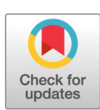

Effects of Phage Endolysin SAL200 Combined with Antibiotics on *Staphylococcus aureus* Infection

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ABSTRACT Phages and their derivatives are increasingly being reconsidered for use in the treatment of bacterial infections due to the rising rates of antibiotic resistance. We assessed the antistaphylococcal effect of the endolysin SAL200 in combination with standard-of-care (SOC) antibiotics. The activity of SAL200 when it was combined with SOC antibiotics was assessed *in vitro* by checkerboard and time-kill assays and *in vivo* with murine bacteremia and *Galleria mellonella* infection models. SAL200 reduced the SOC antibiotic MICs and showed a $\geq$3-$\log_{10}$-CFU/ml reduction of *Staphylococcus aureus* counts within 30 min in time-kill assays. Combinations of SAL200 and SOC antibiotics achieved a sustained decrease of $>$2 $\log_{10}$ CFU/ml. SAL200 significantly lowered the blood bacterial density within 1 h by $>$1 $\log_{10}$ CFU/ml in bacteremic mice ($P < 0.05$ versus untreated mice), and SAL200 and SOC antibiotic combinations achieved the lowest levels of bacteremia. The bacterial density in splenic tissue at 72 h postinfection was the lowest in mice treated with SAL200 and SOC antibiotic combinations. SAL200 combined with SOC antibiotics also improved *Galleria mellonella* larva survival at 96 h postinfection. The combination of the phage endolysin SAL200 with SOC antistaphylococcal antibiotics showed synergistic effects *in vitro* and *in vivo*. The combination of SAL200 with SOC antibiotics could help in the treatment of difficult-to-treat *S. aureus* infections.

KEYWORDS *Staphylococcus aureus*, phage, endolysin, synergism

Staphylococcus aureus is a virulent opportunistic pathogen that commonly causes invasive infections, including bacteremia (1). *S. aureus* bacteremia is frequently complicated by metastatic infections that can occur in various organ systems and that can lead to prolonged antibiotic therapy and a poor prognosis due to serious sequelae (2). Methicillin-resistant *S. aureus* (MRSA) has spread since its initial description in 1961 and remains a major worldwide health problem (3). The rate of mortality from *S. aureus* bacteremia reaches 20 to 30%, while the rate of mortality from MRSA bacteremia is even higher, with clinical cure rates estimated to be 50 to 60% (4–6). Vancomycin remains the first-line agent for the treatment of serious MRSA infections (2, 7). However, global surveillance studies have indicated a gradual rise in the vancomycin MIC, and there is evidence that the increased numbers of treatment failures are related to an elevated vancomycin MIC, raising concerns about its clinical efficacy and prompting the need for alternative therapeutic options (7–9).

Therapy using phages and their derivatives for treating bacterial infections has been reconsidered due to the increasing rates of resistance to standard-of-care (SOC) anti-

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TABLE 1 MICs of SAL200 and SOC antibiotics against *Staphylococcus aureus* isolates

Strain[a]	Median (range) MIC (μg/ml)		
	SAL200	Nafcillin	Vancomycin
ATCC 33591 (MRSA)	1.6 (0.8–1.6)	>64 (>64)	1.5 (1.0–2.0)
ATCC B1707 (MRSA)	1.2 (0.8–1.6)	32 (32)	1.5 (1.0–2.0)
LAC (MRSA)	1.6 (1.6)	32 (32–64)	1.0 (1.0)
Newman (MSSA)	0.8 (0.8–1.6)	0.5 (0.5)	1.0 (1.0–2.0)
ATCC 29213 (MSSA)	1.2 (0.8–1.6)	0.5 (0.5)	1.0 (1.0)

[a]ATCC, American Type Culture Collection; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.

biotics (10). Phage endolysins (lysins) are bacteriophage-encoded peptidoglycan hydrolases that lyse Gram-positive bacterial cell walls from within the bacterial cells in order to release progeny phages after replication in the bacteriophage life cycle (11, 12). In previous studies, exogenously applied endolysins have shown similar rapid lysis of Gram-positive bacteria *in vitro* and also in various animal models of infection (11, 13–18). Endolysins differ from SOC antibiotics, in that they are generally genus/species specific and therefore are likely to cause minimal insult to the normal flora. Endolysins act on contact and thus are able to rapidly lyse bacteria, regardless of their growth phase or antibiotic resistance profile (11, 13, 14).

SAL200 is a new phage endolysin-based candidate antistaphylococcal drug formulated for injection (19, 20). Its active pharmaceutical ingredient is the recombinant phage endolysin SAL-1, derived from the staphylococcus-specific bacteriophage SAP-1 (19, 20). In previous *in vitro* and *in vivo* studies, SAL200 has shown rapid and effective bactericidal activity against various *S. aureus* strains, including MRSA strains (19, 20).

