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Evaluation of a Cationic Antimicrobial Peptide as the New Antibiotic Candidate to Treat *Staphylococcus aureus* Keratitis

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Abstract

Staphylococcus aureus is a most important reason of bacterial keratitis. The emergence of *S. aureus* resistance to methicillin necessitates searching for new antimicrobial components for keratitis. CM11 is a cationic peptide with strong antibacterial activity against a range of bacteria. In this study, in vitro bactericidal activity of CM11 was investigated using the time-killing assay. For this purpose, corneal methicillin-resistant *S. aureus* (MRSA)-infected rabbit models were experimentally developed through intrastromal injection of bacteria. The infected rabbits were treated in three groups by artificial tear, gentamicin, and CM11, and their conjunctiva, iris, and cornea were clinically examined using a slit lamp and histopathological examination. The variance analyses of microbial (colony counts) and pathological examinations on the harvested cornea samples showed a significant improvement in the treated groups (CM11 and gentamicin) compared to the control eyes ($P \leq 0.05$). According to findings, CM11 and gentamicin could significantly reduce the CFU in comparison with the group received artificial tear. The mean bacterial counts (log CFU/ml) from corneal culture were 2.1, 5.02, and 8.89 for gentamicin, CM11, and control group, respectively ($P \leq 0.05$). The above-mentioned findings displayed the efficacy of CM11 cationic peptide for curing the MRSA-mediated keratitis.

Keywords *Staphylococcus aureus* · Eye infection · Keratitis · Antibiotic resistance · Antimicrobial peptide

Introduction

Among the ocular infections, bacterial keratitis is one of the most famous conditions which is mostly due to infection by *Staphylococcus aureus* in various populations around the world (Durrani et al. 2020). Patients suffering from diabetes, HIV, and epithelial trauma caused by foreign bodies as well as elderly persons and individuals wearing contact lenses are the main susceptible groups to *S. aureus* keratitis (Durrani et al. 2020; Sharma 2018). Pathologic and immunologic studies show there is a co-operation between bacterial products and host-associated factors in cornea destruction during keratitis. *S. aureus*-mediated keratitis results in an irreversible corneal scar, severe inflammation, visual acuity loss, pain, and corneal perforation (Robles-Contreras et al. 2013). At present, β-lactam antibiotics such as cefazolin in combination with a fluoroquinolone or aminoglycoside are the selective drugs for treating the keratitis. However, treating *S. aureus* infections are specifically complicated because they have acquired resistance to many antibiotics (Lalitha et al. 2017; Miller 2017). Methicillin-resistant *S. aureus* (MRSA) was unfortunately developed in 1961, leading to a considerable increase in mortality due to MRSA-infection. Consequently, new antimicrobial formulations are required for novel treatments of *S. aureus*-induced keratitis (Lakhundi and Zhang 2018). One of the most promising molecules inferred to be potential antimicrobial and therapeutic agents are antimicrobial peptides (AMPs). Either bacteria,

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viruses, fungi, and protozoa can be killed by AMPs as the essential part of the innate immunity system in eukaryotic hosts (Moravej et al. 2018). One of the most described antimicrobial peptides is the cationic peptides group which are multifunctional agents present and functional on the skin and mucosal surfaces (Pfalzgraff et al. 2018). Generally, cationic peptides disrupt the structure of microbial cell membranes and disturb their function, or disorder the activity of ATP-dependent enzymes via interacting with ATP (Chen and Lu 2020). Cationic peptides exert their antimicrobial function by perturbing the microbial membranes through electrostatic interactions owing to their amphipathic structure and net positive charge. The physical perturbation of microbial membrane infers a direct mechanism for killing bacteria which is independent of the bacterial resistance strategies. Antimicrobial agents with this property provide a combat material against antibiotic-resistant pathogens (Hollmann et al. 2018; Moghaddam et al. 2015). The therapeutic potentials of these peptides motivated us to evaluate the antibacterial effect of a short cationic peptide (CM11 peptide) on bacterial keratitis caused by *S. aureus* in vitro and in vivo. The CM11 peptide (WKLFKKILKVL-NH2), as a hybrid and synthetic cationic peptide is derived from cecropin A (residues 2–8) and melittin (residues 6–9), has been previously addressed for its high antimicrobial activity against several pathogens in human (Amani et al. 2015; Moghaddam et al. 2012).

