POLYENES IN THERAPY OF MYCOSIS – CHARACTERISTICS AND MECHANISMS OF RESISTANCE

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Abstract

Polyenes are one of the most commonly used drugs in the treatment of mycoses. In the polyenes group we can find amphotericin B, nystatin, and natamycin. The resistance of pathogenic fungi is rare, but more visible than a few years ago. Several mechanisms of resistance have been described, concerning ergosterol in cell membrane, biofilm formation, and others. The aim of this paper was to describe the polyenes and try to explain mechanisms which may lead to resistance of fungi.

Key words: antibiotics, pathogenic fungi, fungemia, superficial mycoses

Introduction

Mycoses are very common. Estimated data say that over one billion people suffer because of mycosis [1]. One of the most commonly used groups of antifungal drugs are polyenes, which have been present in medicine from the mid-20th century. Data from Centers for Medicare & Medicaid Services says that in 1991-2009 in the United States of America polyenes were the second most often prescribed group of antifungal drugs (generally azoles were first). They were on about 120 000 prescriptions. In the last 18 years patients in the USA paid for polyenes about 490 mln dollars [2]. Polyenes are effective against most of human's pathogenic fungi. They are used in the treatment of superficial mycosis (nystatin, natamycin), and systemic mycosis as well (amphotericin B) [3]. Amphotericin B is called the "gold standard" in the treatment of systemic mycosis and has the widest range of action among all available antifungal drugs [4].

The aim of this paper is to describe polyene drugs, including their structure and mechanism of action, and explain the processes which are the basis of antifungal resistance for this group of drugs.

Polyenes structure and their antifungal mechanism of action

Polyenes antibiotics are natural products of the aerobic metabolism of Gram-positive bacteria from the genus *Streptomyces spp.* [4, 5]. They are made of a macrocyclic lacton ring, which contains 4-7 conjugated double bonds. The ring is connected by a glycosidic bond with an aminosaccharide [6]. There are also many hydroxyl groups in the polyene structure [7]. These drugs are sensitive to light; that's why they are easily oxidized [6].

The antifungal mechanism of action of all polyenes is based on the disruption of the integrity of the fungal cell membrane. The amphiphilic structure of the molecule lets polyenes bind to ergosterol – the main sterol of fungal cell membranes – creating pores, thereby increasing permeability, for example for potassium and magnesium ions (Fig. 1). As a result, the membrane is destabilized and the cell dies [8, 9].

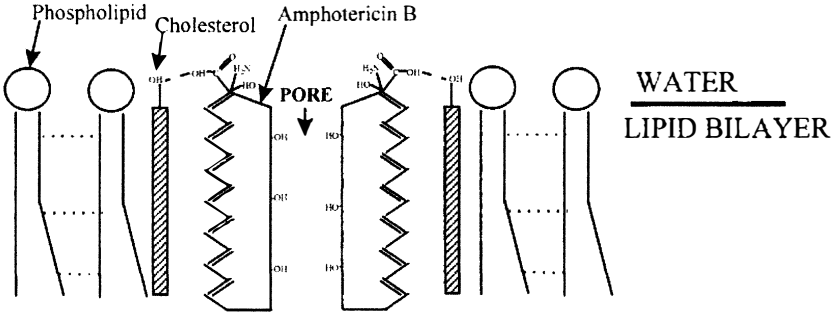

Fig. 1. Mechanism of action of polyenes [10]

Characteristic of selected polyenes

Amphotericin B. Amphotericin B (Fig. 2) has been present in medicine from 1959, and it's called the "gold standard" of mycosis treatment [11, 12]. Despite the development of many new antifungal agents, amphotericin B is still the most commonly used antifungal agent in hospitals due to its strong antifungal activity. It's a life-saving drug in the treatment of serious systemic fungal infections [7]. It's produced by *Streptomyces nodosus* [13]. It has a wide spectrum of activity against most species of the genus *Candida spp.* and *Aspergilus spp.* In addition, it exhibits antifungal activity against *Cryptococcus spp., Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides immitis and posadasii, Paracoccidioides spp.* [4, 6]. Despite many decades of clinical use, resistance to amphotericin B is very rare. It's exhibited by strains such as *Candida lusitanie, Candida guilliermondi, Aspergillus terreus, Trichosporon beigelii, Scedosporium apiospermum* [3, 4, 7,

