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DRUG DEVELOPMENT

Investigation of the Inhibition Action of Antiviral Preparation 1 - Boraadamantane Concerning the Flu Virus

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Abstract

In this work, the surface-active properties of 1- boraadamantane have been studied on the model of monomolecular phospholipid monolayers. Compression isotherms for the phospholipid monolayer are received, before the addition of 1-bromoadamantane in a concentration of 10^{-7}-10^{-6} M.

From the analysis of the compression isotherms generated, it is possible to conclude that at a concentration of 1-bromoadamantane (10^{-6}M), an increase in the area falling on one phospholipid molecule occurs. The increase in the area falling on one single phospholipid molecule leads to a reduction in the frequency of the action of the lateral diffusion of the phospholipid molecules, a potential difference of the monolayer and a reduction in a corner of an arrangement of the phospholipid molecules to the monolayer surface. The specified phenomena can lead to a decrease in the degree of interaction of the viral membrane and a cell membrane.

Also, in this work, study was done on the activity of virion RNA-dependent RNA - polymerase after interaction with various concentrations of 1- boraadamantane.

***Key words**: 1- boraadamantane, flu virus, phospholipid monolayers, compression isotherms, virion RNA-dependent RNA-polymerase.*

Introduction

In our previous researches [1,2], the inhibiting action of 1-boraadamantane with reference to a type of flu virus and certain other viruses was revealed. In fact, 1- boraadamantane treats boric derivatives of adamantane ($C_{10}H_{16}$). It shows a related connection for the known antiviral preparation, Remantadin, interacting with the viral proteins. We have shown that unlike other adamantane derivatives, 1- boraadamantane does not interact with the surface antigenic viral proteins; however, it interacts with the viral membranee and liposomes, apparently possessing a superficial activity. With the application of the liposomes, no toxic concentrations of this connection were defined. Interestingly, 1- boraadamantane is one of the first connections influencing a membrane [3]. In this work, we attempted to study the mechanism of the interaction of BG-12 with a viral membrane by means of a phospholipid monolayer from phosphatidylcholine, using the cellular membranes as models. For the first time, an attempt was made to study the interaction of BG-12 with the virion RNA - dependent RNA - polymerase of a flu virus.

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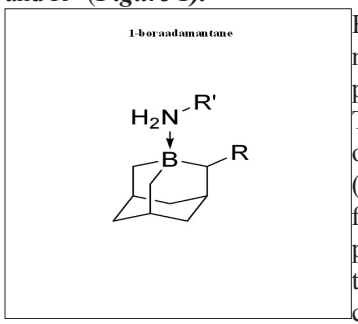
Methods

In this work, we used the derivative 1- boraadamantane – BG-12 connection (A. N. Nesmeyanov Institute of Organoelement Compounds of Russian Academy of Sciences).

In this connection, the fragment adamantane is connected by coordination communication with an atom of the boron atom; however, the radicals are represented by the organic groups – R and R ′ (***Figure 1).***



BG-12 solutions in a 10^{-7}-10^{-6} M non-toxic concentration were prepared using distilled water. To receive the monolayers of the phosphatidylcholine ("Sigma", the USA) with the final concentration of the phosphatidylcholine in 10 mg/mL the original installation was used consisting of a Teflon Langmuir trough, 200x200x1 mm in size, Langmuir's scales, fixed and removal barriers. With the movement of removing a barrier, the monolayer exerts a certain pressure upon the teflon plate of scales, which passes through the torsion thread on the IR sensor (Russian Federation).

The tension with the IR of the sensor and electric drive (the barrier coordinator) is entered into the computer. Installation provides accuracy of the measurement of superficial pressure, F=0.05 mN/m.

The action of the virion dependent RNA - polymerase of a flu virus can find adding to virions the suitable buffer, ribonucleoside triphosphates, bivalent cations and cleaner for a permeabilization of the virion cover. As a priming ApG was used in a concentration of 0.5 mM.

The standard reactionary mix (100 µl) has the following structure: tris-HSL, pH of 8.2 - 50 mM, KCl – 150 mM, MgCl2 - 8 mM, dithiothreitol – 5 mM, NP-40 – 0.5%, ATP-of 2.0 mM, GTP-of 0.2 mM, CTP-of 0.4 mM, [3H] UTP-of 0.4 mM cleared and the concentrated virus – 0.5-1.0 mg/mL.

The reactionary mixture was prepared at 0^0C. Next the test tubes were incubated at 31^0C (temperature optimum for transcription). For research of the kinetics of RNA synthesis from the reactionary mix, through certain periods, aliquots of 10 µl were selected and mixed with 20 µl one part of the chloric acid containing 0.125 M of sodium pyrophosphate.

To test 10 mcg of calf thymus DNA was added and incubated for 10 min at 0 0C. The RNA precipitates were collected on the filters from the fiber glass and 0.88 M of HSL and 0.125 M of sodium pyrophosphate were added. Next, they were washed with ethanol and dried [4]. Radioactivity was determined using the BL-BDB-2 radiometer (Russian Federation).