Material and Methods

Microorganisms and Growth Conditions

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were provided from the clinical diagnostic laboratory of the Khatam-al-Anbia hospital (Tehran, Iran). The standard *S. aureus* ATCC 29213 was obtained from the Iranian Research Organization for Science and Technology (IROST), Karaj, Iran. Both bacterial strains were grown in Mueller–Hinton agar (MHA) (Sigma, USA) at 37 °C and kept at 4 °C. The stokes of bacteria were stored at − 80 °C for future assessments.

MIC Determination of the CM11 Peptide

A solution with 1 mg/ml concentration was prepared from peptides in phosphate buffer saline (PBS: pH 7.2). The antibacterial activity of the peptide was measured by minimal inhibitory concentration (MIC) test according to the procedures outlined by the Clinical and Laboratory Standards Institute (CLSI). For this purpose, the micro-dilution method was performed using an initial inoculum of 1.5×10^8 CFU/ml in the Mueller–Hinton broth medium (Wayne 2009). Bacteria inoculums were added to different concentrations of peptide/PBS from 1 to 128 mg/L and incubated for 18 h in a shaking bath at 37 °C. MIC was defined as the lowest peptide concentration that was able to inhibit bacterial growth. All experiments were performed in triplicate (Amani et al. 2015).

In Vivo Animal Testing

Animals

New Zealand white rabbits, all weighing 2.0–3.0 kg were used. Animals were treated according to the guide for the Care and Use of Laboratory Animals (Albus 2012) and the Association for Research in Vision and Ophthalmology (ARVO). Ethical approval was obtained from the ethics committee of the Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1398.225). For rabbit anesthesia, a mixture of xylazine hydrochloride (100 mg/ml) and ketamine hydrochloride (100 mg/ml) with a 1:5 ratio was subcutaneously administered. One drop of proparacaine hydrochloride ophthalmic solution (0.1%) was instilled into each eye for additional anesthesia (Holve et al. 2013).

Determination of the CM11 Toxicity on Eye

First, the toxicity effect of CM11 peptide was evaluated on the rabbits' eyes via the Draize scoring system. The Draize scale includes scoring the redness, chemosis, and discharge of conjunctivae, the ocular irritation of iris, as well as the opacity degree and involved area of the cornea (Wilhelmus 2001). For this purpose, one drop of CM11 at MIC and 2 × MIC was instilled into one of the rabbits' eyes every 1 h for 10 h. The signs and symptoms of CM11 toxicity effect on the eye were determined by an ophthalmologist. The MMTS irritation scores were used as the following classification: 0.0–0.5 Nonirritating; 0.6–2.5 Practically Nonirritating; 2.6–15 Minimally Irritating; 15.1–25 Mildly Irritating; 25.1–50 Moderately Irritating; 50.1–80 Severely Irritating; 80.1–100 Extremely Irritating; 100.1–110 Maximally Irritating.

Induction of Keratitis

To induce the keratitis, MRSA strain was grown to log phase in MH broth at 37 °C. Then, the bacterial suspension was diluted in PBS to approximately 10,000 CFU/ml. The rabbits were anesthetized as described previously. The corneal epithelium was scratched by an Amoils epithelial scrubber. After that, a quota of bacterial suspension containing approximately 100 CFU (10 µl of 10^4 CFU/ml *S. aureus*) was injected intrastromal into the rabbits' cornea.

Treatment

The rabbits were divided into three groups (one test group, one positive group, and one control group) each consisting of five rabbits. Rabbits in test groups were immediately treated by a single topical drop of CM11 (25 µl equaling to 2 × MIC) every one hour, over a 10-h duration. Treatment was continued on the second and third days with a single topical drop every 4 h. Rabbits in the positive group were treated with gentamicin (10 mg/ml) by a similar time course, and the other group was considered as the control group receiving PBS in the same pattern.

Scoring of the Corneal Improvement

At the end of treatment, the rabbits' eyes were examined and graded using a slit lamp. This evaluation has been previously explained by Beisel and colleagues (Beisel et al. 1983). Accordingly, eye macroscopically identical to the state of the eyes previous to the bacterial injection were considered as grade 0; faint opacity in the injected area was considered as grade 1; dense opacity in the injected area was considered as grade 2; dense opacity covering the entire anterior segment was considered as grade 3, and observing perforation of the cornea was considered as grade 4. Finally, the total clinical score was calculated for all groups and the results of the three groups were compared using Kruskal–Wallis multiple comparisons (K–W test).

