

Antifungal Macrocyclic Antibiotic Amphotericin B—Its Present and Future. Multidisciplinary Perspective for the Use in the Medical Practice

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Abstract—This review is devoted to a broad analysis of the results of studies of the effect of macrocyclic antifungal polyene antibiotic amphotericin B on cell membranes. A detailed study of polyenes has shown that some of them can have not only antifungal, but also antiviral and antitumor effects. Under conditions of global pandemic fungal pathology develops especially quickly and in this case leads to invasive aspergillosis, which contributes to the complication of coronavirus infection in the lungs and even secondary infection with invasive aspergillosis. The treatment of an invasive form of bronchopulmonary aspergillosis is directly related to the immunomodulatory and immunostimulating properties of the macrocyclic polyene drug amphotericin B. The article presents experimental data on the study of the biological activity and membrane properties of amphotericin B and the effect of its chemically modified derivatives, as well as liposomal forms of amphotericin B on viral, bacterial and fungal infections. The mechanism of action of amphotericin B and its analogues is based on their interaction with cellular and lipid membranes, followed by formation of ion channels of molecular size in the membranes. The importance of these studies is that polyenes are sensitive to membranes that contain sterols of a certain structure. The analysis showed that pathogenic fungal cells containing ergosterol were 10–100 times more sensitive to polyene antibiotics than host cell membranes containing cholesterol. The high sterol selectivity of the action of polyenes opens broad prospects for the use of polyene antifungal drugs in practical medicine and pharmacology in the treatment of invasive mycoses and the prevention of atherosclerosis. In this context, it should be noted that polyene antibiotics are the main tool in the study of the biochemical mechanism of changes in the permeability of cell membranes for energy-dependent substrates. Chemical and genetic engineering transformation of the structure of polyene antibiotic molecules opens prospects for the identification and creation of new biologically active forms of the antibiotic that have a high selectivity of action in the treatment of pathogenic infections.

Keywords: polyene antibiotics (PA), amphotericin B, chemical modification

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INTRODUCTION

The study of amphotericin B started in the 60s of the last century, when it was discovered that macrolide polyene antibiotics (PA) could act as fungicidal drugs applicable in practical medicine as drugs against fungal diseases [1]. Moreover, despite the fact that all PAs possess these properties, some of them are the most effective pharmacological agents. These include amphotericin B, levorin, nystatin, trichomycin and candidine, which have a wide range of applications. The study of the biophysical and biochemical properties of PA has shown that these compounds have membrane activity. This is manifested in an increase in ionic conductivity across cell membranes, as well as in different ionic selectivity of these antibiotics [1, 2]. An increase in the ionic conductivity of membranes in the presence of PA is connected with the interaction of the antibiotic with the sterol component of the cell

membrane, which leads to impairments in the membrane structure due to formation of ion pores or channels. As a result, the conductivity of the membrane increases, because of increased flow of ions and low molecular weight compounds. This fact, to a certain extent, suggests that the creation of ion channels in the presence of PA contributes to the effectiveness of the biopharmaceutical properties of these antibiotics [1, 2]. In contrast to animal cells containing cholesterol as a sterol component, fungal cells contain ergosterol. For several decades, PAs have been used in medical practice as fungicidal drugs, however, a number of factors, such as certain toxicity and increasing resistance to these drugs, began to limit the range of their application [3, 4]. The search for new effective drugs at the beginning of the XXI century was performed on the basis of chemical transformation of molecules and genetic engineering, which led to the emergence of

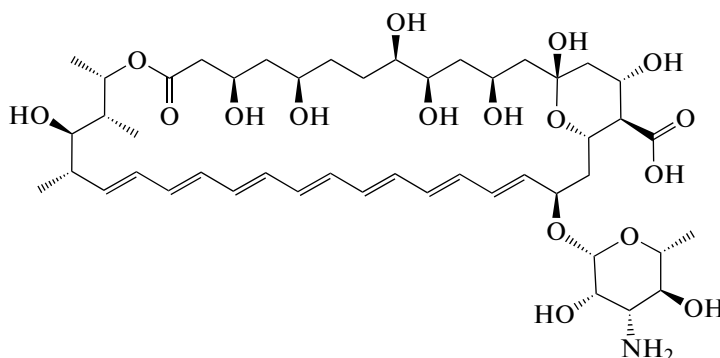


Fig. 1. Chemical structure of amphotericin B.

new generation antibiotics [5]. Experiments have shown that chemically modified PA analogs are more effective against a number of pathogenic diseases, especially candidiasis, the main fungal infection that causes many diseases. A multifaceted study of PA showed that some of them could have not only antifungal, but also antiviral and antitumor effects [6–8]. Studies have shown that among polyene macrolides, amphotericin B, as the most effective and studied drug of this group, can be widely used in medical practice.

1. AMPHOTERICIN B—EFFECTIVE ANTIFUNGAL DRUG. APPLICATION IN PRACTICAL MEDICINE

Representatives of a large class of PA (more than 200 drugs) are products of lower plant organisms of the genus *Streptomyces* or *Actinomyces*. Amphotericin B was isolated from a strain of the microorganism *Streptomyces nodosus* by Gold et al. in 1956 [9, 10]. All antibiotics of this group of drugs have a macrolide ring with hydrophilic and hydrophobic parts of the molecule in their structure. The hydrophilic chain of amphotericin B contains several hydroxyl groups, and the hydrophobic part contains 7 conjugated double bonds. Therefore, this antibiotic belongs to the heptaene subgroup. The molecular chemical structure of amphotericin B is shown in Fig. 1.

