DEVELOPING AND EVALUATING THE EFFECTIVENESS OF WOUND-HEALING COMPOUNDS BASED ON CATIONIC PEPTIDES AND FULLERENE

Galkina AA[1] ✉, Bolyakina DK[1], Shatilova AV[1], Shatilov AA[1], Babikhina MO[1], Golomidova AK[2], Andreev SM[1], Shershakova NN[1], Khaitov MR[1,3]

[1] National Research Center — Institute of Immunology of Federal Medical Biological Agency, Moscow, Russia

[2] Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences, Moscow, Russia

[3] Pirogov Russian National Research Medical University, Moscow, Russia

Skin and soft tissue infections following surgical procedures are usually caused by a broad range of bacteria and are the major cause of septic complications and hospital mortality. Treatment of such wounds is a challenge often resulting from the transition from acute to chronic inflammation due to persistence of pathogenic microflora in the wound tissue. The study was aimed to assess the wound-healing activity of the ointment composition based on the dispersion of fullerene C60 (AFD) in the in vivo model of skin wound, to estimate the effects of AFD on the expression of cytokines as markers of regenerative processes, to determine antibacterial activity of the developed cationic peptides. AFD was obtained by tangential ultrafiltration and used to make an ointment composition. The BALB/c mice were used to model the skin injury. The cationic peptides (CPs) were synthesized by the solid-phase method using the Fmoc technology. Antibacterial effects of CPs and AFD were estimated by colony counting. It was found that the AFD-based ointment exerted wound-healing and anti-inflammatory activity. The minimum bactericidal concentrations (MBC) of the CPs most active against the *E. coli* Dh5α strain, AB-1, AB-2, AB-3, and ST-10, were 1.15, 0.11, 0.74, and 0.74 mM, respectively, while MBC of ampicillin was 0.7 mM. We assume that constructing the hybrid compounds/fullerene C60 conjugates with active CPs will be a promising area of the development of drugs for treatment of wounds complicated by bacterial infection.

Keywords: fullerene C60 aqua dispersion, regenerative activity, cationic peptides, antibacterial activity

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✉ **Correspondence should be addressed:** Anastasiia A. Galkina
Kashirskoe sh., 24, Moscow, 115522, Russia; anastasia.a.galkina@gmail.com

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РАЗРАБОТКА И ОЦЕНКА ЭФФЕКТИВНОСТИ РАНОЗАЖИВЛЯЮЩИХ СОЕДИНЕНИЙ НА ОСНОВЕ КАТИОННЫХ ПЕПТИДОВ И ФУЛЛЕРЕНА

А. А. Галкина[1] ✉, Д. К. Болякина[1], А. В. Шатилова[1], А. А. Шатилов[1], М. О. Бабихина[1], А. К. Голомидова[2], С. М. Андреев[1], Н. Н. Шершакова[1], М. Р. Хаитов[1,3]

[1] Государственный научный центр «Институт иммунологии» Федерального медико-биологического агентства, Москва, Россия

[2] Федеральный исследовательский центр «Фундаментальные основы биотехнологии» Российской академии наук, Москва, Россия

[3] Российский национальный исследовательский медицинский университет имени Н. И. Пирогова, Москва, Россия

Инфекции кожи и мягких тканей при хирургических манипуляциях обычно вызваны широким спектром бактериальных микроорганизмов, и служат основной причиной септических осложнений и госпитальной смертности. Лечение таких ран является очень сложной проблемой, часто обусловленной переходом воспалительного процесса в хроническую стадию в связи с наличием устойчивой патогенной микрофлоры в раневой ткани. Целью работы было проанализировать ранозаживляющую активность мазевой композиции на основе водной дисперсии фуллерена С60 (ВДФ) на модели кожной травмы *in vivo*, оценить влияние ВДФ на экспрессию цитокинов как маркеров регенеративных процессов, определить антибактериальную активность разработанных нами катионных пептидов. ВДФ получали методом тангенциальной ультрафильтрации, а затем на ее основе готовили мазевую композицию. Моделирование кожной травмы проводили с использованием мышей линии BALB/c. Синтез катионных пептидов (КП) осуществляли твердофазным методом, используя Fmoc-технологию. Антибактериальную активность КП и ВДФ оценивали методом подсчета колоний. Установлено, что мазь на основе ВДФ обладала ранозаживляющей и противовоспалительной активностью. У наиболее активных КП, АВ-1, АВ-2, АВ-3 и ST-10 минимальная бактерицидная концентрация (МБК) в отношении бактериального штамма *E. coli* Dh5α составляла 1,15, 0,11, 0,74 и 0,74 мМ, соответственно, при МБК ампициллина 0,7 мМ. Мы предполагаем, что создание гибридных соединений/конъюгатов фуллерена С60 с активными КП будет перспективным направлением в разработке лекарственных средств для терапии раневых поражений, осложненных бактериальной инфекцией.

