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Mucroporin, the First Cationic Host Defense Peptide from the Venom of *Lychas mucronatus*▽

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The misuse of antibiotics has led our age to a dangerous edge, as antibiotic-resistant pathogens appear to evolve more quickly than antibiotics are invented. Thus, new agents to treat bacterial infection are badly needed. Cationic host defense peptides are on the first line of a host defense system and are thought to be good candidates for treating bacterial infection. Here, a novel cationic host defense peptide, mucroporin, was cloned and characterized from the venom of *Lychas mucronatus*. The MIC for *Staphylococcus aureus* was 25 μg/ml, including antibiotic-resistant pathogens. Based on the molecular template of mucroporin, mucroporin-M1 was designed by amino acid substitution. The MIC for *S. aureus* was 5 μg/ml, including the antibiotic-resistant pathogens methicillin-resistant *S. aureus*, methicillin-resistant coagulase-negative *Staphylococcus*, penicillin-resistant *S. aureus*, and penicillin-resistant *S. epidermidis*. Moreover, mucroporin-M1 also inhibited gram-negative bacteria. The modes of action of mucroporin and mucroporin-M1 were both rapid killing by disrupting the cell membrane of bacteria, and the number of surviving bacteria was reduced by about 4 to 5 orders of magnitude immediately after peptide delivery. These results showed that mucroporin could be considered a potential anti-infective drug, especially for treating antibiotic-resistant pathogens.

When a new antibiotic comes into use, resistance will follow. During the development period of antibiotics discovery, when resistance to former antibiotics appeared, drug substitution was used to treat resistant pathogens. However, during the last decade, it seems that the frequency of antibiotic resistance, especially the evolution of multidrug-resistant pathogens, is outpacing the development of new antibiotics (44, 48). Among the existing antibiotic-resistant pathogens, methicillin-resistant *Staphylococcus aureus* (MRSA) is considered the most lethal one. MRSA was first identified as a hospital-acquired pathogen in the 1960s, which was resistant to the entire class of β-lactam agents (20). Over the past 40 years, MRSA infections have become endemic in hospitals worldwide and have recently appeared in communities (13, 20, 38). It was reported that there were roughly 94,000 invasive MRSA cases in the United States, with roughly 19,000 deaths, in 2005, which was higher than the number of deaths caused by AIDS in the same year (29). Facing the so-called "superbug" (29), we have only got one remaining antibiotic weapon: vancomycin. Unfortunately, the overuse of vancomycin in treating MRSA infection and the existence of vancomycin-resistant enterococcus has led to the emergence of vancomycin-intermediate *S. aureus* (2, 4, 36). Therefore, new kinds of antimicrobial drugs are needed (26).

Cationic host defense peptides are produced by many organisms as part of their host defense system (23, 25, 34). These peptides are potent antimicrobial agents against gram-positive and gram-negative bacteria, fungi, parasites, and some viruses (9, 21), and a few of them have been reported to inhibit MRSA growth (42, 43). Studies indicated that the targets of cationic host defense peptides varied from the outer membrane to the signaling pathway (8, 10, 27), which may be the reason why resistance to cationic host defense peptides is more difficult to attain than resistance to the conventional antibiotics. The broad-spectrum activity, low potential to induce resistance, and the huge family of over 1,300 peptides (19) make cationic host defense peptides an attractive family of compounds that have the potential to be developed as therapeutic agents (7, 30).

Cationic host defense peptides are usually very short, ranging from 10 to 50 amino acid residues with a net positive charge of 2 to 9 (27, 37, 49, 51). Despite their common physiological effects, cationic host defense peptides vary in both sequence and secondary structure (6, 22, 27, 49). Structurally, natural cationic host defense peptides can be classified as: (i) amphipathic α-helix, (ii) β-sheet structures stabilized with two or three disulfide bonds, (iii) extended structures, and (iv) loop structures with one disulfide bond (40). Besides the main antibacterial, antifungal, antiviral, and antitumor functions, there is increasing evidence supporting the idea that cationic peptides have diverse functions in modulating immune responses, especially infection and inflammation (10, 24, 50). Several peptides are in clinical trial periods (1), and cationic host defense peptides present the best alternative to conventional antibiotics.

