

PRACA ORYGINALNA
ORIGINAL ARTICLE**THE RESEARCH OF ANTIMICROBIAL EFFICACY OF ANTISEPTICS
DECAMETHOXIN, MIRAMISTIN AND THEIR EFFECT ON NUCLEAR
DNA FRAGMENTATION AND EPITHELIAL CELL CYCLE****ANALIZA AKTYWNOŚCI PRZECIWBAKTERYJNEJ DWÓCH
ANTYSEPTYKÓW, DEKAMETOKSYNY I MIRAMISTYNY,
ORAZ ICH WPŁYWU NA FRAGMENTACJĘ JĄDROWEGO
DNA ORAZ CYKL KOMÓRKOWY ŚRÓDBŁONKA****Oleksandr A. Nazarchuk, Ihor L. Cheresniuk, Halyna H. Nazarchuk**
NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSYA, UKRAINE**ABSTRACT**

Introduction: Nowadays, the study of biological safety of modern cationic surface-active antiseptics with a wide antimicrobial spectrum has acquired particular importance. **The aim** was to study antimicrobial effectiveness of antiseptics decamethoxin, miramistin and their influence on nuclear DNA fragmentation and cellular cycle.

Materials and methods: A comparative microbiological study of antimicrobial efficacy and a cytometric study of the effect of decamethoxin 0,02% and miramistin 0,01% on the cellular cycle were carried out.

Antimicrobial activity of decamethoxin and miramistin was estimated by their minimal inhibitory and minimal microbicidal concentrations against opportunistic microorganisms using serial double dilution technique. Decamethoxin and miramistin cytotoxicity on anterior corneal epithelial cells, after their two-week daily instillation into the eyes of a *Vistar line* male rats was studied using flow cytometry. The parameters of epithelial cellular cycle, nuclear DNA fragmentation and apoptosis under the influence of antiseptics were registered.

Results: High antimicrobial effect of decamethoxin and miramistin against Gram-positive, Gram-negative bacteria with the significant advantages of decamethoxin were found ($p < 0,001$).

Decamethoxin caused minimal influence on anterior corneal epithelial cells, the insignificant decrease of their proliferation index, low increase of apoptosis (0.68%), no difference of mitotic activity ($p > 0.05$). But the use of miramistin resulted in the significant increase of nuclear DNA fragmentation, decrease of proliferative activity ($p < 0.05$).

Conclusions: Higher antimicrobial effect against a wide range of opportunistic pathogens is proved in decamethoxin 0,02% comparably to miramistin 0,01% ($p < 0,001$). In prolonged antiseptic use of the first one there were found no cytotoxic and no pro-apoptotic effects on the epithelium ($p < 0,05$).

KEY WORDS: antiseptics, apoptosis, decamethoxin, decasan, flow-cytometry, miramistin, cytotoxicity

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INTRODUCTION

Antiseptic drugs have gained a prominent place in medicine and pharmacy among the wide range of antimicrobial agents, having been used in medicine for a long period of time. Antiseptics are known to be chemicals and biological agents, which possess the ability to inhibit the reproduction of microorganisms (bacteriostatic action) and to kill them (bactericidal action), providing effective treatment of purulent-inflammatory diseases and the prevention of infectious complications. Antiseptics are medicines containing antimicrobials or their combination and they are permitted in accordance to the law for use in medical practice [1, 2].

Antiseptic drugs are well known to obtain high antimicrobial activity in non-toxic doses to the patient. Comparing the activity of antiseptics and antibiotics, it is necessary

to note the higher antimicrobial effectiveness of antibiotics. But, at the same time, there is essential difference between the minimum effective concentration of the antibiotic in the organism that provides the antimicrobial action and the maximum concentration, which does not cause negative influence on the patient's organism. Such an indicator for antiseptics is known as the index of effectiveness of medicinal antiseptic drugs. It is known that skin, mucous membranes have a higher resistance to the damaging effects of chemicals and drugs in comparison with body tissues. Due to these properties, it is possible to use topical higher concentrations of antiseptic drugs, comparably to biosecurity antibiotic doses for internal organs cells [1, 3, 4].