Ключевые слова: водный раствор фуллерена С60, ранозаживляющая активность, катионные пептиды, антибактериальная активность

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Вклад авторов: А. А. Галкина — постановка методики, лабораторные исследования, написание статьи; Д. К. Болякина — лабораторные исследования; А. В. Шатилова, А. А. Шатилов, М. О. Бабихина — лабораторные исследования; А. К. Голомидова — постановка методики; Н. Н. Шершакова — постановка методики, концепция исследования, написание статьи, научное руководство; С. М. Андреев — постановка методики, концепция исследования, научное руководство; М. Р. Хаитов — концепция исследования, научное руководство.

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✉ **Для корреспонденции:** Анастасия Андреевна Галкина
Каширское ш., д. 24, г. Москва, 115522, Россия; anastasia.a.galkina@gmail.com

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Skin wound healing is a complex process involving cells of various types and multiple regulatory factors, and abnormal wound healing can result in scarring and the transition from acute to chronic inflammation. Scars can restrict movements, cause pain and itching; scars can be the cause of physiological stress in case they remain visible and cannot be hidden by clothing or makeup, thereby seriously affecting the person's self-confidence and quality of life. Removal and treatment of scars remain a pressing issue, since, despite the diversity of available treatment methods, the methods' efficacy is quite low. The total volume of the global market in this field was 19.6 billion dollars in 2019. It is expected that it will grow by 11.5% in the next decade [1].

Comorbidities, such as diabetes mellitus, hypertension, and other vascular and autoimmune disorders, can hinder effective treatment of the wound healing process complications [2]. Bacterial superinfections play an important role in chronification of inflammation.

All wounds are to some extent contaminated with microorganisms being a part of saprophytic skin microflora. The type and abundance of such microorganisms vary depending on the wound type [3]. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Acinetobacter baumannii* are the most common bacterial species causing wound infection [4]. It is well-known that wound infections constitute a third of cases of nosocomial infections in surgical patients and cause 70–80% of deaths from wounds [5]. Chronic wounds affect the patients' quality of life, along with the increased morbidity and mortality, and represent a huge financial burden on health systems all over the world, since such wounds are associated with the expenses for long-term hospital stay, diagnostic tests, antibiotics, and sometimes invasive surgery [6, 7]. That is why wound healing is a serious health problem that requires the development of safe and effective therapeutic agents.

The drugs for wound healing are based on adsorbents, anti-inflammatory components, antibiotics or dexpanthenol that stimulates regeneration processes. The search for new approaches to healing wounds complicated by bacterial infection has become a pressing issue due to the alarming increase in resistance to conventional antimicrobial drugs. Among alternative antimicrobial agents, special attention is paid to cationic antimicrobial peptides (CAMPs) [8].

Cationic peptides (CPs) attract much attention as transporters and biologically active substances due to high affinity for cell membranes and specific structure enabling mass spectrometry analysis and wide possibilities to design the variety of such molecules. CPs are widespread in nature and found in all mammals, especially in the skin, where they play a protective role against pathogenic microorganisms. Cationic antimicrobial peptides, or host defense peptides, are a heterogeneous group of short positively charged peptides, mostly amphiphilic. These peptides are secreted by immune (for example, neutrophils and macrophages [9]) and epithelial cells of vertebrates and invertebrates to ensure protection against microbial invasion [10]. The shortcomings of using peptides as potential antimicrobial drugs for treatment include a very complex structure that hampers synthesis and the CPs' proteolytic instability. It has been assumed, that the CAMP conjugation with other biologically active molecules, such as other peptides, polypeptides, proteins, and antibiotics in general, can contribute to improvement of antimicrobial properties and provide the basis for the development of drugs possessing multiple biological activities. In particular, the development of drugs with low toxicity combining antibacterial and anti-inflammatory effects and showing low probability of developing resistance would contribute significantly to both fundamental research and practical healthcare.

Since CAMPs show proteolytic instability, we believe that it seems promising to use such molecules, as fullerene C60, as carriers for peptides.