Cationic host defense peptides have also been found in scorpion hemolymph (14, 18) and venom, including hadrurin (46), scorpine (15), opistoporins, parabutoporin (39), ISCTs (17), pandinins (16), and BmKn2 and BmKb1 (53). The functions of these scorpion-derived peptides vary from cytotoxic (17) to inhibiting bacteria (15, 46) to inhibiting fungus (18, 39).

In our previous study, we characterized two cationic host defense peptides, BmKn2 and BmKb1, derived from the venom of *Buthus martensii* Karsch (53). Here, we describe a novel cationic defense peptide mucroporin, which is the first

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cationic host defense peptide characterized from the scorpion *Lychas mucronatus*. We found that mucroporin can effectively inhibit standard bacteria, especially gram-positive bacteria. The optimized design of mucroporin-M1 by amino acid substitution resulted in the inhibition of both gram-positive and gram-negative bacteria at low concentrations. We chose *S. aureus* as a model bacteria strain to further explore the mechanism of mucroporin and mucroporin-M1's bioactivity. Some evidence showed that the cell membrane of *S. aureus* was broken immediately after the treatment of mucroporin or mucroporin-M1. The assay revealed that the inhibitory effect of mucroporin and mucroporin-M1 was exerted by the action mode of rapid killing. Moreover, the in vitro treatment of clinically isolated pathogens showed that mucroporin-M1 is highly capable of inhibiting antibiotic-resistant pathogens, including MRSA, methicillin-resistant coagulase-negative *Staphylococcus* (MRCNS), etc. Mucroporin and its analogue present potential anti-infective drugs or lead compounds, especially for treating antibiotic-resistant pathogens.

MATERIALS AND METHODS

cDNA library construction. The *L. mucronatus* scorpions were collected in Hainan province, China. Their glands were collected 2 days after electrical extraction of their venom. Total RNA was prepared from the glands by using TRIzol reagent (Invitrogen). Poly(A)-mRNA was purified by a poly(A) tract mRNA isolation system (Promega). A cDNA library was constructed according to the Superscript plasmid system cDNA library construction kit (Gibco-BRL). cDNAs were cloned into pSPORT1 plasmids and transformed into *Escherichia coli* DH5α cells. Randomly chosen cDNA clones were sequenced to obtain a reliable representation of the toxin content in the venom gland.

Screening of the cDNA library with PCR strategy. A specific primer was designed and synthesized to screen mucroporin gene (homologue of BmKb1/BmKb2) from the venomous gland cDNA library of *L. mucronatus* by PCR method as described previously (52). The specific forward and reverse primers were 5′-TCGACCCACGCGTCCG-3′ and 5′-GCGTTTCCTTCGGCC-3′, respectively, corresponding to the digestion sites of the vector and the conserved processing region of the propeptide.

cDNA sequencing and computer analysis. The plasmids characterized as positive clones were determined by using an ABI Prism 377XL DNA sequencer with a universal T_7 promoter primer. Sequence analysis was performed by using BLASTX, DNAMAN, and GENRUNR. All homologue sequences of mucroporin were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/) by the BlastP method. Multiple sequence alignments of mucroporin proteins were carried out by using CLUSTAL X version 1.83 (http://www2.ebi.ac.uk/clustalw/).

Chemical synthesis. The peptide was synthesized by GL Biochem. (Shanghai), Ltd., China, with a purity of >95%.

Bacterial strains. *E. coli* (AB94012), *Pseudomonas aeruginosa* (AB93066), *Bacillus thuringiensis* (AB92037), *S. aureus* (AB94004), *Bacillus subtilis* (AB91021), and *Micrococcus luteus* (AB93113) were purchased from the China Center of Type Culture Collection.

