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Cationic Antimicrobial Peptide LL-37 Is Effective against both Extra- and Intracellular *Staphylococcus aureus*

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The increasing resistance of bacteria to conventional antibiotics and the challenges posed by intracellular bacteria, which may be responsible for chronic and recurrent infections, have driven the need for advanced antimicrobial drugs for effective elimination of both extra- and intracellular pathogens. The purpose of this study was to determine the killing efficacy of cationic antimicrobial peptide LL-37 compared to conventional antibiotics against extra- and intracellular *Staphylococcus aureus*. Bacterial killing assays and an infection model of osteoblasts and *S. aureus* were studied to determine the bacterial killing efficacy of LL-37 and conventional antibiotics against extra- and intracellular *S. aureus*. We found that LL-37 was effective in killing extracellular *S. aureus* at nanomolar concentrations, while lactoferricin B was effective at micromolar concentrations and doxycycline and cefazolin at millimolar concentrations. LL-37 was surprisingly more effective in killing the clinical strain than in killing an ATCC strain of *S. aureus*. Moreover, LL-37 was superior to conventional antibiotics in eliminating intracellular *S. aureus*. The kinetic studies further revealed that LL-37 was fast in eliminating both extra- and intracellular *S. aureus*. Therefore, LL-37 was shown to be very potent and prompt in eliminating both extra- and intracellular *S. aureus* and was more effective in killing extra- and intracellular *S. aureus* than commonly used conventional antibiotics. LL-37 could potentially be used to treat chronic and recurrent infections due to its effectiveness in eliminating not only extracellular but also intracellular pathogens.

Conventional antibiotics are becoming increasingly ineffective due to rapidly evolving multidrug-resistant bacterial strains. The heavy use of antibiotics is causing bacteria to mutate and emerge as multidrug-resistant "superbugs" such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *S. aureus*, and vancomycin-intermediate *S. aureus* (1–4). Recent studies reported that MRSA is posing a serious health care issue due to treatment failure, higher mortality rates, and increased health care costs (5–7). MRSA is now killing more people in the United States than AIDS (8). In 2009, the U.S. Centers for Disease Control and Prevention reported that bacterial infections, especially those caused by multidrug-resistant *S. aureus*, are on the rise globally (9). Each year, approximately 19,000 people die in the United States alone due to recalcitrant and recurrent bacterial infections (8). Moreover, treating recurrent bacterial infections (10, 11) has become a daunting challenge due to the possible presence of intracellular bacteria (12–14); historically, a high infection recurrence (~17%) was found in combat-related injuries (15). Therefore, the increasing resistance of bacteria to conventional antibiotics and the challenges posed by intracellular bacteria have driven the need for advanced or alternative antimicrobial drugs.

Cationic antimicrobial peptides (CAMPs) have recently emerged as an alternative to conventional antibiotic therapies (16, 17). They are produced by the innate immune system in both vertebrates and invertebrates as a first line of defense against microbial infections (18–20). They have broad-spectrum killing ability against pathogens (21, 22). In addition to their antibacterial and antifungal properties, CAMPs have also been described recently for their role in neutralization of endotoxins, chemokine-like activities, immunomodulating properties, induction of angiogenesis, and wound repair (23–27). Currently, companies like HelixBiomedix are developing arrays of CAMPs in several pharmaceutical programs ranging from topical anti-infective to wound healing and cystic fibrosis (28), and several CAMPs and their derivatives are being investigated in preclinical and clinical trials (28–33).

Conventional antibiotics are relatively large molecules compared to CAMPs and have different types of mechanisms in killing bacteria. Cefazolin, a beta-lactam and frequently used in orthopedic infection treatment (34), has a very low permeability through cell membranes due to its hydrophilic nature and does not accumulate in the cytoplasm because of its rapid efflux (35). However, it binds to bacterial penicillin-binding proteins, thereby disrupting the synthesis of peptidoglycan, the integral part of the bacterial cell wall (36). Doxycycline (tetracycline) and clindamycin (lincosamide) traverse bacterial membranes using the membrane transport system, but they have to cross the threshold limit to interact with the ribosomes (36). Clindamycin was proven effective against intracellular bacteria (36–38).

