STUDY OF THE ANTIVIRAL PROPERTIES OF CHLOROPHYLLIN SODIUM PREPARATIONS

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Preparations of magnesium-chlorophyllin sodium, irrespective of their origin, possess viricidal action against influenza and herpes viruses. A preparation of copper-chlorophyllin sodium has virtually no viricidal properties.

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One of the writers (B. L. K.) previously showed that the compound magnesium-chlorophyllin sodium obtained from conifers has a marked viricidal action on certain viruses [3, 4]. It has also been shown that this preparation gives a marked therapeutic effect in herpetic keratitis, herpes zoster, and herpes simplex [1]. Chlorophyllin preparations used in medical practice are not chemically pure, but are plant extracts enriched with chlorophyllin [5, 6].

The object of the present investigation was to study viricidal properties of chlorophyllin preparations of different origin in order to determine the nature of the viricidal components in this preparation.

EXPERIMENTAL METHOD

The following preparations were used: 1) magnesium-chlorophyllin sodium from conifer needles; 2) magnesium-chlorophyllin sodium from lilac leaves; 3) a copper derivative of chlorophyllin sodium from conifer needles; 4) a derivative of chlorophyllin sodium with no metal in the center of its porphyrin structure; 5) magnesium-chlorophyllin sodium from mint.

The concentration of the preparation in aqueous solutions was determined on the FÉK-M photoelectric colorimeter with a red filter.

Viruses: allantois culture of influenza virus A2 (strain No. 101), 10% brain suspension of herpes simplex virus (strain L2) – reference strains from the D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR. Influenza virus was titrated in 9-11 day chick embryos and herpes simplex virus in albino mice weighing 7-8 g. The titers of the viruses were calculated by Karber's method as modified by I. P. Ashmarin. The standard deviation was determined by the formulas given in [9]:

$$\sigma = \pm \sqrt{\frac{\Sigma P \cdot q}{n}} \cdot d;$$

the significance of differences between single results was assessed by the use of the formula [7]:

$$X_1 - X_2 > 2{,}7 \sqrt{\sigma_1^2 + \sigma_2^2}.$$

Scheme of experiments: aqueous solutions of chlorophyllin were mixed with the virus suspension and the mixture was incubated at room temperature in darkness for a given time interval, after which these mixtures and the corresponding controls were titrated for infectivity.

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TABLE 1. Effect of Different Chlorophyllin Preparations of Infectivity of Herpes Simplex Virus (Strain L2) After Contact for 2 h

Preparation	Concentration of preparation in virus-containing mixture (in μg/ml)	Infective titer of virus (log ED_{50})		Number of infective doses (ED_{50}) per ml virus suspension inactivated by preparation	$X_1 - X_2 > 2{,}7\sqrt{\sigma_1^2 + \sigma_2^2}$
		expt.	control		
Magnesium-chlorophyllin sodium	5	0,5±0,25	3,9±0,33	$3{,}3\times10^{4.4}$	3,4>1,1
	50	0	4,7±0,28	$3{,}3\times10^{5.7}$	4,7>0,756
from conifer needles	100	0	4,5±0,25	$3{,}3\times10^{5.5}$	4,5>0,675
from lilac leaves	5	0	4,9±0,30	$3{,}3\times10^{5.9}$	4,9>0,81
	25	0	3,9±0,33	$3{,}3\times10^{5.7}$	3,9>0,891
	25	0	3,16±0,28	$3{,}3\times10^{5.5}$	3,6>0,728
from mint	100	0	5,5±0,25	$3{,}3\times10^{6.5}$	5,5>0,675
	100	0	5,0±0,25	$3{,}3\times10^{6}$	5,0>0,675
	100	0	5,25±0,21	$3{,}3\times10^{6.25}$	5,25>0,675
Derivative of chlorophyllin sodium without metal in center of porphyrin structure	100	0	5,5±0,25	$3{,}3\times10^{6.5}$	5,5>0,675
	100	0	5,0±0,25	$3{,}3\times10^{6}$	5,0>0,675
	100	0	5,25±0,21	$3{,}3\times10^{6.25}$	5,25>0,567
Copper-chlorophyllin sodium from conifer needles	100	4,25±0,30	5,5±0,25	$3{,}3\times10^{2.25}$	1,25>1,05
	100	3,75±0,30	5,0±0,25	$3{,}3\times10^{2.25}$	1,25>1,05
	100	4,5±0,30	5,25±0,21	$3{,}3\times10^{1.75}$	0,75<0,999