Antibiotic-resistant strains were obtained from the 302nd Military Hospital of Beijing, China, including penicillin-resistant *S. aureus* (PRSA) P1383; penicillin-resistant *S. epidermidis* (PRSE) P1389; MRSA P1381, P1386, and P1374; and MRCNS P1369. MRCNS 1538 was obtained from the Hubei Maternal and Child Health Hospital, Hubei, China.

Antibiotic-sensitive clinical isolates were also obtained from the 302nd Military Hospital of Beijing, China, including penicillin-sensitive *S. aureus* (PSSA) P969 and penicillin-sensitive *S. epidermidis* (PSSE) P1111 and P1368.

MIC determination. Overnight-cultured bacteria were diluted with Luria-Bertani (LB) medium to about 10^4 to 10^6 CFU/ml. This bacterial suspension and serial diluted peptides were added to 96-well plates at ratio of 4:1 in a final volume of 100 μl. The microplates were incubated at 37°C with continuous shaking. After 12 to 16 h, the optical density at 630 nm (OD_{630}) was measured with a microplate reader.

Overnight-cultured clinically isolated strains were diluted with LB medium to 0.5 McFarland. This bacterial suspension and serial diluted peptides were added to 96-well plates at a ratio of 4:1 in a final volume of 100 μl. The microplates were incubated at 37°C with continuous shaking for 12 to 16 h. The OD_{630} was measured with a microplate reader.

Each concentration reading was repeated three times. The MIC was determined at the concentration at which there was no optical density. All of the above experiments were repeated at least twice.

Bactericidal assay. Overnight-cultured *S. aureus* was transferred to LB medium and cultured to exponential phase (OD_{600} of ~0.6). A 300-μl portion of peptide solution was added to a 1,200-μl bacterial suspension, and the mixture was incubated at 37°C with continuous shaking. At each time point, 200 μl of treated bacterial suspension was transferred to a sterilized 1.5-ml tube. After centrifugation at 1,000 × *g* for 5 min, the supernatant was removed, and the pellet was resuspended with 200 μl of LB medium. This bacterial suspension was placed on agar plates and incubated at 37°C until the viable colonies could be counted.

Scanning electron microscopy. Overnight-cultured *S. aureus* was transferred to LB medium and cultured to the exponential phase. A portion (300 μl) of the peptide solution was added to a 1,200-μl bacterial suspension, and the mixture was incubated at 37°C with continuous shaking. At 2 min after incubation, the bacterial suspension was centrifuged at 1,000 × *g* for 5 min, and the pellet was washed with 0.1 M phosphate-buffered saline (PBS) several times and then fixed overnight with 2.5% glutaraldehyde in 0.1 M PBS at 4°C. After fixation, the bacteria were washed with PBS for a minimum of 15 min and then dehydrated by using a series of graded ethyl alcohols (50% for 15 min, 60% for 15 min, 70% for 15 min, 80% for 15 min, 90 for 15 min, and 2 changes of 100% for 10 min each). After this, the samples were mounted on aluminum stubs with adhesive tabs and sputter coated for 3 min by using a polaron. The samples were then ready to view on the Hitachi X650 scanning electron microscope.