The mode of action of CAMPs is different from that of conventional antibiotics and is often more effective in destroying bacteria; they interact with bacteria through electrostatic forces (39, 40). CAMPs, including cathelicidin LL-37, are amphiphilic in nature and are comprised of hydrophobic and hydrophilic residues aligned on opposite sides of the peptides, facilitating their easy penetration through cell membranes (19, 41–44). Their positively charged domain allows CAMPs to bind to bacterial membranes like magnets, and the hydrophobic domain facilitates their pene-

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tration through phospholipid bilayers (45, 46). This mode of action results in bacterial death (47, 48).

Bacteria could develop resistance to conventional antibiotics by altering their antibiotic binding cell membrane receptors through mutations, thereby making the antibiotics ineffective; however, CAMPs target the lipid matrix of cell membranes whose lipid composition is highly unlikely to change due to bacterial mutation (49). Development of resistance against CAMPs by modifying membrane compositions of bacteria would compromise the bacteria's viability (50) and thereby would not likely occur (16, 19, 41, 42). However, CAMPs may suffer proteolytic digestion (43), which could be minimized via a small alteration of the peptide structure to make them not be recognized or degraded by proteolytic enzymes (51).

Cathelicidin LL-37 is a CAMP that has recently attracted great interest (16, 17, 52). The objective of this study was to determine the antimicrobial properties of cathelicidin LL-37 compared to those of conventional antibiotics against extra- and intracellular *S. aureus*. We hypothesized that LL-37 can be effective in eliminating not only extracellular bacteria but also intracellular bacteria.

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MATERIALS AND METHODS

A clinical strain of *S. aureus* obtained from a patient's chronic wound at Ruby Memorial Hospital, Morgantown, WV, and an American Type Culture Collection (ATCC; Manassas, VA) strain (ATCC 25923) of *S. aureus* were investigated in this study. Susceptibility tests showed that the clinical *S. aureus* strain was susceptible to cefazolin, ciprofloxacin, clindamycin, erythromycin, gentamicin, levofloxacin, linezolid, moxifloxacin, oxacillin, rifampin, tetracycline, tigecycline, and vancomycin and was resistant to ampicillin, cefoxitin, and penicillin. The ATCC *S. aureus* strain was susceptible to cefazolin, cefoxitin, ciprofloxacin, clindamycin, erythromycin, gentamicin, levofloxacin, linezolid, moxifloxacin, oxacillin, penicillin, rifampin, tetracycline, tigecycline, and vancomycin and was resistant to ampicillin. *S. aureus* was chosen because it is one of the major pathogens responsible for most bacterial infections, including orthopedic infections (53–57), and is a potential cause of chronic and recurrent infections (15, 58). CAMPs (cathelicidin LL-37 and lactoferricin B) and conventional antibiotics (cefazolin, doxycycline, and clindamycin) were purchased from Sigma-Aldrich (St. Louis, MO). The purity of LL-37 (product no. 94261; molecular weight [MW], 4492) was 98.5% (as determined by high-performance liquid chromatography [HPLC]). Tryptic soy broth was from Becton, Dickinson and Company (Sparks, MD).

Extracellular antimicrobial activities of LL-37 and conventional antibiotics. The killing efficacies of LL-37, lactoferricin B, doxycycline, and cefazolin were determined against extracellular *S. aureus* under the same experimental conditions. Sterile tryptic soy broth, prepared based on the manufacturer's instructions, was used for bacterial cultures. Three colonies of *S. aureus* were inoculated into a sterile tube containing 5 ml of tryptic soy broth and incubated for 16 h at 37°C. The next day, 100 μl of a 16-h-old *S. aureus* culture (stationary phase) was inoculated into a sterile tube containing 20 ml of fresh tryptic soy broth and was subjected to shaking (80 rpm) at 37°C for 2.5 h to acquire log-phase bacteria (exponential bacterial growth) using a reciprocal shaking bath, made by Precision (El Cajon, CA). The log-phase *S. aureus* inocula were diluted to 1.0×10^5 CFU/ml with sterile phosphate-buffered saline (PBS, pH 7.0). The assays were run with a total volume of 1 ml comprising *S. aureus* (1.0×10^5 CFU/ml) and different molar concentrations (ranging from 10 nM to 100 mM) of cathelicidin LL-37, lactoferricin B, doxycycline, and cefazolin, individually. The controls and the treated samples were incubated at 37°C for 30 min in a reciprocal shaking bath. The samples were then diluted and plated on 5% sheep blood agar plates. Dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were made for control and treated samples with sterile PBS. The drop plate method (59, 60) was used for viable bacterial enumeration and was performed as follows. A sheep blood agar plate was divided into six sectors. A 20-μl bacterial suspension was pipetted and placed as a drop in each sector. After the drops dried, the plates were inverted and incubated at 37°C for 24 h. The procedure was repeated for each dilution. CFU were determined using an Acolyte colony counter made by Synbiosis (Frederick, MD). The killing efficacy of each drug was presented in terms of percent killing at different molar concentrations. Percent killing was calculated by dividing the difference between control and treated samples with a control value and then multiplying by 100. Data were averages of four samples.

