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STUDY OF PHYSICAL AND CHEMICAL PROPERTIES OF ION CHANNELS CREATED BY POLYENE ANTIBIOTICS IN LIPID MEMBRANES

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Abstract. Beneficial to achieve the goals set in the research process, the following research activities planned. We studied the ultraviolet absorption spectrum of the complex formed by polyene antibiotics at different concentrations separately and stable with cholesterol. We studied of the properties of ion channels formed conclusion unilateral action of antibiotics and used expulsion of alkyl derivatives of amphotericin B and levorin from membranes by perfusion and study of reduction of membrane integral permeability by kinetic relaxation method. We investigated of physical and chemical properties of single ion channels formed in membranes under the influence of antibiotics of different structures and compare effects of antibiotics on bimolecular and cell membranes.

Keywords: *polyene antibiotics, amphotericin B, cell membranes, ion channels, sterol.*

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1. Introduction

The development of selective permeability for ions and organic compounds in membranes is due to antibiotics synthesized by some microorganisms. Recently, attempts have been made to differentiate pharmacological drugs used in clinical practice according to their end-effects, regardless of the mechanism of action. However, given the different mechanisms of action of the drug, there are extensive problems with their combined use. At present, there is a tendency that the search for new drugs should have a wide range of pharmacological properties. (Kamiński, 2014).

One of the most necessery challenges in the world's leading countries in the development of new drugs that have a highly selective effect against viral, bacterial and fungal infections. The essential purpose is to detect compounds with high biological activity against pathogenic microorganisms. Currently, there is a tendency that a new drug should have a wide range of pharmacological properties (Pashazade, 2020). First of all, they must show their sensitivity to membranes. Therefore, research on this problem should be conducted on membranes. Polyene antibiotics are one of the most effective compounds used in medicine against fungal infections. The bimolecular lipid membrane method is to be used to determine the biological activity of antibiotics. For the first time, a classification of the interaction of polyene antibiotics with membranes depending on their molecular structure was given and their synthesis was theoretically substantiated. By studying the interaction of polyene antibiotics with membranes, it has opened the way for the synthesis of new drugs. (Cavassin *et al*., 2021).

The parameters of single ion channels - selectivity, conductivity, a lifetime of open and closed channels in membranes - are regulated by structural modification of

polyene molecules. It should be noted that the mechanism of selective conduction of ions from polyene channels remains unresolved (Pashazade, 2019). According to a group of scientists, the molecular system that regulates the selective conductivity of ions is located inside the channel and is in the amine or carboxyl groups. According to other authors, the molecular system that regulates the selective conductivity of ions is located in the middle of the channel in the hydrophilic part. In our opinion, the molecular system that regulates the selective conductivity of ions is located in the hydrophilic chain and depends on the number of hydroxyl or carbonyl groups. It is clear that chemically modified antibiotics can provide us with very important information about the channels. PA is one of the most effective compounds used in medicine against fungal infections. Some PAs have the ability to destroy tumor cells. Positive results were obtained with the use of levorin and its derivatives. It depends on the strength of the antibiotic that inhibits the growth of tumor cells. One of the main reasons that prevent the growth of tumor cell strains is the increased permeability to ions in the membranes. Determination of the selective permeability of ions and organic compounds across membranes under the influence of polyene antibiotics using physicochemical methods. One of the main tasks of the work is to study the interaction of PA with membranes, to prove the formation of a complex with cholesterol, and to determine the duration of activity of polyene alkyl derivatives on membranes.

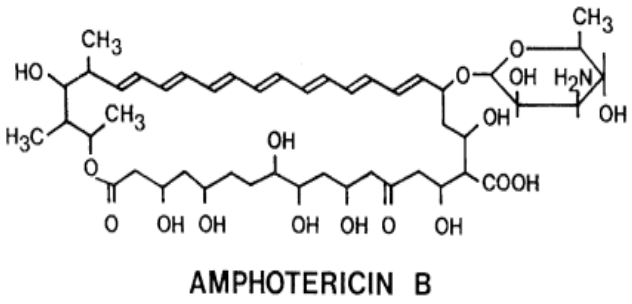

Fig. 1. Chemical structure of the polyene antibiotic AmB

2. Materials and methods

Lipid membranes are to be used to study the effect of PA on membranes at the molecular level. Bimolecular lipid membranes (BLM) are made of phospholipids present in the brain tissues of large and small horned animals. It is one of the methods of reflecting the formation of lipid membranes by inserting phospholipids in the hollow part of a glass made of Teflon material. This method showed that PA is highly sensitive to sterols in the membranes. Polyenes interact with sterols to form molecular-sized ion channels in membranes, and the physicochemical properties of these channels have been studied by the patch-clamp method. (Samedova *et al*., 2018).