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                    tactaggaaaATGAAAGTTAAATTCCTTCTCGCTGTCTTC
                                M  K  V  K  F  L  L  A  V  F      10
TTGATCGTTTTGGTTGTTACTGATCATTGTCATGCATTGTTCGGACTTATCCCTTCGTTG
L  I  V  L  V  V  T  D  H  C  H  A  L  F  G  L  I  P  S  L      30
ATCGGAGGGCTGGTATCAGCATTCAAGGGCCGAAGGAAACGCCAGATGGAAGCTCGATTC
I  G  G  L  V  S  A  F  K  G  R  R  K  R  Q  M  E  A  R  F      50
GAACCCCAAAATAGGAATTACAGGAAACGCGAGCTCGACCTTGAAAAGTTATTCGCAAAT
E  P  Q  N  R  N  Y  R  K  R  E  L  D  L  E  K  L  F  A  N      70
ATGCCTGATTACTGAtctcaattactctttcagtttcatcgcttagcataagtttttcaa
M  P  D  Y  end                                                  74
 gttactgccgctactattgcatcatgatgtgaattggttactttctaatataataaaaaa
 actatacttaatcataaaaaaaaaaaaaaaaaaaaaa
```

FIG. 1. cDNA and protein sequences of mucroporin (accession no. EU669864). The deduced amino acid residues are shown below the corresponding nucleotide sequences. The signal peptide is in italics, and the propeptide is underlined. The potential polyadenylation signal AATAAA is double underlined.

RESULTS

Sequence analysis. After systemic screening of 500 clones from the venomous cDNA library of *L. mucronatus*, we obtained eight positive clones. Sequencing of all of these positive clones revealed a novel precursor, termed mucroporin, with high similarities to BmKb1 and BmKb2 and a number of antimicrobial peptides from other scorpion species (Fig. 1). The mucroporin precursor consists of three parts: a 5′ untranslated region (5′UTR), an open reading frame (ORF), and a 3′UTR. The 5′ and 3′UTRs of mucroporin are 10 and 120 bp in length, respectively. At the 3′UTR end of the cDNA, a single AATAAA polyadenylation signal is found 12 bp upstream of the poly(A) tail. An ORF of 222 bp encodes a precursor of 74 amino acid residues (Fig. 2).

Mucroporin is composed of a putative 22 residues, followed by a presumed 17-residue mature peptide, and an uncommon acidic propeptide at the C terminus. The 35-residue propeptide starts with a conserved posttranslational processing signal,

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AP6_TITCO    MQIKHLITLFFLVLIVADQCSAFFS-LIPSLIGGLVSAIKGRKKREISTQID-QYRNLQKREAELEELLDRLPMY    52.7%
AP5_TITCO    MQIKHLITLFFLVLIGADQCSAFFS-LIPSLIGGLVFAIKGRKKREVSPQID-QYRNFQKREAELEELLDRLPMY    50.0%
AP4_TITCO    MQMKYLIPIFFLVLIVADHCHAFLG-MIPGLIGGLISAFKGRRKRDITSQIE-QYRNLQKREAELEDILANLPVY    56.7%
Caerin-1     MEIKYLLTVFLVLLIVSDHCQAFLFSLIPSAISGLISAFKGRRKRDLNGQID-HFKNFRKRDAELEELLSKLPIY    50.6%
KB2_MESMA    MEIKYLLTVFLVLLIVSDHCQAFLSSLIPSAISGLISAFKGRRKRDLNGQID-HFKNFRKRDAELEELLSKLPIY    50.6%
KB1_MESMA    MEIKYLLTVFLVLLIVSDHCQAFLFSLIPSAISGLISAFKGRRKRDLNGYID-HFKNFRKRDAELEELLSKLPIY    50.6%
Caerin-2     MEIKYLLTVFLVLLIVSDHCQAFLFSLIPSAISGLISAFKGKRRRDLNAQID-QFKNFRKRDAELEELLSKLPIY    50.6%
Mucroporin   MKVKFLLAVFLIVLVVTDHCHALFG-LIPSLIGGLVSAFKGRRKRQMEARFEPQNRNYRKRELDLEKLFANMPDY   100.0%
```

FIG. 2. Protein sequence alignments of mucroporin with AP4_TITCO, AP5_TITCO, AP6_TITCO, caerin-1, caerin-2, BmKb1, and BmKb2. The percentage of sequence similarity relative to mucroporin is indicated to the right of each sequence. The signal peptide is in italics. The propeptide is underlined.

Gly-Arg-Arg, at positions 40 to 42. Conventionally, removal of the propeptide with such processing signal would result in a mature peptide with C-terminal amidation as described previously (17).

