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The therapeutic efficacy of 1-[4-(1,1,3,3-tetramethyl butyl) phenoxy]-3-(n-benzyl hexametylenimino)-2-propanol chloride under *in vivo* models

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Key words: antibacterial activity, experimental bacterial infection, therapeutic efficacy, aryl aliphatic aminoalcohols

The continuous spread of resistant pathogens as well as decrease of the efficacy of current antimicrobial chemotherapy indicates the need of research and development of novel active compounds.

There are varieties of potential antimicrobials amongst such chemical classes as peptides [1–5], antisense compounds [6], pleuromutilins [7], aminobenzimidazoles [8], pyrrolidine diones [9], bis-imidazolinylindoles [10], lantibiotics [11], bis-indoles [12], 3-methoxybenzamides [13], derivatives of barbituric [14], N-alkyl urea hydroxamic acids [15], etc. One of the promising chemical classes is aryl aliphatic aminoalcohols, possessing a pronounced antibacterial properties *in vitro* [16, 17]. However, *in vitro* results yet do not show the clinical perspectives of potential new drugs.

The aim of this study was to investigate the therapeutic efficacy of aryl aliphatic aminoalcohol derivative 1-[4-(1,1,3,3-tetramethylbutyl)phenoxy]-3-(N-benzyl hexametylenimino)-2-propanol chloride (compound KVM-194) using *in vivo* models of generalized and local bacterial infection.

Materials and methods. Therapeutic efficacy of aryl aliphatic aminoalcohol derivative KVM-194 was evaluated in models of generalized infection, topical inflammatory process and bacterial conjunctivitis.

Experimental generalized infection was reproduced in white nonlinear mice (18,0–20,0 g weigh) [18–20]. The overnight culture of *Pseudomonas aeruginosa* was injected intraperitoneally in dosage $4 \cdot 10^7$ CFU/animal. The compound was administered as follows:

- therapeutic regimen – a single intraperitoneal administration of compound 30 min after infection at dosages, equal to 0,1 LD_{50} (51,5 mg/kg), 0,01 LD_{50} (5,15 mg/kg) and 0,001 LD_{50} (0,515 mg/kg) [21];
- preventive regimen – single intraperitoneal administration 24 h prior to infection at dosages, equal to 0,01 LD_{50} and 0,001 LD_{50}.

Clinical signs of infection and survival rate were registered in animals of experimental and control groups. The presence of *P. aeruginosa* was confirmed by its isolation from blood samples and subsequent identification.

The efficacy of compound was examined as well on the rabbit models of topical inflammatory process (n = 10) and conjunctivitis (n = 9), caused by *Staphylococcus aureus.* Chinchilla rabbits (2,0–2,5 kg weight) were used for these experiments.

Topical inflammatory process was reproduced by intradermal injection of 0,5 ml ($1 \cdot 10^9$ CFU) of *S. aureus* inoculum, followed by skin scarification. Compound KVM-194 was applied on the inflamed areas as a 0,25 % hydrophilic ointment (ointment base contained PEO-400 and PEO-1500 in a ratio of 4:1) 2 times a day. The first application was made the next day post infection. Inflamed skin areas of animals of control group were moistened with sterile saline.

Bacterial conjunctivitis was reproduced on background of topical anesthesia by 0,5 % solution of proxymetacaine hydrochloride. Both eyes were administered 2 drops of 2,5 % ammonia solution and 0.1 ml of *S. aureus* inoculum ($1 \cdot 10^9$ CFU/ml) 30 min after [18–20].

Animals were divided into three groups of 3 animals: the first group was treated by 0,1 % solution of compound KVM-194; the second group was treated by 30 % solution of sodium sulfacil («Sulfacil», «JSC "Farmak») as a reference; and finally, the third group included control animals (sterile saline only).

The examination was graded for clinical signs of conjunctivitis (ocular discharge, hyperemia and edema of conjunctiva) using the severity scale (0–3 points).

The presence of *S. aureus* was confirmed by its isolation from discharge samples and subsequent identification. Treatment was started 5 h after infection. Eyes were washed with sterile saline before each instillation of KVM-194 or «Sulfacil» solutions. Drug administration was performed 5 times a day every 2 h for 7 days.

Animal experiments were carried out according to the Directive 2010/63/EU [22].

Fisher's exact test and Mann-Whitney criteria were used for statistical analysis in the «StatSoft Statistica 6.0» [23].