Fullerene C60 is a carbon-based molecule that has a shape of truncated icosahedron and possesses strong antioxidant activity. It is well-known that the fullerene C60 water-soluble forms have multiple biological effects, including antiviral, anti-inflammatory, anti-allergy, and regenerative effects [11, 12]. An *in vivo* model of wound healing process has shown that some covalent fullerene C60 derivatives accelerate wound healing and prevent inflammatory cell infiltration [13].

It is known that fullerene C60 is insoluble in aqueous media, which is clearly a significant obstacle to its extensive use in medicine. We have earlier developed a unique scalable technology to obtain stable aqueous dispersion of fullerene C60, enabling assessment of its biological activity [14]. It should be noted that this technology does not involve the use of organic solvents, ultrasonic processing or heating, which ensures bio-compatibility and safety of the resulting solution. Our method makes it possible to generate highly concentrated stable AFD with the concentration of at least 1 g/l.

The main purpose of the study was to assess the wound-healing activity of the AFD-based ointment composition in the experimental model of wound inflammation, as well as to estimate the effects of AFD on the expression of marker genes involved in regeneration, analyze the CP antibacterial activity, and assess the prospects of creating the CP- and fullerene C60-based complexes for treatment of wounds.

METHODS

AFD-based composition

The AFD-containing ointment (composition: AFD, vaseline, sucrose palmitate in the ratio of 40 : 36 : 24 (by weight)) for *in vivo* treatment of wounds was prepared using the IKA 25 digital homogenizer IKA 25 as a mixing device ("AFD ointment"). The fullerene C60 aqueous dispersion was acquired by the dialysis method [14]. This bio-compatible method does not involve the use of toxic organic solvents, ultrasonic processing or heating. At the same time, it ensures high yield of fullerene C60 during transition from crystals into solution (the concentration of sterile fullerene C60 solution was 1 mg/mL). The hydrodynamic particle size determined by the dynamic light scattering method was 100–200 nm.

In vivo model of wound inflammation

Female BALB/c mice aged 4–6 weeks (Stolbovaya breeding nursery; Moscow, Russia) were used to model the wound healing process [15]. The following housing conditions were used for animals: ambient temperature 18–26 °C; automatic 12 h ligh/dark cycle; relative humidity 30–70%. All animals had unlimited access to drinking water and food. The animals were anesthetized with 4% isoflurane solution for 2 min via airways and topically administered 0.5% lidocaine solution prior to making the incised wounds. To model the surgical wound, a skin fragment (1 × 1 cm) was excised from the back of the BALB/c mouse. The AFD ointment was applied to the wound surface (40 μg of C60/mouse) 24 h after the surgical procedure ("AFD ointment" group). The widely used therapeutic agent, cream for treatment of surgical wounds, was used as positive control ("C+" group). The ointment containing phosphate

buffered saline (PBS) instead of AFD was used as negative control ("PBS" group). The "intact" group (with no skin wounds) was also used as negative control. The listed above preparations were used once a day for 11 days. On day 12 mice were euthanized by cervical dislocation, and skin samples were collected for quantitative RT-PCR.

Assessment of wound healing efficiency

The skin wound healing rate was assessed by measuring the wound area in the longitudinal and transverse directions (mm) every day and calculating the wound area using the following formula:

$$S_{el} = \varpi ab,$$

where S_{el} — area of an ellipse, a — semi-major axis (half of the longer diameter or transverse dimension), b — semiaxis (half of the shorter diameter or longitudinal dimension).

The wound healing efficiency (X) was calculated as percentage using the following formula:

$$X = (1 - S_f/S_i) \times 100\%,$$

где S_f — final wound area, S_i — initial wound area [16].

Real-time polymerase chain reaction

Total RNA was extracted from skin samples using the RNeasy Mini Kit (Qiagen, Courtaboeuf; France) in accordance with the manufacturer's instructions; cDNA was synthesized using the Reverta-L kit (InterLabService; Russia). The reverse transcription product was amplified by the real-time polymerase chain reaction (RT-PCR) using the iCycler iQ real-time PCR detection system (Bio-Rad Laboratories; USA) and the PCR Mix kit (Syntol; Russia).

The calculations to determine relative gene expression were performed using the comparative Ct method (ΔCt) against mHPRT.