LL-37 was next tested from 50 nM to 100 μM (0.05, 0.25, 0.5, 1.0, 2.0, 3.0, 10.0, and 100 μM) concentrations for strain (ATCC strain versus clinical strain; log phase was studied) and phase (log phase versus stationary phase; the ATCC strain was used) comparisons. The inocula were diluted to 1.0×10^5 CFU/ml with sterile PBS. The experiments were carried out with a total volume of 1 ml comprising *S. aureus* (1.0×10^5 CFU/ml) and different molar concentrations (0.05, 0.25, 0.5, 1.0, 2.0, 3.0, 10.0, and 100 μM) of LL-37. The controls and the treated samples were incubated at 37°C for 30 min in a reciprocal shaking bath. The samples were then diluted and plated on 5% sheep blood agar plates using the drop plate method; the CFU were determined and the percent killing of LL-37 was calculated.

In addition, kinetic studies were conducted individually for LL-37 (250 nM), lactoferricin B (25.0 μM), and cefazolin (1.0 mM) at given time intervals (5, 10, 15, and 30 min). LL-37 at 250 nM, lactoferricin B at 25.0 μM, and cefazolin at 1.0 mM had approximately the same percent killing from the aforementioned experiments. The kinetic experiments were run with a total volume of 1 ml comprising log-phase bacteria (1.0×10^5 CFU/ml) and LL-37 (250 nM), lactoferricin B (25.0 μM), or cefazolin (1.0 mM) and incubated separately for 5, 10, 15, and 30 min at 37°C in a reciprocal shaking bath. At the predetermined time, the control and treated samples were diluted and plated on 5% sheep blood agar plates using the drop plate method and the CFU were determined. The percent killing data were calculated and normalized by assuming that LL-37 (250 nM), lactoferricin B (25.0 μM), and cefazolin (1.0 mM) had 100% killing at 30 min. Data were averages of four samples.

Intracellular antimicrobial activities of LL-37 and conventional antibiotics. An infection model of osteoblasts and *S. aureus* (61–65) was used to obtain intracellular *S. aureus*; *S. aureus* can internalize into osteoblasts and survive within them (61–65). The clinical strain of *S. aureus* in the log phase was studied, and a 500:1 ratio of *S. aureus* to osteoblasts was used. Experiments were conducted using a 12-well plate in a laminar-flow hood under aseptic conditions. Dulbecco's modified Eagle's medium–F-12 (DMEM–F-12) and PBS were used for osteoblast culture. One milliliter of osteoblasts (UMR-106, passage 2) with a cell density of 4×10^5 cells/ml was seeded in each well and incubated at 37°C with 5% CO_2 for 36 h to form a confluent monolayer. After 36 h, the wells were washed twice with 1 ml of PBS to remove growth medium. One milliliter of log-phase *S. aureus* (2×10^8 CFU/ml) was then added to each well, and the 12-well plate was incubated at 37°C. After culture for 2 h, the wells were washed twice with 1 ml of PBS; 50 μg of lysostaphin was added to each well, and the plate was incubated for 2 h to eliminate extracellular *S. aureus*. Lysostaphin is an antimicrobial agent that does not penetrate eukaryotic cells, and 50 μg/ml of lysostaphin (Sigma-Aldrich) was found to be effective at eradicating any extracellular *S. aureus* organisms (63, 66). The wells were washed twice with 1 ml of PBS. Osteoblasts in three wells were immediately lysed with 0.1% Triton X-100 in PBS for 10 min at 37°C; the cell lysates were diluted in PBS and plated on blood agar plates overnight, and the count of intracellular *S. aureus* was 4×10^4 CFU. Different molar concentrations (10, 30, 50, and 100 μM) of LL-37 or plain DMEM were added to the remaining wells. After incubation at 37°C for 2 h, osteoblasts were rinsed twice with 1 ml of PBS and then lysed with 0.1% Triton X-100,