1.1. The Amphotericin B Status among Macrocyclic Drugs of the Polyene Group of Antibiotics (PA)

For many decades, amphotericin B has been the main PA used in antifungal therapy for various types of mycoses. The mechanism of its fungicidal action is explained by its interaction of the antibiotic-sensitive fungus with the cell membrane ergosterol. This results in membrane integrity damage followed by increase membrane permeability, and release of intracellular components into the extracellular space and lysis of fungal cells [1, 11, 12]. Based on these facts, it turned out to be possible to modify the antibiotic molecule to

reduce toxicity and develop more effective low-toxic derivatives, as well as to study the mechanism of ion channel formation. From a chemical point of view, amphotericin B belongs to the subgroup of non-aromatic heptaene polyene macrolide compounds (Fig. 1). For a long time, it was used for treatment of candidiasis of various etiologies and mold mycoses [13]. The antibiotic is especially effective for treatment of cryptococcal meningitis, coccidioidomycosis, leishmaniasis, cystic fibrosis, and amoebiasis [14, 15]. During systemic use, it is active, acting on mycelial, yeast-like and dimorphic fungi. When compared with other PA, such as nystatin, pimaricin, partricin, etc., amphotericin B is the only one used for systemic and invasive mycoses [2]. Treatment with amphotericin B is very effective for ophthalmomycosis of fungal etiology, such as keratitis caused by filamentous fungi [16–19], as well as fungal diseases caused by *Candida*, *Aspergillus*, *Fusarium*, *Cryptococcus*, *Mucorales*, *Scedosporium*, *Paecilomyces* [20, 21]. It should be noted that amphotericin B plays a special role (mainly in the treatment using lipid-associated forms of amphotericin B) in treating pulmonary mycoses, especially in aspergillosis, which is the cause of 30% of deaths in patients with aspergillosis and infected with coronavirus; the latter is especially important during a pandemic [22–25]. Treatment with amphotericin B is also very effective in invasive mycoses, such as histoplasmosis, blastomycosis and fungal meningitis, which have local foci of infection [22, 26].

1.2. The Use of Amphotericin B in Medicine. Effectiveness, Toxicity, Resistance. Immunomodulating and Immunostimulating Properties. Features of Its Use for Treatment of Candidiasis

As noted above, among PAs amphotericin B is a very popular pharmacological agent used in medical practice. It is effective against many systemic and invasive mycoses. Fungal infections dramatically weaken the immune system and contribute to immunodeficiency [4, 21, 22]. In the clinics, invasive myco-

ses are concomitant factors of many infectious and oncological diseases [20, 22, 27]. It should be noted that the use of amphotericin B in combination therapy gave quite effective results [28, 29]. However, the systematic use of amphotericin B has revealed a number of factors that limit its clinical application. These include nephrotoxicity, hematotoxicity, poor solubility of the drug in water, as well as antibiotic resistance, reducing the effectiveness of amphotericin B. It is believed that the mechanism of toxicity consists in the binding of PA to cell membrane lipoproteins [3, 4, 22, 30–32]. To eliminate these side effects, a search has been started for highly effective drugs with lower toxicity [5]. According to experimental data, modification of the PA molecule in the region of polar groups is one of the solutions to this problem. For example, amphotericin B methyl ester and nystatin methyl ester are approximately 250 times less toxic than the parent antibiotics [33]. The most common types of mycoses are Candidiasis and Aspergillosis infections. For a long time, amphotericin B has been used in diseases caused by these pathogens [13, 34]. The acquired resistance to amphotericin B is probably associated with changes in the steroid composition of the fungal cell membrane [35, 36]. Several gene mutations in ergosterol biosynthesis (*ERG* genes) are associated with the mechanism of amphotericin resistance. For example, in *C. albicans*, the loss of function of the *ERG11* and *ERG3* genes (encoding lanosterol 14-demethylase and C-5-sterin desaturase, respectively) leads to the ergosterol replacement for alternative sterols, such as lanosterol and 4,14-dimethylzymosterol in the fungal cell membranes [37, 38], but these negative factors, such as toxicity and antibiotic resistance, led to the search for new forms of the antibiotic. In the 21st century, new generation antibiotics were developed and tested against various types of Candidiasis and Aspergillosis infections.

Treatment of candidiasis and invasive forms of bronchopulmonary aspergillosis is directly related to the immunomodulatory and immunostimulating properties of amphotericin B, as new forms of this antibiotic and its derivatives significantly increase their antifungal activity and reduce toxicity as compared to the original drugs [13, 39–42]. Modification of the amphotericin B molecule at the carboxyl group of the macrolactone ring at position C16 or at the amino group of the amino sugar led to the creation of hybrid compounds of amphotericin B with high antifungal activity, especially in derivatives of this antibiotic, in which dimethylaminoethylamide was used at position C16 [5, 43, 44].

2. RESULTS OF BIOPHYSICAL AND MOLECULAR-BIOLOGICAL STUDIES OF AMPHOTERICIN B

The study of biophysical and biochemical properties of PA made it possible to study the mechanism of

permeability of cell membranes for ions and organic compounds. It was found that the presence of PA increased the conductivity of cell membranes. This is due to formation of ion channels (pores) in biological membranes, which are the result of the interaction of the antibiotic and sterol of cell membranes. The antibiotic-sterol complexes are an integral part of functional ion channels. The study of the biological activity of the antibiotic, the mechanism of the permeability of cell membranes, modified by PA, and the functional and structural organization of ion channels makes it possible to transform the antibiotic molecule for its more efficient use [1, 22].