Mucroporin-M1 (LFRLIKSLIKRLVSAFK) was designed based on the protein sequence of mucroporin for the purpose of enhancing the net positive charge of the hydrophilic side. Mucroporin and mucroporin-M1 was synthesized by GL Biochem, Ltd., Shanghai, China, with reliable quality. The molecule weights measured with mass spectrum (1,731.22 and 2,031.57, respectively) matched with the calculated molecule weights of the amidated mucroporin and mucroporin-M1 (1,731.13 and 2,031.58, respectively) very well.

MIC. The effect of mucroporin and mucroporin-M1 on bacteria was studied by the microdilution method. As shown in Table 1, it can be seen that mucroporin was more effective on gram-positive bacteria than on gram-negative bacteria. In all, the MICs of mucroporin were 25 μg/ml for *S. aureus* AB94004, 25 μg/ml for *B. thuringiensis* AB92037, and 50 μg/ml for *B. subtilis* AB91021. The MICs for mucroporin-M1 were 5 μg/ml for *S. aureus* AB94004, 25 μg/ml for *B. thuringiensis* AB92037, and 25 μg/ml for *B. subtilis* AB91021. In addition, *E. coli* AB94012 and *P. aeruginosa* AB93066 were both insensitive to 100 μg of mucroporin/ml, while the MICs of mucroporin-M1 were 12.5 μg/ml for *E. coli* AB94012 and 100 μg/ml for *P. aeruginosa* AB93066. As shown in Fig. 3A, we investigated the growth of *S. aureus* AB94004 8 h after peptide treatment. It was found that *S. aureus* AB94004 treated with mucroporin reproduced faster than *S. aureus* AB94004 treated with mucroporin-M1 at the same concentration. These results indicated that the modification of the mucroporin sequence not only enhanced its in vitro activity but also expanded its antibacterial spectrum.

TABLE 1. Antibacterial effects of mucroporin and mucroporin-M1

Strain	MIC (μg/ml)		
	Mucroporin	Mucroporin-M1	Penicillin
Escherichia coli AB94012	>100	12.5	
Pseudomonas aeruginosa AB93066	>100	100	
Bacillus thuringiensis AB92037	25	25	12.5
Bacillus subtilis AB91021	50	25	25
Staphylococcus aureus AB94004	25	5	2
1538[a]	25	5	25

[a] Strain 1538 was MRCNS, a clinical isolate obtained from the Hubei Maternal and Child Health Hospital.

The antibiotic-resistant pathogens were clinical isolates, all of which were tested with traditional antibiotics to determine the resistance before experiments. As shown in Table 1, mucroporin and mucroporin-M1 can both inhibit MRCNS 1538 growth. Comparing the MICs of penicillin to 1538 and *S. au-*