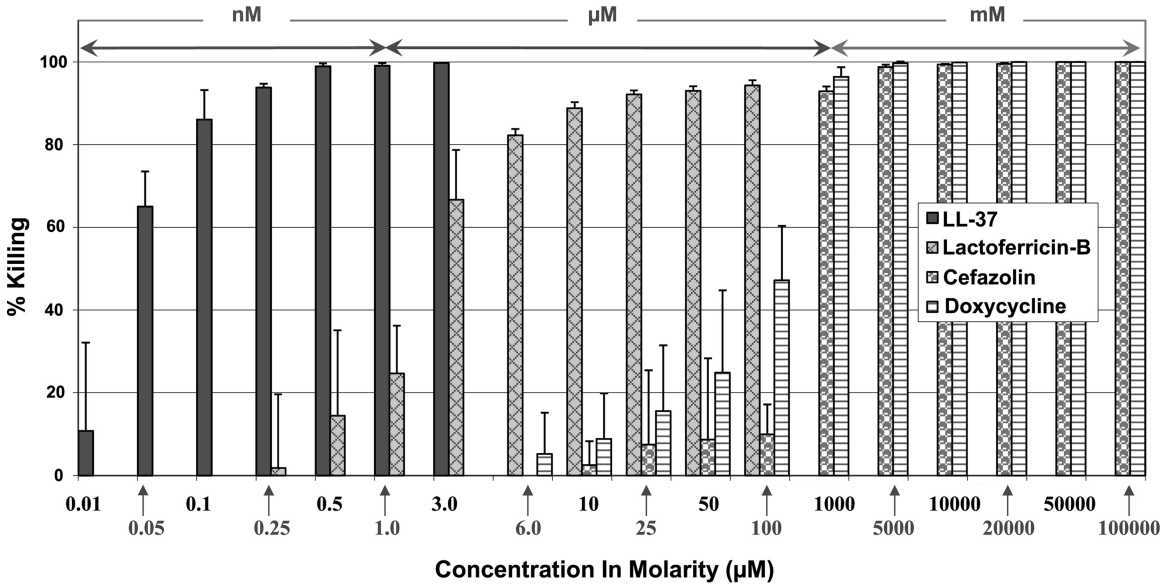

FIG 1 Killing potencies of LL-37, lactoferricin B, and conventional antibiotics (i.e., cefazolin and doxycycline) against extracellular *S. aureus* (clinical strain) in log phase.

and the intracellular *S. aureus* was plated on 5% sheep blood agar plates using the aforementioned drop plate method. Dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were made for control and treated samples with sterile PBS. The colony numbers of viable intracellular *S. aureus* were determined. The same experiments were also carried out with conventional antibiotics, including cefazolin and clindamycin at 100 μM, for comparison; clindamycin was chosen due to its effectiveness against intracellular bacteria (36–38) and cefazolin due to its wide applications in orthopedic infection treatment (34). Data were averages of four samples.

Kinetic studies of LL-37 (100 μM) were also conducted against intracellular *S. aureus* at different time intervals (i.e., 0.5, 2, 12, and 24 h). Log-phase *S. aureus* was internalized within the osteoblasts in a 12-well plate as described above in the osteoblast-*S. aureus* infection model. The extracellular *S. aureus* was eliminated using lysostaphin, and the wells were washed twice with 1 ml of PBS; 100 μM LL-37 was added to each well, and the plate was incubated at 37°C. Controls were run separately for each time point. After 0.5, 2, 12, and 24 h, osteoblasts were rinsed twice with 1 ml of PBS and then lysed with 0.1% Triton X-100; the intracellular *S. aureus* was plated on 5% sheep blood agar plates. Percent killing was calculated; data were averages of four samples.

Statistical analysis. Values of percent killing were expressed as means ± standard deviations. Differences in percent killing of extracellular *S. aureus* between the ATCC and clinical strains and between log phase and stationary phase and differences in percent killing of intracellular *S. aureus* among cefazolin, clindamycin, and LL-37 were analyzed using JMP-V9 statistical visualization software (SAS Institute Inc., Cary, NC). The data were transformed as the arcsin of the square root of percent killing, and a *t* test was run to compare the two groups; in the case where there were three groups, an analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test was used to determine significance. A P value of <0.05 was considered statistically significant.