Membrane leaching of the antibiotic by the perfusion method and the reduction of membrane permeability has observed by the kinetic relaxation method. Ultraviolet spectra of amphotericin B, levorin, and dimethyl sulfoxide molecules were obtained using T92 + UV / VIS Spectrometer. The purposeful synthesis of polyene antibiotics and the study of their physicochemical properties in membranes opens a wide way to define theoretical ways and create new antibiotics. All experiments were performed at a

room temperature 23^0C. The stock AmB solution was obtained by dissolving AmB in DMSO. The conclusive pH was calibrated with a pH mini-electrode. Changes in pH were conducted by addition of small volumes of concentrated solutions of either HCl or KOH. The analysis was made using pClamp 8.2.

3. Results

PA is characterized by a maximum of three absorptions due to the presence of a double-bonded molecule in the chromophore part in water and organic solutions. Changes in the UV absorption spectrum observed during the interaction of PA with cholesterol. (Pashazade, 2021). An increase in the number of double bonds in a polyene molecule causes a change in the UV absorption spectrum. The addition of cholesterol or other sterols to an antibiotic solution leads to a decrease in the UV absorption spectrum, resulting in the formation of a complex between cholesterol and PA. The presence of sterols does not change the UV absorption wavelength of polyenes and only changes the maximum UV absorption. It has been shown that the addition of cholesterol to the aqueous solution of the Philippines changes the UV spectrum, but this member of the spectrum does not change in the solvents. No change in the UV spectrum occurs when cholesterol is added to an organic solvent. According to the effectiveness of interaction with cholesterol, PA is in the following order: Philippine> amphotericin B> etruscomycin> pimarisin> nystatin. The structure of sterols often determines their interaction with polyenes. Thus, sterols containing the 3β-OH group are more sensitive to polyenes than sterols containing 3α-OH or 3-keto groups. For polyenes to interact with sterols, the antibiotic C17 must form a hydrogen bond with the 3β-OH group of the sterins molecule. The maximum UV absorption depending on the number of double bonds of the polyene.

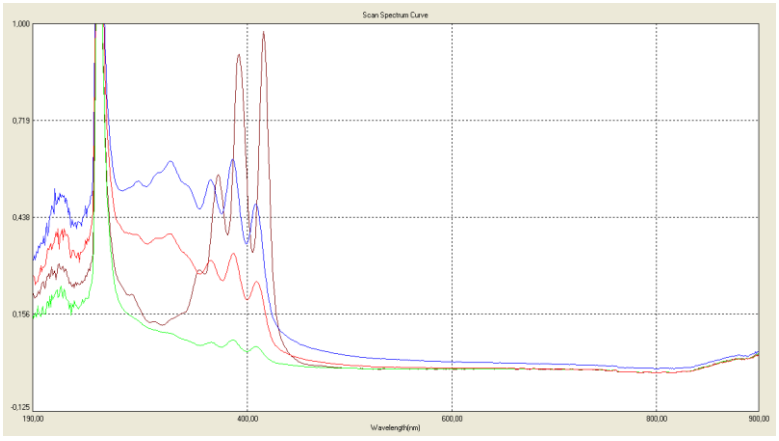

Fig. 2. UV absorption spectra of interaction of amphotericin (AmB) with B cholesterol (Pashazade, 2021)