9]. Amphotericin B is ineffective in the treatment of monosporiosis and trichosporiosis [14]. However, it's characterized by its effective action in the treatment of candidiasis: oral cavity, digestive system, urinary system, lungs, meninges, pancreas or spine. Amphotericin B is present in various pharmaceutical forms: lozenges, washing suspensions, suspensions for intravenous administration [15]. Due to its insolubility in water, it's practically not absorbed from the digestive system, therefore it's usually administered intravenously. Chemical modification of the antibiotic by combining it with sodium deoxycholate allowed obtaining a water-soluble pharmaceutical – Fungizone [13]. The daily dose of amphotericin B for the treatment of systemic fungal infections ranges from 0.5 to 1 mg / kg / day (in severe cases up to 1.5 mg / kg / day). In central nervous system infections, the antibiotic is given intrathecally due to its poor penetration into the cerebrospinal fluid [13, 14]. This antibiotic has a high toxicity associated with similarity in the structure of fungal and human cell membranes and with the mechanism of cytokine activation [3, 6]. During therapy it can cause many side effects, e.g. fever, chills, or shortness of breath, and in almost every patient after long-term therapy causes kidney damage [14]. It's characterized by a long half-life and high degree of spread in tissues [3]. Amphotericin B, despite a wide spectrum of activity, causes many serious side effects. It's the most toxic compound among polyene antibiotics. It's characterized by high neurotoxicity. During therapy, many patients have adverse reactions, such as fever, nausea, headache, muscle and joint pain, chills, hypotension, dyspnoea, hepatotoxicity, hypokalaemia, hypomagnesemia and anemia. Long-term therapy almost always ends in impaired renal function [6, 7, 14]. Too high doses can bind to the cholesterol of human cell membranes, disrupting their integrity and structure [6]. There are many contraindications regarding the use of amphotericin B, including renal failure, hypocalcaemia, or liver damage [3]. This limits the possibilities of its use in long-term therapies [14]. Research shows an antagonism between amphotericin B and azoles. It's likely that azoles inhibit the effectiveness of amphotericin B as a result of lowering the sterol content in fungal cell membranes [7]. In addition, the mechanism of amphotericin B and other toxicity may be associated with the activation of cytokines and the secretion of TNFα (tumor necrosis factor) by macrophages [3].

To reduce side effects, amphotericin B is often combined with other substances, such as flucytosine or fluconazole [14]. In addition, to minimize cytotoxicity – and increase its penetration into tissues – lipid forms of amphotericin B were introduced: AmBisome (liposomal amphotericin B), Amphocil (colloidal suspension, amphotericin B with cholesteryl sulfate), Abelcet (amphotericin B in the form of a lipid complex). Because of the use of those preparations, higher doses of the active substance (AmBisome – 3mg/kg bw/d, Amphocil – 3-6 mg/kg bw/d, Abelcet – 5 mg/kg bw/d) can be used [7, 14]. Research is also being carried out on nanoparticles as carriers of amphotericin B, which after interaction with macrophages would release the drug gradually, thereby reducing side effects [3].

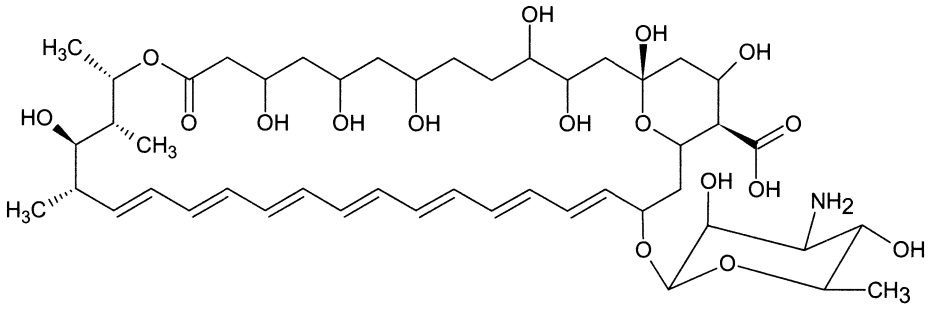

Fig. 2. Chemical structure of amphotericin B

Nystatin. Nystatin (Fig. 3) was the first antifungal drug from the polyenes group in treatment. It's been used since 1949 [7]. It's used in treatment of superficial infections of the skin, nails, vagina, mucosa of the digestive system, mouth and eyeball [3, 6, 15]. It's produced by *Streptomyces noursei* [8]. This antibiotic works mainly against fungi of the genus *Candida spp.*, *Cryptococcus spp.*, *Fusarium spp.*, *Aspergillus spp.* It's not effective against dermatophytes [7, 8]. Nystatin is insoluble in water, therefore it's used for prophylaxis after antibiotic therapy, in cancer patients, or before surgery [6]. This drug is present in different forms: gel, ointments, rinse suspensions, and vaginal balls. It has different trade names; it's sold e.g. as Moronal or Mycostatin. It's the only antifungal drug used in the treatment of vaginal and vulvar candidiasis in the first trimester of pregnancy [15, 16]. It has high toxicity after parenteral administration. The developed liposomal form of nystatin has higher vitro activity than standard nystatin [7].