Antiseptics, which possess a wide spectrum of antibacterial, antifungal, antiviral action, and effectively eradicate resistant pathogens of infectious diseases, are acquired of

particular importance. Detergents (surface-active substances) occupy an important place in antiseptics among the well-known assortment of antiseptic agents of different groups. Quaternary ammonium compounds (QACs), which during the last century were characterized by high antimicrobial efficacy in the fight against pathogens of infectious complications, are worthy of particular attention [1, 5]. The high index of biocompatibility of antiseptics, containing quaternary nitrogen in its structure, opens up new perspectives of their in-depth comprehensive study and multi-vector application [6-9].

That is why, an in-depth study of the biological safety of modern QAC antiseptics with high microbicidal properties, is relevant too. Among them modern QAC antiseptic drug decamethoxin (DCM; 1,10-Decamethylene bis (N, N-dimethylmethoxycarbonylmethyl) ammonium dichloride) is known. Decamethoxin has been developed and introduced into medical practice by the Ukrainian scientists [3, 10, 11].

Nowadays, many years of experience of the effective clinical use of this antiseptic have been accumulated. But, there is a need for in-depth study of DCM effect on the eukaryotic cell. There is still the lack of the knowledge of molecular mechanisms of the QAC effects on the fragmentation of the genetic material (nuclear DNA) and the cycle of epithelial cells. Therefore, these studies are important for broadening the perceptions about the properties of the agents, along with the study of the antimicrobial properties of antimicrobial agents on the basis of quaternary ammonium.

One of the contemporary research areas is the flow cytometry method, which, in contrast to the classical microbiological methods, can provide information on molecular changes at the level of the eukaryotic cell, assessing the cytotoxic properties of the drugs. This method is suitable for the analysis of drugs, including antiseptics, because it allows one to accurately determine the content of nuclear DNA in individual cells and the distribution of cells in accordance with the phases of the cell cycle [12, 13].

THE AIM

The aim of our research work was to study of antimicrobial effectiveness of modern antiseptic drugs as decamethoxin and miramistin, based on the QAC, and their influence on nuclear DNA fragmentation and cycle of eukaryotic cell.

MATERIALS AND METHODS

In the work, a comparative microbiological study of antimicrobial efficacy and a cytometric study of the effect on the cell and its cycle of surface-active antiseptic drugs based on DCM and miramistin (MR) produced by the pharmaceutical industry of Ukraine were carried out. In the research there was used the ready-made dosage form of an antiseptic based on DCM, containing decamethoxin 200 µg/ml.

The other QAC antiseptic miramistin (Benzyltrimethyl[3-myrystoilamine]propyl]ammonium chloride

monohydrate), containing miramistin 100 µg / ml, was chosen as a comparison preparation. Both drugs for the experiment were purchased at the pharmacy network at our own expense.

The microbiological study of the antimicrobial activity of antiseptic drugs required determination of minimal inhibitory (MIC) and minimal microbicidal (MmCC) concentrations of DCM and MR against clinical strains of opportunistic isolates of microorganisms as *Staphylococcus aureus* (n=126), *Enterococcus faecalis* (n=18), *Escherichia coli* (n=89), *Enterobacter spp.* (n=39), *Klebsiella pneumoniae* (n=12), *Acinetobacter baumannii* (n=209), *Pseudomonas aeruginosa* (n=127), *Proteus mirabilis* (n=34), *Candida albicans* (n=35). There was used serial double dilution technique in accordance with standard methodological recommendations [14]. The analysis of antimicrobial effectiveness of ready-made dosage forms of an antiseptics DCM and MR, were carried out by means of comparison of indexes of antiseptic activity (IAA). In accordance with the well-known method, IAA, which indicates the efficacy of the drug, should be at least higher than "4". This index was defined for both antiseptics in accordance with the well-known standard method by the ratio of the concentration of the active antiseptic substance in its official medicinal form to the MIC of the same drug for the relevant pathogen [4].

The effect of DCM and MR antiseptic drugs on nuclear DNA fragmentation and epithelial cell cycle was studied by flow cytometry in the experiment *in vivo*. Experimental studies were performed on male rats of the *Vistar line*, at the age of 3 months with a body weight of 150 g, which were kept under standard vivarium conditions in National Pirogov Memorial Medical University, Vinnytsya. Animals were kept in the light mode: from 7:00 to 19:00 – light period (12 hours), from 19:00 to 7:00 – dark period (12 hours).

