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MECHANISM OF ACTION OF MACROCYCLIC ANTIBIOTIC FILIPIN ON CELL AND LIPID MEMBRANES

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The mechanism of action of macrolide pentaene antibiotic filipin on cell and bilayer lipid membranes was investigated. The sterol hypothesis of the filipin mechanism of action is confirmed experimentally. The data of filipin action on bilayer lipid membranes (BLM) are presented. Single ionic channels with conductance of 15—20 pS were showed on cholesterol-containing membranes by the action of filipin at low concentration ($1 \cdot 10^{-8}$ M). Combined ionic channels of filipin and amphotericin B with conductance of 25—30 pS, that by1.5—2 times lower than of the clean filipin channels and by 5 times more than of the clean amphotericin B channels were discovered. The selectivity of filipin channels is mainly cationic. The potential of the penetrating ion on 10-fold gradient is +18 mV. It is shown the practical aspects of filipin application in medicine and pharmacology.

***Keywords**: polyene antibiotics (PA), filipin, bilayer lipid membranes (BLM), ionic channels, filipin-cholesterol complexes, conductivity of membranes*

Introduction

Polyene antibiotics (PA) are most efficient substances at the treatment of fungal infection [1]. Their application bases on detail study of molecular and biological mechanism of their interaction with cell membrane. There is known more than 200 representatives of PA group [2]. Among them nystatin, amphotericin B,mycoheptin and levorin. It is determined that PA have membrane-active function[3]. PA interact with sterols located in lipid membranes and formed channels that permeable for ions and organic substances [2]. Nevertheless molecular aspects of PA-membrane interaction is unknown yet. The relationship between structure and function of PA molecules in lipid membranes is one of very important problems for molecular biology. Filipin is very interesting antibiotic as neutral one. There is information that this PA disrupts cell membranes [4]. However we search that this preparation increases conductivity of cell and bilayer membranes in small concentration(2,5). Mechanism of filipin action is based on the interaction between antibiotic and sterol which are located in cytoplasmic membranes and BLM. This interaction leads to the formation of ionic channels with molecular sizes that are permeable for ions and organic substances. There was detected monotonic kinetics of increasing of conductivity in membrane without inactivation for PA-filipin (Fig.2).

Chemical structure and chemical characteristics was shown on Fig.1.and Table. This article is described physical and chemical properties of filipin and mechanism of action on the lipid membranes.

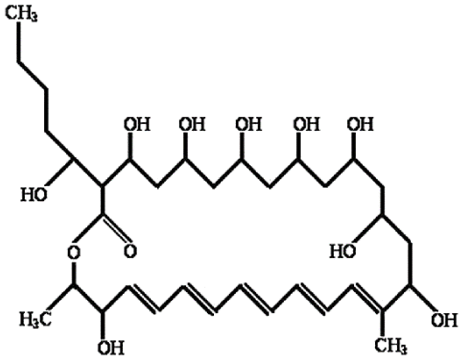

Fig.1. Chemical structure of filipin

Table

Chemical characteristics of filipin

Chemical name: *[3R-[3R*(R*),4S*,6S*,8S*,10R*,12R*,14R*,16S*,17E,19E,21E,23E,25E, 27S*,28R*]]-4,6,8,10,12,14,16,27-Oktahydroxi-3-(1-hydroxylhexil)-17,28-dimethyloxatsiklooktoza-17,19,21,23,25-pentaene2-1*

Classification: Oxacyclooktozan; 14-Deoxilagozin;15-Deoxilagozin;Filimarizin

Nomenclatural number: 480-49-9:

Chemical composition: $C_{35}H_{58}O_{11}$

Molecular mass: 654,83

Materials and methods

Filipin was produced by soil actinomycetes (*Streptomyces filipensis*). The total phospholipids were extracted from bull brain by the chloroform- methanol solution (initial concentration 20 mg/ml) and stored in heptane. The main part of this work was connected with membrane from total phospholipids extracted from bull brain. For membrane preparation the mix of membrane solutions with cholesterol and its derivatives in different ratios was used. Before experiments initial membrane mixtures transformed to heptane solution by evaporation and then formed BLM. Filipin was kindly provided by Scientific-Research Institute of antibiotics and enzymes (St.Petersburg, Russia). Filipin in the concentration of 1 mg was dissolved in 1 ml dimethylsulfoxide (DMSO) and then added to salt-water solution. Antibiotic's solution was updated every week. It is provided the storage and efficiency of antibiotics and stability of results. The research of integral conductivity and measurement of membrane potential was done by the comparison of voltage decrease of equivalent and membrane.