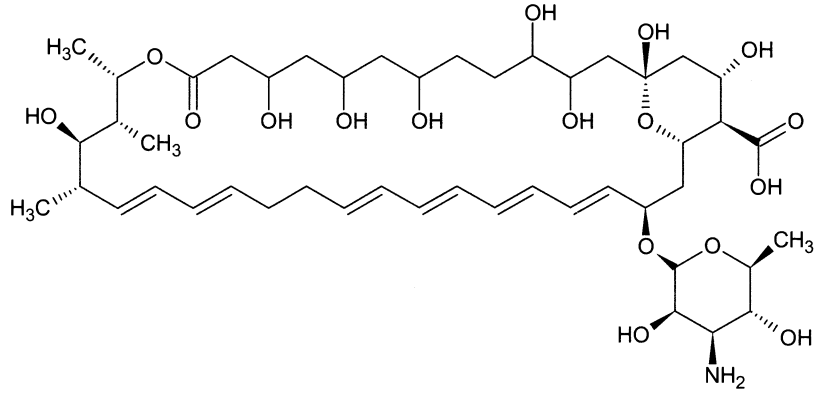

Fig. 3. Chemical structure of nystatin

Natamycin. Natamycin (Fig. 4) was first isolated in 1955 from the bacterium *Streptomyces natalensis* [8, 17]. It's used locally in vaginal and vulvar candidiasis, onychomycosis of the feet, nails, mucous mebranes, urinary and sex organs in men, yeast skin folds or keratitis [13, 16]. It has in vitro activity against fungi

from genus *Aspergillus* spp., *Candida* spp., *Penicilium* spp., *Cephalosporium spp., Fusarium spp., Cladosporium spp., Scopulariopsis spp.* [18]. Natamycin is insoluble in water and it can't be absorbed from the digestive system. It's applied in the form of creams (in combination with e.g. glucocorticosteroids), globules, ointments or solutions [13, 16]. Commercial preparation appears as Pimafucin. Natamycin is highly sensitive to ultraviolet light. The daily dose of this drug is 0.3 mg / kg / day. Besides in medicine, natamycin is used in the food industry as a preservative in food (such as cheese, yogurt, sausages, wine and juice). Maximum concentration of this antibiotic can be 52 mg / l, but the growth of fungi is inhibited for 16 weeks in four times lower concentrations [18].

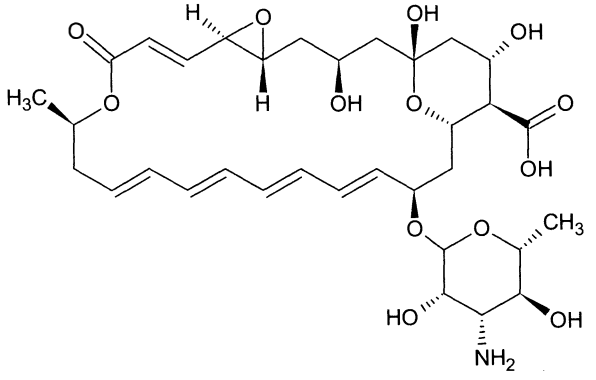

Fig. 4. Chemical structure of natamycin

Mechanisms of resistance of fungi to polyenes

Changes in ergosterol content in fungal cell membrane. The mechanism of the action of polyenes is combined with the content of ergosterol in cell membranes. That's why one of the basic mechanisms of resistance is connected with the content of this sterol in fungal cell membranes, or even its complete absence. In the results drugs can't be stuck to the compounds of a cell and make pores in it. It has been shown that ergosterol in cell membranes is replaced by episterol or fecosterol (intermediates of the ergosterol biosynthesis pathway) [19]. Extremely significant, genetic changes underlying the resistance of fungi to azoles, may also be appropriate for resistance to polyenes [20-22]. The most common mutations connected with resistance to polyenes are point mutations in the *ERG3, ERG6, ERG4* or *ERG11* genes [20, 23-24], coded enzymes compatible with the synthesis of some intermediates of the ergosterol biosynthesis pathway.

