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THE ANTIMICROBIAL ACTION OF DECAMETHOXINUM SUBSTANCE AT DIFFERENT PH VALUES

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Key words: *decamethoxinum*; "Decasan"; antimicrobial action; antifungal action; pH

This study considers the effect of pH on the specific activity of decamethoxinum substance under experimental conditions. It has been shown that the minimum inhibitory concentration (MIC) of this substance depends on the microorganism species and pH; the lowest MIC values for S. aureus, P. aeruginosa, E. coli are determined in the basic medium. The significant antimicrobial activity in both acidic and basic media was shown against S. paratyphi, and the antifungal effect against C. albicans increased in the basic medium. It has been determined that decamethoxinum inhibits formation of biofilms by E. coli and P. aeruginosa, the effect depends on the concentration and pH (the maximal inhibitory effect is at pH 8.0±0.1 in the concentration of 10.0). Decamethoxinum-induced inhibition of the microorganism biomass accumulation is also pH-dependent. Thus, inhibition of the growth and reproduction of P. aeruginosa was observed in 6 hours of incubation with the substance at pH 6.3±0.4; inhibition of the vital activity of E. coli was registered in the basic medium (pH 8.0±0.1) in 1 hour of incubation; while inhibition of S. paratyphi was in 12 hours of incubation at pH 5.1±0.2; 6.3±0.4; 8.0±0.1. The most significant activity of decamethoxinum against the gram-positive bacteria (S. aureus) was revealed in 6 hours of incubation at pH 5.1±0.2. In the acidic medium mentioned the antifungal action against C. albicans was also observed with inhibition of the cell accumulation in 12 hours after the substance introduction.

Intestinal diseases of the microbial etiology remain a global problem today because of high prevalence and a severe course, especially at a young age, in immunocompromised persons and patients with chronic pathologic processes in the gastrointestinal system. Development of resistance of infectious agents determines a need in broadening the spectrum of antimicrobial agents [9, 12]. Since biological films cause about 65-80% of all chronic infectious diseases, including the diseases of the gastrointestinal system [13], the effect of antibacterial agents on the biofilm formation and viability is especially important. In addition, the pH of the biological medium can determine the antimicrobial activity of substances and should be taken into account during the drug development.

To use the known antimicrobial drug decamethoxinum was recognized to be expedient for developing peroral medicinal forms for the treatment of acute intestinal diseases of the microbial etiology. Being a surface-active cationic detergent, decamethoxinum exhibits the antibacterial, antiviral, antifungal activity of a broad spectrum; it is practically not adsorbed from the gastrointestinal tract, and can break microbial toxins and inhibit inflammation [2, 4, 7]. "Decasan" is 0.02% decamethoxinum solution for peroral use developed by "Yuria Pharm" (Kyiv, Ukraine); it is widely prescribed in infectious diseases in surgery, dentistry, gynaecology, urology, otolaryngology, pulmonology [2, 7]. The pharmacological studies of the drug activity in the intestinal infections, as well as its toxicological properties have recently started and shown the promising results [3, 8].

The aim of the study is to determine the effect of pH on the specific activity of decamethoxinum substance, including its ability to inhibit the biofilm formation.

Materials and Methods

The experiments were conducted at the Department of Antimicrobial Agents of the SI "Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine". Decamethoxinum substance ("Yuria Pharm," Kyiv, Ukraine) was studied after its dissolution in distilled water. Clinical test strains of bacteria and fungi with different sensitivity to the antibacterial agents were used, namely *Escherichia coli* 1512, *Salmonella paratyphi* B 252, *Pseudomonas aeruginosa* 2094, *Staphylococcus aureus* 042012, as well as *Candida albicans* 1486. Such growth media as Müller-Hinton agar, meat peptone agar, growth media No.8, Sabouraud medium (fluid and solid) were used depending on the species, in accordance with the current recommendations [6, 11]. Different pH values were used: 8.0 ± 0.1 (basic); 7.15 ± 0.2 (neutral); 6.3 ± 0.4 (weakly acidic), 5.1 ± 0.2 and 4.1 ± 0.3 (acidic).