2.1. Membrane Activity of Amphotericin B. Integral Membrane Conductivity and Selectivity

The classical molecular model of the ion channel as a conductive structure of cell membranes was proposed by the Dutch scientists de Kruijff and Demel in 1974 [45]. Cholesterol was used as a sterol component, and amphotericin B was used as PA. Later, American scientists (Anderson et al.) slightly modified the classical model of the canal and used ergosterol instead of cholesterol, believing that it was a more sensitive sterol component for amphotericin [46]. Figure 2 shows both amphotericin B ion channel models. Both models demonstrate complex formation of amphotericin B with a sterol component in lipid membranes. The classical channel model (Fig. 2a) shows that the ion channel (pore) consists of two half-pores, each of which has the same number of antibiotic and sterol molecules. Figure 3 shows a schematic model of the half-pore ion channel of amphotericin B-cholesterol. According to this model, antibiotics interact with cholesterol to form half pores on both sides of the membrane. The half-pore radius is 4 Å. Two half-pores, located along a common axis across the membrane, form an ion channel (pore) that spans the membrane. This pore induces permeability in membranes for non-electrolytes, water and ions. Each semi-pore consists of 8 molecules of amphotericin B and cholesterol. In the complex, one amphotericin B molecule binds to two cholesterol molecules. The hydrophilic sides of the antibiotic are located inside the pore [45–48]. Two half pores formed on opposite sides of the membranes form an ion channel. Hydroxyl groups at C35 of one half-pore form hydrogen bonds with the corresponding groups of the other half-pore, forming a complete conducting pore through the entire hydrophobic part of the membrane. Figure 4 shows a modern molecular model of the channel formed by the interaction of amphotericin B with ergosterol and cholesterol in the cell membranes of fungi and mammals [22]. Amphotericin B and sterol molecules are approximately the same in length. Figure 4 shows the location of the mycosamine group in the molecule of amphotericin B and ergosterol in the lipid bilayer of the membrane. At the entrance to the channel, formed by amphotericin B and ergosterol, there is a hydroxyl

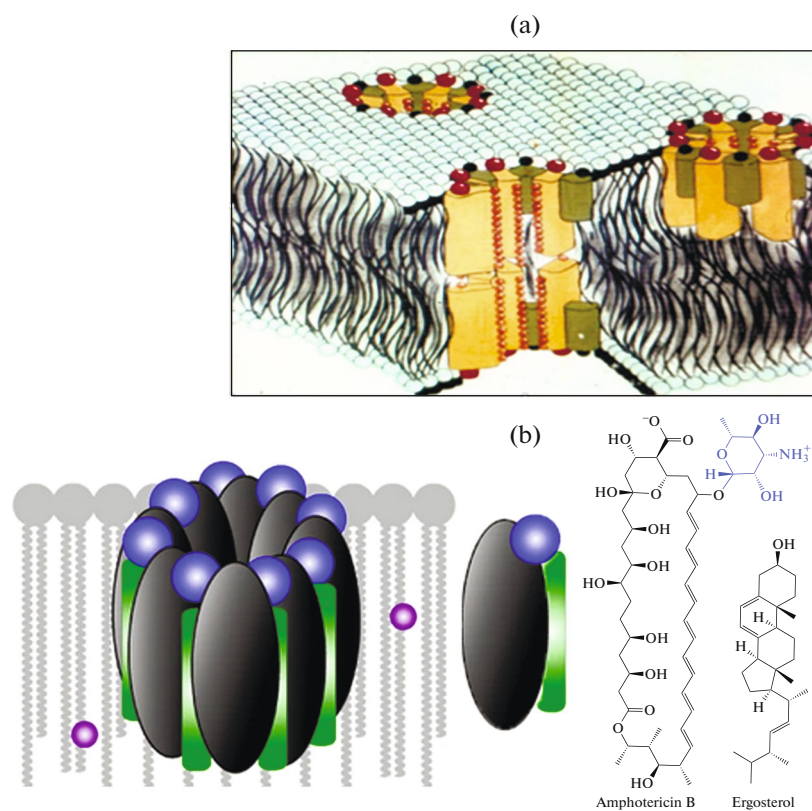


Fig. 2. Molecular models of the amphotericin channel: (a) the classical model of the amphotericin B–cholesterol channel (adapted from [46]). (b) The model of the channel amphotericin B–ergosterol (adapted from [46, 48]).

