The Effect Of Some Polyene Antibiotics On Cancer Cells

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The action of alkyl derivatives of amphotericin B and levorin that are modified in the particular parts of lactone ring on lipid and biological membranes are researched. It is found that methyllevorin has higher biological activity than the initial compound. Antitumor effect of methyllevorin and initial amphotericin B was shown*in vitro* at the researching of alkyl derivatives of levorin and amphotericin B on clonogenic cellular cultures Hela (carcinoma of the cervix) and C6 (rats glioma).

Keywords: *Amphotericin B, levorin, alkyl derivatives of polyene antibiotics, tumor cells, rat's glioma, carcinoma of the cervix*

INTRODUCTION

Polyene antibiotics (PA) effeciently interact with cell and lipid membranes and increase membrane permeability for ions and organic substances (Récamier et al., 2010). Main representatives of PA are amphotericin B, nystation, micoheptin and levorin. PA are produced by soil gram-positive microorganisms from Streptomyces group. There is macrolidering with number of conjugated double bonds that define chromofor properties of this substances in the chemical structure of all PA. There are aminosugar (micosamin), carboxyl, carbonyl and hydroxyl groups with amphoteric and specific properties in the PA molecules (Kasumov, 2009).

PA form structural ionic channels of molecular sizes in lipid and cell membranes (Cohen, 2010; Samedova, 2018). Most of these antibiotics have high biological activity against yeast-like fungi that are used for the treatment of systemic fungal diseases (Baginski, Czub, 2009; Recamier et al., 2010; Solovyova et al., 2011; Gray et al., 2012). It became clear later that antibiotics of polyene structure have important property to inactivate some viruses (AIDS virus, enteroviruses), interfere of their penetration into the cell and inhibit their reproduction (Waheed et al., 2008; Xu et al., 2016). However, their relative toxicity, low solubility in the water and resistance of microflora limits the application of PA in medical practice. In this regard, it needs the search of new more effective pharmaceuticals with high antifugal activity. Synthesis and slection of antibiotics with new properties and non-toxic for humans is actual problem. It was shown that some chemically modified PA interfere the growth of malignancies (Sultanova et al., 2017). It is possible to establish the connection between structure and function of polyenes in membranes in the research of new PA on bilayer lipid membranes (BLM) and to thereby concretize directions for theoretical approaches to targeted synthesis of new antibiotics with particular therapeutic properties.

MATERIAL AND METHODS

PA were prepared as solutions of different concentration in dimethylsulfoxide (DMSO). Native levorin has mainly two biologically active compounds: levorin A and levorin B. Levorin A exceed levorin B by biological activity (Kasumov, 2009). Biologically active concentrations of antibiotics were defined by BLM method with ultraviolet (UV) (Kasumov, 2009). UV-spectrum of amphotericin B and levorin are received by the spectrophotometer T 92+ UV/VIS Spectrometer. There were used oncogenous cellular lines HeLa (carcinoma of cervix) and C6 (rats glioma). Cells were cultivated on growth medium DMEM (Sigma, USA) containing 10% of embryo serum of calf. Cytotoxicity was defined by MTT-test (colorimetric test for estimation of digital material carried out by methods of nonparametric statistics in program «STATISTICA 7.0»).

RESULTS AND DISCUSSION

Alkyl radical can replace protons on the amino-group (N-replacement with intensification of positive charge on the amino-group) and on the carboxyl group (etherification with blocking of acid function) at the alkylation of polyene amphoteric antibiotics by iodic ether or dialkylsulfate at the presence of alkaline acceptor in the DMSO medium or dimethyl formamide. The degree of N-replace-

ment in limits may be 4 if alkyl has small size (methyl) that is shown on scheme 1. At the big sizes of radical R (from ethyl till butyl) the degree of replacement decreases because of steric factor. For example it is averages 2.2 for ethyl radical and 1.2 for butyl one. Carboxyl group at the every researched alkyl replacements practically total replaces and it defines enough colloid solubility in water for methyl-, ethyl-, propyl-, butyl-replaced.

There is a chemical structure of alkyl ethers N-alkyl derivatives of amphotericin B and levorin in fig.2. Biological activity of polyenes increases by the addition of DMSO solution. The DMSO action on BLM of alkyl derivatives of amphotericin B and levorin modified by amino- and carboxyl group was studied.