Corneal epithelial cells (CEC) were selected as a model of a single layer of epithelial cells in the whole organism for the research of *in vivo* cytotoxic action of drugs and their effects on the cell cycle. The use of CEC to study the effects of drugs, in particular antiseptics, on cell division according to phases of cell cycle and nuclear DNA fragmentation was based on the natural ability of the anterior epithelium of the cornea to complete the cycle of cell changes for 14 to 21 days, which in turn allowed us to investigate with the highest accuracy the smallest changes in the vital functions of individual cells of the whole organism, their proliferative activity and apoptotic death [13, 15].

Depending on the studied antiseptic drug, the animals were divided into two experimental groups per 10 animals in each there were used: DCM – in the main monitoring group and MR – in the comparison group. Both antiseptics were installed to the right of the rats' eyes 4 times a day for 14 days. In all rats, involved in the research, left eyes were intact ones.

To reduce the influence of the circadian rhythm on the parameters of the cellular cycle of the corneal anterior epithelium, the collection of material for the cytometric study was carried

Table I. The characteristics of antimicrobial activity of antiseptic drugs, based on decamethoxin and miramistin

Clinical isolates of microorganisms	Number	Decamethoxin (0,02 %)		Miramistin (0,01 %)	
		MIC* (µg/ml)	MCC** (µg/ml)	MIC* (µg/ml)	MCC** (µg/ml)
<i>S.aureus</i>	126	1,76±0,12	3,40±0,27	3,32±0,21	6,61±0,42
<i>E.faecalis</i>	18	1,50±0,19	3,65±0,42	5,03±0,75	10,07±1,44
<i>E.coli</i>	89	4,15±0,29	7,30±0,40	8,75±0,63	16,50±0,69
<i>A.baumannii</i>	209	27,92±1,58	57,51±2,97	41,93±2,16	75,00±3,36
<i>P.aeruginosa</i>	127	48,99±3,41	97,70±5,73	65,51±3,22	87,86±2,58
<i>P.mirabilis</i>	34	56,67±4,78	88,33±3,93	47,50±3,25	91,67±3,46
<i>Enterobacter spp.</i>	39	10,70±0,99	20,83±1,86	12,51±1,42	23,43±1,49
<i>K.pneumoniae</i>	12	11,06±1,44	21,15±1,67	14,11±2,40	24,63±2,48
<i>C.albicans</i>	35	8,39±0,51	15,36±0,90	17,50±1,05	28,57±1,50

*- minimal inhibitory concentrations; **- minimal cidal concentration.

out in the morning – from 10:00 to 11:00, and then the animals underwent euthanasia according to the ethical principles.

Under anaesthesia (10 mg/kg propofol intraperitoneally), under the control of the binocular microscope, a blade of a disposable microsurgical knife keratoma was used to collect the entire cornea of the CEC limited by limb. Subsequently, the preparation of samples of CEC cell suspensions for performing flow cytometric analyses was performed using a special kit CyStain DNA Step 2 (Partec, Germany). In each resulting sample of nucleic suspensions, 20.000 events were recorded at one and the same flow rate (2 ml/sec). Fluorescence control was performed using a special DNA standard of trout erythrocytes marked DAPI (Partec, Germany). During the flow cytometry of nuclear suspensions of PE on DNA histograms, a number of peaks were recorded. The first peak was placed in Channel 200 at 1024 channels for the most suitable for analysing the content of DNA on a linear scale, by adjusting the gain in the desired channel and scale [14]. To exclude unwanted alarm, the limit 20 was set. The analysis of the received DNA histograms was performed using multi-functional scientific-research flow-cytometer Partec Pas and analytical program FloMax (Partec, Germany).

The flow cytometry method allows to determine the percentage values of the cells that are in the phase of DNA synthesis (S-phase), as well as in the phases G0G1 (with DNA content = 2 s, post-mitotic cell), G2 + M (tetraploid/cells in which directly mitosis and there was a replication of DNA). The analysis of the special indices obtained during flow cytometry allowed to evaluate the proliferative activity of the cells under study and the peculiarities of their death by apoptosis under the influence of QAC antiseptic agents [12, 13, 16, 17]. The statistical processing of the received CEC cell cycle parameters was performed separately for the right and left eyes using the Student's parametric t-criterion and the Man-Whitney nonparametric criterion [18].

