 22 (b) independent experiments are given. Response of intact cells was taken as 100%.

A comparison of the effects of GA and $PbGA_2$ on neutrophil oxidase activity showed some differences in their effects for the two methods of activation. The action of $PbGA_2$ was practically the same as that of GA itself on production of AFO by neutrophils after activation by PMA (Fig. 1a). However, the inhibiting effect of $PbGA_2$ on NADPH-oxidase activity was slightly less than that of GA after activation by FMLP (Fig. 1b). Thus, the effect of $PbGA_2$ (1 μM) was 21±3% vs. the control whereas GA reduced AFO production in this instance by 51±6%.

The inhibition of neutrophil oxidase activity is not due to the antiradical properties of the compounds, addition of which to the cell at the time of maximum activity of neutrophils activated by PMA did not change the chemiluminescence intensity even at the maximal concentration (100 μM). Furthermore, we performed a series of experiments in an acellular model system using Fenton's reaction. The results have also shown that the studied compounds do not supress luminol-dependent chemiluminescence.

Two mechanisms apparently are responsible for the suppression of neutrophil NADPH-oxidase activity by GA and $PbGA_2$. On one hand, the effect may be due to the known inhibiting action of GA on PKC, since it has been established that AFO generation by PMA occurs as a result of direct activation by PKC [5]. On the other, GA may interact with the FMLP receptor because GA can bind nonspecifically with the kidney mineral-corticoid receptor [7], estrogen receptors [8], and EGF-receptors [9]. Evidently the decrease in the inhibiting activity of $PbGA_2$ after activation of AFO production by FMLP is due to the lower affinity of this compound for the FMLP receptor than that of GA.

EXPERIMENTAL

IR spectra of $PbGA_2$ were recorded on a Spekord-757R instrument using KBr disks.

We used the reagents luminol, phorbol-12-myristate-13-acetate (PMA), Hanks medium, the chemotoxic peptide N-formylmethionylleucylphenylalanine (FMLP), HEPES, horseradish peroxidase (type VI), sodium azide, zymosan, and H_2O_2 (all Sigma, USA).

Synthesis of $PbGA_2$. A solution of potassium glycyrrhizate (0.02 mole) in aqueous-alcohol (70%, 50 mL) was heated, stirred, and treated with $Pb(NO_3)_2$ (0.01 mole). After 2-3 h, the precipitate of $PbGA_2$ was separated and washed twice with alcohol. Yield 67%, mp 218-220°C.

Neutrophils were obtained from male mice (NMRI line, 25-30 g mass). Peritoneal neutrophils were isolated by the literature method [10].

AFO production by neutrophils was estimated by luminol-dependent luminescence [11] measured by a CL-111 chemiluminometer that was developed and built in the Laboratory of Nerve-Cell Biophysics of the Institute of Cell Biology of the Russian Academy of Sciences. Cells were incubated for 10 min with GA and $PbGA_2$ at a controlled temperature of 37°C. A respiratory burst of the neutrophils was activated using FMLP (10 μM) and PMA (1 μM).

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