

Recommended by Doctor of Pharmacy, professor L.S.Strelnikov

UDC 615.281:577.112.34:547.466: 547.233.1/4

THE EFFECT OF SUBSTITUENTS IN THE MOLECULES OF N-, R-ALKYL AMINES ON SOME GRAM-POSITIVE STRAINS OF MICROORGANISMS

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Key words: N-, R-alkylamines; antimicrobial activity

The intensive use of antibiotics in patient care institutions often without determining specificity and sensitivity to them leads to more rapid development of resistance to pathogens of nosocomial infections; that is why it is one of the urgent problems of healthcare of Ukraine. The most common gram-positive pathogens of nosocomial infections are Staphylococcus aureus, coagulase-negative staphylococci and enterococci. In order to determine dependence of the microbiological action on the nature of substituents in the molecules of N-, R-alkylamines some methyl, ethylamine, aminoalcohols, N-hydroxymethyl- and N-methyl-N-carboxymethylamines have been tested. In accordance with the WHO recommendations to assess the antibacterial activity of N-, R-alkylamines the gram-positive test strains – Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 6633 were used. The aliphatic amines and aminoalcohols studied show a weak or moderate activity in relation to strains of Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 6633. Compounds containing a carboxyl group and methyl radicals in the molecule exhibit the greatest antimicrobial activity in relation to the gram-positive strains of microorganisms under research.

Antibacterial agents are practically the only group of drugs which effectiveness decreases with time due to development of resistance. The intensive use of antibiotics in patient care institutions often without determining specificity and sensitivity to them leads to more rapid development of resistance to pathogens of nosocomial infections; that is why it is one of the urgent problems of healthcare of Ukraine. The main factors that contribute to the increased disease incidence are: shortage of medicines, antiseptics, detergents and disinfectants, medical instruments, linen, sterilization equipment since medical preventive institutions are forced to work in conditions of the extremely limited funding; a significant growth in the number of hospital strains that are resistant to antibiotics and disinfectants, etc. [4].

The most common gram-positive pathogens of nosocomial infections are *Staphylococcus aureus*, coagulase-negative staphylococci and enterococci. The results of the multicentre randomized trial SCOPE (USA) published in 2004 indicate the predominance of gram-positive cocci in the etiological structure of nosocomial bacteriemias [14].

This tendency creates significant problems since the choice of antimicrobial agents intended to combat drug-resistant gram-positive microorganisms is limited. It should be also noted the fact that antibiotics are less than 5% of the drugs being currently at the stage of drug development [5], and as resistance to the medicines used develops, there is a need in both creation of new drugs, and correction of methods for using the existing ones. Therefore, the research in development of drugs, including

vaccines and diagnostic agents, are of vital importance for protection of future generations.

It is known that tertiary amine salts or quaternary ammonium bases containing radicals with a large number of carbon atoms exhibit a strong bacteriostatic and bactericidal action, as well as possess pronounced disinfectant properties [1, 8]. In literature there are data that compounds with the number of carbon atoms from 5 to 16 are the most effective against microorganisms [6].

The aim of this work was to determine the effect of various functional groups containing in the molecules of N-, R-alkylamines derivatives on their antibacterial activity in relation to some gram-positive strains of microorganisms.

Materials and Methods

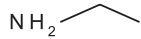
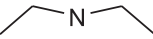
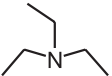
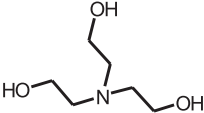
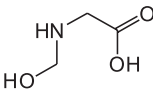
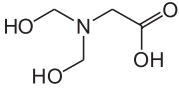


In order to determine dependence of the microbiological action on the nature of substituents in the molecules of N-, R-alkylamines the following groups were tested: alkylamines (compounds I-VI), aminoalcohols (compounds VII-IX), N-hydroxymethyl-N-carboxymethylamines (compounds X-XII), and N-methyl-N-carboxymethylamines (compounds XIII-XV) (see Table).

The compounds under research were obtained from commercial sources or synthesized according to the synthetic schemes previously developed [2, 3, 6, 9, 11-13]. Reagents were purchased from "Sigma-Aldrich" (USA) and used without further purification. 1% Aqueous solutions of compounds I-XV were tested.

