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Received: March 3, 2017. Accepted: May 25, 2017.
Published online: September 11, 2017.
DOI: 10.7883/yoken.JJID.2017.090
The antibacterial effects of antimicrobial peptides OP-145 against clinically isolated multi-resistant strains

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Abstracts

OP-145 is a synthetic antimicrobial peptide developed from the human cathelicidin LL-37. The purpose of this investigation was to evaluate the effect of the antimicrobial peptide OP-145 against clinically isolated drug resistant strains. Ten Methicillin-resistant Staphylococcus aureus (MRSA) strains were obtained from our hospital's clinical inspection center and the activity of OP-145 on growth and biofilm formation on these strains was evaluated by colony counts and scanning electron microscopy. The antimicrobial peptide OP-145 showed significant antibacterial activity against 9 MRSA strains. For the biofilm experiments, MRSA counts in the biofilms decreased significantly after 24h (P < 0.05). OP-145 strongly reduced growth and biofilm formation of clinically isolated drug resistant strains in vitro, and the use of this class of antimicrobial agents may be an important new approach in controlling bacterial infections.

Keywords: Antimicrobial peptides, Biofilm, Antibacterial effects, Resistance isolates, Bacteriostatic test
Introduction

With the development of modern medicine and the use of antibiotics, there has also been an increased trend towards greater numbers of hospital acquired infections (1). With increasing bacterial resistance, there is also an increased risk of treatment failure, recurrent infections and death (2).

The misuse of antimicrobials is a major cause of increased bacterial resistance in hospitals (3), which has led to increased prevalence of drug resistant strains (4). Thus, the proper choice of antimicrobial agents is of great importance in combating drug-resistant bacteria (5). Antimicrobial peptides (AMPs) are a type of antimicrobial agents to treat infection diseases (6). Natural antibacterial peptides have a wide antimicrobial spectrum (7), particularly with the ability to kill multidrug-resistant bacteria (8), and meanwhile antibacterial peptides have good thermal stability and good solubility in water. One of the best-studied AMPs is human LL-37, which is the only cathelicidin peptide found in humans. Studies have shown that LL-37 could inhibit the growth and biofilm formation of the pathogenic bacteria (9, 10). As a synthetic antimicrobial peptide developed from the human cathelicidin LL-37, OP-145 shows strong antibacterial activities against pathogens which suggests a novel method for treating infectious diseases (11). Although the relative efficacy of LL-37 in inhibiting the in vitro growth of various pathogenic bacteria has been determined (12), there is a lack of information on the activity of OP-145 against pathogenic bacteria, especially drug-resistant bacteria, such as MRSA.
In this study, we investigated the antibacterial effect of the antimicrobial peptide OP-145 on MRSA. We measured the effects of OP-145 on biofilm formation of MRSA and aimed to verify the effects of OP-145 in the treatment of infectious diseases in vivo.

**Subjects and Methods:**

**Peptide**

OP-145 (acetyl-IGKEFKRIVERIKRFLRELVRPLR-amide) peptide was synthesized by Shanghai Apeptide Co. Ltd (Shanghai, China). OP-145 was purified by high-performance liquid chromatography, and the identity of the peptide was confirmed by SDS-PAGE and its purity (> 95%) and mass were confirmed by electrospray ionization mass spectrometry(11).

**Strains and culture conditions**

Ten Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were obtained from our hospital's clinical inspection center. All isolates were sent to the CDC for identification, testing, and storage by the methods of Klevens et al (13). The strain *S. aureus* 99308 was used as a reference strain in this study. The *S. aureus* strains used to screen for antibacterial activity were grown overnight in brain heart infusion broth (BHI, Difco, USA) at 37°C with gentle agitation and maintained on blood agar plates (bioMérieux, France).
Interference test

OP-145 was diluted to 1 mg/mL in PBS. *S. aureus* clinical isolates were grown on Columbia sheep blood agar under microaerophilic conditions at 37 °C for 24 h. After incubation, the *S. aureus* culture concentration was adjusted to a 0.1 McFarland standard \((3 \times 10^7 \text{ CFU/ml})\) with PBS using a Densicheck analyzer (BioMérieux, France). OP-145 (500 µl) was mixed with 10 ml of BHI agar in petri dishes. Finally 100 µl of the *S. aureus* cultures were evenly spread on BHI plates and incubated under microaerophilic conditions at 37 °C for 24 h. 100 ul PBS were used as blank control.

After 24h incubation, the colonies were counted using image analysis software (Image Pro).