RESULTS

The research of antimicrobial activity has demonstrated high bacteriostatic and bactericidal action of antimicrobial drugs

based on DCM and MR against a wide range of opportunistic pathogens. As evidenced by the investigated relevant minimal microbicidal concentrations of DCM and MR, there was found high antimicrobial activity of both drugs against prominent Gram-positive microorganisms (*S.aureus*, *E.faecalis*). Predominantly, *Enterobacteria* (*E.coli*, *Enterobacter spp.*, *K.pneumoniae*), as well as non-fermentative gram-negative representatives of the *Acinetobacter* genus, has been found to have high sensitivity to both of the studied antiseptic drugs (table I).

Also, DCM and MR have possessed effective fungistatic and fungicidal effects on yeast-like fungi of the genus *Candida*. Clinical isolates of *Proteus spp.* and *Pseudomonas spp.* were found to had obtained lower susceptibility to DCM and MR. Corresponding high values of MCC of DCM (88,33±3,93 µg/ml and 97,70±5,73 µg/ml) and MR (91,67±3,46 µg/ml and 87,86±2,58 µg/ml) indicated their low bactericidal properties against *P.mirabilis* and *P.aeruginosa*. The analysis of the antimicrobial activity of antiseptic agents DCM and MR, by an IAA, has demonstrated significant advantages of the dosage form containing the first one.

The IAA of DCM, was 6 times higher than the same criteria in MR-based antimicrobial agent against enterococci ($p<0,001$); predominated 4 times IAA of MR against *S.aureus* ($p<0,001$), 4 times – in the case of *E.coli*, ($p<0,001$), 3,3 times – *Acinetobacteria* ($p<0,001$), 2,2 times – in the case of *Klebsiella* ($p<0,01$), 2 times higher – other *Enterobacteria* ($p<0,001$). The advantages of 4 times higher antifungal effectiveness of DCM-based antiseptic was found against *C.albicans* (fig. 1; $p<0,001$).

Despite vivid resistance of Gram-negative pathogens as *P.mirabilis* and *P.aeruginosa* to DCM and MR, the sufficient activity of an antiseptic medicinal product containing 0,02% DCM has been established. According to the data of IAA, predominating criteria IAA, which exceeded minimum acceptable threshold and were respectively 1,8 and 2,8 higher than the same ones in antiseptic medicinal product containing 0,01% of MR ($p<0,001$).

As a result of this study, the absence of objective changes in the eyes of rats was observed in the long-term use of DCM and MR. Following a cytometric study after two

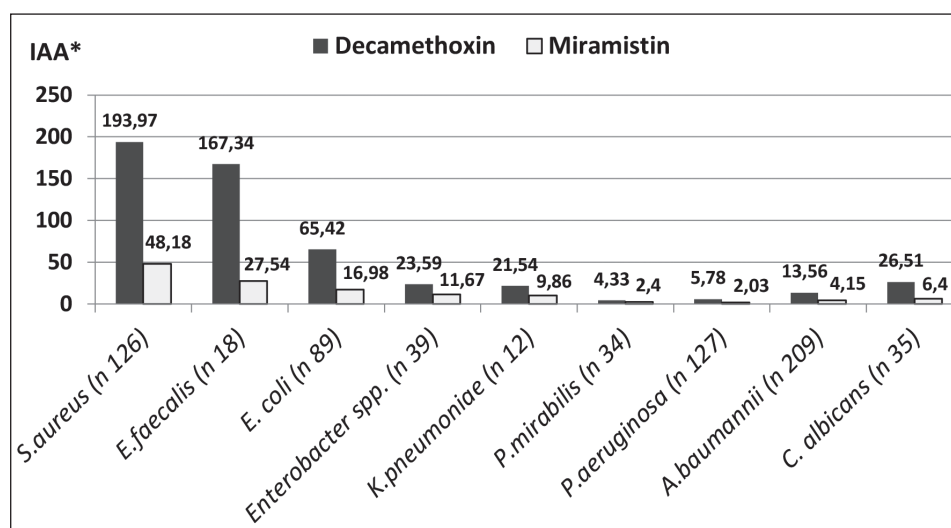


Fig. 1. Comparative characteristics of antimicrobial efficacy of decamethoxin and miramistin by the index of antiseptic activity (IAA*).