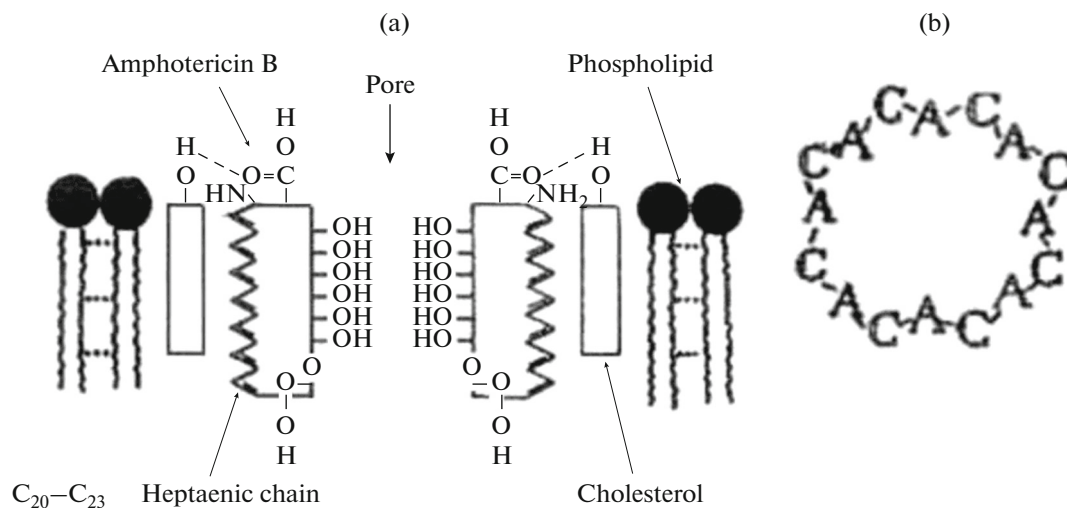


Fig. 3. Schematic model of the half-pore of the ion channel of amphotericin B–cholesterol. In the part of figure (b): C—cholesterol, A—amphotericin. In the complex, they form an ion-conducting channel (adapted from [45]).

group at C₁₅ of the antibiotic molecule. On the inner surface of the membrane, NH₂– and COOH– groups are localized. Hydrogen bonds are formed between the

OH group of the cholesterol molecule and the oxygen atom of the carboxyl COOH group of the antibiotic [1, 22, 46]. Since amphotericin B has a lethal effect on

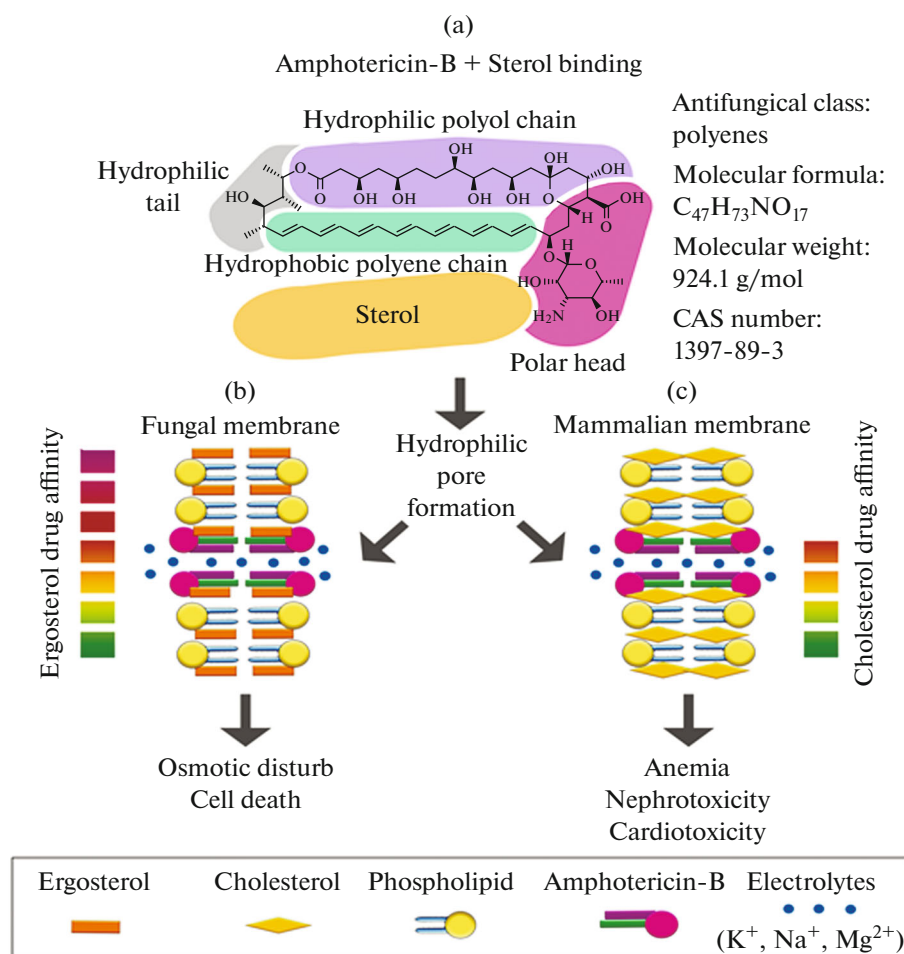


Fig. 4. Molecular model of the ion channel amphotericin B–ergosterol in the cell membrane of fungi and amphotericin B–cholesterol in the membrane of mammalian cells (Reproduced from [22] under the license CC BY-SA 4.0).

yeast-like fungi, it is very important to develop antibiotic derivatives with an improved therapeutic index [22].