Relative quantification by RT-PCR was used to detect changes in the expression of target genes relative to the reference gene represented by murine *hprt* gene. Quantitative PCR results for mRNA expression were compared as ΔCt values calculated using the following formula: ratio (reference/target) = $2^{Ct(hprt)-Ct(target\ gene)}$ [17].

Assessment of antibacterial effects of AFD and cationic peptides

Antibacterial activity of AFD and the synthesized peptides was assessed *in vitro* on the example of the *E. coli Dh5*α strain by colony counting relative to the well-known antibiotic ampicillin selected as positive control. In the method the bacterial suspension was incubated with various CAMP concentrations in the LB liquid medium for 4 h at 37 °C, then it was applied dropwise to the surface of the dried agar medium. The culture was incubated overnight at 37 °C.

It is important to note that the abovementioned *E. coli* strain is not pathogenic and shows no resistance to antibiotics. Activity of peptides against the selected strain was assessed based on the determined minimum bactericidal concentration.

Cationic peptide synthesis

Peptides were synthesized by the solid-phase method in the automated PS3 Peptide Synthesizer (Gyros Protein Technologies Inc.; USA) according to the Fmoc-chemistry protocol using the N-hydroxybenzotriazole and diisopropylcarbodiimide (HOBt/DIC) mixture as a condensing agent. The starting Fmoc-aminoacyl polymers and the Rink Amide ChemMatrix gel-type resin were used for synthesis. The side carboxyl and hydroxyl groups of amino acids were protected by the tert-butyl group (t-Bu), the lysine ε-amino group by Boc, the cysteine SH-group by Trt, the arginine guanidinium functional group by Pbf, and the carboxyl and hydroxyl groups of amino acids by tert-butyl ethers. The standard cycle included washing (DMF), removal of Fmoc protection (20% 4-methylpiperidine in DMF), preliminary Fmoc amino acid (DIC/HOBt) activation and condensation on the DMF/N-methylpyrrolidone medium with the twofold carboxyl component excess (~0.5–1 h). The extent of the reaction was controlled using the Kaiser Test (ninhydrin test), the condensation reaction was repeated when necessary (0.5 h). The terminal peptides were cleaved from the polymer with trifluoroacetic acid in the presence of scavengers (triisopropylsilane, ethanediol, water, dimethyl sulfide). The raw product was precipitated with the dry methyl tert-butyl ether, then the peptide was extracted with the acetic acid aqueous solution, and the extract was lyophilized (VirTis AdVantage 2.0 EL freeze dryer; SP Scientific, USA). The peptides were purified by preparative HPLC chromatography (LC-20 Shimadzu; Japan) on the reversed phase column (C18) using acetonitrile — 0.1% trifluoroacetic acid aqueous solution as a mobile phase (gradient elution). The resulting peptides were tested for homogeneity by capillary zone electrophoresis in the Kapel-105M system (Lumex; Russia) with photometric detection at 226 nm. Molecular weight was analyzed using the Microflex™ LT MALDI-TOF mass spectrometer (Bruker Daltonic; USA).

Statistical analysis

Statistical analysis was performed using the Statistica 8.0 software (StatSoft Inc.; USA). Significance was determined based on Student's t-test. The differences were considered significant at $p < 0.05$. The data were presented as mean ± standard error.

RESULTS

In vivo assessment of the AFD regenerative effect in the model of wound inflammation

Analysis of the AFD-based ointment regenerative activity relative to commercial drug ("C+", positive control) was performed using the model of wound healing. Visual assessment of the wound healing process by measuring the lesion area was carried out individually for each mouse. The baseline average lesion area was 143.5 ± 6.1 mm^2 ("before treatment" group). On the last day of the experiment the lesion area by groups was as follows: "no treatment" — 44.4 ± 6.5 mm^2, "AFD ointment" — 14.8 ± 2.7 mm^2, "C+" — 26.0 ± 2.6 mm^2. These values show that healing of wounds treated with AFD was effective, and the healing rate was comparable with that of the positive control group and even slightly superior to the latter. Since visual assessment of healing was a subjective parameter, statistical analysis of wound area was performed in the groups that revealed significant differences between the values of the "AFD ointment" and "C+" groups, which, in turn, differed from the values of the "PBS" group. It was found that the residual wound area was the least when the AFD-based ointment was used for surgical wound treatment.