In this study, we show that SAL200 acts synergistically when combined with SOC antibiotics *in vitro*. We also show that SAL200 enhances the antistaphylococcal activities of SOC antibiotics in a murine bacteremia model as well as an invertebrate model using larvae of *Galleria mellonella*.

(This study was presented in part as a mini-oral presentation at the 27th Annual Meeting of the European Congress of Clinical Microbiology and Infectious Diseases, 2017, Vienna, Austria.)

RESULTS

SAL200 is rapidly bactericidal and synergizes with SOC antibiotics *in vitro*. The MICs of the tested *S. aureus* isolates are summarized in Table 1. SAL200 MICs were between 0.8 and 1.6 μg/ml, regardless of methicillin resistance. MICs for the combination of SAL200 with SOC antibiotics are summarized in Tables 2 and 3. No SAL200 and SOC antibiotic combinations resulted in antagonism. Synergy was seen for the combination of SAL200 and nafcillin in the *S. aureus* strains ATCC B1707, LAC, and Newman. Synergy was also seen for the combination of SAL200 and vancomycin in the *S. aureus* strains ATCC B1707, Newman, and ATCC 29213.

A bactericidal effect was seen with exposure to SAL200 within 30 min in all *S. aureus* strains, while minimal bactericidal activity was observed for SOC antibiotics alone (Fig. 1; see also Fig. S2 in the supplemental material). Although SAL200 was rapidly bactericidal alone even at sub-MIC doses, the effect did not last long and bacterial regrowth was observed in

TABLE 2 MICs for combination of SAL200 and nafcillin

Strain[a]	MIC (μg/ml)			Lowest FIC index	Interpretation
	SAL200	Nafcillin	SAL200-nafcillin		
ATCC 33591	0.781	256	0.391/4	0.516	Indifferent
ATCC B1707	0.781	16	0.195/2	0.375	Synergistic
LAC	0.781	16	0.195/4	0.5	Synergistic
Newman	1.563	0.5	0.391/0.125	0.5	Synergistic
ATCC 29213	0.781	0.5	0.391/0.016	0.533	Indifferent

[a]ATCC, American Type Culture Collection.

TABLE 3 MICs for combination of SAL200 and vancomycin

Strain[a]	MIC (μg/ml)			Lowest FIC index	Interpretation
	SAL200	Vancomycin	SAL200-vancomycin		
ATCC 33591	1.563	2	0.781/0.031	0.515	Indifferent
ATCC B1707	1.563	2	0.391/0.5	0.5	Synergistic
LAC	1.563	1	0.781/0.063	0.563	Indifferent
Newman	1.563	2	0.391/0.5	0.5	Synergistic
ATCC 29213	0.781	2	0.195/0.25	0.375	Synergistic

[a]ATCC, American Type Culture Collection.

all *S. aureus* isolates tested. However, SAL200 synergistically inhibited bacterial growth when combined with sub-MIC doses of SOC antibiotics, leading to a sustained reduction of the viable bacterial count of ≥3 $\log_{10}$ CFU/ml.

SAL200 with SOC antibiotics synergistically reduces bacteria in the bloodstream and in metastatic foci in mice. Mice with intraperitoneally induced *S. aureus* bacteremia received either SAL200 alone, SOC antibiotics alone, SAL200 and SOC antibiotics in combination, or phosphate-buffered saline (PBS) as a negative control. Mice treated with SAL200 alone showed a significantly lower bacterial density in blood at 1 h after treatment compared to the negative controls (Fig. 2a and b; 2.202 versus 3.385 $\log_{10}$ CFU/ml [$P = 0.009$], respectively, for LAC-infected mice and 2.284 versus 3.437 $\log_{10}$ CFU/ml [$P = 0.025$], respectively, for Newman-infected mice), and mice receiving the combination of SAL200 and SOC antibiotics showed the lowest median blood bacterial density at 1 h after treatment (2.125 and 1.635 $\log_{10}$ CFU/ml for LAC- and Newman-infected mice, respectively).