The minimum inhibitory concentration (MIC) of the substance under study was determined by serial dilution in the liquid nutrient medium according to [1, 5, 6]. MIC was determined as the maximum dilution of the substance, in which there was no growth of microorganisms visually observed within the specified time (concentrations used in this study were within the range of 50.0-0.312 mcg/ml). The density of the bacterial inoculum was 10⁵ colony forming units (CFU) per 1.0 ml of medium, for yeasts this value equalled 10⁵ fungal elements per 1.0 ml. Bacteria

Table 1

The antibacterial activity of decamethoxinum substance

Microorganisms	Minimum inhibitory concentration, mcg/ml			
Gram-positive bacteria				
<i>S. aureus</i> 042012	pH 5.1±0.2	pH 6.3±0.4	pH 7.15±0.2	pH 8.0±0.1
	1.25	1.25	0.62	0.62
Gram-negative bacteria				
<i>E. coli</i> 1512	12.5	6.25	6.25	3.12
<i>S. paratyphi</i> B 252	0.62	1.25	1.25	0.62
<i>P. aeruginosa</i> 2094	50.0	50.0	50.0	25.0

were cultivated for 24-48 hours (depending on the species) under aerobic conditions at 35-37°C. Fungi were cultivated using Sabouraud medium for the same period at 30-35°C. The density of the bacterial inoculum was measured using a KFK-2 photocolorimeter at 590 nm (bacteria) and 540 nm (fungi), it was equalled to 5×10^8 CFU/ml for bacteria and 1×10^6 - 5×10^6 for fungi.

The sensitivity of microorganisms to the action of decamethoxinum was studied by the agar diffusion assay measuring the diameters of zones of the microorganism growth inhibition [1]. The concentration of decamethoxinum in the solutions was equalled to 100.0; 50.0; 25.0; 10.0 mcg/ml.

The intensity of biofilm formation [10] and accumulation of the microbial biomass in the presence of decamethoxinum at the different pH levels were registered using an "Absorbance Microplate Reader ELx800" microbiological analyser (BioTek, USA). The optical density was registered at 405 nm (samples) and 630 nm (reference).

Biofilms of the gram-negative bacteria *P. aeruginosa* and *E. coli* (24-h cultures) were studied. Decamethoxinum (in the concentrations equalled 10.0 MIC and 1.0 MIC in accordance with the species) and inocula of microorganisms (10^7 CFU/ml) were added to the growth media simultaneously. In 24 hours of incubation at 37°C the test objects were treated with 0.1% solution of gentian violet, washed with distilled water, the dye was extracted with ethanol.

The process of the microbial biomass accumulation was studied in the liquid nutrient media using 24-h cultures of *E. coli* 1512, *S. paratyphi* B 252, *S. aureus* 042012, *P. aeruginosa* 2094, *C. albicans* 1486 (the inoculation dose was equalled to 10^6 colony forming units per 1.0 ml for bacteria and 10^5 fungal elements per 1.0 ml for fungi). After adding decamethoxinum in the concentrations mentioned above the plates were kept in a PST-60HL-4 termoshaker (Biosan, Latvia) at 37°C with mixing (500 rpm) for 48 hours. The optical density was registered in 1, 6, 12, 24, 48 hours as mentioned above. The individual control data for the growth medium sterility, as well as the culture growth were provided for each pH value.

The data were processed using the Microsoft Excel standard software; Student's t-test was applied. The level of significance was taken as $p \leq 0.05$.