Assessment of the expression of pathogenetically significant genes

Expression of a number of genes was analyzed to assess the AFD capability of affecting the pathogenetically significant factors of

Table. List of cationic peptides showing antibacterial activity against *E. coli Dh5*α

Peptide	Structure	Charge	Molecular weight, Da
AB–1	Linear	+ 8	1736
AB–3	Linear	+ 12	3328
AB–4	Dendrimeric	+ 11	2758
ST–10	Dendrimeric	+ 8	2749

regenerative process. The expression of genes in murine skin with wounds treated/not treated with AFD was determined by real-time PCR. It was shown that the expression of such pro-inflammatory factor, as *tnf*α produced in response to pathogen entry and tissue damage that stimulates local inflammatory response, was significantly lower in all experimental groups, where animals received AFD, than in the group of animals that received no therapy ("PBS"). The expression of genes encoding other pro-inflammatory cytokines, such as *il6* and *il1a*, was also significantly lower in mice with wounds treated with AFD than in animals that received no therapy. Furthermore, we have revealed the fullerene C60 capability of enhancing the expression of HMGB1 factor that violates the collagen synthesis and can ensure scarless tissue healing observed when treating the wound with AFD.

Thus, our findings suggest that fullerene C60 can inhibit the expression of genes encoding pro-inflammatory cytokines, which results in the pro-inflammatory effect of this substance that is likely to contribute to the healing process acceleration.

Cationic peptide design

CPs are widespread in nature and produced by almost all organisms as part of the nonspecific immune system. These compounds were initially considered as potential substitutes for antibiotics, however, it was found out that the compounds had a broader spectrum of therapeutic effects, including the effects on viruses, bacteria, and microbial biofilms. The naturally occurring CPs are linear molecules consisting of up to 50 amino acids having a high share of hydrophobic and cationic residues. This makes the molecules to fold into amphipathic structures to form α-helices and β-sheets. Such peptides form specific loop conformations due to high cysteine content and disulfide bond formation. The charges of the vast majority of natural antimicrobial CPs vary between +3 and +9. The mechanism underlying their effects is associated primarily with the cell membrane damage [18]. Today, technology makes it possible to build the structures which are quite different from natural constructs in terms of topology, including the non-naturally occurring dendrimeric structures. The amino acid sequence construction included building the construct with low toxicity, which was stable in the serum medium. Furthermore, the structure had to demonstrate high efficiency of transfection stimulation. The plan also involved building modular constructs. One of such modules is the N-terminal supercationic fragment represented by the arginine and/or lysine residues, which is essential for interaction with the nucleic acid and the cell surface. The central module is represented by the hydrophobic core consisting of the lysine residues and short hydrophobic/amphiphilic inserts. The C-terminal module also forms a hydrophobic fragment ensuring additional affinity for cell membrane, it contains the cysteine residue with a free thiol group intended for reporter label attachment.

Hydrophobic interactions between the aliphatic chains of lipid membranes and the peptide hydrophobic residues play an important role in the mechanism underlying membrane damage, which contributes to its incorporation in the membrane bilayer through various interactions, such as pore formation. In the carpet model, the cationic peptide is directed in parallel to the cell to cover it and saturate via interaction with the outer phospholipid layer of the membrane. After the threshold value is achieved, the peptides start spinning and embed in the membrane causing its permeabilization. The branched structures, dendrimeric CPs, are of special interest. It should be noted that these show higher resistance to proteolytic enzymes along with lower toxicity compared to the linear peptides with similar amino acid composition. At the same time these show stronger binding to cells due to cooperative effects resulting from the fact that the molecule has several chains. Our early experiments demonstrated their high permeability through cell membranes enabling using such peptides as carriers for cell transfection, transfer of genes and other biologically active compounds [19].

Assessment of antibacterial effects of AFD and cationic peptides

When assessing antibacterial activity of AFD and CPs, we showed that AFD possessed no bactericidal activity and was unable to inhibit bacterial growth. Then we analyzed a number of CPs, which were expected to possess potential antibacterial activity based on their structural features. Thus, among 35 linear and dendrimeric CAMPs with the molecular weight not exceeding 4.5 kDa we had synthesized, four cationic peptides, specifically linear peptides AB-1, AB-3 and dendrimeric peptides AB-4, ST-10, to various extent inhibited growth of the *E. coli Dh5a* microbial culture and showed some bactericidal activity against this strain (Table).

Minimum bactericidal concentrations (MBC) of the cationic peptides, i.e. the lowest concentrations killing all bacteria under standard experimental conditions, were determined by colony counting.