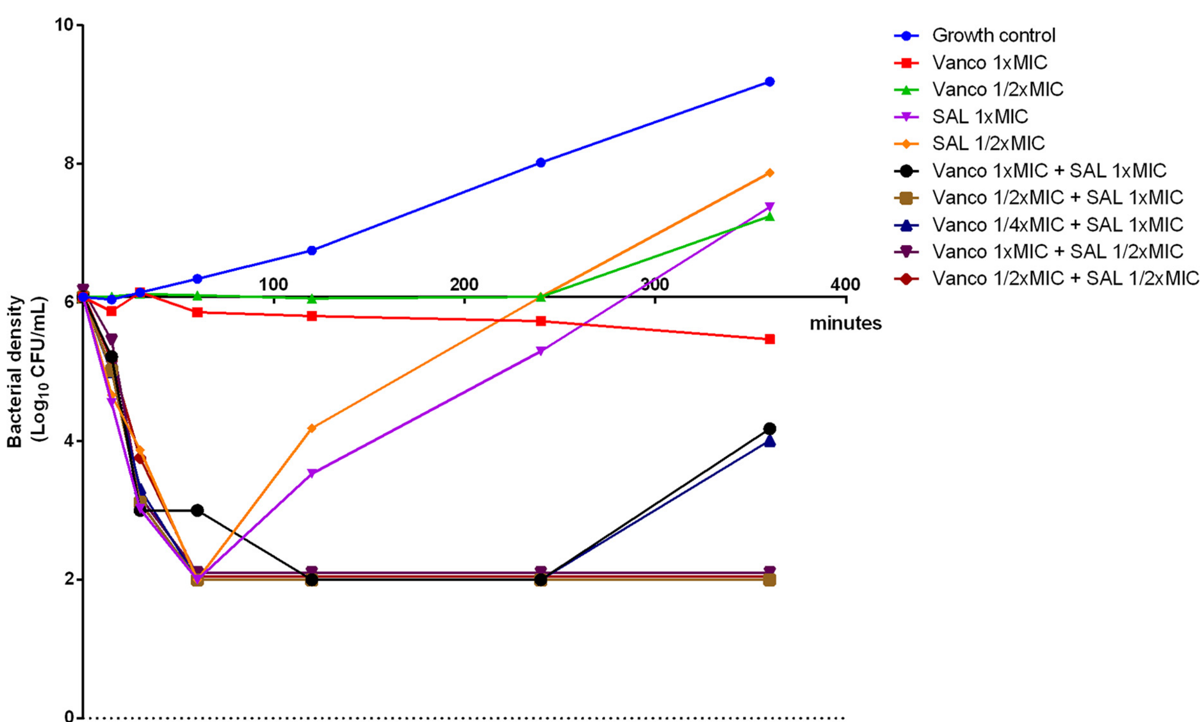
The extent of metastatic infections, as assessed by the splenic bacterial density at 72 h after infection, was similar to the acute posttreatment bacterial densities observed in blood; the median number of $\log_{10}$ CFU per gram in the combination treatment group was significantly lower than that in the group treated with the SOC alone (Fig. 2c and d; 3.771 versus 4.794 $\log_{10}$ CFU/g [$P = 0.008$], respectively, for LAC-infected mice and 4.087 versus 5.416 $\log_{10}$ CFU/g [$P = 0.015$], respectively, for Newman-infected mice) and was the lowest among all active treatment groups.

Treatment with SAL200 with SOC antibiotics improves the survival of *Galleria mellonella* larvae. Treatment with SOC antibiotics or the combination of SAL200 and SOC antibiotics showed significantly improved the survival rates for infected larvae at 96 h postinfection compared to those for the negative controls (6.7% [$P = 0.0002$] and 33.3% [$P < 0.0001$] survival, respectively, for LAC-infected larvae and 46.7% [$P < 0.0001$] and 73.3% [$P < 0.0001$] survival, respectively, for Newman-infected larvae) (Fig. 3).

DISCUSSION

Our findings show that the endolysin SAL200 enhances the antibacterial activity of SOC antibiotics against *S. aureus* both *in vitro* and *in vivo*. Regardless of their antibacterial resistance profile, well-characterized *S. aureus* strains have consistently shown low SAL200 MICs, which are even lower when SAL200 is combined with SOC antibiotics in a checkerboard assay. The rapid bactericidal effect of SAL200 was demonstrated by a time-kill assay, which further showed the synergistic activity of SAL200 when combined with SOC antibiotics. This synergistic activity was also demonstrated *in vivo* with both a murine bacteremia model and a *Galleria mellonella* larva infection model.

All combinations of SAL200 with SOC antibiotics led to a consistent decrease in the MICs of SOC antibiotics in all *S. aureus* strains tested in the checkerboard assays, showing that the presence of SAL200 increases the susceptibility of *S. aureus* strains to SOC antibiotics. Vancomycin MICs decreased by 4 to 64 times in individual strains, and nafcillin MICs decreased to 2 to 4 μg/ml in MRSA strains, suggesting that SAL200 can facilitate antibiotic therapy in nonsusceptible *S. aureus* strains with SOC antibiotic MICs close to the breakpoint for resistance. Although some *S. aureus* strains failed to show definite synergism (defined when the lowest fractional inhibitory concentration index