Results and Discussion

1. The minimum inhibitory concentrations of decamethoxinum substance at different pH values

The strains of bacteria and fungi tested were characterized by different sensitivity to antimicrobial agents. *E. coli* 1512 was sensitive to aminoglycosides, fluoroquinolones, cephalosporins (cephtriaxone), carbapenems, co-trimoxazole and chloramphenicol; moderately sensitive to tetracycline; resistant to ampicillin and cephepime. *P. aeruginosa* 2094 was sensitive to aminoglycosides, fluoroquinolones, carbapenems, monobactams and co-trimoxazole; moderately sensitive to cephalosporins (cephthasidime, cepthriaxone); resistant to cephepime, ampicillin and tetracycline. *S. aureus* 042012 was sensitive to aminoglycosides, fluoroquinolones, carbapenems, monobactams, lincosamides, cephalosporins (cephthasidime) and chloramphenicol; *S. paratyphi* B 252 – to all agents used (namely gentamycin, imipinim, tobramycin, ciprofloxacin, cepthriaxone). *C. albicans* 1486 was sensitive to azoles; moderately sensitive to amphotericin B and terbinafin.

As the data in Tab. 1 indicate, changes in the pH of the media affected the sensitivity of the test strains to decamethoxinum substance. Thus, the inhibitory action against gram-positive bacteria such as *S. aureus* increased in the basic medium. In relation to the gram-negative bacteria the most significant inhibition of *E. coli* was observed at pH 8.0±0.1 with reduction of the activity in more acidic medium; *S. paratyphi* was sensitive to the substance effect in the basic and acidic media, while at the levels of pH close to the neutral one the sensitivity was slightly decreased. *P. aeruginosa* appeared to be less sensitive to decamethoxinum with the less evident effect in the acidic medium. The activity of the substance studied against yeasts (*C. albicans*) increased with the increase of the pH values and was equalled to 25.0 mcg/ml at pH 4.1±0.3; 6.25 mcg/ml at pH 5.1±0.2; pH 6.3±0.4; pH 7.15±0.2.

2. Sensitivity of microorganisms to the decamethoxinum action in the agar diffusion assay at different pH values

As seen from Tab. 2, the inhibitory effect of decamethoxinum substance against gram-positive bacteria (*S. aureus*) increased in the basic medium. The same phe-

Table 2

Diameters of the zones of the microorganism growth inhibition in the presence of decamethoxinum substance at different pH values, (M±m)

Microorganisms	Concentrations	Zone of the growth inhibition (d, mm)			
		pH 8.0±0.1	pH 7.15±0.2	pH 6.3±0.4	pH 5.1±0.2
<i>E. coli</i> 1512	100.0 mcg	24.2±1.08	23.8±2.58	21.2±0.08	22.3±0.25
	50.0 mcg	22.7±0.33	22.2±1.08	18.7±0.08	20.0±0.08
	25.0 mcg	20.7±0.33	19.7±0.08	17.2±0.08	17.5±0.05
	10.0 mcg	18.5±0.05	17.2±0.08	13.3±0.33	13.7±0.25
<i>P. aeruginosa</i> 2094	100.0 mcg	24.8±0.08	20.3±0.33	11.7±0.33	–
	50.0 mcg	21.5±0.25	17.8±0.08	–	–
	25.0 mcg	18.7±0.08	14.7±0.58	–	–
	10.0 mcg	14.0±3.0	–	–	–
<i>S. aureus</i> 042012	100.0 mcg	29.8±1.12	30.2±0.08	29.2±1.08	30.5±0.25
	50.0 mcg	29.3±1.12	29.0±1.75	27.8±0.08	28.5±0.25
	25.0 mcg	28.0±0.5	27.5±0.75	26.0±0.05	26.3±0.58
	10.0 mcg	26.0±0.5	25.2±0.58	24.0±0.25	23.6±0.58
<i>S. paratyphi</i> B 252	100.0 mcg	29.2±0.08	28.7±0.58	27.7±0.58	32.8±0.58
	50.0 mcg	27.0±0.05	26.7±1.33	26.2±0.58	30.3±0.58
	25.0 mcg	25.3±0.33	24.5±0.75	24.5±0.25	28.0±0.25
	10.0 mcg	23.8±0.08	22.8±0.58	22.3±0.08	25.5±0.08
		pH 7.15±0.2	pH 6.3±0.4	pH 5.1±0.2	pH 4.1±0.3
<i>C. albicans</i> 1486	100.0 mcg	21.8±0.08	20.3±0.08	20.0±0.05	*
	50.0 mcg	20.2±0.08	18.7±0.08	19.3±0.33	*
	25.0 mcg	18.0±0.25	16.8±0.08	17.5±0.75	*
	10.0 mcg	17.3±1.33	15.3±2.33	15.8±1.08	*