Biofilm formation

For this assay, 5 of the 10 clinically isolated *S. aureus* strains were randomly selected as test strains. They were grown on Columbia sheep blood agar under microaerophilic conditions at 37 °C for 24 h and were then diluted in PBS at a concentration of 0.5 McFarland standard \((1.5 \times 10^8 \text{ CFU/ml})\). The bacterial solutions were transferred to sterile 9 cm petri dishes with 10 ml BHI broth, and 0.1ml OP-145 suspension (0.1mg/ml) was added to the petri dishes with coverglass. The petri dishes were incubated under microaerophilic conditions at 37 °C for 24 h. Biofilm formation was examined after 24 h incubation[14].
Scanning electron microscopy (SEM) of Biofilms

After 24h incubation, coverslips were then fixed with 2.5% glutaraldehyde at 4°C for 1 h were observed Scanning electron microscopy with a scanning electron microscope (Hitachi SU-70) in high-vacuum mode at 3 kV.

Statistical Analysis

All experiments were performed three times and expressed as mean ± SD. SPSS 14.0 software for Windows was used for data analysis. Data were analyzed for statistical significance using Student's test. p < 0.05 was considered statistically significant.

Results

Interference test

Among the 10 clinically isolated S. aureus strains, OP-145 showed significant antibacterial activity against 9 strains (P < 0.05, Fig 1). OP-145 showed no significant antibacterial effect against strain 9 (P > 0.05, Fig 1). The response of the S. aureus strains to OP-145 and PBS loaded onto disks is shown in Fig 3.

Inhibition of biofilm formation

After 24 h of biofilm formation, the number of bacteria in the biofilm formed by strains 1, 3, 4, and 5 was significantly decreased compared to the control group (P < 0.05, Fig 2), while there was no obvious difference in the bacterial count from the strain 2 biofilm (P >0.05, Fig 2). The images of scanning electron microscopy were shown in Fig 4.
Discussion

Antimicrobial peptides (AMPs) are small peptides that exist widely in nature (15). They form an important part of the innate immune system of an organism. Antimicrobial peptides have a wide range of activities against bacteria, fungi, parasites, viruses, as well as tumors (16). Antimicrobial peptides usually possess high efficiency, broad-spectrum and potential antitumor activity, and can accelerate the body's immune defenses and wound healing, making them an alternative to traditional antibiotics to treat infectious diseases. Our study demonstrated that the AMP OP-145 can inhibit growth and biofilm formation of \textit{S. aureus in vitro}, which is consistent with previous (11, 17).

\textit{S. aureus} is the most important infectious disease pathogen (18), and invasive \textit{S. aureus} infections often lead to death (19). In the 1940’s, penicillin was used to treat \textit{S. aureus} infections, however, it was not long before penicillin resistant strains, such as methicillin-resistant \textit{S. aureus} evolved (20). MRSA then appeared in many hospitals all over the world. It was reported that the detection rate of the 37,227 clinical isolates of \textit{S. aureus} and coagulase negative methicillin-resistant \textit{Staphylococcus} strains rose from 9% to 78 in China's 149 hospital joint. This increase in the rate of MRSA cases suggests that the last line of defense for the treatment of MRSA seems be on the verge of collapse. MRSA has been well studied in recent years due to its heterogeneity and multiple drug resistance.
In addition, any additional potential mechanisms of action of AMPs have not yet been thoroughly studied. Therefore, further studies on the antibacterial effects of AMPs using in vivo animal models are still necessary to fully understand the role that AMPs may play in the prevention of implant-associated infections. In the meantime, our experiment serves to confirm the short-term effects of AMPs on clinically relevant strains; however, further studies are required to determine their long-term effects.

**Conclusion**

The antimicrobial peptide OP-145 coating holds promise for further clinical development as an alternative to coatings that release conventional antibiotics that are associated with the development of resistance. In addition, the present coating has the potential for rapid translation to humans, particularly since all compounds present in the coating, including OP-145, have already been approved for human use.

**Disclosure of conflict of interest:**

None.

**References**


Fig.1. Viable *S. aureus* cells in the presence of OP-145 or PBS (1-10: isolated *S. aureus* strain No.1-10, C: control), * P<0.05, ** P<0.01 compared with PBS controls (paired-t test).

Fig.2. Viable *S. aureus* cells in the biofilms in the presence of OP-145 or PBS (1-5: isolated *S. aureus* strain No.1-5, C: control), * P<0.05, ** P<0.01 compared with PBS controls (paired-t test).
controls (paired-t test).

Fig. 3. Response of OP-145 and PBS with disks containing 1ml of OP-145 solution and PBS (1-5: isolated *S. aureus* strain No.1-5, C: control)

Fig. 4. SEM images of the biofilm of MRSA (1-10: isolated *S. aureus* strain No.1-10, C: control),