2.2. Single and Combined Ion Channels of Amphotericin B

Chemical modification of charged groups of amphotericin B molecules, as well as blocking the charge of these groups by a shift in the pH of solutions with a high electrolyte concentration are not accompanied by a noticeable change in the conductivity of single channels [1, 49–51]. Figure 5 shows discrete changes in membrane current in the presence of amphotericin B. The selectivity of amphotericin single ion channels is the same as for multiple channels. The number of amphotericin channels does not depend on the value of the potential applied to the membrane (i.e. it is not a voltage-dependent parameter) [49–51]. Amphotericin B forms single ion channels with various types of sterols (cholesterol, ergosterol, stigmasterol). Sterol type does not change the anionic selec-

tivity of the amphotericin channel [1]. The most effective sterol is ergosterol. Combined ion channels of amphotericin B were obtained with some PA: levorin, nystatin, nystatin components and filipin [1, 49–51]. Figure 6 shows the combined ion channels of amphotericin B and filipin. The conductivity of the hybrid channels of filipin and amphotericin B is 25–30 pS, which is 1.5–2 times higher than the conductivity of “pure” filipin channels and about 5 times higher than the conductivity of “pure” amphotericin channels. Analysis of the structural and functional properties of amphotericin B makes it possible to perform target modification of the antibiotic molecule to obtain more effective compounds with desired properties.

3. CHEMICAL TRANSFORMATION OF AMPHOTERICIN B AND ITS ANALOGUES BY USING GENETIC ENGINEERING

Being metabolic products of lower yeast-like fungi, PA, like many other antibiotics, lose their biological

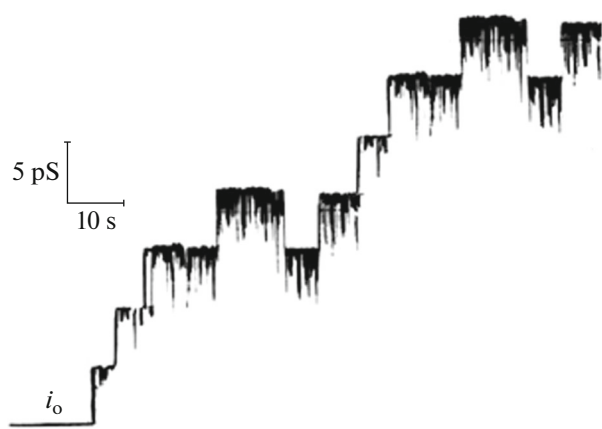


Fig. 5. Discrete changes in the membrane current in the presence of 2×10^{-7} M amphotericin B. The membrane potential was 100 mV. Membranes were prepared from a phospholipid : cholesterol solution in a volume ratio of 20 : 1. The aqueous solution contained 3 M KNO_3 , pH 6.5, $T = 23^\circ\text{C}$ (modified from [49]).

activity over time. In this context, the use of PA in medicine does not give the previous results, especially during the irrational use of antibacterial antibiotics with a wide spectrum of antifungal action, where the development of resistance to pathogens of deep mycoses is the main indicator [11, 52]. Highly effective derivatives of amphotericin B can be synthesized from the original natural antibiotic [53, 54]. The original amphotericin B (its clinical analogue, amphotericin B deoxycholate) is one of the most used antifungal macrolide antibiotics in practical medicine. Amphotericin B is the only PA approved for the use in systemic mycoses [22]. For the treatment of invasive mycoses, a search for new drugs with high efficiency and low toxicity was carried out using new biotechnological methods [55, 56]. The production of such drugs is carried out mainly by chemical modification of natural antibiotics. The development of biosynthetic analogues of antibiotics became possible with the development of genetic engineering [53, 54, 57, 58]. These analogues can be further used as basic compounds in the synthesis of new antifungal drugs with improved pharmacological characteristics. Thus, the creation of new generation macrolide antibiotics is based on a combination of methods of chemical synthesis and genetic engineering. Genetic engineering methods are based on data obtained during the complete deciphering of the gene clusters responsible for biosynthesis of the antibiotic by the producer strain. The main stages of the biosynthesis of amphotericin B include formation of a 37-membered macrolactone ring, which involves six polyketide synthetases, and oxidation of the methyl group by methyloxidase. In the process of biosynthesis, mycosamine is attached to the carbon atom C8 or C10, controlled by transferase and hydroxylase. The biosynthetic route of antibiotic biosynthesis may be changed using the genetic mate-

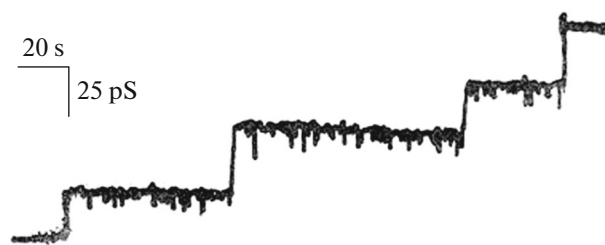


Fig. 6. Combined channels formed in lipid membranes at the same concentrations of filipin and amphotericin B (2×10^{-8} M), injected on opposite sides of the membrane. The composition of the membrane: a mixture of total bovine brain phospholipids and cholesterol in a weight ratio phospholipid : cholesterol = 20 mg : 1 mL, 2 M KCl, pH 7.0, $T = 22^\circ\text{C}$. The potential across the membrane was 200 mV (modified from [49]).