Results and discussion. Antimicrobial activity of compound KVM-194 *in vivo* was studied on the murine model of generalized *P. aeruginosa* infection. The data obtained are shown in Table 1 and Table 2.

Clinical sighs of infection (weakness, tail and ear cyanosis) were noted on the 1st day post infection. All animals were observed during 14 days and mortality was registered to the 3rd day.

Mortality rate of the control animals in the study of therapeutic efficacy was 50 %. Administration of KVM-194 in the dosage, equal to 0,1 LD_{50} did not improve the survival, furthermore, resulted in 100 % lethality. This effect could be due to distinct bactericidal properties of compound. Simultaneous lysis of bacteria led to the release of a significant amount of endotoxins, and consequently – to animal death. These effects are also observed in officinal bactericidal antibiotics [24].

Single intraperitoneal injection of 0,01 LD_{50} of KVM-194 prevented the death of 70 % of infected animals, but showed no statistically significant differences comparatively to control. Compound at a 0,001 LD_{50} demonstrated no advantages; mortality rate was equal to control (50 %).

Table 1

Therapeutic efficacy of the compound KVM-194 in a generalized P. aeruginosa infection

Group	Dosage	Animals	
		Total	Survived
KVM-194	51,5 mg/kg (0,1 LD_{50})	10	0*
	5,15 mg/kg (0,01 LD_{50})	10	7
	0,515 mg/kg (0,001 LD_{50})	10	5
Control	–	10	5

*Note. *p < 0,05 comparatively to control.*

Table 2

Preventive efficacy of the compound KVM-194 in a generalized P. aeruginosa infection

Group	Dosage	Animals	
		Total	Survived
KVM-194	5,15 mg/kg (0,01 LD_{50})	10	5*
	0,515 mg/kg (0,001 LD_{50})	10	0
Control	-	10	0

*Note. *p < 0,05 comparatively to control.*

Further studies revealed preventive efficacy of KVM-194 (Tab. 2).

The data obtained suggest, that mortality rate in this experiment was 100 %. Single administration of KVM-194 at a dosage 0,01 LD_{50} reduced lethality to 50 %. Compound at a 0,001 LD_{50} demonstrated no advantages; mortality rate was equal to control (100 %).

Thus, investigation of the compound KVM-194 action on the model of generalized *P. aeruginosa* infection showed the distinct preventive efficacy of compound at a dosage equal to 0,01 LD_{50}.

The rabbit model of topical inflammatory process was used to investigate the therapeutic efficacy of compound in the case of topical administration on the skin (Tabl. 3). Hyperemia, edema and infiltration of affected areas were observed 24 h post infection. Abscess formation was registered on 3^{rd}–4^{th} day of experiment in treated animals, and on 5^{th}–6^{th} day – in untreated control.

Administration of KVM-194 ointment on the skin surface 2 times a day reduced hyperemia and accelerated abscess formation and rupture. Skin necrosis and wound were not observed. Complete recovery was noted in 8 days for treated animals, while sighs of inflammation in control animals were registered for 16 days. Statistically significant differences in size of inflammation zones between control and treated animals were registered starting from the 1^{st} day of experiment.

Bacterial conjunctivitis model was used to investigate the therapeutic efficacy of topical administration of compound on the mucosa. Inflammation of conjunctiva of untreated animals lasted for 7 days (Tabl. 4).

Instillation of KVM-194 solution, as well as instillation of «Sulfacil», led to reduction of inflammation symptoms in conjunctiva of experimental animals, starting from the 1^{st} day. Complete recovery of treated animals was registered on the 4^{th} day of the experiment.

The experimental data suggest the efficacy of 0,1% solution of KVM-194 on the model of staphylococcal conjunctivitis, compound did not concede the reference drug «Sulfacil».