TABLE 2. Effect of Different Chlorophyllin Preparations on Infectivity of Influenza Virus A2 (Strain No. 101) After Contact for 2 h, M± σ

Preparation	Concentration of preparation in virus-containing mixture (in μg/ml)	Infective titer of virus (log ED_{50})		Number of infective doses (ED_{50}) per ml virus suspension inactivated by preparation	$X_1 - X_2 > 2{,}7\sqrt{\sigma_1^2 + \sigma_2^2}$
		expt.	control		
Magnesium-chlorophyllin sodium	200	0	7,0±0,25	5×10^{7}	7,0>0,675
	200	0	5,75±0,33	$5\times10^{5.75}$	5,75>0,891
from conifer needles	200	0	7,5±0,30	$5\times10^{7.5}$	7,5>0,81
	200	0	7,75±0,39	$5\times10^{7.75}$	7,75>1,05
	200	0	7,0±0,25	$5\times10^{7.0}$	7,0>0,675
	200	0	5,25±0,21	$5\times10^{5.25}$	5,25>0,567
from lilac leaves	100	1*±0,25	6,75±0,21	$5\times10^{5.75}$	5,75>0,864
	200	0	7,0±0,25	$5\times10^{6.0}$	7,0>0,675
	200	0	7,0±0,25	5×10^{7}	7,0>0,675
from mint	200	0	5,75±0,33	$5\times10^{5.75}$	5,75>0,891
	200	0	7,5±0,30	$5\times10^{7.5}$	7,5>0,81
	200	0	7,75±0,39	$5\times10^{7.75}$	7,75>1,05
	200	0	7,0±0,25	$5\times10^{7.0}$	7,0>0,675
	200	0	5,25±0,21	$5\times10^{5.25}$	5,25>0,567
Derivative of chlorophyllin sodium without metal in center of porphyrin structure	200	0	7,5±0,30	$5\times10^{7.5}$	7,5>0,81
	200	0	7,75±0,39	$5\times10^{7.75}$	7,75>1,05
	200	0	7,0±0,25	$5\times10^{7.0}$	7,0>0,675
	200	0	5,25±0,21	$5\times10^{5.25}$	5,25>0,567
Copper-chlorophyllin sodium from conifer needles	200	6,0±0,45	7,5±0,30	$5\times10^{1.5}$	1,5>1,485
	200	7,0±0,25	7,75±0,39	$5\times10^{0.75}$	0,75<1,242
	200	6,25±0,33	7,0±0,25	$5\times10^{0.75}$	0,75<1,1
	200	5,0±0,25	5,25±0,21	$5\times10^{0.25}$	0,25<0,891

*Duration of contact between virus and preparation 1.5 h.

EXPERIMENTAL RESULTS

The experimental results are given in Tables 1 and 2. Analysis of these data reveals that all tested preparations contain a common antiviral component.

The experimental results also show that the viricidal activity of the preparation correlates with the strength of binding of the metal ion in the center of the porphyrin structure of the chlorophyllin. Magnesium preparations of chlorophyllin and its derivatives without metal in the center of the porphyrin structure possessed well marked viricidal activity, while the copper-chlorophyllin sodium, in which the copper atom is much more strongly bound than the magnesium atom with the nitrogen atoms of the pyrrole rings [2], showed virtually no viricidal action. This suggests that the active component in the preparation is the chlorophyllin molecule, since changes in the bonds of nitrogen atoms in the porphyrin ring have a considerable effect on the biochemical viricidal activity of the preparation. These results also indicate that the functional group in the chlorophyllin molecule is evidently formed by the nitrogen atoms of the pyrrole rings.

LITERATURE CITED

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