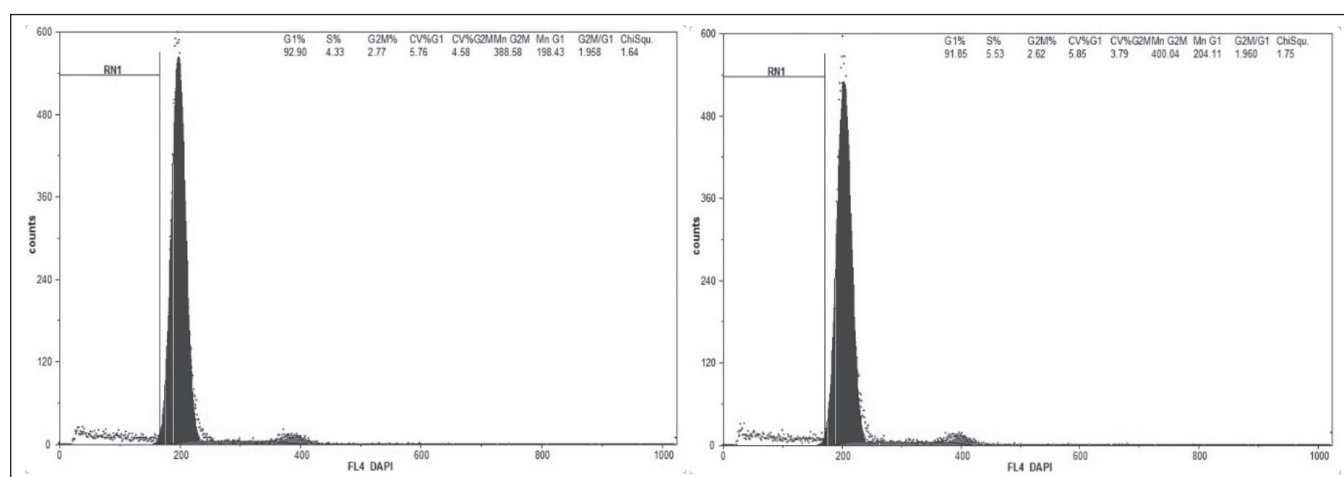


Fig. 2. DNA-histogram of nuclei suspension of the corneal epithelium: a) the administration of DCM-based antiseptic for 14 days (OD); b) intact comparison eye (OS).

weeks of application of the antiseptic drugs, an evaluation of the distribution of cells in the phases of the cell cycle was performed. In the observation study group after daily instillations of DCM in conjunctival cavity for 14 days there were evaluated further criteria of the distribution of cells in the phases of the cell cycle as G0G1 (93,13±0,99) %, S (4,12±0,57) %, G2+M (2,75±0,43) % (fig. 2; 3).

After the administration of antiseptic DCM, there has been found no significant difference between the percentages of CEC cells (4,12±0,57) %, which were registered in their phase of DNA synthesis (S-phase) in comparison with the intact eye (4,88±0,39) % ($p>0,05$). After the course of DCM administration, the average values of the number of CEC cells present in the G0G1 phase, which characterized mitotic activity, did not differ from those of the G0G1 in the CEC of the eyes which did not receive antiseptic instillations ($p>0,05$). The percentage rate of G2+M was lower by 1,04% when DCM having been used than in control eye. The observation demonstrated insignificant difference with this indicator of intact eyes. A detailed analysis of DNA-histograms has shown the complete acceptability of the variation coefficient (CV), which was (5,94±0,16)% for G0G1 peak of CEC when DCM

had been used. And CV did not differ from the same criterion in CEC of intact eyes (5,87±0,15) % ($p>0,05$; fig.3).

The results of cytometric studies showed that that the index of proliferation of IP (6,87 ± 0,99%) in 1,3 times was lower under two-weekly daily every six hours instillation of DCM than in epithelial cells of CEC intact eyes. Under the 14 days influence of DCM on CEC cells, the minimal signal changes were recorded on DNA histograms that corresponded to nuclei of cells containing DNA <2 seconds in the range of SUB-G0D1. An insignificant increase in the value of the apoptosis index (APO) of CEC cells treated with DCM for 14 days was found to be 0.68% in comparison with this indicator in the epithelial cells of the cornea of the intact eye. The difference was statistically insignificant ($p>0.05$), which seemed the absence of nuclear fragmentation of DNA in corneal epithelium with long-term use of DCM.