Note: "–" – the zone of the growth inhibition was not registered; * – the effect was not studied.

nomena were registered for *E. coli*, and the dependence of the effect on the pH was the most apparent after addition of decamethoxinum in the amount of 10 mcg (that might be connected with the specificity of the substance penetration into the cell of the gram-negative bacteria). In relation to *P. aeruginosa* the most significant effect was observed at pH 8.0±0.1, the decrease of the pH value to 7.15±0.2 was accompanied with reduction of the inhibitory effect, while at pH 5.1±0.2 the zones of the growth inhibition were not registered at all. Somewhat different results were obtained after the experiments with *S. paratyphi*: the antibacterial activity of decamethoxinum increased with the increase of the pH value, still the sufficient activity was present in the neutral and acidic medium. The antifungal effect of decamethoxinum did not change significantly with the pH and, after addition of decamethoxinum in the amount of 10 mcg the zones of the growth inhibition were within the range of 15.3–17.3 mm.

3. The effect of decamethoxinum on the process of biofilm formation

E. coli biofilms were not sensitive to the substance action in the concentration of 1.0 MIC, but the increase of concentration up to 10.0 MIC allowed obtaining a significant effect: inhibition of biofilm formation reached 48% at pH 8.0±0.1; 41% at pH 7.15±0.2; 39% at pH 6.3±0.4. Biofilm formation of *P. aeruginosa* slightly re-

duced in the presence of decamethoxinum in the concentration of 1.0 MIC (1.7% at pH 8.0±0.1; 5.4% at pH 6.3±0.4; there was no effect at pH 7.15±0.2); at higher concentrations the increase in the activity was also registered with inhibition of 54% at pH 8.0±0.1; 39% at pH 7.15±0.2; 37% at pH 6.3±0.4. Thus, decamethoxinum could inhibit the process of biofilm formation by the gram-negative bacteria.

4. The effect of decamethoxinum on the biomass accumulation of the microorganisms

Decamethoxinum was able to inhibit the growth and reproduction of the cultivated *P. aeruginosa* (Fig. 1). The inhibitory effect was registered in 6 hours of incubation at pH 6.3±0.4; it reached a significant level in 12 hours in all media and subsequently increased. Therefore, in 48 hours the biomass accumulation of *P. aeruginosa* was blocked at all pH values studied. The effect of the substance studied on *E. coli* is shown in Fig. 2. This strain appeared to be sensitive to decamethoxinum with the inhibitory action seen as early as in 1 hour of incubation in the basic medium with the increase in the subsequent hours and the highest activity in 48 hours. Decamethoxinum also inhibited the growth and reproduction of the cultivated *S. paratyphi* B 252 with the effect being dependent on the pH and the time of incubation (Fig. 3). The most active inhibition of these bacteria in the bio-

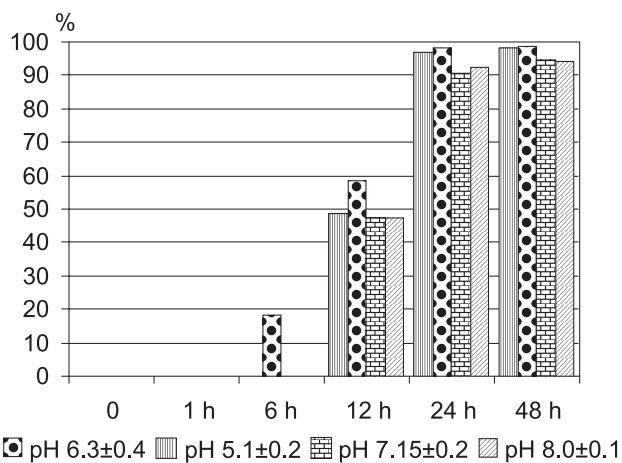


Fig. 1. The effect of decamethoxinum on the biomass accumulation of *Pseudomonas aeruginosa* 2094 at different pH values. The data are given as % of inhibition (the individual control data are taken as 100%), decamethoxinum is used in the minimal inhibitory concentration (1.0 MIC).