In Figure 2, a dark red line - UV absorption spectrum was obtained by adding 0.03 ml of amphotericin B dissolved in 1 mg/ml DMSO to 3 ml of DMSO in the first tub. 3 ml of ethanol solution was added to the second tub. The blue line in Figure 2 shows that the UV absorption spectrum was obtained by adding 0.03 ml of amphotericin B dissolved in 1 mg/ml DMSO to the first tub and 0.5 mg of cholesterol in 3 ml of DMSO. 3 ml of ethanol solution was added to the second tub. In Figure 2, a bright red line - UV absorption spectrum was obtained by adding 0.03 ml of

amphotericin B dissolved in 1 mg/ml DMSO to the first tub and 1 mg of cholesterol in 3 ml of DMSO. 3 ml of ethanol solution was added to the second tub. In Figure 2, the green line - UV absorption spectrum was obtained by adding 0.03 ml of amphotericin B soluble in 1 mg/ml DMSO to the first tub and 2 mg of cholesterol in 3 ml of DMSO. 3 ml of ethanol solution was added to the second tub (Pashazade, 2021).

The results in Figure 2 show that amphotericin interacts with B cholesterol to reduce the concentration of amphotericin B, which is reflected in the UV absorption spectra.It is known that DMSO molecules facilitate the delivery of drugs from biological membranes to the cell. However, the effects of DMSO on membranes have not yet been fully studied (Lee *et al*., 2016).

It recently has been shown by molecular-dynamic modeling that DMSO creates water pores in biological membranes (Pinisetty *et al*., 2012). The effect of DMSO on the diffusion of Ca2 + ions from cell membranes has been studied(Jacl et al., 2014). The increase in DMSO-induced Ca2 + permeability did not alter the increase in Ca2 + permeability due to the effects of K-channel blockers and K-Na-ATF-aza. This means that water pores form in the cell membranes induced by DMSO, and Ca2 + ions are transferred to the cells through these pores. In addition, the permeability of Ca2 + ions increases significantly due to the high concentration of DMSO, which indicates the selectivity of water pores induced by DMSO. Thus, these studies suggest that DMSO can induce water pores in cell membranes and, in turn, facilitate the transport of biologically active substances to cells (Pashazade, 2021).

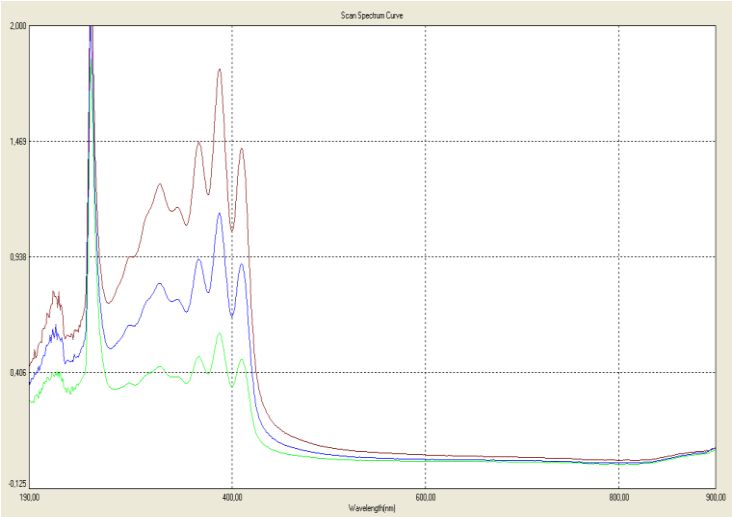

Fig. 3. UV absorption spectra obtained from the interaction of levorin with cholesterol (Pashazade, 2021)

In Figure 3, a dark red line - 0.03 ml of levorin solution dissolved in 1 mg/ml DMSO added to 3 ml of DMSO in the first tub. 3 ml of ethanol added to the second tub.

The blue line in Figure 3 - 0.03 ml of levorin solution dissolved in 1 mg/ml DMSO and 1 mg of cholesterol in 3 ml of DMSO added to the first tub. 3 ml of ethanol added to the second tub.

The green line in Figure 3 - 0.03 ml of levorin solution dissolved in 1 mg/ml DMSO and 3 mg of cholesterol in 2 ml of DMSO added to the first tub. 3 ml of ethanol added to the second tub.