It is known that steroid homeostasis of oncological patients significantly changes (Kit et al., 2011). As there is more cholesterol at the membranes of tumor cells than in normal ones it is reasonable to act on the development of tumor process by the PA. It became clear at the research of PA that these substances may stop the development of metastasis of experimental animals (Ibragimova, Aliyev, 2002). It was detected that PA are capable to reduce the toxic action of some cytostatics and increase their antitumor effect in the experiments with laboratory animals and a lot of models in reinjection malignancies (sarcoma, leukosis, ascyte tumors) (Ibragimova, Aliyev, 2002; Zotchev, 2003). It is apparently connected with capability of PA to increase selectively the permeability of tumor cells and sensitivity of cells to action of chemotherapeutic agents. Structural changes in the polyene molecules act on format of formed pores in membranes and biological activity of antibiotics. In this regard, *in vitro* experiments were carried out with the purpose of study of cytostatic action of some alkyl derivatives of aromatic PA levorin – methyllevorin, butyllevorin, isolevoridon (transform of levorin) and also initial amphotericin B on the cellular cultures HeLa (cancer of cervix) and C6 (rat's glioma). These researches showed antitumor action of initial levorin in the concentration 40 mkg/ml and methyllevorin in concentrations 20 and 40 mkg/ml. There are data of cytostatic action of some PA on tumor cells HeLa and C6. Amphotericin B in 40mkg/ml concentration essentially decreases the proliferation of tumor cells HeLa in comparison with the same antibiotic in concentration 20 mkg/ml.

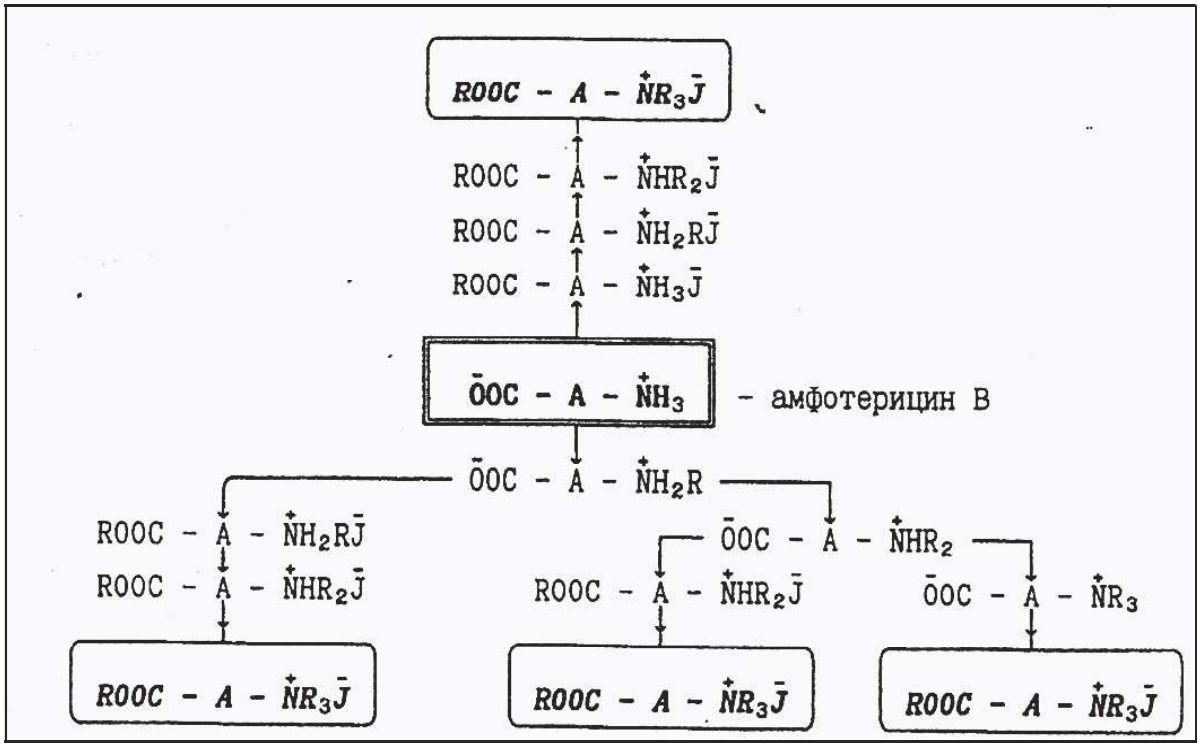
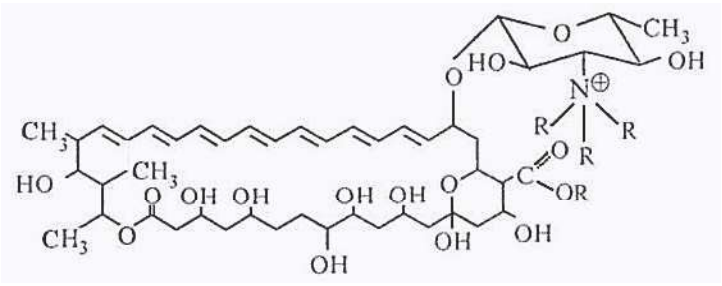
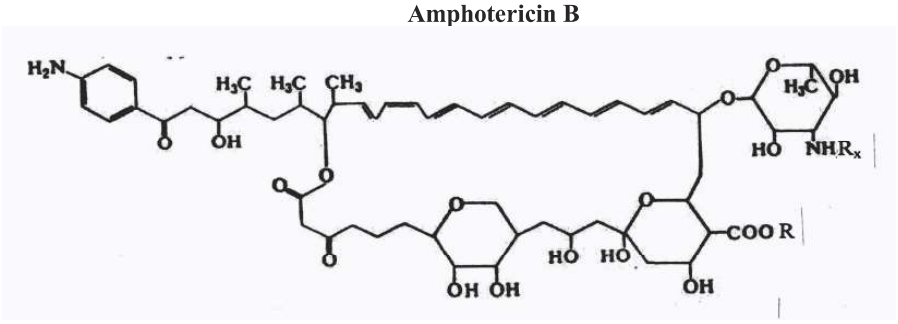