In accordance with the WHO recommendations to assess the antibacterial activity of N-, R-alkylamines the gram-positive test strains – *Staphylococcus aureus*

Table

Data of the study results of the antibacterial activity of 1% solutions of the samples under research

Compound	The structural formula	Test strains/inhibition zones, mm	
		Staphylococcus aureus ATCC 25923	Bacillus subtilis ATCC 6633
I	$\text{H}_2\text{N}-$	10 (l.s.)* ± 0.1	10 (l.s.) ± 0.1
II	$-N-$	10 (l.s.) ± 0.1	10 (l.s.) ± 0.1
III	$\begin{array}{c} \\ -N- \\ \end{array}$	15 (l.s.) ± 0.1	15 (l.s.) ± 0.1
IV	NH_2 	10 (l.s.) ± 0.1	10 (l.s.) ± 0.1
V		12 (l.s.) ± 0.1	10 (l.s.) ± 0.1
VI		10 (l.s.) ± 0.1	10 (l.s.) ± 0.1
VII	$\text{H}_2\text{N}-\text{CH}_2-\text{CH}_2-\text{OH}$	10 (l.s.) ± 0.1	10 (l.s.) ± 0.1
VIII	$\text{HO}-\text{CH}_2-\text{CH}_2-\text{N}-\text{CH}_2-\text{CH}_2-\text{OH}$	13 (l.s.) ± 0.1	10 (l.s.) ± 0.1
IX		20 (s.) ± 0.2	21 (s.) ± 0.2
X		20 (s.) ± 0.2	17 (s.) ± 0.2
XI		20 (s.) ± 0.2	25 (s.) ± 0.2
XII	$\left[\begin{array}{c} \text{HO}-\text{CH}_2 \\ \\ \text{HO}-\text{CH}_2-\text{N}^+ \\ \\ \text{HO}-\text{CH}_2 \end{array} \right] \text{Cl}^-$	21 (s.) ± 0.2	25 (s.) ± 0.2
XIII		40 (h.s.) ± 0.3	39 (h.s.) ± 0.3
XIV		40 (h.s.) ± 0.3	39 (h.s.) ± 0.3
XV	$\left[\begin{array}{c} \text{O} \\ \\ -\text{N}^+ \\ \end{array} \right] \text{Cl}^-$	42 (h.s.) ± 0.3	39 (h.s.) ± 0.3

* Note: l.s. – low sensitivity of the culture to the given concentration of the test substance; s. – sensitivity of the culture to the given concentration of the test substance; h.s. – high sensitivity of the culture to the given concentration of the test substance.

ATCC 25923 and *Bacillus subtilis* ATCC 6633 were used. The suspension of the test microorganism was prepared according to the method [10]. Standardization of the bacterial suspension of microorganisms prepared was carried out using a Densi-La-Meter device (manufactured by PLIVA-Lachema, Czech Republic). Synchronization of cultures by changing the cultivation temperature was achieved with a single effect of low temperature

(4°C). Microbial load was 10^7 microbial cells per 1 ml of the medium and set up according to McFarland standard. We worked with 18-24 hour culture of microorganisms.

For studies Mueller-Hinton agar (“Himedia Laboratories, Pvt. Ltd India” the shelf life of the medium to XI 2016, manufactured by India) was used.

Diffusion of the drug into the agar was conducted by the “wells” method [7]. When assessing the activity

of compounds I-XV, as well as when studying antibiotic-resistant strains the following criteria were used:

- the absence of inhibition zones of microorganisms around the well, the diameter of the inhibition zone to 10 mm indicates that the organism is insensitive to the drug introduced into the well or to the antibiotic concentration;
- inhibition zones with the diameter of 10-15 mm indicate a low sensitivity of the culture to the given concentration of the test substance;
- inhibition zones with the diameter of 15-25 mm assessed as an indicator of the sensitivity of a microorganism to the test substance;
- inhibition zones, which diameter exceeds 25 mm, indicates the high sensitivity of microorganisms to the test substance.

Results and Discussion

A low sensitivity of the set of microorganisms used to the action of aliphatic amines I-VI was determined. The antimicrobial activity of aminoalcohols VII-IX was slightly higher, and its enhancement is observed with increasing the number of hydroxyethylene radicals in the molecule. Compounds X-XII containing a carboxyl and hydroxymethyl group in the molecule was more

active, with the increasing number of hydroxyethylene groups the inhibition zones were 20-21 mm for *Staphylococcus aureus* and 17-25 mm for *Bacillus subtilis*.