RESULTS

Extracellular bacterial killing efficacy versus concentration of LL-37, lactoferricin B, and conventional antibiotics. *S. aureus* was treated with two CAMPs (i.e., cathelicidin LL-37 and lactoferricin B), and their killing efficacies were compared with those of cefazolin and doxycycline, two commonly used antibiotics, under the same experimental conditions. Overall, LL-37 was effective in killing *S. aureus* at nanomolar concentrations, while lactoferricin B was effective at micromolar concentrations and doxycycline and cefazolin were effective at millimolar concentrations (Fig. 1). LL-37 was found to exhibit over 90% killing efficacy at as low as 250 nM, over 99% at 500 nM, and 100% at 3.0 μM (Fig. 1). Lactoferricin B had approximately 2% killing potency at 250 nM, 15% at 500 nM, 67% at 3.0 μM, and over 90% at 25 μM. On the other hand, doxycycline and cefazolin were found to have significant killing abilities only at much higher concentrations; they had no killing efficacy at 3.0 μM, more than 90% killing efficacy at 1.0 mM, and 100% killing potency at 10 mM or higher (Fig. 1).

Extracellular bacterial killing efficacy of LL-37 against *S. aureus* strains. LL-37 was tested on both the clinical and ATCC *S. aureus* strains with different molar concentrations, ranging from 0.05 μM to 100 μM for strain comparison. LL-37 exhibited 100% killing against both strains at higher concentrations (10 and 100 μM). However, at concentrations lower than 3 μM, LL-37 was surprisingly more effective in killing the clinical strain than the ATCC strain (Fig. 2). There was a 24% increase in the ability to kill

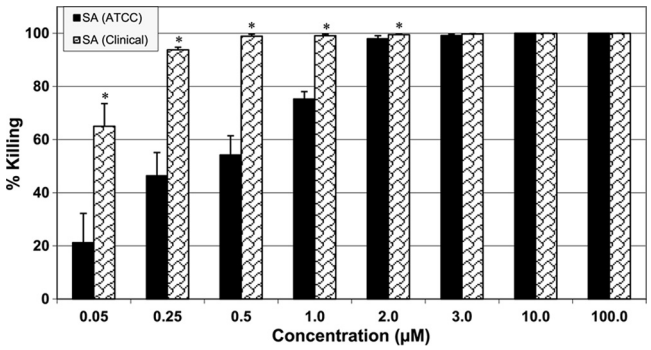

FIG 2 Strain-specific killing efficacy of LL-37 against *S. aureus* (ATCC and clinical strains) in log phase. Incubation time was 30 min. *, $P < 0.05$ compared to ATCC strain at the same concentration.

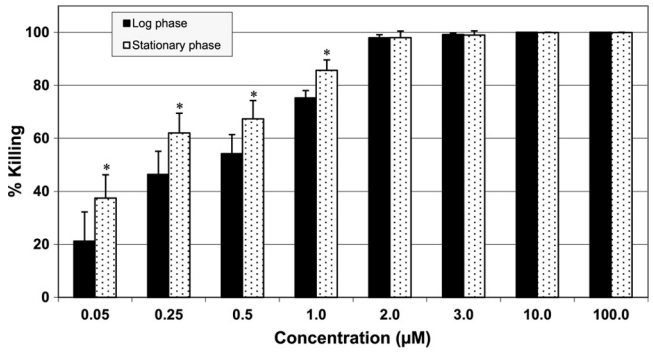

FIG 3 Killing efficacy of LL-37 against *S. aureus* in log and stationary phases. Incubation time was 30 min. *, $P < 0.05$ compared to log phase at the same concentration.

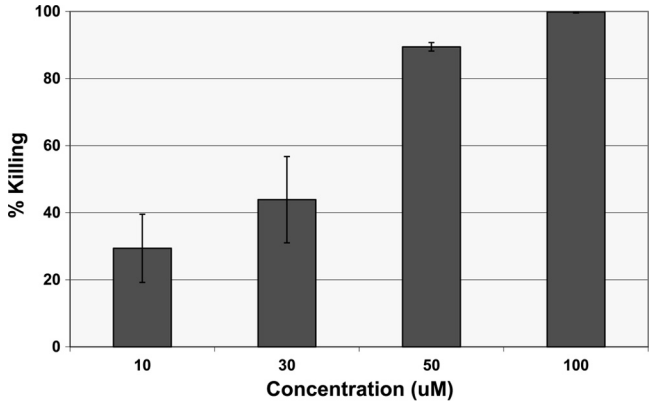

FIG 5 Intracellular killing efficacy of LL-37 against *S. aureus* (clinical strain) within osteoblasts. Incubation time was 2 h.

the clinical strain compared to the ATCC strain at 1.0 μM; the difference was more prominent (over 40%) at lower concentrations, e.g., 0.5, 0.25, and 0.05 μM (Fig. 2).