Under the experimental study of the daily installation of MR antiseptic for a two-week period the CEC cell cycle was characterized by a distribution of cells which differed slightly from those of intact cells. According to received data there was found the significant difference between

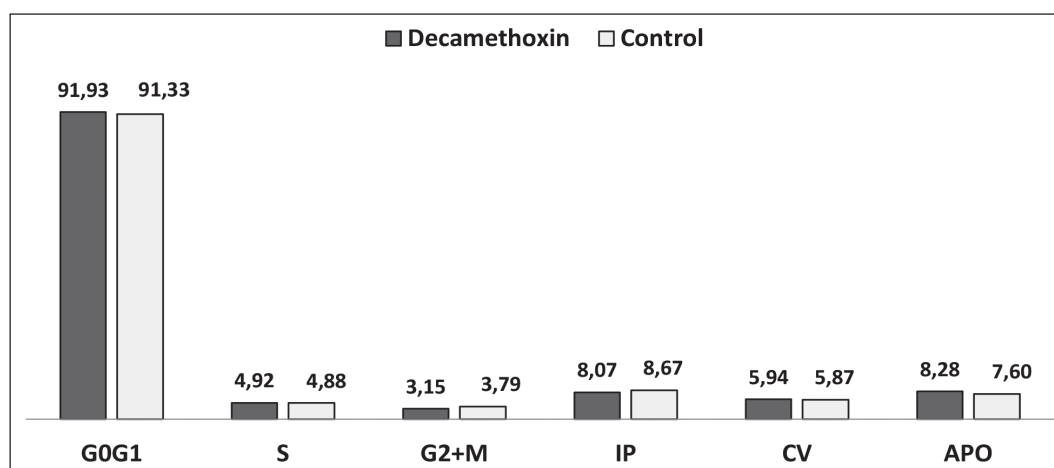


Fig. 3. The cytometric characteristics of the parameters of the cell cycle of CEC with in vivo long-term use of antiseptic decamethoxin in the experiment.

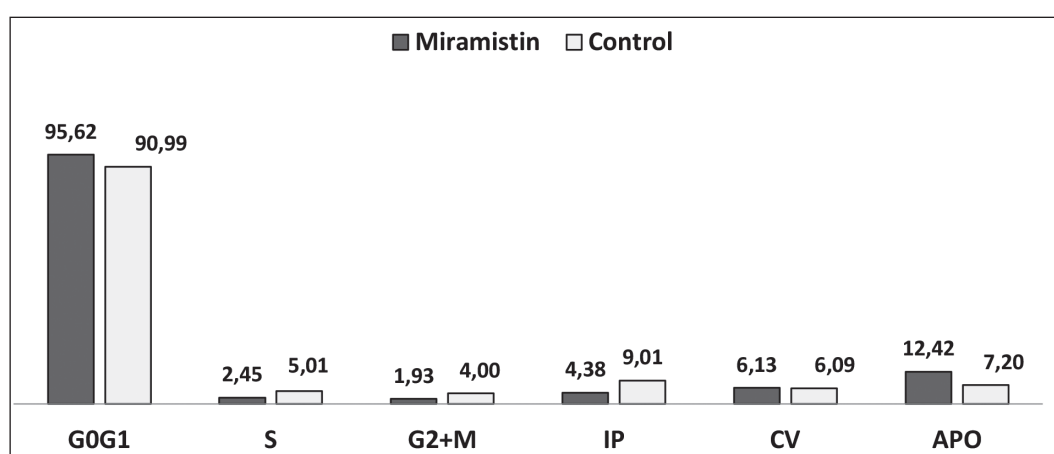


Fig. 4. The cytometric characteristics of the parameters of the cell cycle of CEC with in vivo long-term use of antiseptic miramistin in the experiment.

the values of the cell cycle phases G0G1 ($95,62 \pm 0,19$ %), S ($2,45 \pm 0,17$ %), G2+M ($1,93 \pm 0,04$ %) of the experimental eyes and of comparison ones ($p < 0,05$). The prolonged use of MR resulted in the significant increase of cells in the G0G1 phase (4.63%) in comparison with intact ones and 3.69% compared to cells managed with DCM.