Fig. 1. The scheme of possible directions of alkylation of amphoteric polyene macrolide antibiotics by iodide alkyl or alkyl sulfates



Amphotericin B



Levorin

Fig. 2. Chemical structure of alkyl ethers N-alkyl derivatives amphotericin B and levorin. Alkylation of molecules of native antibiotics by iodide alkyl in the DMSO takes place in amine and carboxyl groups. Letter R designates alkyl radical-replaced: R-CH_3 methyl-, R-C_2H_5 ethyl-, R-C_3H_7 propyl- , R-C_4H_9 butyl-derivatives.

Table. Estimation of cytostatic action of some PA on tumor cells in line C6 and HeLa

№	Substance	Dose, mkg	Survival of cells on the relation to control%	
			C6(rats glioma)	HeLa (carcinoma of cervix)
1	Levorin A	20	124,16±1,14(p<0,01)	101,22±2,42 (p>0,05)
		40	118,0±1,56 (p<0,01)	106,69±2,41 (p<0,01)
		200	94,76±1,62 (p<0,01)	64,12±1,57 (p<0,01)
2	Levorin A (methyllevorin)	20	87,30±2,01p<0,01	91,44±2,51 (p<0,01)
		40	38,35±2,85 (p<0,01)	31,40±1,23 (p<0,01)
3	Levorin (native)	20	97,91±0,99 (p<0,01)	105,68±1,39 (p<0,01)
		40	123,34±1,66 (p<0,01)	117,44±3,76 (p<0,01)
4	Amphotericin B	20	158,33±3,56 (p<0,01)	137,4±3,94 (p<0,01)
		40	106,04±4,33 (p>0,05)	76,11±0,7 (p<0,01)

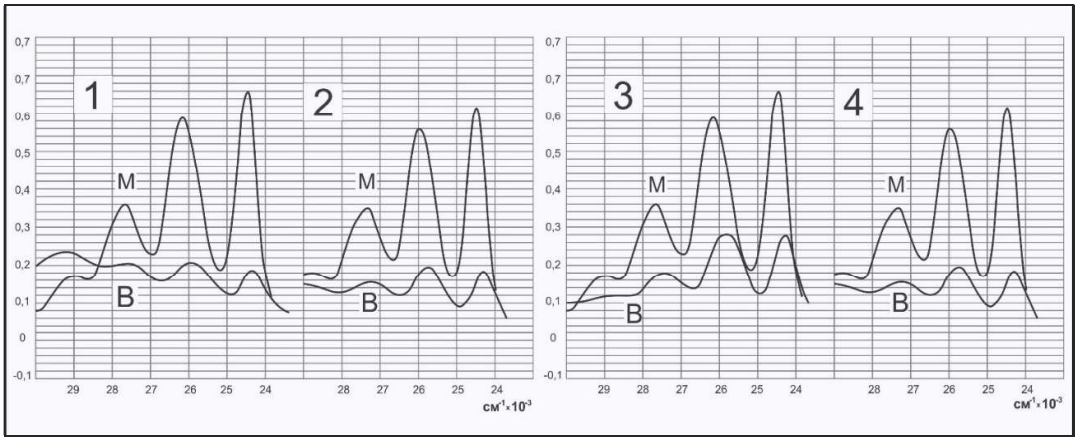


Fig. 3. UV spectrum of initial of amphotericin B (1) and its alkyl derivatives: methyl-(2), ethyl- (3), propyl- (4) at the concentrations $3 \cdot 10^{-5}$ M in the methanol (M) and in the water (B).