To determine the enzyme activity, BG-12 was used in concentrations of 10 mkg/ml and 5 mkg/mL. In the control, the defined activity of the virion RNA – dependent RNA - polymerase was done in the absence of BG-12.

Results and discussion

Experimentally, certain values of the superficial pressure (F) and the area falling on one molecule of phospholipid (A) isotherms of compression (Fig. 2) were constructed.

The addition of BG-12 in a concentration of 10^{-7}-10^{-6} M authentically changed the area falling on one molecule of the phospholipid.

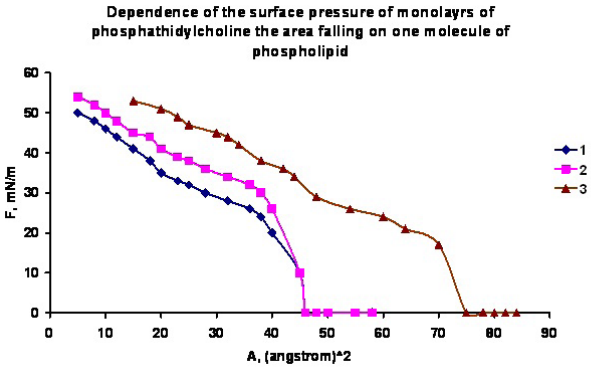


Figure 2
Curves 1, 2 were received at a BG-12 concentration of 10^{-7} M, and curve 3 – at a concentration of 10^{-6} M.

The increase in the area falling on one molecule of phosphatidylcholine occurred only at the BG-12 concentration of 10^{-6} M; this was observed as an increase in size of A, which could lead to a change in the frequency of the action of the lateral diffusion of the phospholipids in the cellular membrane.

Proceeding from dependence (1):

$$\nu = 2\sqrt{\frac{3D}{A}} \qquad (1)$$

where - the frequency of the action of the lateral diffusion, D - diffusion coefficient, A – the area falling on one molecule of the phospholipid; it is possible to draw a conclusion on the reduction in the frequency of the lateral diffusion by the increase in the area falling on one molecule of the phosphatidylcholine. As observed from [5], the increase in size of A shows a potential difference in the reduction on the monolayer as on the condenser and corner facings, under which the phosphatidylcholine molecules are located at the monolayer surface. At the point where the viral membrane merges with the cell membrane, superficial antigenic glycoproteins of the flu virus are noted to be distributed on the membrane by lateral diffusion; however, because of the reduction in the number of actions of lateral diffusion it does not occur. The membrane appears to be in an «unprofitable» condition with respect to superficial energy. It leads to a bending of the membrane inside the cell and subsequently causes its damage. Any violation of the morphological integrity of the virus leads to a decrease in its infectious activity, until its total disappearance.

A reduction in the frequency of the actions of lateral diffusion and a reduction in the corner of the arrangement of the phospholipid molecules in relation to the superficiality of the monolayer lead to conformational changes of the phosphatidylcholine molecules in the monolayer, which results in the impossibility of the merger of the viral membrane and the cell membrane. Apparently, as observed by us in the earlier studies, the inhibition action is caused by the specified conformational

changes in the flu virus membrane.

The following investigation phase was done to study the interaction of BG-12 with the virion RNA – dependent RNA - polymerase by means of determination of the enzyme activity. The kinetic dependences of enzyme activity (Fig. 3) were received.

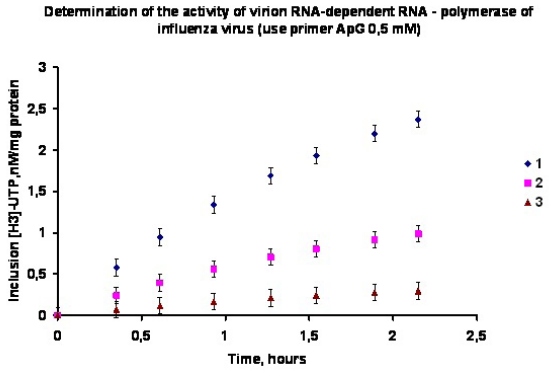


Figure 3

Curve 1 control of the enzyme; curve 2 – addition of BG-12 in the final concentration of 5 mkg/mL; curve 3 – addition of BG-12 in the final concentration of 10 mkg/mL.

From the results received, it is possible to conclude that the preparation processes inhibit the activity related to the virion RNA - dependent RNA – polymerase at concentrations of 10 mkg/ml and 5 mkg/mL, causing the enzyme activity to decrease dependably, [3H] UTP being determined by the inclusion.

The decrease in the enzyme activity can be due to the BG-12 interaction with its active center.

In future, it is necessary to further study the kinetic mechanism of the BG-12 enzyme inhibition (virion RNA - dependent RNA - polymerase).

Conclusion

Thus, the antiviral activity of the derivative 1-bromoadamantane BG-12, can be connected with the conformational changes of the membrane of the enveloped viruses. It provides universality of this preparation when used in the treatment of infections caused by enveloped viruses, as the phospholipid structure of the viral membrane, unlike the ones of the antigenic viral proteins, remains unchanged in the course of the evolution of the enveloped viruses.

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