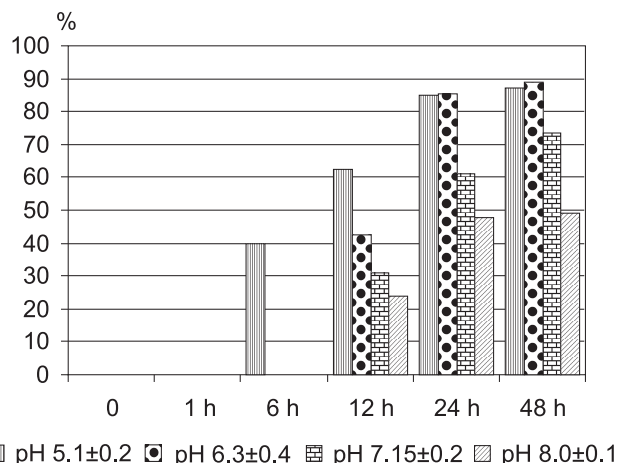


Fig. 4. The effect of decamethoxinum on the biomass accumulation of *Staphylococcus aureus* 042012 at different pH values. The data are given as % of inhibition (the individual control data are taken as 100%), decamethoxinum is used in the minimal inhibitory concentration (1.0 MIC).

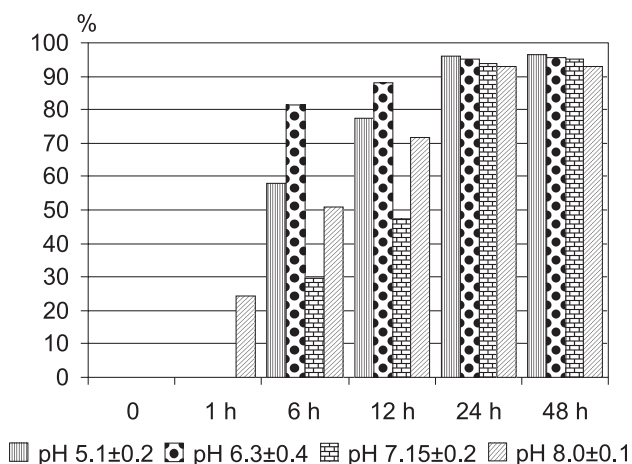


Fig. 2. The effect of decamethoxinum on the biomass accumulation of *Escherichia coli* 1512 at different pH values. The data are given as % of inhibition (the individual control data are taken as 100%), decamethoxinum is used in the minimal inhibitory concentration (1.0 MIC).

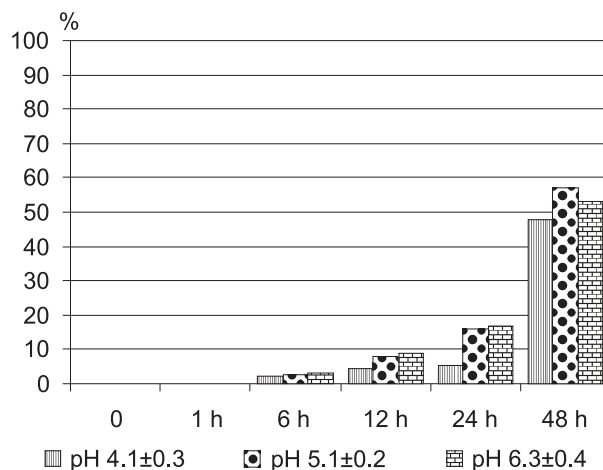


Fig. 5. The effect of decamethoxinum on the biomass accumulation of *Candida albicans* 1486 at different pH values. The data are given as % of inhibition (the individual control data are taken as 100%), decamethoxinum is used in the minimal inhibitory concentration (1.0 MIC).