Extracellular bacterial killing efficacy of LL-37 against *S. aureus* phases. LL-37 seemed to kill significantly more *S. aureus* organisms in the stationary phase than *S. aureus* organisms in the log phase at concentrations at or lower than 1.0 μM; no differences in percent killing were observed at concentrations higher than 2.0 μM (Fig. 3).

Extracellular bacterial killing kinetics of LL-37. The extracellular bacterial killing kinetics of LL-37 were compared with those of lactoferricin B and cefazolin. Incredibly, LL-37 was able to eliminate more than 70% of *S. aureus* organisms within just 5 min and more than 90% within 15 min (Fig. 4). In contrast, lactoferricin B and cefazolin had much slower kinetics and showed almost no bacterial killing within the first 5 min and less than 40% killing within 15 min (Fig. 4).

Intracellular antimicrobial activities of LL-37. The killing potency of LL-37 against intracellular *S. aureus* was determined at different molar concentrations (10, 30, 50, and 100 μM). LL-37 was found to be very effective in eliminating intracellular *S. aureus*. The intracellular bacterial percent killing increased with increasing LL-37 concentration, and 100 μM LL-37 completely killed the intracellular *S. aureus* organisms (Fig. 5). In contrast, at the same concentration (i.e., 100 μM), cefazolin and clindamycin eliminated only 2% and 23% of the intracellular *S. aureus* organisms, respectively (Fig. 6). Kinetic studies further showed that LL-37 killed approximately 50% of the intracellular *S. aureus* organisms within 30 min and all bacteria within 2 h (Fig. 7).

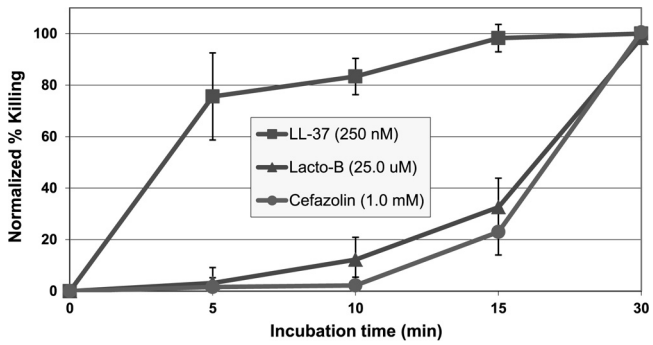

FIG 4 Kinetics of LL-37 killing against extracellular *S. aureus* (clinical strain) in log phase.

DISCUSSION

It is well known that a wide variety of pathogens, including bacteria and viruses, are capable of internalizing into human cells, thereby causing intracellular diseases like human immunodeficiency virus/AIDS (HIV/AIDS), hepatitis, and tuberculosis (TB) (reviewed in reference 67). One of the critical challenges in treating these types of infections is the intracellular nature of the pathogens, which may protect the pathogens from a variety of antibiotic therapies and host immune responses. Antibiotics such as aminoglycosides and beta-lactams have limited cellular penetration, whereas antibiotics like fluoroquinolones or macrolides have poor retention within cells and therefore are inefficient at killing intracellular pathogens (68). Moreover, some bacteria such as *S. aureus*, which has long been considered an extracellular pathogen, have now been found to be able to internalize and survive within host cells, e.g., osteoblasts (13, 64, 69–72), and may contribute to chronic and recurrent infections (54). Therefore, advanced drugs for effectively destroying both extra- and intracellular pathogens are needed in order to reduce or prevent chronic and recurrent infections. In this study, the potential bacterial killing activities of LL-37 against intracellular *S. aureus* were examined and compared with those of conventional antibiotics. The bacterial killing activities of LL-37 against extracellular bacteria were also investigated and compared with those of conventional antibiotics.

Our studies indicated that LL-37 is very potent and fast (Fig. 1 and 4) at eliminating extracellular *S. aureus*, the common culprit of many bacterial infections. Among LL-37, lactoferricin B, doxycycline, and cefazolin, LL-37 was apparently foremost in eliminating extracellular *S. aureus*. LL-37 was remarkably potent in killing more than 90% of *S. aureus* organisms even at 250 nM (Fig. 1). Our experiments showed that a substantially smaller quantity of LL-37 (100 times less than lactoferricin B and 4,000 times less than doxycycline and cefazolin) was needed to eliminate extracellular *S. aureus* (Fig. 1). Moreover, LL-37 was not only potent but also expeditious in eliminating extracellular *S. aureus*. LL-37 was found to be much faster in killing extracellular *S. aureus* than were lactoferricin B and cefazolin (Fig. 4).