A comparative analysis of the G0G1 peak variation coefficients in the use of MR ($CV = 6.13 \pm 0.14$ %) and intact CECs ($CV = 6.09 \pm 0.36$ %) showed valid CV values, and a lack of a significant difference between their values ($p > 0,05$) confirmed the proper quality of DNA-histograms in accordance with generally accepted requirements. In the use of MR during the S-phase of the epithelial cell cycle, suppression was found to be 2.55% of DNA synthesis in the nuclei of the CEC cells (2.45 ± 0.17 %), which was significantly lower than in intact epithelium ($p < 0,05$; fig. 4).

In the study group, after 14 days of MR administration on DNA histograms there were observed peaks, indicating a decrease in the percentage of cells in the G2+M phase in 2.1 times less than in intact CEC cells ($p < 0,01$). According to the obtained values of the interval SUB-G0G1 ($12,42 \pm 0,46$ %), a significant increase in the level of DNA

fragmentation in the nuclei of epithelial cells has been demonstrated, which was subjected to 5.22% daily MR exposure over a two-week period. At the same time, in intact cells, the level of nuclear DNA fragmentation did not exceed (7.2 ± 0.63 %), which was 1.73 times less than with this antiseptic. It was found, that the average values of the index of proliferation (4.38 ± 0.19) in the use of MR were significantly lower, than the proliferative activity of intact cells (6.09 ± 0.36 %) only in 1.3 times ($p < 0,05$; fig. 4).

After the application of the MR-based antiseptic drug solution, there was found no significant difference between the percentage content of CEC cells ($4,12 \pm 0,57$ %) that were in the phase of DNA synthesis (S-phase) compared with the intact eye ($4,88 \pm 0,39$ %) ($p > 0,05$).

DISCUSSION

QAC antiseptic agents contain a nitrogen atom, which is directly linked to four alkyl groups of varying complexity, which have a variable structure. The unique chemical structure, physical and chemical properties of the DCM realize its high therapeutic, prophylactic antimicrobial activity. Bioequivalence of DCM is significantly dependent on the

presence in the composition of the molecule of L-menthol, which could be as of synthetic origin so as obtained by the biological extraction method from peppermint. [3, 5, 10, 11]. The mechanism of antimicrobial effect of DCM and MR is realized by reducing the surface tension and violation of the permeability of the cell membrane of the microbial cell, transmembrane transport of molecules, osmotic balance ions, nitrogen and phosphorus exchange, activation of proteolytic enzymes, lysis and autolysis of microorganisms [1, 3].

A comparative study of the sensitivity of 689 clinical strains of opportunistic pathogenic microorganisms, causative agents of infectious complications in severely ill patients with surgical pathology, who were treated at the intensive care units showed a high sensitivity of *Staphylococci* and *Enterococci* to DCM and MR antiseptic drugs.

The high bacteriostatic and bactericidal effectiveness of these antiseptics against the Gram-negative representatives of enterobacteria (*E.coli*, *Enterobacter spp.*, *K.pneumoniae*) and non-fermentative Gram-negative microorganisms as *A.baumannii*.

The advantages of the DCM-based antiseptic were found on the basis of the values of IAA, which exceeded 2-4 times the corresponding parameters of MR ($p < 0,001$). In spite of the high concentrations of DCM and MR, needed for ensuring bactericidal action against clinical strains of microorganisms *P.mirabilis* and *P.aeruginosa*, there were found relevant values of IAA of drug, containing 0,02% of DCM, to exceed the minimum allowable threshold, having proved its efficacy against mentioned pathogens. IAA of DCM also were 1,8-2,8 times higher than similar values of the antiseptic, containing 0,01% MR ($p < 0,001$).

In the process of developing, manufacturing and investigation of antiseptic drugs for medical purposes, the issue of biocompatibility of a medicinal product always poses a challenge, leading to the need for modern research with quantitative and qualitative analysis of cell cycle performance through flow cytometry along with traditional microbiological, biochemical, pharmacological and pharmaceutical techniques.