Table 3

Therapeutic efficacy of 0,25 % KVM-194 ointment on the model of topical inflammatory process caused by S. aureus, M ± m

Parameter	Term of observation					
	Day 1	Day 3	Day 6	Day 8	Day 14	Day 16
Hyperemia zone, sm^2						
- treated	4,14 ± 1,04*	1,15 ± 0,20*	0,73 ± 0,19*	0*	0*	0
- control	9,67 ± 2,37	2,08 ± 0,46	1,75 ± 0,20	1,80 ± 0,29	0,85 ± 0,07	0
Infiltrate, sm^2						
- treated	1,12 ± 0,18	1,03 ± 0,18*	0,73 ± 0,19*	0*	0*	0
- control	1,10 ± 0,24	2,23 ± 0,22	1,75 ± 0,20	1,80 ± 0,29	0,85 ± 0,07	0

*Notes. *p < 0,05 comparatively to control group; M – mean value; m – standard error.*

Table 4

Therapeutic efficacy (total score) of a 0,1 % KVM-194 solution on a model of staphylococcal conjunctivitis

Group	Prior to treatment	Term of observation				
		Day 1	Day 2	Day 3	Day 4	Day 7
I group (KVM-194)	36	26	9	6	0	0
II group («Sulfacil»)	36	28	10	6	0	0
III group (Control)	36	36	36	36	20	0

Conclusion

Our studies have shown a distinct preventive efficacy of 1-[4-(1,1,3,3-tetramethylbutyl)phenoxy]-3-(N-benzyl hexametylenimino)-2-propanol chloride (compound KVM-194) on the murine model of generalized *P. aeruginosa* infection at a dosage, equal to 0,01 LD_{50}. Aryl aliphatic aminoalcohol derivative also demonstrated therapeutic effect on the rabbit models of topical inflammatory process and conjunctivitis, caused by *S. aureus*; administrations of compound reduced symptoms and shorten the course of the pathological processes. The data obtained suggest prospects of further animal studies to establish the spectrum of *in vivo* activity and rational dosage regimens of the compound KVM-194.

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Терапевтична ефективність 1-[4-(1,1,3,3-тетраметилбутил)фенокси]-3-(N-бензилгексаметиленіміній)-2-пропанол хлориду на моделях *in vivo*

Похідні арилаліфатичних аміноспиртів – новий перспективний клас сполук, що проявляють виразну антимікробну активність в умовах *in vitro*. *Мета дослідження* – встановлення терапевтичної ефективності похідного арилаліфатичних аміноспиртів 1-[4-(1,1,3,3-тетраметилбутил)фенокси]-3-(N-бензилгексаметиленіміній)-2-пропанол хлориду (шифр КВМ-194) на моделі генералізованої інфекції та при місцевих ураженнях бактеріального генезу.

Експериментальну генералізовану інфекцію відтворювали на білих нелінійних мишах з використанням *Pseudomonas aeruginosa*. Інфікуюча доза складала 4 • 10^7 КУО/тварину. При моделюванні місцевого патологічного процесу кролям внутрішньошкірно вводили 1 • 10^9 КУО *Staphylococcus aureus* (0,5 мл) з наступною скарифікацією шкіри. Для відтворення кон'юнктивіту в око кролям вносили по 2 краплі 2,5 % розчину аміаку на фоні анестезії 0,5 % розчином проксиметакаїну гідрохлориду. Через 30 хв на кон'юнктиву наносили 0,1 мл культури *S. aureus* зі щільністю 1 • 10^9 КУО/мл.

Результати проведених досліджень свідчать про те, що при генералізованій синьогнійній інфекції найвиразнішу ефективність сполука КВМ-194 проявляє в дозі, що відповідає 0,01 $ЛД_{50}$. Дослідження впливу сполуки при місцевому застосуванні показало, що нанесення 0,25 % мазі КВМ-194 сприяло зменшенню гіперемії, прискорило формування абсцесу та його розрив. Повне зникнення ознак запалення в дослідних тварин відмічали на 8 добу, у тварин контролю – на 16 добу. На моделі бактеріального кон'юнктивіту сполука у вигляді 0,1 % розчину також проявила ефективність, спостерігалось зменшення проявів запалення та скорочення терміну перебігу патологічного процесу.

Проведені дослідження на тваринах свідчать про необхідність подальших поглиблених досліджень ефективності сполуки на моделях інфекційного процесу *in vivo* для встановлення широти спектра дії та дозування сполуки КВМ-194.

Ключові слова: антибактеріальна активність, експериментальна бактеріальна інфекція, терапевтична ефективність, арилаліфатичні аміноспирти

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Терапевтическая эффективность 1-[4-(1,1,3,3-тетраметилбутил) фенокси]-3-(N-бензилгексаметилениминий)-2-пропанол хлорида на моделях *in vivo*

Производные арилалифатичних аминоспиртов – новый перспективный класс соединений, проявляющих выраженную антимикробную активность в условиях *in vitro*. *Цель исследования* – установление терапевтической эффективности производного арилалифатичних аминоспиртов 1-[4-(1,1,3,3-тетраметилбутил)фенокси]-3-(N-бензилгексаметилениминий)-2-пропанол хлорида (шифр КВМ-194) на модели генерализованной инфекции и при местных поражениях бактериального генеза.