Research has been done that includes this correlation of fungal resistance levels and content of ergosterol and other sterols in cell membranes. Analysis of the content of *Cryptococcus neoformans* cell membrane before and after amphotericin B treatment in the analysis of infection in AIDS patients shows fungi

isolated before treatment had 75% ergosterol content in membranes, and after treatment - only 4%.

Furthermore, the accumulation of intermediates of the ergosterol biosynthesis pathway, e.g. fecosterol [23] has been demonstrated. Studies which were done on *Candida glabrata* with nonsense mutation in the *ERG6* gene showed that this kind of mutation leads to increased resistance and reduced content of ergosterol in cells [25]. Other studies concerned *S. cerevisiae* and *C. albicans*. Scientists have shown that changes in the composition of *ERG11* affect the reduced sensitivity to amphotericin B, as well as being needed in the increasing range of accumulate intermediates of the ergosterol biosynthesis pathway [23, 26]. Studies carried out on *C. albicans* in 2003 by Geraghty and Kavanagh showed that mutants that had changes at the level of the respiratory chain were also less sensitive to the effects of amphotericin B [27]. In addition, these mutants had a significantly lower ergosterol content in the cells than the other variants [27], which may be due to the association of this organelle with the ergosterol biosynthesis pathway [28]. A decrease in mitochondrial activity may also reduce the production of free radicals. Thanks to this, the damage they cause will be reduced [28].

Biofilm formation. A biofilm is a structure that is created not only by bacteria, but also by fungi. It is believed that fungal organisms are relatively easily organized into structures composed of many layers of cells and extracellular substances. The structure of individual biofilms as well as the chemical composition of the extracellular matrix determines the level of sensitivity of these structures to the effects of drugs [29]. They usually cover solid surfaces, but there are also biofilms appearing on the surface of the water (liquid-air interfaces) [30]. In studies on *Candida* fungi it has been shown that biofilms had about 8 times higher resistance to amphotericin B than cells that don't form these kinds of structures [31]. Unfortunately, biofilm-forming cells may be resistant to most of the currently used drugs (the exception among polyenes are amphotericin B lipid compounds) [32]. In addition, patient-safe drug levels do not guarantee sterilization, which is likely to cause biofilm regrowth [31]. Monitoring the development of resistance of these structures is also important for patient safety.

The biofilm structure determines the reduced availability of fungicidal compounds to cells. The extracellular matrix includes, among others, polymers of glucan, which is responsible for the retention of antimicotic molecules outside the cell, which completely or partially prevents them from entering the cells. As a result, it is necessary to use a higher concentration of the drug for biofilm-forming fungi than for cells that don't form them [31]. In studies conducted on biofilms of *Candida albicans* mutants, it was noted that changes in the expression of the *FKS1* gene affect changes in sensitivity, both to amphotericin B as well as to other antimicotics [33]. Increased expression of this gene reduces the sensitivity of these structures to the compounds used [33]. In addition, in the biofilm structure persisters are presented, which have high tolerance to antimicotics [34]. The reduced sensitivity of these cells to amphotericin B may be due to changes in the level of signaling pathways. In studies on *S. cerevisiae*, a correlation was observed in the increase in resistance to this compound and the inhibition of TORC1, EGO and RAS complexes [35]. In addition, it was shown

that mutants having mutations in the *ras1* and *ras2* genes had definitely more persisters than wild forms [35]. These cells can form the basis for a new biofilm that is being formed [36]. Due to their location, these cells receive a much lower dose of drugs than cells in the outer part of the biofilm. In addition, because of their presence, structures can survive in high concentrations of antifungal drugs [37]. Due to these features, biofilms are considered to be the main mechanisms of resistance of fungi in chronic infections [38].

In the case of biofilms and their resistance to polyenes, attention is also paid to the rate of its growth. It's believed that the low growth rate in relation to non-biofilm-forming cells may reduce the sensitivity of these structures to the effects of antimicotics [39]. Comparing cell density *of C. glabrata, C. tropicalis* and *C. parapsilosis* in biofilms, the first species usually shows higher resistance to amphotericin B than the others, which can be correlated with higher density in the structure being created [30].