As shown in Figure 2 and Figure 3, amphotericin B and levorin form a complex with cholesterol, reducing the maximum amplitude of UV absorption spectra. Increased cholesterol further lowers the maximum of the UV absorption spectra. The results show that cholesterol molecules combine with the double communication systems of amphotericin B and levorin to gradually lower the maximum absorption spectra of UV. These studies confirm the complex formation of amphotericin B and levorin with cholesterol and the molecular model of the channel (Kaminski, 2014; Cavassin *et al*., 2021).

We examined at the single-channel level the effects of pH changes on the biophysical properties of AmB channels inserted in bilayer lipid membranes (Figure 4). Our information showed the pH-induced modulation of AmB channels open probability, and conductance. Although the ion selectivity did not change. The acidity greatly decreased the open probability of the channel. The capacitance of the bilayers was smaller in a solution of lower pH than in a solution of medium pH. We discovered that AmB channels become even more cationic selective in the acidic pH and can even change to anionic in the alkaline pH. This information is useful to sufficiently comprehend the mechanism of action of AmB and the toxic side effects manifestations. Single-channel current amplitude was decreased after lowering pH in the voltages of 40 and 20. The single-channel current did not change by expanding pH from 7 to 8. Theoretical current-to-voltage (I/V) relationships were obtained for currents at three different pHs, and single-channel conductance for each pH was calculated from the slope of the I/V curves. The conductance for pH 7 and 8 were 505 and 570 pS. By reduction of cis pH to 6, the single-channel conductance decreased to 240 pS. As seen in this figure the reversal potential in the pH of 6, 7, and 8 were 36, 37, and 40 mV. The calculation of ionic selectivity founded on the middle values of related reversal potentials indicated a highly cation-selective channel. By changing the pH between 6-8 the ion selectivity did not significantly change.

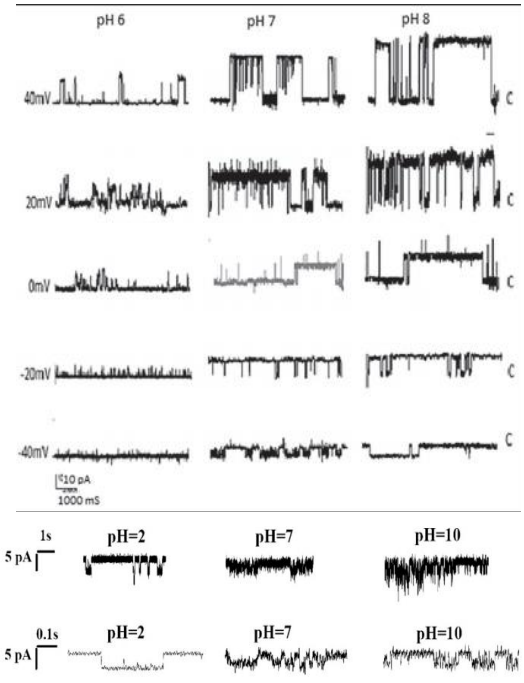

Fig. 4. Typical current recordings of single ion channels formed by amphotericin B in bilayer membranes measured at different potential

4. Conclusion

In significance, we conclude that:

The ultraviolet spectra of amphotericin B and levorin show that amphotericin B differs from 370 nm to 420 nm and levorin from 368 nm to 410 nm with three essential absorption spectra, which are due to the presence of a chromophore chain in the molecule. Amphotericin B and levorin complex with cholesterol reduce the maximum amplitude of UV gain spectra. The results show that cholesterol molecules combine with the double communication systems of amphotericin B and levorin to reduce the maximum amplitude of UV gain spectra. The ultraviolet spectrum of dimethyl sulfoxide molecules was obtained. Its gain spectrum ranges from 240 nm to 250 nm. The gain spectrum of dimethyl sulfoxide molecules in these waves is due to the presence of the disulfide S = O group (Pashazade, 2021).

The pH-value dependent reversal potential measured under such condition is an indicator of the fact that pH modulates of the selectivity of AmB channels, so that AmB oligomers are mostly anionic-selective in low-pH solutions and cationic-selective at neutral and alkaline solutions. By reduction of pH to 6,2, the open probability significantly decreased in all voltages.

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