Экспериментальную генерализованную инфекцию воспроизводили на белых нелинейных мышах с использованием *Pseudomonas aeruginosa*. Инфицирующая доза составляла 4 • 10^7 КОЕ/животное. При моделировании местного патологического процесса кроликам внутрикожно вводили 1 • 10^9 КОЕ *Staphylococcus aureus* (0,5 мл) с последующей скарификацией кожи. Для воспроизведения конъюнктивита в глаза кроликам вносили по 2 капли 2,5 % раствора аммиака на фоне анестезии 0,5 % раствором проксиметакаина гидрохлорида. Через 30 мин на конъюнктиву наносили 0,1 мл культуры *S. aureus*, содержащей 1 • 10^9 КОЕ/мл.

Результаты проведенных исследований свидетельствуют о том, что при генерализованной синегнойной инфекции наибольшую эффективность соединение КВМ-194 проявляет в дозе, соответствующей 0,01 $ЛД_{50}$. Исследование влияния соединения при местном применении показало, что нанесение 0,25 % мази КВМ-194 способствовало уменьшению гиперемии, ускорило формирование абсцесса и его разрыв. Полное исчезновение признаков воспаления у опытных животных отмечалось на 8 сут, у животных контроля – на 16 сут. На модели бактериального конъюнктивита

соединение в виде 0,1 % раствора также проявило эффективность, наблюдалось уменьшение воспаления и сокращение длительности патологического процесса.

Проведенные исследования на животных свидетельствуют о необходимости дальнейших углубленных исследований эффективности соединения на моделях инфекционного процесса *in vivo* для установления широты спектра действия и дозировки соединения КВМ-194.

Ключевые слова: антибактериальная активность, экспериментальная бактериальная инфекция, терапевтическая эффективность, арилалифатические аминоспирты

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The therapeutic efficacy of 1-[4-(1,1,3,3-tetramethyl butyl) phenoxy]-3-(N-benzyl hexametylenimino)-2-propanol chloride under *in vivo* models

Aryl aliphatic aminoalcohol derivatives are a new promising chemical class, possessing a pronounced antibacterial properties *in vitro*. The aim of this study was to investigate the therapeutic efficacy of aryl aliphatic aminoalcohol derivative 1-[4-(1,1,3,3-tetramethylbutyl)phenoxy]-3-(N-benzylhexametylen imino)-2-propanol chloride (compound KVM-194) using *in vivo* models of generalized and local bacterial infections.

Experimental generalized infection, caused by *Pseudomonas aeruginosa* was reproduced in white nonlinear mice; infecting dose was $4 \cdot 10^7$ CFU/animal. Topical inflammatory process was reproduced by intradermal injection of $1 \cdot 10^9$ CFU (0,5 ml) of *Staphylococcus aureus* inoculum, followed by skin scarification. Bacterial conjunctivitis was reproduced on background of topical anesthesia by 0,5 % solution of proxymetacaine hydrochloride. Both eyes were administered 2 drops of 2,5 % ammonia solution and 0,1 ml of S. aureus inoculum ($1 \cdot 10^9$ CFU/ml) 30 min after.

Investigation of the compound KVM-194 action on the model of generalized *P. aeruginosa* infection showed the distinct efficacy of compound at a dosage equal to 0,01 LD_{50}. Topical administration of KVM-194 (0,25 % ointment) on inflamed skin surface reduced hyperemia and accelerated abscess formation and rupture. Complete recovery was noted in 8 days for treated animals, while sighs of inflammation in control animals were registered for 16 days. Compound showed efficacy on the model of bacterial conjunctivitis as well. Instillation of 0,1 % KVM-194 solution resulted in reduction of inflammation symptoms and shortening the course of the pathological process.

The data obtained suggest prospects of further animal studies to establish the spectrum of *in vivo* activity and rational dosage regimens of the compound KVM-194.

Key words: antibacterial activity, experimental bacterial infection, therapeutic efficacy, aryl aliphatic aminoalcohols

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