LL-37 furthermore exhibited a strain-specific, higher ability to

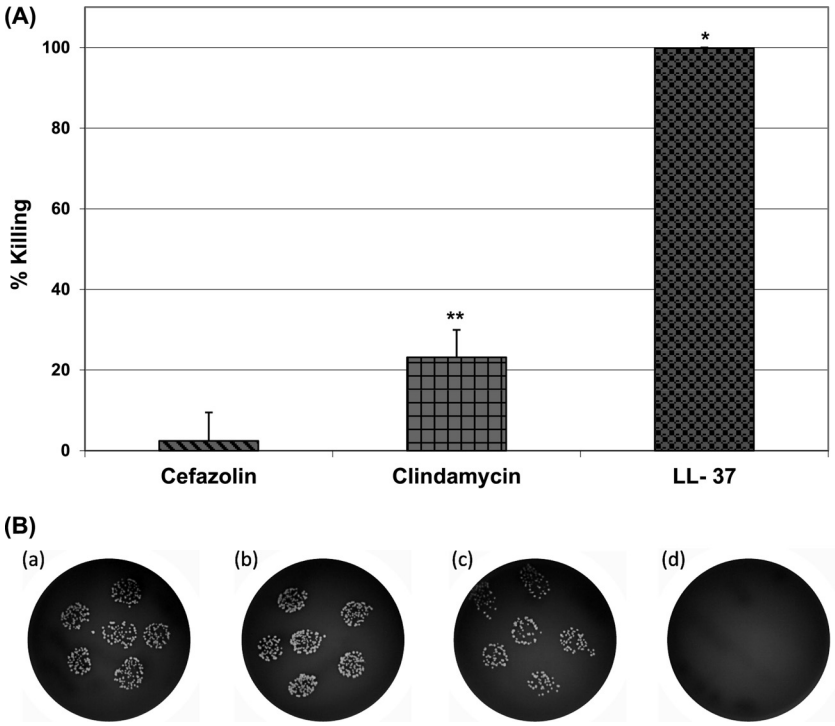

FIG 6 Intracellular killing efficacies of cefazolin, clindamycin, and LL-37 against *S. aureus* (clinical strain) within osteoblasts. The concentration of cefazolin, clindamycin, and LL-37 was 100 μM; incubation time was 2 h. (A) Percent killing; (B) images at 10^{-1} dilution: control (a), cefazolin (b), clindamycin (c), and LL-37 (d). *, $P < 0.05$ compared to cefazolin and clindamycin; **, $P < 0.05$ compared to cefazolin.

kill the clinical strain than the ATCC strain at concentrations lower than 3.0 μM (Fig. 2). These findings indicated that the *S. aureus* clinical strain was surprisingly more susceptible to LL-37 than the ATCC strain; the reason is unknown. In our previous *in vivo* studies, we found that the *S. aureus* clinical strain was much more virulent in inducing infections than the ATCC strain (73). LL-37 also presented a phase-specific response (Fig. 3) at concentrations lower than 1.0 μM, with a higher ability to kill bacteria in the stationary phase than in the log phase. This may suggest that it is relatively easier to eliminate stationary-phase bacteria than log-phase bacteria.

More interestingly, we found that LL-37 was very effective in eliminating intracellular pathogens. LL-37 had remarkable intracellular killing ability against *S. aureus* compared to conventional antibiotics like cefazolin and clindamycin; clindamycin was reported to have potent antimicrobial properties against intracellular *S. aureus* due to its good penetration, retention, and distribution properties in eukaryotic cells (36, 37). Our results indicated that a 100 μM concentration of LL-37 completely eliminated intracellular *S. aureus* within just 2 h, whereas cefazolin and clindamycin eliminated only 2% and 23%, respectively (Fig. 6). However, due to the intracellular nature of the pathogen, a much higher (100 μM versus 3 μM) concentration of LL-37 was needed (Fig. 1 and 5), and relatively slower kinetics were observed (Fig. 4 and 7) in killing intracellular *S. aureus* than for extracellular *S. aureus*. Note that 10 mM concentrations of cefazolin and doxycycline were needed to completely eliminate extracellular *S. aureus* alone (Fig. 1).