(b) MSSA Newman

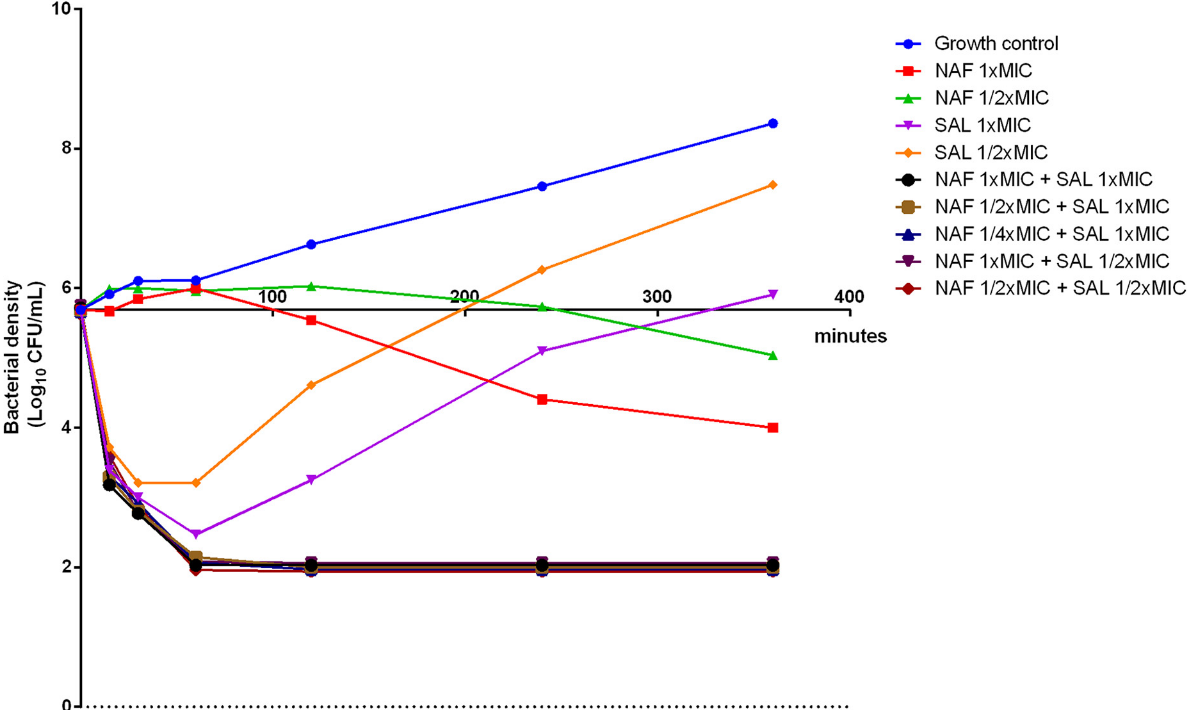


FIG 1 Time-kill curves for MRSA LAC (a) and MSSA Newman (b) treated with buffer and MICs and sub-MICs of either SAL200, SOC antibiotics, or combinations of SAL200 and SOC antibiotics. The curves were jittered vertically by adding random numbers within the range of −0.1 to 0.1 unit to the bacterial densities at times of 60, 120, 240, and 360 min after culture. Vanco, vancomycin; NAF, nafcillin; SAL, SAL200.