Thus, MBC of the AB-1 peptide was 1.15 mM. It should be noted that the AB-1 peptide concentration of 0.23 mM (five times lower compared to bactericidal concentration) showed no significant activity against this bacterial culture, which was indicative of the extremely narrow operative range of peptide concentrations (Fig. 1).

The AB-3 peptide has higher bactericidal activity against the *E. coli Dh5*a strain than AB-1 (Fig. 2).

MBC of this peptide was 0.11 mM. Meanwhile, activity of appropriate ampicillin doses was about 6 times lower. MBC of ampicillin was 0.74 mM.

The studied AB-4 peptide exerted considerable bactericidal activity that was comparable with that of control antibiotic (ampicillin). MBC of this peptide was 0.74 mM (Fig. 3).

MBC of the ST-10 dendrimeric peptide was 0.74 mM (Fig. 4).

The above concentration was the least concentration that killed almost 100% of cells. It should be noted that the level of activity exerted by the ST-10 peptide was slightly higher compared to the control sample of ampicillin antibiotic. Thus, with comparable concentrations of 0.15 mM and the same *E. coli* dilution (1 : 10) it is evident that the number of bacterial colonies detected in the culture treated with the ST-10 peptide

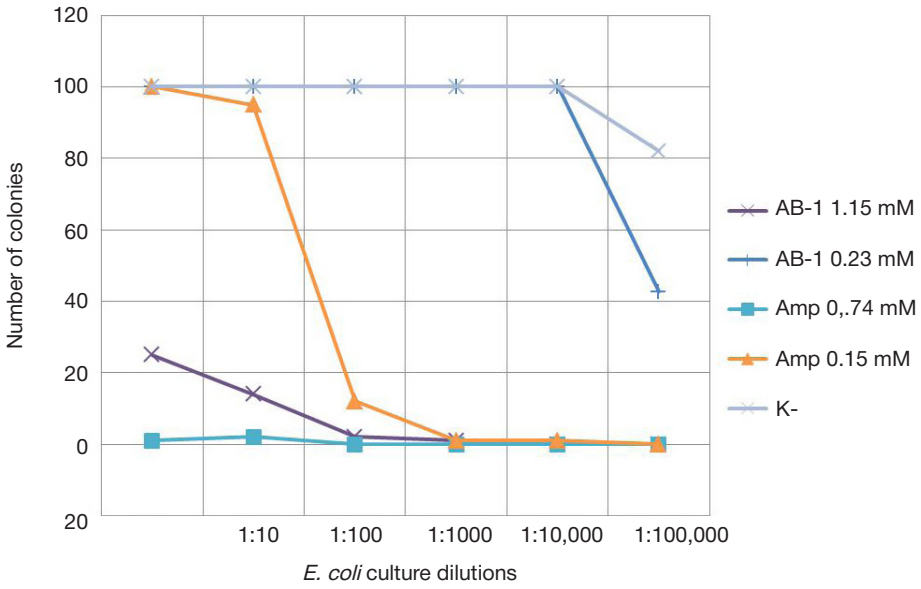

Fig. 1. Bacterial growth intensity under exposure to the AB-1 peptide

is considerably lower than in the culture treated with ampicillin (16 and 95, respectively). Therefore, the effect of bacterial culture growth inhibition under exposure to 0.15 mM of peptide and antibiotic was more prominent in ST-10.

DISCUSSION

In the majority of cases wounds are associated with bleeding from the damaged blood vessels and the release of inflammatory mediators, such as serotonin, histamine, vasoactive substances, and cytokines, into the surrounding tissues. Normal wound healing includes the following phases: inflammation, proliferation, maturation, and remodeling. To determine the AFD regenerative effects, we assessed the expression of a number of marker genes, such as *tnf*α, *il-6* and *il-1*α, involved in regenerative process by RT-PCR. Elevated expression of these cytokines is observed during the inflammatory phase of wound healing. It is well-known that TNFα stimulates production of not only IL1, IL6, but also other pro-inflammatory cytokines [20, 21]. IL6 is one of the most important mediators of the acute phase of inflammation. It is known that delayed wound epithelization is observed in mice with IL6 deficiency. However, excess IL6 levels serve as a signal for fibroblast proliferation suppression during the late wound healing phase and lead to scar formation [22]. As for IL1a, it has been earlier shown that it stimulates collagenase production, and overexpression of this cytokine can be associated with abnormal wound healing due to collagen breakdown. The moderate *il1a* expression increase later mediates keratinocyte proliferation in the wound area [23]. Low levels of *il1a* are observed in the wound fluid from acute wounds, while fluid from surgical wounds shows elevated levels of *il1a*.