The Microbial Load of Treated Eyes

Control and infected corneas were prepared for bacterial quantification after treatment. In brief, corneas were retrieved under aseptic conditions and vortexed in 1 ml of sterile PBS using a tissue homogenizer (8–10 strokes for 10 s with 3 repeat). Next, a serial dilution was prepared from the 100 µl aliquot of homogenate in PBS. Duplicate aliquots were cultured on plates containing MH agar. After incubation for 24 h at 37 °C, the CFU number was counted and results were reported for each eye.

Histopathological Study

After seven days, the rabbits were sacrificed and the corneal samples from control and infected animal models were collected and prepared for histopathology characterization. For this purpose, tissues were formalin-fixed in neutral buffered formalin (10%, pH 7.4) and embedded in paraffin wax. Then, samples were cut into sections with 4 mm thickness and stained by hematoxylin/eosin method. Finally, the stained sections were investigated for tissue abnormalities.

Statistical Analysis

The corneal improvement data were analyzed using the K–W test (a nonparametric one-way analysis of variance) and Wilcoxon's test. The bacterial count data were analyzed by the one-way ANOVA and Student's *t* tests. *P* values ≤ 0.05 were considered as significant.

Results

MIC of the CM11 Peptide

In the first stage, we evaluated the sensitivity of MRSA *S. aureus* isolate to CM11 using MIC determination. The incubation of *S. aureus* in the presence of CM11 peptide revealed the dose-dependent effect of CM11 on reducing bacterial growth. Accordingly, the MIC of CM11 for the standard and MRSA isolates was evaluated 2 and 4 µg/ml, respectively.

Determination of the CM11 Toxicity on the Rabbit's Eye

The toxicity effect of the peptide on the rabbit's eye was evaluated using the Draize method. Redness, chemosis, and discharge were not observed in the conjunctiva. Iris was normal and corneal epithelium defect was not visible in neither of rabbits. Generally, the Draize score for cornea, iris, and conjunctiva was almost near zero. Rabbits did not show any blinking or immediate wiping of the eye after instillation. The maximum mean total scores (MMTS) of the rabbits' eyes treated with MIC (4 µg/ml) and 2 × MIC (8 µg/ml) concentrations of CM11 both were near zero (Fig. 1). No severe

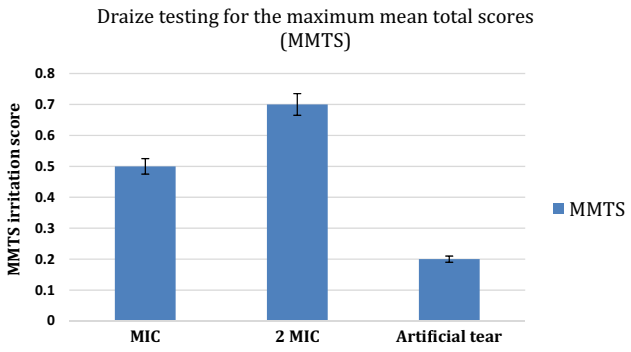

Fig. 1 The result of the Draize testing demonstrating the maximum mean total scores (MMTS) of rabbits' eyes treated with MIC (4 µg/ml) and 2 × MIC (8 µg/ml) concentrations of CM11 and artificial tear. Score 0.0–0.5: nonirritating; score 0.6–2.5: practically nonirritating

reaction was observed in the rabbits' eyes after the CM11 application. Also, there was no delayed toxicity in the treated groups 4 days after peptide instillation.

Efficacy of CM11 for Treatment of Infected Cornea

To evaluate the effectiveness of CM11 in treating the ocular infections, the rabbits' eyes were infected with a clinical MRSA isolate and then treated with 2 × MIC of the peptide (8 µg/ml). Variance analysis was performed after the calculation of the total clinical score for all treatment. The median corneal opacity scores of the test, positive, and control groups at the end of the treatment period (4 days) are shown in Fig. 2. The variance analysis showed a significant difference between the two treatment groups (CM11 and gentamicin) and the control group ($P \leq 0.05$). Comparing the two treatment groups also showed a significant difference ($P \leq 0.05$) as the peptide-treated group rabbits' eyes displayed better scores (Fig. 2). As shown in Fig. 3, the rabbits' eyes which were treated with CM11 were clear without any complications. While, in the control group which was treated with PBS, eyes were completely opac and the cornea was melted, and redness and hemorrhage were observed. On the other hand, only one rabbit in the gentamicin group showed a small opacification 4 days after treatment.