The research of filipin activity on bilayer lipid membranes

Filipin in concentration $4x10^{-5}$M decreases the life-time (stability) of the membranes with the same molar quantity of lecithin and cholesterol. In this case life-time is 5 min, but the life-time of cholesterol-free membranes is 60 min [4]. It was supposed that filipin-cholesterol interaction doesn't give pore-formation but ruptures of cell membranes[5]. Stoichiometry of filipin-cholesterol interaction and formation of filipin-cholesterol complexes shows that one filipin molecule associates with the one molecule of cholesterol. By this assumption hydrophobic part of cholesterol interacts with double bonds system of filipin molecule in bilayer. There are two possible ways of filipin-cholesterol orientation in membrane. By the first version hydroxyl groups of antibiotic molecule may be located on the membrane-water surface, but double bonds system of filipin molecule interacts with the cholesterol and located in parallel to bilayer surface. According the second version filipin-cholesterol complex may settles down perpendicularly to bilayer surface [3-5].

In this case hydrophilic side of filipin molecule takes place in hydrophobic part of membrane and interacts with the hydrophilic part of the second complex and there is formation of some of these complexes. Absence of the charged groups in filipin molecule may possible lateral dislocation of every filipin-cholesterol complexes[4,5] and this is the reason of membrane rupture. Nevertheless detail study of this antibiotic shows that membranes are enough stabile during two hours at the membrane potential 200 mV after treatment by filipin [5].Filipin in concentration of $2x10^{-6}$M from the both sides of membrane increases conductivity by some orders but doesn't increase conductivity of membranes if it is added with one side of membrane in concentration $4x10^{-5}$M.

There are researched kinetics of conductivity and selective permeability of membranes at the addition of pentaene PA-filipin to membranes(Fig.2).

It is showed the monotonic kinetics of increasing membrane conductivity with the exit on stationary level without further inactivation takes place for the filipin[5]. There is shown the kinetics of increasing conductivity of membranes modifying by filipin (Fig.2). The increasing of membrane conductivity depends of ion types in solution and doesn't depend from the value of membrane potential. The membrane current follows consecutive changes of conductivity in the time. There is also fixed the increasing of conductivity if on the one side of membrane there is filipin but on the another side –nystatinA_1 or amphotericin B. There are detected single ionic channels at the small concentrations of filipin in the salt water solution ($1x10^{-8}$M) on the cholesterol-containing membranes. Conductance of these channels is 15-20 pS, it is more than by 3-4 times in comparison with conductance of amphotericin B channels (Fig.3). It is researched that filipin and amphotericin B channels have two main states: conducting and non-conducting. During life-time of the channel in membrane you can see short-time transitions from conducting state to non-conducting one. At lower concentration of amphotericin B and filipin were fixed the formation of combined ionic channels in the membranes. Research of filipin shows that addition of this antibiotic to one side and amphotericin B or nystatin to another side in the equal concentrations ($1x10^{-6}$M) leads to the interaction between antibiotics and also increasing of integral conductance of membranes. Conductivity of filipin –amphotericin B combined ionic channels is 25-30 pS. İt is more than 1,5-2 times in comparison "pure" filipin channels and by 5 times in comparison with "pure" amphotericin B channels. Selectivity of filipin is cationic (+18 $\pm$ 2 mV).

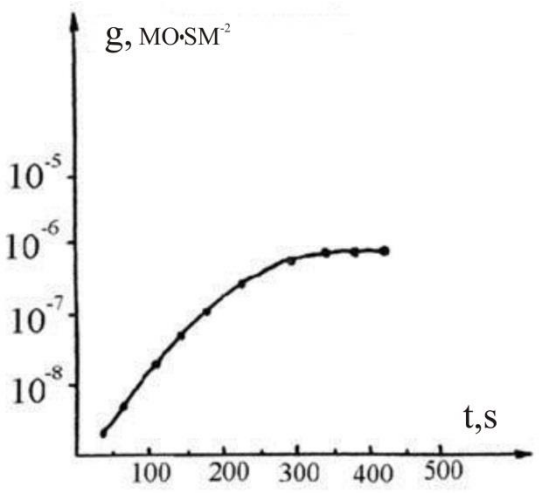

Fig.2.The kinetics of conductance of BLM at the presence of filipin in concentration $1x10^{-6}$M.Membrane composition: phospholipid:cholesterol=2:1; 2MKCl, pH=7,0; $t^0=22^0$C. Membrane potential=200mV.