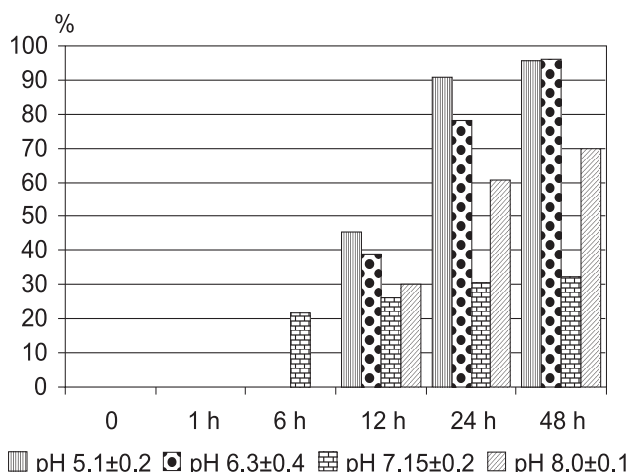


Fig. 3. The effect of decamethoxinum on the biomass accumulation of *Salmonella paratyphi* B 252 at different pH values. The data are given as % of inhibition (the individual control data are taken as 100%), decamethoxinum is used in the minimal inhibitory concentration (1.0 MIC).

mass accumulation in 6 hours was observed at pH 7.15±0.2, in other periods under study – at pH 5.1±0.2 and pH 6.3±0.4 (less but still significant effect was also seen at pH 8.0±0.1). In relation to *S. aureus* 042012 the inhibitory activity of decamethoxinum was registered in 6 hours at pH 5.1±0.2 and subsequently increased in all media, especially at pH 5.1±0.2 and pH 6.3±0.4 (Fig. 4). When the bactericidal concentration of 10.0 MIC was used, decamethoxinum exerted a significant antimicrobial effect from the first hours without a dependence on the pH. The antifungal action of the substance studied against *C. albicans* 1486 began in 6 hours of incubation and reached the values close to inhibition of 50% in 48 hours in all media. In the concentration of 10.0 MIC decamethoxinum completely blocked the biomass accumulation of *C. albicans* 1486 at all pH values studied.

CONCLUSIONS

1. Decamethoxinum substance exhibits significant antimicrobial properties against *P. aeruginosa*, *E. coli*, *S. aureus*, *S. paratyphi*, and *C. albicans* with the depen-

dence of the inhibitory effect on the microorganism species and pH of the medium. Determination of MIC values shows that the antimicrobial activity of decamethoxinum against bacteria and fungi increases in the basic medium.

2. In the agar diffusion assay decamethoxinum substance demonstrates the antimicrobial action in the concentrations of 100.0; 50.0; 25.0; 10.0 mcg/ml. Diameters of the zones of the microorganism growth inhibition depend on the microorganism species and the pH of the medium: the highest activity against *P. aeruginosa*, *S. aureus*, *E. coli* is observed at pH 8.0±0.1, against *C. albicans* – at pH 7.15±0.2, against *S. paratyphi* – at pH 8.0±0.1 and pH 5.1±0.2.

3. Decamethoxinum substance inhibits formation of biofilms by *P. aeruginosa* and *E. coli*, this effect also depends on the concentration and pH. The most active inhibition of biofilm formation is achieved in the concentration of 10.0 MIC and pH 8.0±0.1.

4. In the concentration of 1.0 MIC decamethoxinum substance decreases the biomass accumulation of *E. coli* (in 1 hour at pH 8.0±0.1, in the other periods the activity under research is increased in all of the media studied), *S. paratyphi* (in 24 and 48 hours of incubation in both basic and acidic media), *P. aeruginosa* (in 12 and, especially 24 hours, as well as 48 hours of incubation in all of the media studied), *S. aureus* (in 1 hour at pH 5.1±0.2, in 24 and 48 hours at pH 5.1±0.2, and in 12 hours at pH 6.3±0.4 with a less significant effect). In the bactericidal/fungicidal concentration of 10.0 MIC decamethoxinum inhibits the biomass accumulation of bacteria/fungi from the first hours without a dependence on pH. The sensitivity of *C. albicans* to the antifungal action of decamethoxinum has not also been determined by pH in the concentration of 1.0 MIC (the significant activity in this case is registered in 48 hours of incubation).