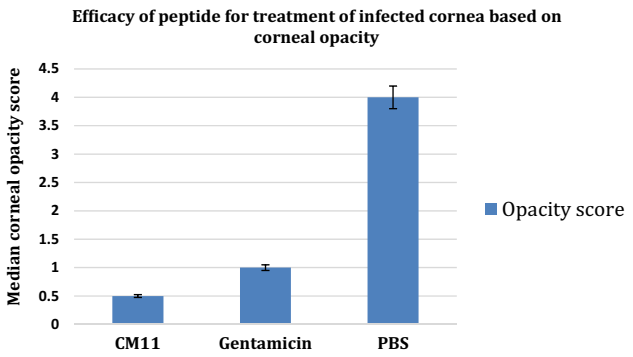

Fig. 2 Median opacity scores for all treatment groups achieved at the end of the treatment period. The variance analysis showed a significant difference between the two treatment groups (CM11 and gentamicin) and the control group ($P \leq 0.05$)

Bacterial quantification in treated eyes

As shown in Fig. 4, CM11 has significantly reduced the CFU (2.02 $\log_{10}$ CFU) in the test group compared to the control-group eyes (8.89 $\log_{10}$ CFU) ($P \leq 0.05$). Besides, in the gentamicin-treated group compared to the control group, the mean bacterial count in corneal culture was reduced (4.1 $\log_{10}$ CFU), while in comparison with the peptide-treated group, this reduction was two units greater.

Histopathological Study

The results of histopathological examinations showed the effectiveness of peptide in treating the keratitis (Fig. 5). Such that after 8 days, the histopathologic outcomes demonstrated that vascularization was inhibited in the CM11- and

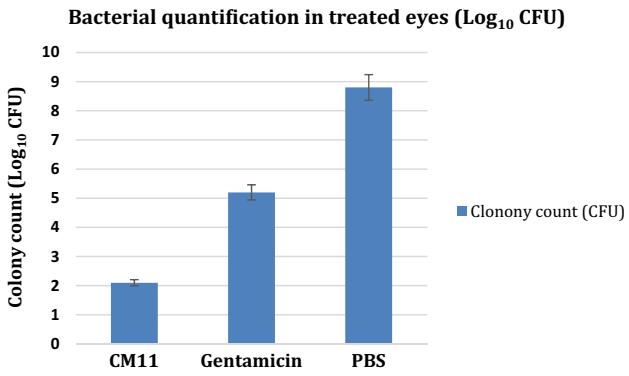

Fig. 4 Colony count of bacteria in infected rabbits' cornea after treatment with peptide (2 × MIC: 8 µg/ml), gentamicin, and PBS. CM11 has significantly reduced the CFU (four units) compared to the negative control group treated by PBS ($P \leq 0.05$), while in comparison with the gentamicin-treated group, this reduction was two units greater

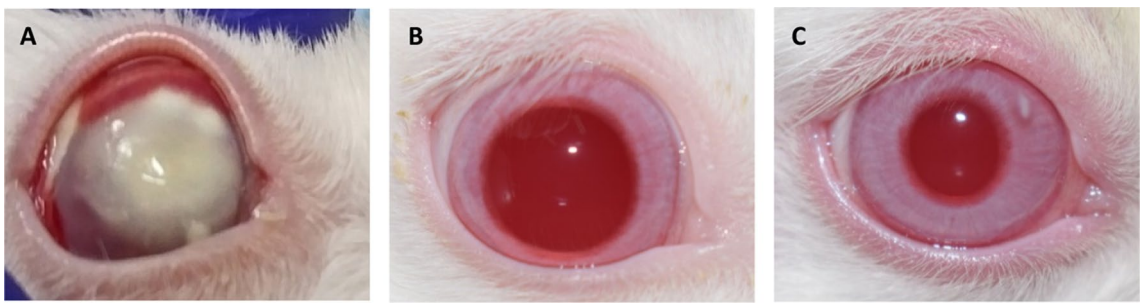

Fig. 3 Rabbits' eyes 4 days after treatments: **a** PBS, **b** CM11 (2 × MIC: 8 µg/ml), **c** gentamicin. As shown in the figure, similar to gentamicin, the peptide leads to the elimination of infection