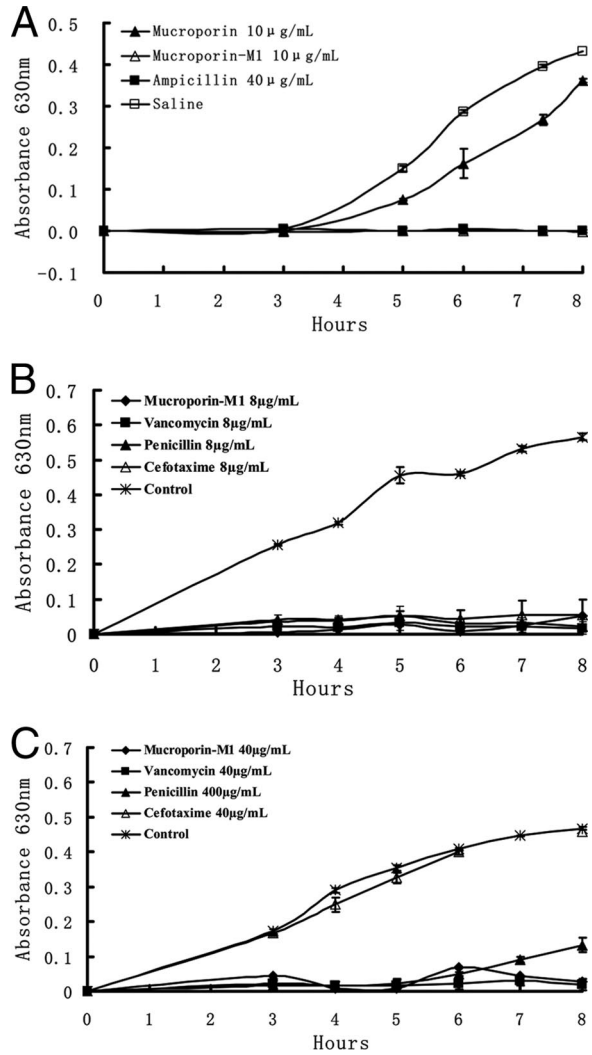

FIG. 3. Time growth curves of *S. aureus* treated by mucroporin or mucroporin-M1. The growth of *S. aureus* was measured at 630 nm. (A) Comparison of mucroporin and mucroporin-M1 activities against *S. aureus* AB94004. (B) Comparison of mucroporin-M1 and antibiotics activities against clinical isolate MRSA P1386. (C) Comparison of mucroporin-M1 and antibiotics activities against clinical isolate PSSA P969.

TABLE 2. MICs of mucroporin-M1 for clinical isolates

Strain[a]	MIC (μg/ml)			
	Mucroporin-M1	Vancomycin	Penicillin	Cefotaxime
Penicillin resistant				
P1383 (PRSA)	10	8	10,000	5
P1389 (PRSE)	8	8	10,000	2
Methicillin resistant				
P1381 (MRSA)	20	8	5,000	400
P1386 (MRSA)	25	4	10,000	100
P1374 (MRSA)	8	8	4,000	400
P1369 (MRCNS)	8	8	20,000	400
Penicillin sensitive				
P969 (PSSA)	40	8	4	5
P1111 (PSSE)	20	8	40	5
P1368 (PSSE)	8	8	40	10

[a] All strains were clinical isolates obtained from the 302nd Military Hospital, Beijing, China.

reus AB94004, we found that MRCNS 1538 was resistant to penicillin. However, the MIC of mucroporin for 1538 was fivefold greater than that of mucroporin-M1. This result further demonstrated that the modification of the mucroporin amino acid residues enhanced its in vitro activity.

As shown in Table 2, mucroporin-M1 was as effective against antibiotic-resistant pathogens (MRSA, MRCNS, PRSA, and PRSE) as it was against antibiotic-sensitive pathogens (PSSA and PSSE). The MICs of mucroporin-M1 for penicillin-resistant strains P1383 (PRSA) and P1389 (PRSE) were 10 and 8 μg/ml, respectively, while the MICs of mucroporin-M1 were 40 μg/ml for penicillin-sensitive strain P969 (PSSA) and 20 μg/ml for P1111 and 8 μg/ml for P1368 (PSSE). In addition, the MICs of mucroporin-M1 for methicillin-resistant strains were 20 μg/ml for P1381 (MRSA), 25 μg/ml for P1386 (MRSA), 8 μg/ml for P1374 (MRSA), and 8 μg/ml for P1369 (MRCNS), which was at the same level as for methicillin-sensitive strains. The MICs of penicillin G salt, cefotaxime, and vancomycin against penicillin-sensitive strains, penicillin-resistant strains, and methicillin-resistant strains were all determined to define susceptibility. Furthermore, as shown in Fig. 3B and C, 400 μg of penicillin/ml and 40 μg of cefotaxime/ml cannot effectively inhibit P1386 strain (MRSA) growth during the first 8 h after drug delivery, whereas growth was totally inhibited by a 40-μg/ml dose of vancomycin and mucroporin-M1. Comparatively, an 8-μg/ml dose of each antibiotic and mucroporin-M1 effectively inhibited P969 (PSSA) growth. These results indicated that mucroporin-M1 can effectively inhibit antibiotic-resistant pathogens, including MRSA.