rial of the original strains, cloned with a specially designed vector, which makes it possible to replace the fragments of genes encoding biosynthesis of the enzymes. Such manipulations with genetic material replacement result in appearance of mutant strains with an altered biosynthesis routes, producing the desired antibiotic derivatives. For example, the gene construction of a mutant strain of the bacterium *Streptomyces nodosus*, in which the stage of methyl group oxidation to the carboxyl group at the C16 atom by the action of the corresponding oxidase is turned off, made it possible to obtain a derivative of amphotericin B, 16-decarboxy-16-methylamphotericin B [57, 58]. This compound was obtained by a multistage chemical synthesis [57–59]. During new drug creation, it is especially important to have an optimal combination of high antifungal activity and low toxicity. Chemical modification of polyene macrolides is significantly complicated by their lability in acidic and alkaline media, dimerization, ease of oxidation with atmospheric oxygen, as well as the tendency to light-induced isomerization of *trans*-diene fragments into the *cis*-form. The amphotericin B derivative, 16-decarboxy-16-hydroxymethylamphotericin B, exhibits insignificant nephrotoxicity, while its antifungal activity remains at the level of the parent antibiotic. Another derivative, 13-deoxy-13-14-didehydroamphotericin B, was a low-toxic compound, but it exhibited low antifungal activity. It should be noted that amphotericin B methyl ester retains antifungal activity. Esterification of the initial antibiotic molecule and its conversion into the methyl ester of amphotericin B (functionalizing the carboxyl group at position C16) resulted in an amphotericin B derivative exhibiting high antifungal activity and low toxicity [60–62]. Amphotericin B has been used for many years to create new effective antimycotics with high biological activity and lower toxicity for their use in practical medicine. In this context the high antifungal activity of new hybrid antibiotics based on benzoxaborols and amphotericin B should be mentioned. Benzoxaborols

are chemical compounds containing oxygen and boron atoms, which occupy an intermediate position between metals and non-metals. An effective way to create new drugs includes the development of hybrid antibiotics, which combine all the positive properties of the components of these compounds [62–64]. A chemical modification of the original amphotericin B was carried out. In particular, the corresponding amido derivatives substituted at the C16 carboxamide group were obtained using such methods as reductive alkylation and aminoacylation [5, 7]. Other amphotericin B derivatives were obtained via the amino group of mycosamine and the carboxyl group at the C₁₆ atom substitution. Modification of amphotericin B with benzoxaborols was carried out at the carboxyl group of the macrolactone ring at position C16 and at the amino group of the amino sugar was very effective [5, 61–64]. Hybrid compounds, mono- and dimodified derivatives of amphotericin B, have also been investigated [57–59]. The study of their biological activity revealed in most hybrids a high antifungal activity *in vitro* against the *Candida* fungal cultures. The most active were dimodified borol derivatives of amphotericin B (they were modified at the carboxyl group C16 by dimethylaminoethylamide). In terms of activity, these derivatives exceeded the activity of the original antibiotic amphotericin B [62, 63]. The introduction of the benzoborol component in many cases leads to a decrease in cytotoxicity and hemolytic activity with a high antifungal activity, which was tested on fungal strains of *Candida*, *Aspergillus*, and *Fusarium*. The membrane activity of benzoborol semisynthetic derivatives of amphotericin B is also very high in ergosterol-containing membranes, which correlates with data on antifungal activity and toxicity [64]. These derivatives, like the original antibiotic, exhibit the ability to form pores (ion channels) in bilayer lipid ergosterol-containing membranes, alternative to the membranes of fungal cells [64]. However, in cholesterol-containing membranes, the ability to form pores is very low; this is explained by the sensitivity of amphotericin B mainly to ergosterol. Analysis of the selective modification of amphotericin B at the mycosamine amino group and the carboxyl group at the C16 atom shows that the derivatives obtained by the method of genetic engineering, in comparison with the original molecule, show differences, when the group at the C16 atom is replaced and in the arrangement of hydroxyl groups in the C6–C10 fragment. The study of the structural and functional dependence of new semi-synthetic macrocycles revealed a certain pattern. In particular, it has been found that antibiotics and their semi-synthetic analogues with two hydroxyl groups in the region of the specified fragment of the molecule at C₈ and C₉ or C₇ and C₁₀, exhibit the greatest activity. In a series of semisynthetic derivatives, they form ion channels in the membranes with ergosterol in fungal cells and have a lower hemolysis index than original amphotericin B [5, 57, 58].