Among the studied PA most efficient one was methyllevorin which in concentration 20 mkg/ml suppressed the growth of malignant cells in lines C6 and HeLa by 35 % and in concentration 40mkg/ml by 70%, respectively. Biological activity of PA depends of medium where antibiotic dis-

solves. Macrolactone ring of amphotericin B includes polyene chromophor with 7 conjugated double bonds that is reflected in the UV spectrum of amphotericin B. This antibiotic has three main maximum of absorption at the wavelengths: 358-360, 378-380 и 400-403 nm. UV spectrum of absorption for amphotericin B and its derivatives in the methanol and water solution was presented in fig.3.

Analysis of the research results shows that most molecular dispersion of amphotericin B is in "good" organic solvents – methanol (Fig.3, spectrum M) and DMSO. Probably, bioavailability of antibiotic would be maximal in the same solvents. Antibiotics in the same concentration in the water form highly dispersive colloid solution with reference maximums but with small optical density (fig.3, spectrum B). This fact explains smaller activity in comparison with DMSO solutions. Thus antibiotics are in the molecular form in DMSO and methanol solutions. They have highest biological activity in this form (İbragimova et al., 2006). Polyenes are in associated form in the water solutions. So they are not so active (Kasumov, 2009). Synthesis and study of new membrane-active antibiotics on BLM promotes the definition of interrelation between structure and biological activity of molecules and makes possible to carry out the synthesis of new PA derivatives with improved the therapeutic properties and efficiently use them in the treatment of endogenous diseases.

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Bəzi Polyen Antibioklərin Xərçəng Hüceyrələrinə Təsiri

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Makrosiklik polyen antibioklərin pimarisin, nistatin, lyusensomisin, amfoterisin B və levorinin kimyəvi yolla yaradılmış törəmələrinin bioloji obyektlərə təsirinin təsviri analizi nəticəsində göstərilmişdir ki, qeyd olunan antibiotiklərin arasında ən yüksək bioloji aktivliyi ilə seçilən amfoterisin B və levorin törəmələridir. Amfoterisin B və levorin molekullarının müəyyən lakton hissələrində aparılan kimyəvi dəyişiklərinin nəticəsində yaradılan törəmələrin bioloji və lipid membranlarına təsiri tədqiq edilmişdir. Müəyyən olmuşdur ki, ilkin levorinə nisbətən metillevorin ən yüksək bioloji aktivliyi ilə seçilir. Levorin və amfoterisin B alkil törəmələrinin C6 sıçan qliomalarına və Hela karsinomaya *in vitro* təsiri nəticəsində metillevorinin və ilkin amfoterisinin xərçəng hüceyrələrinə məhvedici təsiri aşkar edilmişdir

***Açar sözlər**: Amfoterisin B, levorin, polen antibiotiklərin alkil törəmələri, xərçəng hüceyrələri, siçovulların qliomaları, uşaqlıq boynu karsinoması*

Эффект Некоторых Полиеновых Антибиотиков На Опухолевые Клетки

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Сравнительный анализ действия химически трансформированных полиеновых антибиотиков пимарицина, нистатина, люцензомицина, амфотерицина В и леворина на биологические объекты *in vivo* и *in vitro* показывает, что из указанных антибиотиков наибольшей биологической активностью обладают исходный амфотерицин В, леворин и его производные. Исследовано действие алкильных производных амфотерицина В и леворина, модифицированных в определенных частях лактонного кольца молекул, на липидные и биологические мембраны. Установлено, что метилированный леворин обладает высокой биологической активностью, чем исходный антибиотик. При действии алкильных производных леворина и амфотерицина В на клеточные культуры С6 (глиома крысы) и HeLa (карцинома шейки матки) *in vitro* был обнаружен противоопухолевый эффект метилированного леворина и исходного амфотерицина В.

***Ключевые слова**: Амфотерицин В, леворин, алкильные производные полиеновых антибиотиков, опухолевые клетки, глиома крысы, карцинома шейки матки*