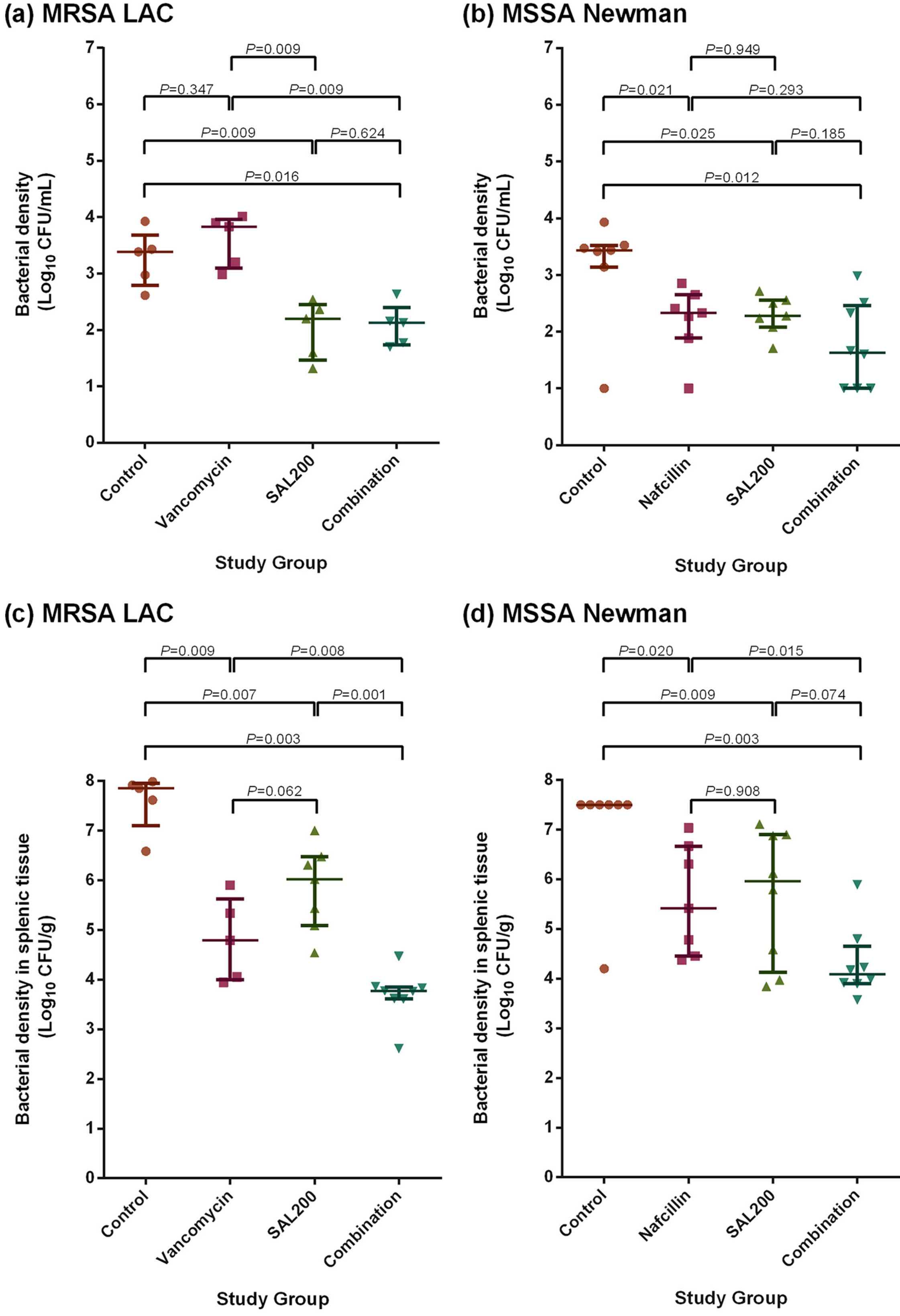
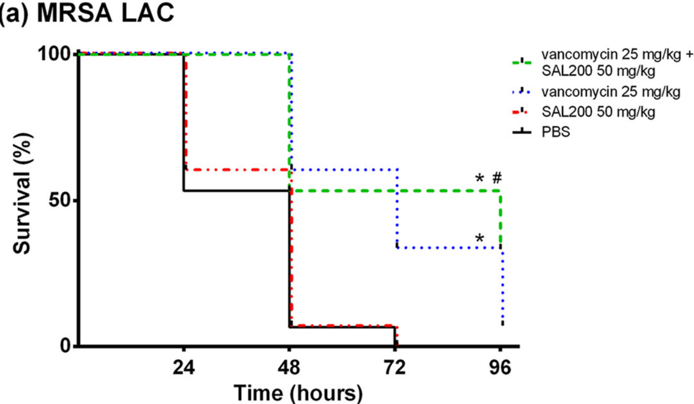


FIG 2 Bacterial density in blood from MRSA LAC-infected mice at 1 h after treatment (a), blood from MSSA Newman-infected mice at 1 h after treatment (b), the spleen at 72 h after MRSA LAC infection (c), and the spleen at 72 h after MSSA Newman infection (d). As only 1 mouse in the negative-control group survived beyond 72 h after MSSA Newman infection, a direct comparison with the control group was not possible. For comparison, a hypothetical splenic bacterial density was presumed to exceed 7.5 log_{10} CFU/g in the dead mice.



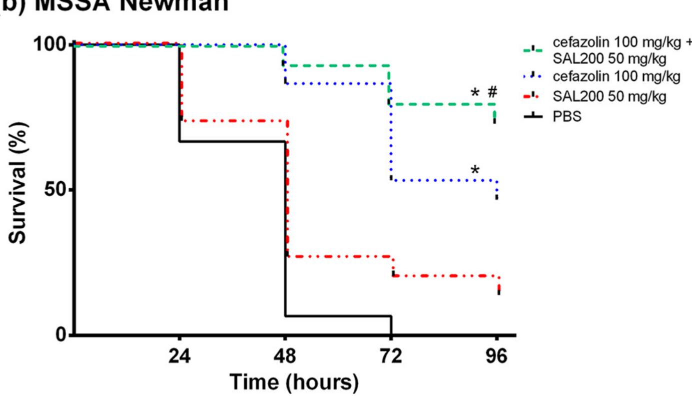


FIG 3 Effect of SAL200 combined with SOC antibiotics on survival of *Galleria mellonella* larvae infected with MRSA LAC (a) and MSSA Newman (b). The survival lines were jittered to prevent overplotting. *, $P < 0.01$ for the tested antibiotic compared with the PBS-treated control; #, *P* values for the combination compared to SOC antibiotics ($P = 0.153$ for vancomycin-SAL200 versus vancomycin and $P = 0.140$ for cefazolin-SAL200 versus cefazolin).