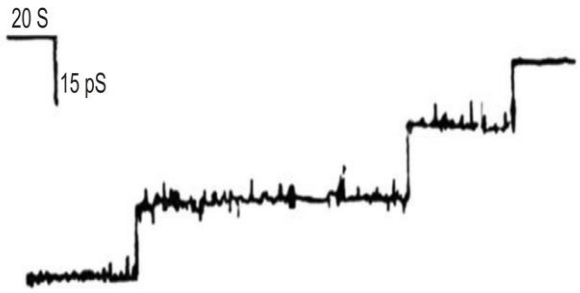

Fig.3. Single filipin ionic channels in BLM. Membrane composition: phospholipid –cholesterol 20:1; water solution - 2M KCl, pH=7,0; $t^0=22^0$C. Membrane potential=200mV. Concentration of filipin – $2x10^{-8}$M.

Conclusion

Application of PA as antifungal drugs in practical medicine is based on the study of molecular and biological properties of these antibiotics in cell and bilayer membranes. PA are membrane-active substances and increase ion conductivity of membranes.

Neutral PA filipin has particular place and position. This drug increase conductivity of cell and bilayer membranes in small concentrations. Mechanism of filipin action is based on the interaction between antibiotic and sterol which are located in cytoplasmic membranes and BLM. This interaction leads to the formation of ionic channels with molecular sizes that are permeable for ions and organic substances. There was detected monotonic kinetics of increasing of conductivity in membrane without inactivation for PA-filipin. There were detected filipin-sterol complexes in plasmatic membranes of different cells. Study of filipin conductivity in BLM showed that single ionic filipin channels have conductivity 15-20 pS but com-

bined ionic channels amphotericin B-filipin-25-30 pS[5]. We would like to notice that filipin has large spectrum for usage in pharmacology and medicine. The research of this preparation continues and gives the new possibilities for its application in future.

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MAKROSİKLİK ANTİBİOTİK FİLİPİNİN HÜCEYRƏ VƏ LİPİD MEMBRANLARA TƏSİR MEXANİZMİ

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Təqdim edilən məqalə pentaene makrolid antibiotic filipinin hüceyrə və ikiqat lipid membranlara təsir mexanizmi barədə məlumat verir. Elmi tədqiqatlar (ion kanalları, inteqral keçiricilik, seçicilik və birləşdirilmiş ion kanalları) "sterin" nəzəriyyəsini sübut etmişdir. Aşkar edilmişdir ki, filipin antibiotikin keçiriciliyi ikiqat lipid membranlarda 15-20 pS. Filipinin iştirakı ilə membranın birləşdirilmiş filipin-amfoterisin ion kanalları – 25-30 pS, elektrik potensial isə +18 mV təşkil edir. Filipin antibiotikin istifadəsi tibb və farmakologiya sahəsində nəzərdə tutulur.

Açar sözlər: *polien antibiotiklər (PA), filipin, bimolekulyar lipid membranları (BLM), ion kanalları, filipin-xolesterin kompleksləri, keçiricilik*

МЕХАНИЗМ ДЕЙСТВИЯ МАКРОЦИКЛИЧЕСКОГО АНТИБИОТИКА ФИЛИПИНА НА КЛЕТОЧНЫЕ И ЛИПИДНЫЕ МЕМБРАНЫ

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Данная статья посвящена исследованию механизма действия макролидного пентаенового антибиотика филипина на клеточные и бислойные липидные мембраны. Экспериментальные данные (зафиксированные ионные каналы, интегральная проводимость, селективность, комбинированные ионные каналы) подтверждают «стериновую» гипотезу, основанную на взаимодействии полиеновых антибиотиков (ПА). Показано, что одиночные ионные каналы филипина в БЛМ обладают проводимостью в 15-20 пС, а гибридные ионные каналы, образованные филипином и амфотерицином в 25-30 пС. Избирательная проницаемость мембран в присутствии филипина составляет +18 мВ. Рассматриваются практические аспекты использования филипина в медицине и фармакологии.

Ключевые слова: *полиеновые антибиотики (ПА), филипин, бислойные липидные мембраны (БЛМ), ионные каналы, филипин-холестериновые комплексы, проводимость*