Killing assay. To study the functional mechanisms of mucroporin and mucroporin-M1, we performed a killing assay for mucroporin and mucroporin-M1 on the selected model strain *S. aureus*. One-, two-, or fivefold the MIC of mucroporin or mucroporin-M1 for *S. aureus* was directly added into exponential-phase bacteria, and the survival rate was then determined by counting the viable colony on the plates. The results revealed that the viable colony was decreased dramatically in a very short time, and the reaction started as soon as the bacteria mixed with these peptides. The killing curves (Fig. 4) show that the killing rate corresponded with the increasing peptide concentration. In contrast, the fivefold greater MIC of ampicillin sodium (40 μg/ml) did not make a dramatic decrease in survival rate, a finding which was consistent with its molecular mechanism of inhibiting bacterial wall synthesis. This result suggested that mucroporin and mucroporin-M1 exerted killing effects on microorganisms, which was probably the reason it can effectively inhibit the growth of microorganisms.

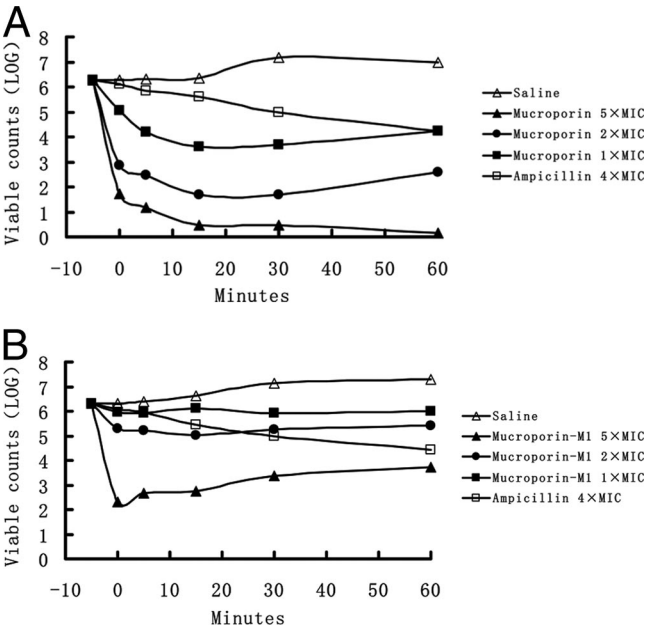

FIG. 4. Killing assay. A killing assay was conducted to determine the count of the surviving bacteria without the supernatant. The set point in the picture refers to the initial bacterial count: 0 min was defined as the time of the first sample collection, which was immediately after mixing bacteria and mucroporin or mucroporin-M1, and the other samples were collected at 5, 15, 30, and 60 min. All of the counts represent the average of three dishes. The experiment was repeated and showed the same trend. (A) Mucroporin at different concentrations; (B) mucroporin-M1 at different concentrations.

Scanning electron microscopy. *S. aureus* cells treated with mucroporin or mucroporin-M1 were observed with a scanning electron microscope to evaluate individual bacterium changes (Fig. 5). As shown in Fig. 5B and C, *S. aureus* cells were found broken, and the cell contents were assembled as small particles, which was direct evidence supporting the idea that the bacterial membrane was broken after treatment with mucroporin or mucroporin-M1.

The results of the in vitro bactericidal activity and scanning electron microscopy provided solid evidence that the bacterial membrane was broken immediately after mucroporin or mu-

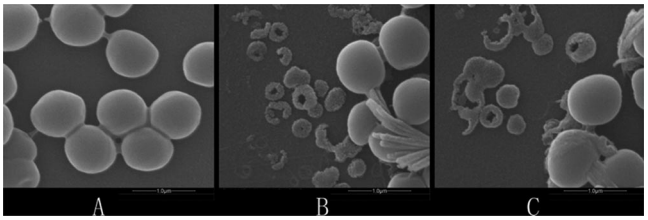

FIG. 5. Scanning electron microscopic images of *S. aureus* treated with mucroporin or mucroporin-M1. (A) Negative control; (B) 10 min after mucroporin treatment; (C) 10 min after mucroporin-M1 treatment.

croporin-M1 treatment and that the inhibitory effect of mucroporin and mucroporin-M1 on bacteria was through the action mode of rapid killing.