[∑FIC] was ≤0.5), an ∑FIC of <1 was noted in all strains tested. Therefore, we believe that the addition of SAL200 to SOC antibiotics can potentiate SOC antibiotic activity.

SAL200 was rapidly bactericidal in time-kill assays, in which a ≥3-$\log_{10}$ reduction in the number of bacterial CFU per milliliter was observed within 30 min even when it was used at sub-MIC doses, in contrast to the gradual growth inhibition by SOC antibiotics, which took hours to achieve a 1-$\log_{10}$ reduction. The rapid bactericidal effect of SAL200 appeared to be dose dependent, as bacterial regrowth occurred faster with lower doses of SAL200 in most strains. A higher bacterial inoculum or a lower endolysin dose is likely to result in an incomplete bactericidal effect and leave a higher residual bacterial burden, thereby resulting in regrowth following the initial rapid decrease in the number of viable bacteria. The regrown *S. aureus* strains were still susceptible to SAL200 with unchanged MIC values (data not shown). Effective growth inhibition was observed with the combination of SAL200 and SOC antibiotics, in which the reduction in bacterial growth was sustained in most strains even with sub-MIC doses.

The rapid bactericidal effect of SAL200 was also observed *in vivo*. Within 1 h of treatment, LAC- and Newman-infected mice treated with SAL200 or the combination of SAL200 plus SOC antibiotics showed a significantly lower level of bacteremia than untreated mice. LAC-infected mice treated with SAL200 showed a significantly lower bacteremia level than mice treated with vancomycin, a possible consequence of the different killing rates of SAL200 and vancomycin. Mice treated with the combination of SAL200 and SOC antibiotics had the lowest posttreatment bacterial levels of the groups tested, suggestive of synergy between SAL200 and SOC antibiotics. We note that the synergistic interaction between SAL200 and SOC antibiotics persisted over time and

effectively decreased metastatic infections. Specifically, at 72 h after infection, mice receiving the combination of SAL200 plus SOC antibiotics showed a significantly lower splenic bacterial density than mice treated with SOC antibiotics alone.

Although all of the described experiments suggested synergism between SAL200 and SOC antibiotics, the effect size differed among assays. The rapid action time and short half-life described in previous studies of SAL200 (19–21) are likely to be responsible for the different results seen in our assays. The checkerboard assays and the overall survival of *G. mellonella* larvae were assessed at 18 h and 96 h postinfection, respectively. Therefore, rapid bacteriolytic effects are possibly masked by the regrowth of residual bacteria over time, minimizing the overall effect of SAL200. However, in time-kill assays, the killing activity of each drug and combination is quantitated in real time, reflecting the killing rates and extent during the measurement time. Similarly, the bacterial density in blood was measured 1 h after treatment in *S. aureus*-infected mice, reflecting the early bacteriolytic effects of SAL200.

These results are consistent with those of previous studies reporting synergistic interactions between other endolysins and antibiotics (22–25), but a definite mechanism of synergism is still not well defined (11). Daniel et al. used a checkerboard assay and a murine MRSA septicemia model to show that the chimeric endolysin ClyS interacted synergistically with oxacillin (22). They hypothesized that oxacillin increases internal peptidoglycan hydrolases and autolysins by inhibiting cell wall assembly enzymes and that the addition of an endolysin further shifts the balance of the cell wall toward increased degradation, causing bacteriolysis (22). Schuch et al. demonstrated synergism between the endolysin CF-301 and SOC antibiotics (vancomycin and daptomycin), and the authors hypothesized that endolysins increase cell wall permeability by cleaving a sufficient number of peptidoglycan bonds, which further enhances antibiotic penetration (25). Our results may indicate that the initial reduction of the inoculum by SAL200 could have further enhanced the subsequent antibacterial effect of vancomycin and nafcillin. As suggested in other studies, mechanisms beyond an inoculum effect are also likely to take part in and increase antibiotic potency.

Given their distinctive mechanisms of action, endolysins are an attractive new therapeutic option to overcome antibacterial resistance (11, 14). However, their mechanism of bacteriolysis also raises several concerns. Intracellular bacterial materials, toxins, and peptidoglycans are likely to be secreted into the host environment, elevating cytokines and causing inflammatory reactions. Entenza et al. showed that rats receiving higher doses of endolysins induced a higher level of circulating cytokines as a result of rapid bacterial lysis in a rat endocarditis model (26). Although our mouse study was not designed to assess cytokine release after endolysin treatment, we did observe that mice treated with SAL200 appeared to be more ill immediately after treatment than their negative controls treated with buffer alone. This could be a result of increased cytokine release induced by the rapid and extensive bacteriolysis by SAL200, but further studies are needed.