Antioxidant protection. Polyenes have a significant effect on the level of oxidative stress within cells. In addition to forming pores in the cell membrane, polyenes also increase the lipid peroxidation in this structure, which increases the therapeutic effect of these compounds. Disorders within the cell membrane may be the result of the action of hydroxyl radicals or hydrogen peroxide [23]. The exact role of amphotericin B in cell oxidative stress is not entirely known. However, it is recognized that this compound may also have antioxidant activity [28]. It is currently believed that one of the mechanisms that may underlie polyene resistance is greater activity of antioxidant enzymes (for example catalase [19]). Studies on *C. albicans* have shown that resistant strains produced greater amounts of catalase, both intra- and extracellular, than control [23]. In addition, studies conducted on strains of *Candida* fungi resistant and sensitive to amphotericin B showed higher activity of both catalase and superoxide dismutase in strains resistant to the tested drug [40]. In studies on *Trichophyton rubrum*, induction of genes coding for GSS (glutathione synthetase) or AOX (alternative oxidase) proteins was observed in response to amphotericin B [41]. In the case of glutathione peroxidase (GPX), amphotericin B reduced the transcription of the gene encoding this protein [41].

Other mechanisms of resistance to polyenes. Studies carried out on *T. rubrum* have shown diverse expression of genes encoding enzymes involved in the metabolism of lipid compounds [41]. In response to this compound, both an increase (e.g. IVD gene) and a decrease in expression of these genes (e.g. ELO2 or MVD1 genes) has been observed [41]. Further analysis of the differentiation in expression of these genes will allow a broader look at the cellular response of fungi to polyenes as well as at the problem of resistance.

It turns out that the cell wall structure can affect the sensitivity of fungal cells to polyenes. Studies on *C. albicans* have shown that the low content of chitin in the cell wall was associated with increased resistance to amphotericin B [42-43]. Research on the chemical composition of the *A. flavus* cell wall has shown that strains sensitive to amphotericin B had a lower content of glucans than in resistant strains [44]. However, in studies carried out on *T. rubrum*, amphotericin B inhibited the expression of the *FKS1* gene encoding 1,3-glucan synthase [41].

These results may suggest that increased 1,3-glucan synthase activity may be one of the mechanisms of fungal resistance to polyenes, e.g. as a result of brief contact with amphotericin B. However, the effect of cell wall structure on the sensitivity of fungi to polyenes, as well as the role of the compounds, isn't entirely clear and requires further research. The role of heat shock proteins (HSPs) in building resistance to polyenes also requires additional analysis. Currently, attention is on the potential impact of their presence and synthesis on reduced sensitivity to drugs from this group [45]. Studies on *T. rubrum* have shown that amphotericin B induces a number of genes that are responsible for the response of cells to stress, including gene coding for HSP70 or HSP104 proteins [41]. In the case of the *HSP10* gene, a reduction in transcription was observed under the influence of this drug [41]. Researchers also point out the importance of yeast life cycle stages to their sensitivity to amphotericin B. Research shows that cells in the steady-state growth phase are more resistant than in the exponential phase [23].

Conclusion

Mycosis appears very often. In treatment, we can use only a few groups of antifungals. One of the most commonly used are polyenes. Those compounds work very well, but using them has many side effects. The aim of using polyenes is to disrupt the integrity of the fungal cell membrane (by binding molecules of the drug to ergosterol in cell membrane and creating pores). The membrane becames destabilized which causes the death of the cell. Nowadays, resistance of pathogenic fungi is rare, but many serious infections are treated by polyenes. Amphotericin B is called the "gold standard" in treatment of systemic mycosis and has the widest range of action among all available antifungal drugs. Polyenes are made of a macrocyclic lacton ring, which contains a few conjugated double bonds. The ring is connected with aminosaccharide. In the structure polyenes have many hydroxyl groups.

Knowledge about the possible mechanisms of resistance to polyenes is very narrow. However, few papers describe the several potential mechanisms of increased sensitivity of fungi to polyenes. Scientists underline the role of ergosterol content in cell membrane as one of the major mechanisms. Lower amount of ergosterol (or even lack of it) in cell membrane protects cells from creating pores in this structure. Polyenes binding becomes impossible or very unsuccessful. A second major mechanism of resistance of fungi is the formation of biofilms. The structure of biofilms has a big impact on the method of treatment. Unfortunately, resistance of fungal biofilms is higher than resistance of cells which don't make it. That's why it's necessary to use a higher concentration of the drug to fight with infection. Biofilms have the ability to regrow, which can be a basis for losing sensitivity to used drugs. These and other mechanisms have been basically described, but there is much more to do. Especially, because amphotericin B is often "the last chance drug". Creating new compounds, or using old ones

but in another way, may be a possible useful modification of treatment of fungal infections.

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