3.1. Liposomal Substances of Amphotericin B

Some derivatives of amphotericin B have been developed as liposomal formulations. These are lipid complexes and colloidal dispersed forms [5, 44, 62, 63]. New liposomal derivatives of amphotericin B have low toxicity and high resistance [22, 44, 62, 63, 65–74]. By modifying the structure of amphotericin B, it is possible to obtain a derivative that has the ability to bind to ergosterol, but not cholesterol. The following liposomal preparations based on amphotericin B have been developed: ambisome, abelset, and amphocyl [22, 67]. Liposomal formulations of amphotericin B were formed on the basis of egg lecithin, a natural antioxidant increasing plasticity of cell membranes. They were investigated against *Candida* fungi at the molecular genetic level after incubation of biofilms with amphotericin B solution and its experimental liposomal form [30, 62, 63]. Expression of the *MET3* gene, which plays an important role in the process of biofilm formation, was checked by the microtiter method. As a result, it was found that the use of the liposomal form of amphotericin led to a decrease in its minimum inhibitory concentration by 8–12 times and a more effective inhibition of *Candida albicans* fungi in comparison with the original amphotericin B [44, 62, 64, 69, 70]. The inhibitory effectiveness depended on the composition of liposomes and their charge. The study of the molecular genetic mechanism of action of liposomal amphotericin B showed that suppression of biofilms of *Candida albicans* occurred simultaneously with blocking the expression of the *MET3* gene. After a 24-hour incubation of biofilms with liposomal amphotericin, *MET3* RNA was absent, thus indicating blockade of this gene expression. The experimental results have shown that the use of liposomal antimycotics is highly effective against *Candida albicans* fungi and makes it possible to predict their use to increase the effectiveness of the pharmacological action of antifungal drugs and reduce their therapeutic dose. The method for determining the *MET3* gene expression can be used during treatment of candidal infections [70, 71]. Invasive mycoses caused by strains of *Aspergillus*, *Zygomycetes* and *Candida* in patients with hematological diseases and bone marrow transplantation are the cause of co-infectious concomitant factors complicating the patient's condition [34]. In these cases, invasive pulmonary infections caused by *Aspergillus* and *Zygomycetes* are very dangerous and are known as opportunistic mycoses [23]. In the context of a global pandemic, fungal pathology develops especially rapidly and in this case leads to invasive aspergillosis, which contributes to the complication of coronavirus infection in the lungs and even secondary infection with invasive aspergillosis. The process of treatment of the invasive form of bronchopulmonary aspergillosis is directly related to the immunomodulatory and immunostimulating properties of the macrocyclic polyene drug amphotericin B, which has the status of an antifungal antibiotic [5, 74, 75].

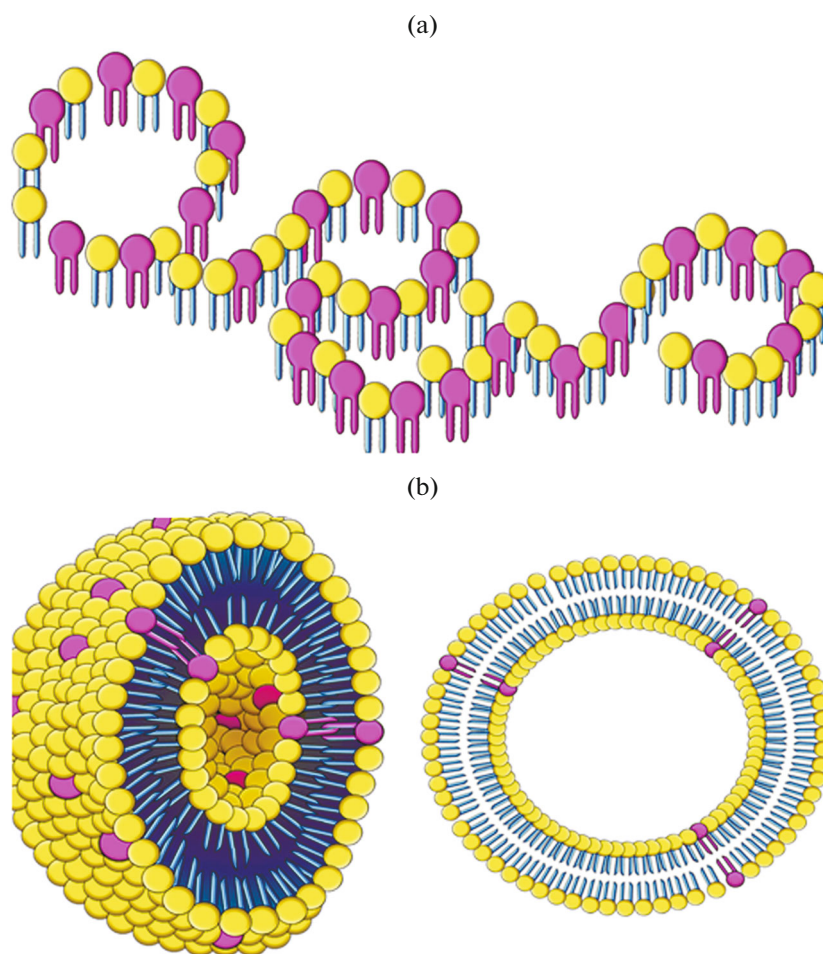


Fig. 7. The structure of lipid-associated forms of amphotericin B (Reproduced from [22] under the license CC BY-SA 4.0): (a) lipid complex of amphotericin B; (b) liposomal amphotericin B.

The main advantage of the lipid forms of amphotericin B is the reduction in the number and severity of side effects characteristic of amphotericin B [22, 30, 70, 76–78]. The molecular structure of the lipid complex and liposomal amphotericin B is shown in Fig. 7. If it is necessary to prescribe the drug, the choice should be based, first of all, on the clinical efficacy of using lipid-associated forms of amphotericin B (Fig. 7) [22]. Works in this direction help to determine the relationship between the structure of molecules and their biological activity and, ultimately, can lead to the synthesis of new antibiotics with improved therapeutic properties, and new liposomal preparations of amphotericin B will effectively fight infectious diseases [76, 78, 79].