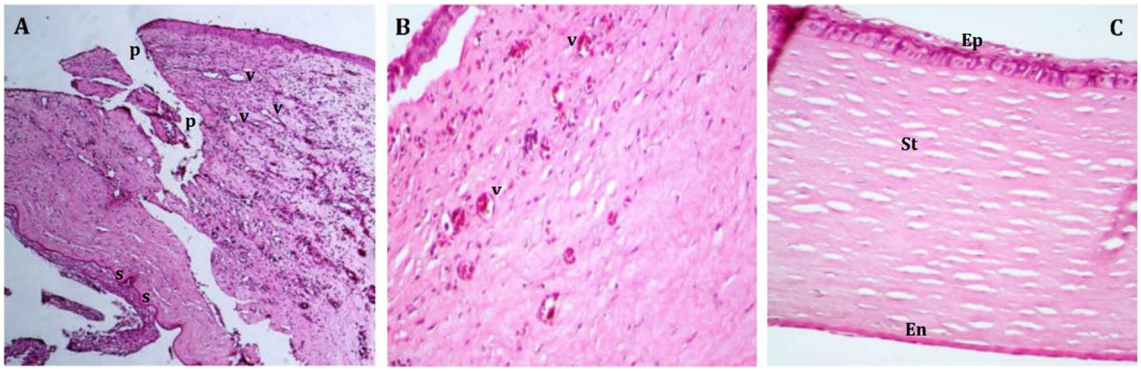

Fig. 5 Histological finding of infected cornea after treatment. **a** Control: corneal tissue with ulcer, full thicknes chronic keratitis and vascularization (v), post descemevt membrane scare tissue formation (s) and, corneal performation (p) (H&E staining; × 100); **b** Gentamicin tissue with focal vascularization (v), and mild chronic keratitis in the superficial and mid stroma (H&E staining; × 400); **c** peptide: normal cornea: epithelial (Ep), stroma (St) and endothelium (En) layers are intact without histopathological findings. (H&E staining; × 400)

gentamicin-treated groups compared to the control group. Additionally, the corneal epithelial loss was reduced in the CM11-treated group compared to the control.

Discussion

Acute or chronic infections of conjunctiva (conjunctivitis) and cornea (keratitis) are among ocular infections with a wide range of consequences from conjunctival scarring to severe keratitis which may lead to loss of vision. These complications usually appear due to the insufficient care and cleaning of contact lenses or occurring an injury in the ocular surface (Teweldemedhin et al. 2017; Yeu and Hauswirth 2020). The increasing emergence of microbial resistance to antibiotics has arisen serious obstacles in the antibiotic therapy of ocular surface infection as a classically efficient treatment in clinical alleviation of these infection types (Brown 2007; Wang et al. 2015). *Staphylococcus aureus* (*S. aureus*), the coagulase-negative staphylococci (CNS) species (e.g., *S. epidermidis*), and *Pseudomonas aeruginosa* are the most reported pathogenic agents underlying the ocular surface infection. *S. aureus* is the main cause of keratitis around the world being responsible for one-quarter of all cases (Chang et al. 2015; Deguchi et al. 2018). The two main empirically-proved antibiotics used against keratitis are fluoroquinolones and cephalosporins but they are losing their efficacy in treating or preventing the infection simultaneously with increasingly occurring methicillin-resistant isolates (Egrilmez and Yildirim-Theveny 2020). At present, MRSA constitutes over one-third of *S. aureus* isolates from the ocular sites embedding multiple mutations that also confer fluoroquinolone-resistance (Suzuki 2011). This alarming incidence of antibiotic-resistant species of *S. aureus* from different anatomical sites vindicates the extensive researches exploring for novel compounds with antibiotic potential. One of the recently of interest compounds with antibiotic properties is antimicrobial peptides (AMPs). These biomolecules can damage the pathogens via stimulating the innate immunity of hosts (Moravej et al. 2018). The advantages of AMPs in comparison with the conventional antibiotics include less incidence of induced resistance, acting against an extensive spectrum of pathogens, specificity for Gram-positive or Gram-negative bacteria, being less toxic for the hosts, and having a synergistic antimicrobial effect with other antibiotics as well as exerting their bactericidal effects fast (Epand and Epand 2009; Lei et al. 2019). A popular subclass of AMPs is the cationic antibacterial peptides (ABPs) with a size of 15–45 amino acids that kill bacteria by attacking their membranes. ABPs are believed to be generally a constituent of the innate immune system in different eukaryotic organisms including plants and animals (Moravej et al. 2018). Some AMPs act specifically against a Gram-type of bacteria based on the presence of lipopolysaccharide (LPS, in Gram-negative bacteria) or lipoteichoic acids (LTA, in Gram-positive bacteria) (Epand and Epand 2009). LPS and LTA impact on the lipid composition and electrical charge of the bacterial outer membrane which favors the interaction between the cationic AMPs and the negatively charged membrane of bacteria in a selective manner. In contrast, the mammalian membranes contain zwitterionic or dipolar ions which repel AMPs leading to their selectivity and specificity for bacterial cells rather than host cells (Griffith et al. 2017).