Thus, suppression of the *tnf*α, *il6*, and *il1a* expression under exposure to fullerene C60 suggests that it not only possesses anti-inflammatory effect, but is also capable of preventing wound chronization [15]. We have earlier shown antiallergy effects of AFD with inhibition of Th1 cytokines in the model of atopic dermatitis, along with the elevated expression of genes *Foxp3* and *FLG* (filaggrin) [24]. Therefore, AFD has shown its ability to suppress inflammation associated with not

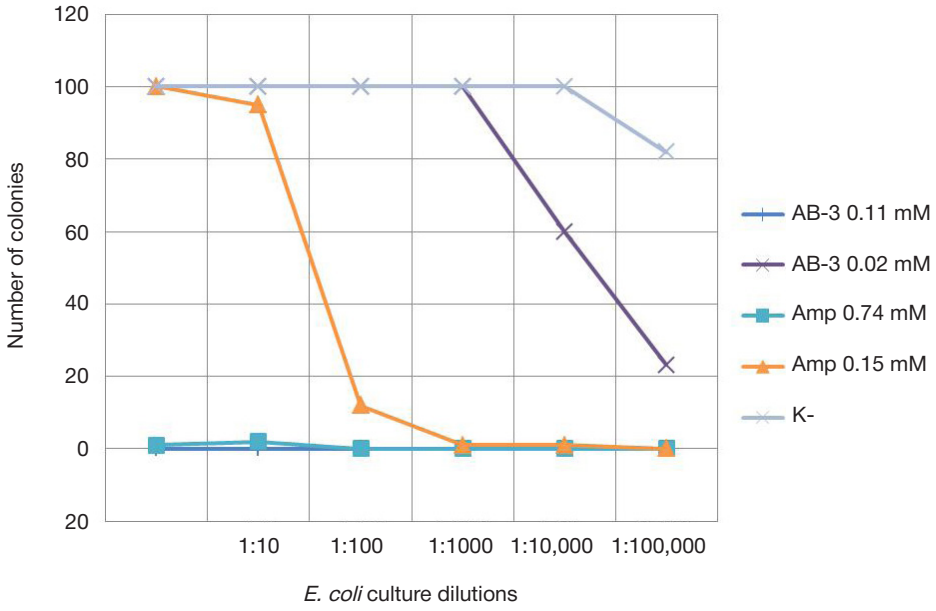

Fig. 2. Bacterial growth intensity under exposure to the AB-3 peptide

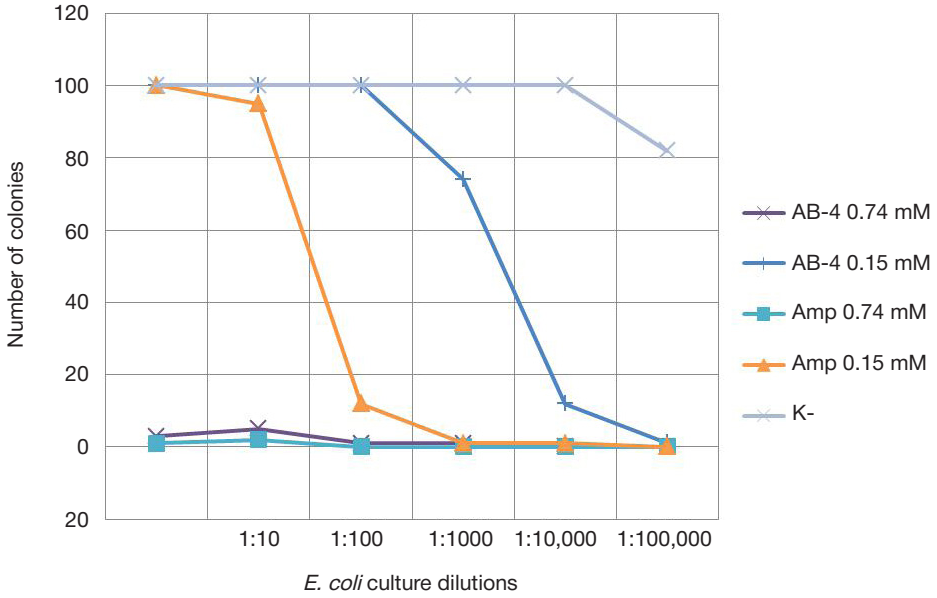

Fig. 3. Bacterial growth intensity under exposure to the AB-4 peptide

only wounds, but also allergy. This makes AFD a promising compound for treatment of inflammatory skin disorders.