Compounds containing a carboxyl group and methyl radicals appeared to be the most promising among the compounds tested. It is this combination of substituents that contributes to a high microbicide activity in relation to *Staphylococcus aureus* and *Bacillus subtilis*. Increase of the number of methyl radicals in the molecules of compounds XIII-XV leads to a significant increase in activity – the inhibition zones are 39-42 mm.

CONCLUSIONS

1. The effect of various functional groups containing in the molecules of N-, R-alkylamines derivatives on their antibacterial activity in relation to some gram-positive strains of microorganisms has been determined.

2. The aliphatic amines and aminoalcohols studied show a weak or moderate activity in relation to strains of *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633.

3. Compounds containing a carboxyl group and methyl radicals in the molecule exhibit the greatest antimicrobial activity in relation to the gram-positive strains of microorganisms under research.

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ВПЛИВ ЗАМІСНИКІВ В МОЛЕКУЛАХ N-, R-АЛКІЛАМІНІВ НА ДЕЯКІ ГРАМПОЗИТИВНІ ШТАМИ МІКРООРГАНІЗМІВ**М.Ю.Голик, М.А.Комісаренко, С.Г.Леонова, Т.П.Осолодченко****Ключові слова:** N-, R-алкіламіни; антимікробна активність

Інтенсивне застосування антибіотиків у лікарських установах, часто без визначення специфіки та чутливості до них, призводить до більш швидкого розвитку стійкості до збудників внутрішньолікарняних інфекцій, що є однією з актуальних проблем охорони здоров'я України. Найбільш розповсюдженими грампозитивними збудниками внутрішньолікарняних інфекцій є *Staphylococcus aureus*, коагулазонегативні стафілококи та ентерококи. З метою встановлення залежності антимікробної дії від природи замісників в молекулах N-, R-алкіламінів були протестовані деякі метил-, етиламіни, аміноспирти, N-гідроксиметил- та N-метил-N-карбоксиметиламіни. У відповідності до рекомендацій ВООЗ для оцінки антибактеріальної активності N-, R-алкіламінів використовували грампозитивні тест-штами *Staphylococcus aureus* ATCC 25923 та *Bacillus subtilis* ATCC 6633. По відношенню до штамів *Staphylococcus aureus* ATCC 25923 та *Bacillus subtilis* ATCC 6633 досліджувані алифатичні аміни та аміноспирти виявляють слабку або помірну активність. Найбільшу антимікробну активність до досліджуваних грампозитивних штамів мікроорганізмів проявляють сполуки, що містять у молекулах карбоксильну групу та метильні радикали.

ВЛИЯНИЕ ЗАМЕСТИТЕЛЕЙ В МОЛЕКУЛАХ N-, R-АЛКИЛАМИНОВ НА НЕКОТОРЫЕ ГРАМПОЛОЖИТЕЛЬНЫЕ ШТАММЫ МИКРООРГАНИЗМОВ**Н.Ю.Голик, Н.А.Комиссаренко, С.Г.Леонова, Т.П.Осолодченко****Ключевые слова:** N-, R-алкиламины; антимикробная активность

Интенсивное использование антибиотиков в лечебных учреждениях, зачастую без определения специфики и чувствительности к ним, приводит к более быстрому развитию устойчивости к возбудителям внутрибольничных инфекций, что является одной из актуальных проблем здравоохранения Украины. Наиболее распространенными грамположительными возбудителями внутрибольничных инфекций являются *Staphylococcus aureus*, коагулазонегативные стафилококки и энтерококки. С целью установления зависимости антимикробного действия от природы заместителей в молекулах N-, R-алкиламинов были протестированы некоторые метил-, этиламины, аминспирты, N-гидроксиметил- и N-метил-N-карбоксиметиламины. В соответствии с рекомендациями ВОЗ для оценки антибактериальной активности N-, R-алкиламинов использовали грамположительные тест-штаммы – *Staphylococcus aureus* ATCC 25923 и *Bacillus subtilis* ATCC 6633. По отношению к штаммам *Staphylococcus aureus* ATCC 25923 и *Bacillus subtilis* ATCC 6633 исследуемые алифатические амины и аминспирты проявляют слабую или умеренную активность. Наибольшую антимикробную активность к исследуемым грамположительным штаммам микроорганизмов проявляют соединения, содержащие в молекулах карбоксильную группу и метильные радикалы.