Accordingly, AMPs are addressing as highly promising and innovative alternatives with the potential of solving the increasing problem of multi-drug resistance. However, some AMPs embed side effects or are problematic because of their size which needs to be overcome. Referring to these problems, synthetic hybrid and short peptide analogs such as insect peptide cecropin and the bee venom peptide melittin are designed, produced, well-investigated, and introduced to be potent (Boman et al. 1989; Cao et al. 2010; De La

Fuente-Núñez et al. 2016). As mentioned earlier, CM11 peptide is also a short hybrid derived from cecropin and melittin which was demonstrated in our previous study that has a strong antibacterial activity against multidrug-resistant pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (Amani et al. 2015; Azad et al. 2017). But the side effect of toxicity still has remained a major problem with this powerful antimicrobial peptide. Regarding this problem, an in vivo study recently investigated the toxicity and histopathological effects of the CM11 by intraperitoneal injection to mice. In this study, different concentrations of this peptide were studied in terms of histopathological effects on the liver, kidney, and jejunum. Both histopathological and physiological analyses showed different histological changes based on a concentration-dependent manner while no change was observed in physiological indexes such as aminotransferase activity, total serum bilirubin, and albumin levels (Ramandi et al. 2017). In the study of Amani et al., the MIC of CM11 was evaluated to be in the range of 4–16 µg/ml for most antibiotic-resistant bacteria and the concentration of > 32 µg/ml was shown to be cytotoxic against the eukaryotic cells (Amani et al. 2015). In the current study, the MIC of the peptide was measured as 4 µg/ml and the 2 × MIC (8 µg/ml) was used for treat keratitis animal models because of giving the possibility of interfering factors present in vivo. At the same time, these concentrations (4 & 8 µg/ml) were shown in previous assessments to have no significant cytotoxic effects. In the same regard, the Draize analyses revealed no significant eye complications and delayed toxicity in the peptide-treated group after 4 days of peptide instillation. Therefore, 2 × MIC (8 µg/ml) peptide concentration was selected to be used as the eye drop dose for treating the experimental infection models. Compared to the gentamycin, our results demonstrated a significant infection control by CM11 peptide treatment leading to complete disappearing of infection in the rabbits' eyes from the test group. Also, the bacterial counting assessment showed that the number of bacteria was greatly reduced in the rabbits' eyes of the peptide-treated group compared to the gentamicin-treated group (positive group). Additionally, the histopathological evaluations displayed a high similarity between the infection models treated with gentamicin and CM11. For example, the leukocyte infiltration was inhibited in both treated groups with the CM11 peptide and gentamycin. These findings confirms that CM11 can be used as an proper alternative to conventional antibiotics for effectively treating the MRSA keratitis.

In recent years, several animal studies have been conducted for treating different bacterial keratitis models using a variety of antimicrobial peptides. For instance, in a report by Edward Clemens et al., eleven designed peptides were synthesized and studied in term of healing effect on the bacterial keratitis in the murine model (Clemens et al. 2017). Their synthetic peptides were derived from host defense peptides (HDPs)—cecropins and magainins—and first evaluated for their bactericidal effectiveness against some Gram-positive and Gram-negative bacteria, as well as some antibiotic-resistant species (e.g., MRSA and *P. aeruginosa*). These peptides showed bactericidal potentials with MICs ranging from 2 to > 64 µg/ml. Among all peptides they investigated, the best results belonged to the RP444 peptide especially against *P. aeruginosa* and *S. aureus* showing the MICs of 4 and 8 µg/ml, respectively. Therefore, the therapeutic potential of this peptide on *P. aeruginosa* keratitis was further evaluated which demonstrated to be considerably efficient in the murine model. The results of the latter study demonstrated that RP444 could decrease the ocular clinical scores of *P. aeruginosa* keratitis models in a significant dose-dependent manner, reduce the bacterial load, and decrease the inflammatory cell infiltrates to a great extent. These observations are similar to those of our study with the difference that CM11 (11-amino acid) is shorter than RP444 (23-amino acid) which makes CM11 more economically viable for therapeutic purposes. In another study by Kolar et al., the anti-*Pseudomonal* activity of esculentin-1a(1-21)-NH_2 was investigated in vitro and in vivo. The in vitro studies on this synthetic 21-amino acid peptide, which originates from a frog skin antimicrobial peptide, showed a MIC of 2–16 µM against different strains, including the antibiotic-resistant *P. aeruginosa* (Kolar et al. 2015). In addition, esculentin-1a(1-21)-NH_2 did not show cytotoxic effects on the epithelial cells of human cornea up to the concentration of 50 µM. Whereas, the drop installation of esculentin-1a(1-21)-NH_2 (40 µM) significantly reduced the mean ocular clinical scores (2.89 ± 0.26 compared to 3.92 ± 0.08 for the control group), recruitment of inflammatory cells (50%), as well as the *Pseudomonal* infection of the ocular surface in the *P. aeruginosa* keratitis murine models. The corneal level of viable bacteria significantly decreased in the treated group (4 $\log_{10}$ CFU) compared to the control group (7.7 $\log_{10}$ CFU). This is while the CM11 peptide in our study could reduce the microbial load by a quarter which is a less outcome compared to the esculentin-1a(1-21)-NH_2 with the microbial load reduction by half. This difference between the results of CM11 and esculentin-1a(1-21)-NH_2 peptides can be attributed to the fewer amino acids of CM11. In another study by Mannis, the antibacterial activity of COL-1 (in concentrations up to 50 µg/ml) against *P. aeruginosa* was investigated in the rabbit keratitis model (Mannis 2002). But this twenty-amino acid peptide showed no antimicrobial activity while exhibited cytotoxic effects on the rabbits' cornea. These results emphasize the importance of further researches on the bactericidal efficacies and cytotoxic consequences of newly introduced AMPs.