The unique selective antimicrobial membran-directed action of QAC antiseptics has been proved due to many *in vitro* studies on protoplast and spheroplast cell-models. Besides of high biosafety, there was proved the cytotoxic effect and suppression of proliferation of fibroblasts in some QAC and cationic-active surfactant antiseptics (octenisept, polyhexamethylene biguanid, chlorhexidine digluconate) [19]. The ability of some of them (chlorhexidine digluconate) to stimulate apoptosis of cells, autolytic and necrotic cell death were proved [20].

According to the research data some scientists estimated *in vitro* cytotoxic effect of therapeutic antimicrobial concentrations of such cationic active detergents as cetylpyridinium chloride and miramistin on L929 fibroblast and keratinocyte cell line HaCaT. There is insufficient data on the cytotoxic action on human cells *in vivo* and the need for further research in the use of some QAC surface-active antiseptic agents [21].

According to the experimental data there was estimated absence of a significant difference between the number of epithelial cells in the phase of DNA synthesis after durable application of DCM and in the intact CEC ($p > 0,05$). This proved the absence of any negative effect of DCM antiseptic drug, with its prolonged use, on the state of proliferative activity of the cells of the anterior corneal epithelium.

There was found, that after the daily administration of an antiseptic DCM, the number of cells, capable for the synthesis of DNA during the S-phase, did not change and corresponded to the number of CEC cells in the inactive eye. At the same time, it was found that the number of epithelial cells, which did not lose the ability to synthesize DNA in the S-phase after prolonged use of an antiseptic based on MR, significantly had decreased in 2.04 times than in intact epithelium ($p < 0,05$).

Flow-cytometric studies have shown that after long-term use of the DCM antiseptic drug, there was not found its side effect on the mitotic activity of CEC. This was proved, by insignificant difference between the number of postmitotic CEC cells after administration of DCM and isotonic sodium solution (intact eyes) respectively ($p > 0,05$). After daily action of DCM for 14 days, the tetraploid cells, in which the replication of DNA had been finished and mitosis took place, were proved to have no significant difference from CEC cells, in which antiseptic had not been used ($p > 0,05$).

As a result of flow-cytometric analysis of apoptotic activity in CEC cell, there was proved a significant increase in 1.73 times of the rate of DNA fragmentation in the nucleus of epithelial cells, which had been undergone two-week everyday effect of miramistin ($p < 0,05$). Insignificant features of nuclear DNA fragmentation, registered in the nucleus of CEC cells, strictly affirmed that prolonged use of DCM-based antiseptic did not provide pro-apoptotic effect in the epithelium.

As a result of experimental imaging of the daily two-week exposure of the DCM-based and MR-based antiseptics into CEC cells, there was no significant difference in the proliferation index ($p > 0,05$), when long duration of DCM administration had been used. This indicated no inhibitory effect of mentioned antiseptic agent on the proliferative activity of the epithelium. The minimal insignificant differences in the corresponding parameters of the cell cycle of the CEC have been experimentally determined by the equally strictly synchronized rate of proliferation and apoptotic death of the epithelial cells in intact eyes and eyes with two-weekly introduction of DCM.

CONCLUSIONS

1. The antiseptic drug, containing 0.02% decamethoxin, has high antimicrobial properties against a wide range of pathogens of infectious complications (*S.aureus*, *E.faecalis*, *E.coli*, *Enterobacter spp.*, *K.pneumoniae*, *A.baumannii*, *P.aeruginosa*, *P.mirabilis*) as evidenced by the corresponding indexes of activity of antiseptics, which exceeded the minimum acceptable threshold, and also were 1.8 – 6 times higher than similar indexes of antiseptic drug miramistin (0.01%; $p < 0,001$).

2. The daily two-week use of an antiseptic drug decamethoxin (0.02%) *in vivo* does not affect the cytometric parameters of the cell cycle, in particular on the mitotic activity of the epithelium and the number of cells, capable to synthesize the DNA during the S-phase, while at the same time the antiseptic drug miramistin (0.01%) reliably leads to the 2.04 times decrease of the number of epithelial cells and is accompanied by 1.73 times increase in the rate of nuclear DNA fragmentation ($p < 0.05$).
3. Prolonged use of an antiseptic drug decamethoxin is characterized by a strictly synchronized rate of proliferation and apoptotic death of epithelial cells, which indicates the absence of cytometric signs of cytotoxic action and pro-apoptotic effects.

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According to the order of the Authorship.

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