Although some differences in effect size were noted among the strains tested, SAL200 demonstrated several properties related to overcoming antibacterial resistance and therapeutic failure, suggesting its usefulness as a therapeutic option for difficult-to-treat *S. aureus* infections. SAL200 is rapidly bactericidal, is effective against *S. aureus* strains regardless of antibacterial resistance, shows low levels of resistance, interacts synergistically with and potentiates the activity of SOC antibiotics, and effectively reduces metastatic infections. In previous studies, SAL200 rapidly and effectively lysed planktonic *S. aureus* and proved effective against biofilms (20).

This study has some limitations. First, SAL200 was given only in single doses in the *in vivo* studies. As SAL200 is a protein of microbial origin, there is a possibility that it might be immunogenic in humans, inducing antibody formation, which would limit its use. Further studies regarding its immunogenicity are necessary, and a phase I clinical trial to evaluate multiple dosing of SAL200 is currently in preparation. Second, we used mice and *G. mellonella* larvae to assess SAL200's *in vivo* antibacterial activity; therefore, its effectiveness against human infections has not been assessed. As indicated by

Pincus et al., it is possible that the observed bactericidal effect may not be reproduced in human blood (27), and lysin activity might be influenced by host immunity and the infection site. As the human body and immune system are very complex and different from those in *in vivo* animal models, it is necessary to investigate SAL200's effect against human infections in a clinical trial. A phase II clinical trial to assess the efficacy and safety of SAL200 in *S. aureus* bacteremia is under way (ClinicalTrials.gov identifier NCT03089697). Finally, the proinflammatory potential of SAL200 was not assessed, and further studies regarding SAL200-induced cytokine production are required.

In conclusion, the combination of the phage endolysin SAL200 with SOC antistaphylococcal antibiotics showed synergistic effects *in vitro* and *in vivo*. The combination produced an enhanced reduction of bacteremia as well as metastatic foci, suggesting that SAL200 could be useful as an adjunctive therapy in the treatment of difficult-to-treat *S. aureus* infections, including persistent bacteremia or metastatic *S. aureus* infection.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and antibiotic preparation. Five *S. aureus* strains, two methicillin-susceptible strains (Newman and ATCC 29213) and three methicillin-resistant strains (LAC, ATCC 33591, and ATCC B1707), were used in this study. The bacterial strains were cultured in cation-adjusted Mueller-Hinton broth (CAMHB; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) supplemented with NaCl (Sigma-Aldrich, Saint Louis, MO, USA). All strains were stored at −70°C in cryobeads, and fresh cultures were used for each experiment.

The standard-of-care antistaphylococcal antibiotics were nafcillin or cefazolin for methicillin-susceptible *S. aureus* (MSSA) strains and vancomycin for MRSA strains. The SAL200 formulation contained 18 mg/ml SAL-1, 1.56 g/liter L-histidine (pH 6.0), 50 g/liter D-sorbitol, 1.47 g/liter $CaCl_2 \cdot 2H_2O$, and 1 g/liter poloxamer 188, as previously described (19), and was provided by iNtRON Biotechnology, Inc. (Seongnam, Republic of Korea).

***In vitro* antibacterial combination assays.** MIC values for SAL200 and the SOC antibiotics were independently determined three times, using the broth microdilution method with an inoculum dose of 5×10^5 CFU/ml, according to Clinical and Laboratory Standards Institute (CLSI) guidelines (28).

Checkerboard assays were performed to test for synergism between SOC antibiotics and SAL200 using the broth microdilution method with a final inoculum dose of 5×10^5 CFU/ml. The final concentration ranges were 0.016 to 16 mg/liter nafcillin (for MSSA) or 1 to 512 mg/liter nafcillin (for MRSA), 0.031 to 32 mg/liter vancomycin, and 0.098 to 6.25 mg/liter SAL200. After 18 h of incubation at 37°C in air, we determined the MICs for each antibiotic alone and in combination and calculated the fractional inhibitory concentration (FIC) as the MIC of drug A in combination with drug B divided by the MIC of drug A alone. The $\sum$FIC was calculated by adding the FIC values for each drug in the combination, and the lowest $\sum$FIC for each combination was used to interpret the interaction between the two drugs: an $\sum$FIC of $\leq$0.5 was defined as synergistic, an $\sum$FIC of $>$0.5 but $\leq$4 was defined as indifferent, and an $\sum$FIC of $>$4 was defined as antagonistic (29, 30).