Conclusion

These findings suggest that using antimicrobial peptides have the potential to be considered as a significant therapeutic method for treating keratitis caused by antibiotic-resistant bacteria. Accordingly, using cationic peptide seems to be a simple and highly successful method for healing the infected ocular surface. In the same regard, considering its short sequence and effectiveness at low concentrations, CM11 peptide displays to be highly advantageous for application against MRSA in the clinics.

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Compliance with Ethical Standards

Conflict of interest None of the authors has conflict of interest with this submission.

References

Albus U (2012) Guide for the care and use of laboratory animals, 8th edn. SAGE Publications, London

Amani J, Barjini AK, Moghaddam M, Asadi A (2015) In vitro synergistic effect of the CM11 antimicrobial peptide in combination with common antibiotics against clinical isolates of six species of multidrug-resistant pathogenic bacteria. Protein Pept Lett 22:940–951

Azad ZM, Moravej H, Fasihi-Ramandi M, Masjedian F, Nazari R, Mirnejad R, Moghaddam MM (2017) In vitro synergistic effects of a short cationic peptide and clinically used antibiotics against drug-resistant isolates of *Brucella melitensis*. J Med Microbiol 66:919–926

Beisel K, Hazlett L, Berk R (1983) Dominant susceptibility effect on the murine corneal response to *Pseudomonas aeruginosa*. Proc Soc Exp Biol Med 172:488–491

Boman H, Wade D, Boman I, Wåhlin B, Merrifield R (1989) Antibacterial and antimalarial properties of peptides that are cecropin-melittin hybrids. FEBS Lett 259:103–106

Brown L (2007) Resistance to ocular antibiotics: an overview. Clin Exp Optom 90:258–262

Cao Y, Yu RQ, Liu Y, Zhou HX, Song LL, Cao Y (2010) Design, recombinant expression, and antibacterial activity of the cecropins–melittin hybrid antimicrobial peptides. Curr Microbiol 61:169–175

Chang VS, Dhaliwal DK, Raju L, Kowalski RP (2015) Antibiotic resistance in the treatment of *Staphylococcus aureus* keratitis: a 20-year review. Cornea 34:698

Chen CH, Lu TK (2020) Development and challenges of antimicrobial peptides for therapeutic applications. Antibiotics 9:24

Clemens LE et al (2017) Designed host defense peptides for the treatment of bacterial keratitis. Invest Ophthalmol Vis Sci 58:6273–6281

De La Fuente-Núñez C, Cardoso MH, de Souza Cândido E, Franco OL, Hancock RE (2016) Synthetic antibiofilm peptides. Biochim Biophys Acta (BBA) Biomembr 1858:1061–1069

Deguchi H et al (2018) The trend of resistance to antibiotics for ocular infection of *Staphylococcus aureus*, coagulase-negative staphylococci, and Corynebacterium compared with 10-years previous: a retrospective observational study. PLoS ONE 13:e0203705

Durrani AF, Atta S, Bhat A, Mammen A, Dhaliwal D, Kowalski RP, Jhanji V (2020) Methicillin-resistant *Staphylococcal aureus* keratitis: initial treatment, risk factors, clinical features, and treatment outcomes. Am J Ophthalmol. https://doi.org/10.1016/j.ajo.2020.03.017