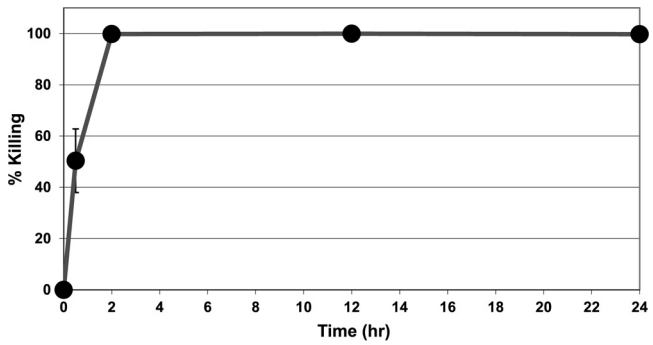

FIG 7 Kinetics of LL-37 killing against intracellular *S. aureus* (clinical strain) within osteoblasts. The concentration of LL-37 was 100 μM.

The current study therefore demonstrated that LL-37 is very potent and fast at eliminating both extra- and intracellular *S. aureus* compared to conventional antibiotics. Moreover, LL-37 may exhibit synergistic antibacterial activities with β-defensin and lysozyme in both neutral and acidic environments (74). However, the antibacterial properties of LL-37 may be reduced by serum proteins. It was reported that certain biological fluids containing glycosaminoglycans and serum may hamper the antibacterial properties of LL-37 (75). Serum proteins such as apolipoproteins could bind to LL-37 and reduce its antimicrobial efficacy (75–77). Interestingly, the removal of N-terminal hydrophobic amino acids from LL-37 may reduce the effect of serum without compromising its antimicrobial properties (78).

One limitation of this study is that the potential toxicity of LL-37 was not examined. It was reported that LL-37 could prevent sepsis in neonatal rats (79), and a low dose (100 μg/kg of body weight) of LL-37 did not induce observable toxicity, but a high

dose (3,000 μg/kg) resulted in adverse effects and appeared to be toxic to organs affected by sepsis (79). It is noteworthy that studies on human cathelicidin analogs reveal that removal of hydrophobic amino acids from the N-terminal end of native LL-37 could decrease its cytotoxicity without compromising the peptide's antimicrobial efficacy toward both Gram-positive and Gram-negative bacteria (78). Wang et al. (80) recently mapped and unmasked the potential roles of cationic residues of human cathelicidin LL-37 against different bacterial strains. The cationic side chains of the major antimicrobial region of human cathelicidin LL-37 were fragmented, and their functional roles were studied in detail. The GF-17 fragment, comprising residues 17 to 32, was found to be more potent against methicillin-resistant *S. aureus in vitro* than was intact LL-37. It also indicated that the conversion of amino acids from lysines (K) to arginines (R) increased the ability of the peptide to kill *S. aureus*. Therefore, the use of the GF-17 fragment of LL-37 may lead to lower dosages and therefore reduced toxicity (80).

In summary, *S. aureus* and *S. aureus* internalized within osteoblasts were treated with LL-37 and conventional antibiotics. LL-37 was found to have rapid and robust killing efficacy against both extra- and intracellular *S. aureus*, one of the most common causes of bacterial infections. In eliminating extracellular *S. aureus*, LL-37 is 100 times more potent than lactoferricin B and 4,000 times more potent than conventional antibiotics such as doxycycline and cefazolin. LL-37 also eliminates the majority (more than 70%) of *S. aureus* organisms within just 5 min, compared to almost no killing by lactoferricin B and cefazolin at the same time point. The efficacy of LL-37 was found to be bacterial strain and phase specific. Surprisingly, LL-37 was more effective at killing the clinical strain than the ATCC strain of *S. aureus*. In eliminating intracellular *S. aureus*, 100 μM LL-37 killed approximately 50% of intracellular *S. aureus* organisms within the first 30 min and completely eradicated the bacteria within 2 h. However, at the same concentration, cefazolin and clindamycin only eliminated 2% and 23% of the intracellular *S. aureus* organisms, respectively, within 2 h. Therefore, we conclude that LL-37 has rapid and remarkable killing abilities toward both extra- and intracellular *S. aureus* compared to conventional antibiotics. In future studies, we will examine the *in vivo* antimicrobial activities of LL-37 in our animal model (81–83) and may evaluate *in vitro* whether LL-37 will induce resistance.

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We declare that we have no conflicts of interest.

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