Wound healing is often accompanied by accession of secondary bacterial infection. It is well-known that CAMPs have a broad range of antimicrobial and immunomodulatory effects against Gram positive and Gram negative bacteria, biofilms, viruses, fungi, and parasites; CAMPs are also effective against multidrug-resistant strains. It is important to note that the likelihood of developing resistance to cationic peptides is extremely low and requires multiple mutations, including that mediating changes in the cell wall structure, due to fast bactericidal action of CAMPs and the diversity of mechanisms of action and targets [25–27]. The above makes CAMPs promising compounds for the development of the CAMP-based antibacterial drugs. Understanding of how the antimicrobial peptide properties depend on the amino acid sequence will make it possible to timely respond to the emergence of new antibiotic-resistant bacterial strains in the future due to targeted reconstruction of peptide sequences [28].

We have created the library of CAMPs supposed to possess high antimicrobial activity and have low toxicity within the framework of current research. When developing the CP panel, we relied on the databases of already known peptides and the literature data on the CP biological (antibacterial) activity. Thus, the peptide sequences were constructed considering the content of positively charged amino acids, hydrophobic amino acids. Furthermore, both linear and dendrimeric molecules were obtained when constructing the sequences.

Thus, by defining some rules for creating cationic peptides showing antibacterial activity we look forward to creating more active CPs. The AFD anti-inflammatory activity makes the idea of developing hybrid molecules based on CPs and AFD perspective.

CONCLUSIONS

As result of the research, we have developed and synthesized CPs possessing antibacterial effects. Thus, we believe that the AB-1, AB-2, AB-3, and ST-10 peptides are promising in terms of developing antimicrobial drugs on their basis. During further studies we plan to develop hybrid compounds based on the CPs and fullerene C60 to combine the anti-inflammatory and wound-healing effects with antibacterial activity. Fullerene can

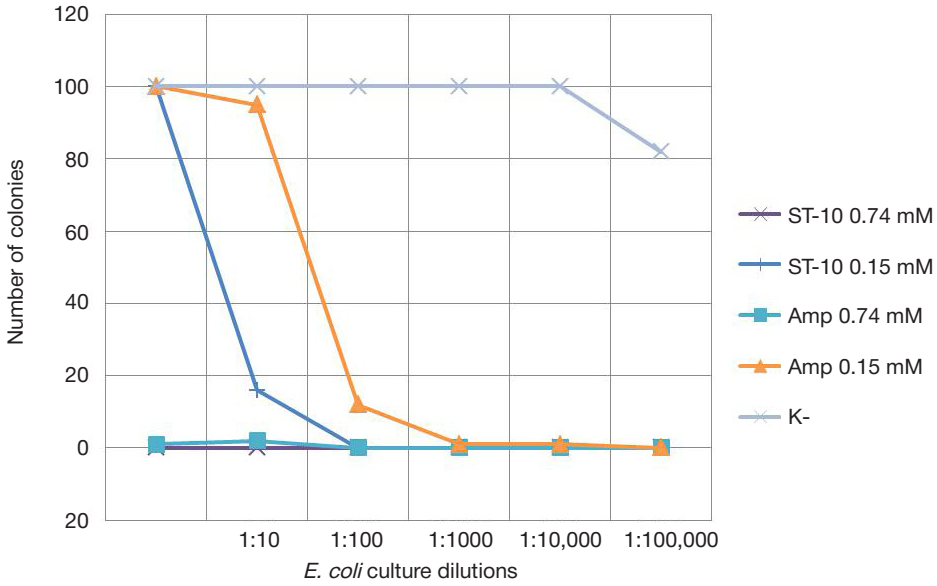

Fig. 4. Bacterial growth intensity under exposure to the ST-10 peptide

play a role of carrier platform for CPs. Since the area of the spherical fullerene molecule is rather large, up to 4–8 peptide molecules can be attached to it. Such multivalent structure is less prone to biodegradation, and antimicrobial activity can be increased due to cooperative effect, simultaneous attachment of several CP chains to the bacterial cell membrane. It should also be considered that fullerene C60 itself and its amino acid adducts can permeate through biological membranes.

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