Egrilmez S, Yildirim-Theveny Ş (2020) Treatment-resistant bacterial keratitis: challenges and solutions. Clin Ophthalmol 14:287

Epand RM, Epand RF (2009) Lipid domains in bacterial membranes and the action of antimicrobial agents. Biochim Biophys Acta Biomembr 1788:289–294

Griffith GL, Kasus-Jacobi A, Pereira HA (2017) Bioactive antimicrobial peptides as therapeutics for corneal wounds and infections. Adv Wound Care 6:175–190

Hollmann A, Martinez M, Maturana P, Semorile LC, Maffia PC (2018) Antimicrobial peptides: interaction with model and biological membranes and synergism with chemical antibiotics. Front Chem 6:204

Holve DL, Gum GG, Pritt SL (2013) Effect of sedation with xylazine and ketamine on intraocular pressure in New Zealand white rabbits. J Am Assoc Lab Anim Sci 52:488–490

Kolar SSN, Luca V, Baidouri H, Mannino G, McDermott AM, Mangoni ML (2015) Esculentin-1a (1-21) NH_2: a frog skin-derived peptide for microbial keratitis. Cell Mol Life Sci 72:617–627

Lakhundi S, Zhang K (2018) Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. Clin Microbiol Rev 31:e00020

Lalitha P et al (2017) Trends in antibiotic resistance in bacterial keratitis isolates from South India. Br J Ophthalmol 101:108–113

Lei J et al (2019) The antimicrobial peptides and their potential clinical applications. Am J Transl Res 11:3919

Mannis MJ (2002) The use of antimicrobial peptides in ophthalmology: an experimental study in corneal preservation and the management of bacterial keratitis. Trans Am Ophthalmol Soc 100:243

Miller D (2017) Update on the epidemiology and antibiotic resistance of ocular infections Middle East. Afr J Ophthalmol 24:30

Moghaddam MM, Abolhassani F, Babavalian H, Mirnejad R, Barjini KA, Amani J (2012) Comparison of in vitro antibacterial activities of two cationic peptides CM15 and CM11 against five pathogenic bacteria: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholerae*, *Acinetobacter baumannii*, and *Escherichia coli*. Probiot Antimicrob Proteins 4:133–139

Moghaddam MM, Aghamollaei H, Kooshki H, Barjini KA, Mirnejad R, Choopani A (2015) The development of antimicrobial peptides as an approach to prevention of antibiotic resistance. Rev Med Microbiol 26:98–110

Moravej H, Moravej Z, Yazdanparast M, Heiat M, Mirhosseini A, Moosazadeh Moghaddam M, Mirnejad R (2018) Antimicrobial peptides: features, action, and their resistance mechanisms in bacteria. Microbial Drug Resistance 24:747–767

Pfalzgraff A, Brandenburg K, Weindl G (2018) Antimicrobial peptides and their therapeutic potential for bacterial skin infections and wounds. Front Pharmacol 9:281

Ramandi F, Piranfar V, Nadoushan M, Sarshoori J, Misialek M, Heiat M, Moosazadeh Moghaddam M (2017) Dose-response effects of the CM11 as a short cationic antimicrobial peptide on histopathological and biochemical changes in mice. Curr Chem Biol 11:150–157

Robles-Contreras A, Perez-Cano HJ, Babayan-Sosa A, Baca-Lozada O (2013) Bacterial keratitis infection: a battle between virulence factors and the immune response. Common Eye Infect 85:842–847

Sharma A (2018) The pharmacist's role in managing microbial keratitis. US Pharm 43:27–30

Suzuki T (2011) A new target for *Staphylococcus aureus* associated with keratitis. Cornea 30:S34–S40

Teweldemedhin M, Gebreyesus H, Atsbaha AH, Asgedom SW, Saravanan M (2017) Bacterial profile of ocular infections: a systematic review. BMC Ophthalmol 17:212

Wang N, Yang Q, Tan Y, Lin L, Huang Q, Wu K (2015) Bacterial spectrum and antibiotic resistance patterns of ocular infection: differences between external and intraocular diseases. J Ophthalmol 2015:813979

Wayne P (2009) Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically approved standard CLSI document M07-A8, 8th edn. Clinical and Laboratory Standards Institute (CLSI), Wayne

Wilhelmus KR (2001) The Draize eye test. Surv Ophthalmol 45:493–515

Yeu E, Hauswirth S (2020) A review of the differential diagnosis of acute infectious conjunctivitis: implications for treatment and management. Clin Ophthalmol 14:805