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АНТИМІКРОБНА АКТИВНІСТЬ СУБСТАНЦІЇ ДЕКАМЕТОКСИНУ ЗА РІЗНИХ ЗНАЧЕНЬ pH Н.М.Деркач, З.С.Суворова

Ключові слова: декаметоксин; «Декасан»; антимікробна дія; протигрибкова дія; pH

Роботу присвячено визначенню впливу pH на специфічну активність субстанції декаметоксину в експерименті. Показано, що мінімальна інгібуюча концентрація (МІК) даної субстанції залежить від виду мікроорганізму та pH із найнижчими значеннями МІК для *S. aureus*, *P. aeruginosa*, *E. coli* в лужному середовищі. Значну антимікробну активність відносно *S. paratyphi* верифіковано як у кислому, так і в лужному середовищі, тимчасом як протигрибковий ефект відносно *C. albicans* зростає в лужному середовищі. Встановлено, що декаметоксин пригнічує процес плівкоутворення *E. coli* та *P. aeruginosa*, ефект є залежним від концентрації та pH (максимально виражений при pH 8,0±0,1 в концентрації 10,0 МІК). Декаметоксин інєбує накопичення біомаси мікроорганізмів, що також залежить від pH. Так, стримування росту та розмноження *P. aeruginosa* виявляється через 6 год інкубації з субстанцією при pH 6,3±0,4; пригнічення життєдіяльності *E. coli* реєструється в лужному се-

редовищі (рН 8,0±0,1) вже через 1 год інкубації, тимчасом як інгібування *S. paratyphi* реалізується через 12 год інкубації при рН 5,1±0,2; 6,3±0,4; 8,0±0,1. Найбільш виражена активність декаметоксину відносно грампозитивних бактерій (*S. aureus*) виявляється через 6 год інкубації при рН 5,1±0,2. У зазначеному кислому середовищі також встановлено противгрибкову активність декаметоксину відносно *C. albicans* із інгібуванням накопичення клітин через 12 год після внесення субстанції.

ПРОТИВОМИКРОБНАЯ АКТИВНОСТЬ СУБСТАНЦИИ ДЕКАМЕТОКСИНА ПРИ РАЗЛИЧНЫХ ЗНАЧЕНИЯХ рН

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Ключевые слова: декаметоксин; «Декасан»; антимикробное действие; противогрибковое действие; рН

Работа посвящена определению влияния рН на специфическую активность субстанции декаметоксина в эксперименте. Показано, что минимальная ингибирующая концентрация (МИК) данной субстанции зависит от вида микроорганизма и рН, наименьшие значения МИК для *S. aureus*, *P. aeruginosa*, *E. coli* определяются в щелочной среде. Значительная антимикробная активность относительно *S. paratyphi* верифицирована как в кислой, так и в щелочной среде, тогда как противогрибковый эффект относительно *C. albicans* возрастает в щелочной среде. Установлено, что декаметоксин подавляет процесс пленкообразования *E. coli* и *P. aeruginosa*, эффект зависит от концентрации и рН (максимально выражен при рН 8,0±0,1 в концентрации 10,0 МИК). Декаметоксин ингибирует накопление биомассы микроорганизмов, что также зависит от рН. Так, подавление роста и размножения *P. aeruginosa* выявляется через 6 ч инкубации с субстанцией при рН 6,3±0,4; угнетение жизненной активности *E. coli* регистрируется в щелочной среде (рН 8,0±0,1) уже через 1 ч инкубации, тогда как ингибирование *S. paratyphi* реализуется через 12 ч инкубации при рН 5,1±0,2; 6,3±0,4; 8,0±0,1. Наиболее выраженная активность декаметоксина относительно грамположительных бактерий (*S. aureus*) проявляется через 6 ч инкубации при рН 5,1±0,2. В упомянутой кислой среде также установлена противогрибковая активность декаметоксина относительно *C. albicans* с ингибированием накопления клеток через 12 ч после внесения субстанции.