4. THE STUDY OF AMPHOTERICIN B AND ITS DERIVATIVES IN VIROLOGY AND ONCOLOGY

In this chapter we consider the effects of amphotericin B on the reproduction of some viruses and on clo-

nogenic cell cultures *in vitro*. PAs are capable of inhibiting the activity of a number of viruses at certain stages of reproduction. Amphotericin B is one of these PA based drugs; in parallel with antifungal properties, it has antiviral and antitumor effects [6–8, 80]. Amphotericin B affects the reproductive properties of influenza, herpes, HIV, hepatitis and enterovirus viruses. The effect of this antibiotic on the reproduction of viruses has been studied. For example, the inhibitory effect of amphotericin B on the replication process of enterovirus has been shown. Amphotericin B significantly reduced expression of enterovirus EV 71 RNA and viral proteins in rhabdomyosarcoma cells and HEK 293 cells (a cell line obtained from human embryonic kidneys) [81]. Amphotericin B inhibited the production of enterovirus [81]. Studies have shown that amphotericin B acts at an early stage of infection: when enterovirus enters the cell amphotericin B prevents binding and incorporation of the virus into the host cell. As an effective antifungal drug, amphotericin B may be a new therapeutic agent for the treatment of enterovirus infection. Amphotericin B and its

methyl ester also exhibit antiviral activity against vesicular stomatitis virus (VSV), herpes simplex virus (HSV-1), hepatitis, Sindbis virus, vaccinia virus and human immunodeficiency virus type 1 (HIV-1) [8, 81–91]. These drugs have been used in combined therapy for the treatment of fungal and viral infections. It turned out that the methyl ester of amphotericin B is much less toxic than the original antibiotic. It exhibits a significant antiviral effect against vesicular stomatitis virus, HSV-1 and Sindbis virus, which have a viral envelope. Based on these data, it can be concluded that the sterol components of the host cell membrane are integrated into the envelope of the virus, and this area of the envelope becomes the site of action of the methyl derivative of amphotericin B. When the methyl derivative of amphotericin B acts on HIV-1, the antibiotic bound to cholesterol and suppressed viral particle formation [83]. Amphotericin B methyl ester destroys the morphology of virions. Membrane-bound cholesterol in its complex with the antibiotic inhibits the process of HIV replication. Changes caused by the drug during replication are also associated with viral infectivity, which influences formation of viral particles, although in this case the antibiotic itself does not affect the infectious properties of the virus [88, 89]. Analyzing the inhibitory ability of amphotericin B on the RNA-containing influenza virus upon contact of the virus with the cell, it should be noted that in this case amphotericin B suppresses the replicative ability of the virus, as it participates in the process of changing the pH in the endosome after the virus enters the cell and, accordingly, into the endosome. In this case, the drug acts on proton ATPases, reducing the activity of this enzyme. The acidity of the cell and endosome is regulated by the activity of proton channels in the viral ATPase. Endosomal pH rises, becomes alkaline under the action of amphotericin B, which is unacceptable for viral replication and development. As a result, the virus loses its activity and the infection does not spread further [88, 89, 91]. The antiviral activity of amphotericin B is also manifested in the case of the hepatitis B virus [87, 91]. Treatment of hepatitis B virus particles with amphotericin B (5–250 µg/mL) showed that the DNA polymerase activity of the hepatitis B virus increased with an increase in the concentration of the antibiotic. In addition, under the action of amphotericin B, antigen particles are transformed into less active subparticles (i.e. the virus antigen loses its properties). Amphotericin B and its methyl ester increase interferon production and inhibit virus penetration and its action in cells in vitro. In this case, the antibiotic affects the work of polyribocytidylic acid, which influences interferon synthesis [86, 87, 91]. A study and analysis of the effect of amphotericin B on the influenza virus was carried out [88]. The influenza virus is an RNA virus. It is relatively simple in structure. Data on the effect of amphotericin on early influenza virus replication obtained in [89–91] indicate that amphotericin B acts

as a pre-replication inhibitor on the influenza virus. Penetrating through the cell membrane, the virus released from the protein spikes of hemagglutinin in the viral envelope, penetrates into the endosome (cytoplasmic vesicle), where the acidity of the endosomal fluid changes (normally the pH of the medium decreases), which leads to the appearance of amphiphilic domains (new surface structures). As a result, the endosome membrane and viral envelope fuse. Amphotericin B is involved in pH modulation by acting on proton ATPases and reducing the activity of this enzyme. It is known, the acidity of cells and endosomes is regulated by the activity of proton channels in viral ATPase. Under the influence of amphotericin B, endosomal acidity changes, and pH rises and this leads to a loss of viral activity and a decrease in infectivity. The virus is activated only in an acidic environment, and in an alkaline environment it loses its infectivity [88]. Thus, the antiviral effect of amphotericin B has been experimentally proven [81–91]. The antitumor effect of amphotericin B together with levorin A was studied on clonogenic HeLa (cervical cancer) and C6 (rat glioma) cell cultures [92]. Amphotericin B was found to have a beneficial effect at certain concentrations compared to levorin and its methyl derivative. Amphotericin B at a concentration of 40 µg/mL reduced survival of HeLa and C6 tumor cells [92, 93].

CONCLUSIONS

Analysis of the biochemical, biophysical and pharmacological properties of the macrolactone PA, amphotericin B, gives a good ground for further use of this antibiotic as a promising pharmacological agent [22, 50]. The amphotericin B molecule may be modified for more effective use, and the sphere of application of both amphotericin B and also its derivatives may be extended as an antifungal, antiviral and antitumor drug [7, 22, 50, 91–93]. In this regard, the use of the liposomal complex of amphotericin B is most preferable for invasive mycoses, which are a coinfective pathology in bacterial, viral and oncological diseases, as well as for postoperative complications.

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COMPLIANCE WITH ETHICAL STANDARDS

Authors declare that they have no conflict of interest.

This article does not contain any research involving humans or the use of animals as objects.

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