Time-kill assays were performed by the macrodilution method according to CLSI guidelines (31) in CAMHB inoculated with each isolate to a final concentration of 5×10^5 CFU/ml. The isolates were tested with nafcillin (MSSA) or vancomycin (MRSA) and SAL200 alone and in combination at concentrations of 1/4×, 1/2×, 1×, and 2× MIC. The tubes were cultured at 37°C with continuous shaking at 200 rpm, and subcultures for inoculum quantitation were taken at 0, 15, 30, 60, 120, 240, and 360 min after culture. Time-kill curves were plotted as time against the logarithm of the inoculum (in number of CFU per milliliter). Bactericidal activity was defined as a $\geq$3-$\log_{10}$-CFU/ml reduction of viable bacteria. Synergism was defined as a $\geq$2-$\log_{10}$-CFU/ml reduction when the bacteria were treated with the combination compared to that when they were treated with the most active constituent alone at 360 min, and antagonism was defined as an $\geq$2-$\log_{10}$-CFU/ml increase (31, 32). Each assay was performed in duplicate.

Murine bacteremia model. Specific-pathogen-free female BALB/c mice that were 5 to 6 weeks of age and that weighed 16 to 19 g (Orient Bio, Seongnam, Republic of Korea) were used. Bacteremia was induced by intraperitoneal injection of 500 μl of an exponential-phase bacterial inoculum with a concentration of 2×10^9 CFU/ml. The mice were divided into four treatment groups, as follows (5 to 8 mice per group): (i) an inactive control group (which received 200 μl intravenous phosphate-buffered saline), (ii) an SOC antibiotic treatment group (which received either 25 mg/kg of body weight subcutaneous vancomycin or 200 mg/kg intramuscular nafcillin), (iii) an SAL200 treatment group (which received 25 mg/kg intravenous SAL200 plus 25 mg/kg intraperitoneal SAL200), and (iv) a combination treatment group (which received SOC [either 25 mg/kg subcutaneous vancomycin or 200 mg/kg intramuscular nafcillin] plus SAL200 [25 mg/kg intravenous SAL200 plus 25 mg/kg intraperitoneal SAL200]). The mice were treated according to study group 1 h after infection, and blood was collected from the tail vein for culture 1 h after treatment. Postinfection survival was assessed every 24 h until 72 h following infection. All mice were sacrificed, and their spleens were harvested for culture 72 h after infection (see Fig. S1 in the supplemental material). The experiment was performed with MRSA LAC and MSSA Newman and repeated 2 to 3 times for each isolate. All *in vivo* studies were performed in accordance with the guidelines of the Seoul National University Hospital Institutional Animal Care and Use Committee.

***Galleria mellonella* infection model.** Healthy *Galleria mellonella* larvae (Ecowin, Daegu, Republic of Korea) that weighed 200 to 250 mg and that were in their final instar stage were selected, food deprived, and stored at 15°C for 24 h before infection (33). The larvae were injected via the last left proleg with 10 μl of a bacterial suspension containing 5×10^8 CFU/ml of the MRSA LAC strain or the MSSA Newman strain. This dose was previously determined to yield 80% mortality at 72 h postinfection. All injections were delivered using 2-in., 26-gauge needles (Hamilton syringe needle; catalog number 7779-04; Hamilton Company, Reno, NV, USA) attached to a 500-μl Hamilton syringe (1750 RN; catalog number 81230) and mounted on a dispenser (Hamilton PB600 repeating dispenser; catalog number 83700). Infected larvae were split into four experimental groups (15 larvae per group): (i) an inactive control group (which received phosphate-buffered saline), (ii) an SOC antibiotic treatment group (which received either 25 mg/kg vancomycin for MRSA LAC-infected larvae or 100 mg/kg cefazolin for MSSA Newman-infected larvae), (iii) an SAL200 treatment group (which received 50 mg/kg SAL200), and (iv) a combination treatment group (which received SOC plus SAL200 treatment). All antimicrobial drugs were delivered into the hemocoel via injections of 10 μl into the last right proleg at 1 h following infection. Larvae were incubated in vented 12-well culture plates (SPL Life Sciences, Pocheon, Republic of Korea) at 37°C in air for 96 h and inspected and scored every 24 h for death, failure to move in response to touch, and melanization. All experiments were independently performed 2 to 3 times.

Statistical methods. Statistical analyses were performed using the IBM SPSS Statistics (version 22.0) software package (SPSS Inc., Chicago, IL, USA). Survival data were plotted using the Kaplan-Meier method, and a log-rank test was used for comparisons between groups. Bacterial density data were analyzed using the Mann-Whitney U test and are presented as the median and interquartile range. A *P* value of $<$0.05 was considered statistically significant.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00731-18.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

S. Y. Jun is employed by the commercial company iNtRON Biotechnology, Inc. The phage endolysin SAL200 used in this study is produced by iNtRON and is currently in use in a human clinical trial (NCT03089697). None of the other authors has a conflict to declare.

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