DISCUSSION

Toxins are specific poisonous substances developed by venomous animals for predation and defense and are composed of various proteins and/or polypeptides. Recently, the output of data by cDNA library sequencing and proteomics profiling research (28) has revealed large numbers of peptide toxins from animal venoms, including ion channel modulators, bradykinin potentiating peptides, cationic host defense peptides, etc. Some of these have been developed and have shown favorable in vivo activity (33), which suggests that the large number of venom peptides is a rich source of potential therapeutic drugs.

Scorpion toxins have been recognized as potential therapeutic drugs for many years, especially as ion channel modulators. For example, margatoxin, from the scorpion *Centruroides margaritatus*, was the first peptide to be tested in vivo and is a potent blocker of the voltage-gated potassium channels Kv1.1, Kv1.2, and Kv1.3 (5). Margatoxin depolarizes the T cells of both pigs and humans in vitro and also inhibits the delayed-type hypersensitivity reaction to tuberculin in mini-pigs, as determined by both the size of induration and the extent of T-cell infiltration (31). Another toxin, chlorotoxin, derived from the venom of the scorpion *Leiurus quengestriatus*, displays an extraordinary feature that specifically targets glioma cells through MMP-2, the primary receptor highly expressed on the glioma cell membrane. The radioactive ^{131}I-labeled chlorotoxin analogue has cytolytic activity and therefore the potential to selectively affect tumors and gliomas of neuroectodermal origin (35, 45). On the basis of these findings, TransMolecular, Inc., is running trials with ^{131}I-TM-601 (^{131}I-chlorotoxin) as an investigational new drug for the treatment of gliomas.

Thus far, several cationic host defense peptides of scorpions have been isolated and characterized (16–18, 39, 46). In our present study, the first cationic host defense peptide from *L. mucronatus* was characterized. It has been shown that mucroporin exerted an inhibitory effect, especially on gram-positive bacteria strains, including clinically isolated pathogens. However, the activity of mucroporin was not very high; this is why we designed the sequence for mucroporin-M1. The principal design aim was to replace the amino acid residues at the hydrophilic site with positively charged residues. As a result, the antibacterial activity was improved, including the activity against gram-positive bacteria, gram-negative bacteria, and clinically isolated antibiotic-resistant pathogens (MRSA, MRCNS, etc.). This result showed us that mucroporin was an ideal template for anti-infective drug design.

Vancomycin is considered the most effective drug for the treatment of MRSA infection, but vancomycin-resistant *S. aureus* strains have also been identified (2, 3). Thus, the treatment of MRSA infection will be a difficult problem in the near future. Cationic host defense peptides may offer us a solution. The results showed that the in vitro effect of mucroporin-M1 on MRSA and MRCNS were at the same level as vancomycin. Clinical trials of cationic host defense peptides have been ongoing for many years (11, 12, 47), including a phase IIIa trial utilizing these peptides in topical treatment (32, 40). However, none of them has obtained U.S. Food and Drug Administration approval for their various clinical indications thus far (21, 41). Designed peptides with high effectiveness and low toxicity are our future goal.

In conclusion, we showed here that mucroporin and mucroporin-M1, a native cationic host defense peptide and its analogue, have shown specific effects on inhibiting bacteria. These peptides kill *S. aureus* very quickly. Mucroporin-M1 can effectively inhibit hospital-acquired MRSA, MRCNS, PRSA, and PRSE. This antibacterial activity suggests that mucroporin may be a good template for anti-infective drug design.

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