Lysostaphin as a treatment for systemic *Staphylococcus aureus* infection in a mouse model

John F. Kokai-Kun*, Tanya Chanturiya† and James J. Mond

Biosynexus Incorporated, 9298 Gaither Rd, Gaithersburg, MD 20877, USA

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**Objectives:** With the isolation of clinical strains of *Staphylococcus aureus* carrying the gene that confers vancomycin resistance, the need for novel antistaphylococcals has become more urgent. Lysostaphin, an example of such a novel therapeutic, is an endopeptidase that rapidly lyses *S. aureus* through proteolysis of the staphylococcal cell wall. We evaluated its efficacy as a therapeutic agent for treatment of systemic *S. aureus* infection in a mouse model.

**Methods:** Mice (5–10 per group) challenged with methicillin-susceptible *S. aureus* developed bacteraemia and organ infections while mice challenged with methicillin-resistant *S. aureus* (MRSA) developed organ infections. The challenged mice received various intravenous doses of recombinant lysostaphin, administered once a day for 1–3 days when compared with treatment with oxacillin or vancomycin. Some mice also received treatment with lysostaphin combined with oxacillin or vancomycin. Following treatment, bacteraemia was determined, and mice were sacrificed and organ infection was determined.

**Results and conclusions:** Lysostaphin administered at 5 mg/kg once a day for 3 days consistently cleared *S. aureus* from the blood and the organs of infected mice. Furthermore, the combination of lysostaphin and oxacillin or vancomycin demonstrated increased efficacy against MRSA over lysostaphin alone allowing the therapeutic dose of lysostaphin to be reduced to 1 mg/kg. These results demonstrate that lysostaphin is an effective treatment for eradicating *S. aureus* from the blood and from the organs of infected mice.

**Keywords:** oxacillin, vancomycin, dosing regimen

**Introduction**

*Staphylococcus aureus* causes a wide range of infections, from skin infections to disseminated systemic infections leading to organ failure and death.1 Vancomycin has been the antibiotic of choice for treatment of methicillin-resistant *S. aureus* (MRSA), but accumulating mutations in *S. aureus* have led to intermediate resistance to vancomycin (VISA).2 and recently, several fully vancomycin-resistant strains of *S. aureus* carrying the vanA gene, apparently acquired from enterococci, have been isolated.3 This has left us with the specter of very few effective antibiotics being available to treat *S. aureus* infections and with the probability that resistance to the remaining antibiotics will likely occur.

Lysostaphin was discovered in 1960 and there was a flurry of early research which focused on lysostaphin as an antistaphylococcal agent.4–14 The lack of available preparations of lysostaphin with consistently high specific activity and purity in the face of the ready availability of other effective antibiotics for use against *S. aureus* relegated lysostaphin to a laboratory reagent useful for lysis of *S. aureus*. The cloning of the lysostaphin gene from *Staphylococcus simulans* into expression systems like *E. coli*,15 as well as new purification procedures13 have led to production of highly purified recombinant lysostaphin with consistently high specific activity. The availability of recombinant lysostaphin, in the face of a vanishing list of effective antistaphylococcal agents, has generated renewed interest and research into lysostaphin as a potential antistaphylococcal agent.14–20

Mature lysostaphin is a 27 kDa glycyl–glycine zinc endopeptidase that cleaves the staphylococcal cell wall, loss of osmotic equilibrium and rapid lysis of the staphylococci, often within seconds.22 Lysostaphin is highly effective against both rapidly dividing *S. aureus* as well as quiescent...
convenient mouse model of carditis in rabbits14,17,26,27 and neonatal sepsis in suckling rats.20

were challenged with either strain of Staphylococcus aureus. Neutropenic mice were challenged with either strain of Staphylococcus aureus in a post-bacterial challenge. Briefly, 100 μL samples of blood were drawn and mixed with 900 μL of PBS containing 500 U of heparin (Sigma), and 100 μL of this mixture was plated on blood agar. The limit of detection for bacteraemia was 100 cfu/mL of blood. Mice that survived until the end of the experiment were sacrificed on either day 4 or 6 of the experiment by CO2 asphyxiation, and various organs were removed to determine organ infection. The whole organ was removed and placed in 900 μL of sterile PBS and then mechanically disrupted by macerating the organ with a sterile Pasteur pipette and then vortexing vigorously. Note, preliminary experiments determined that further disruption of the organ by sonication did not lead to additional recovery of bacteria (data not shown). Mechanically disrupted organs were further diluted in PBS as needed. Aliquots (100 μL) of supernatant from disrupted organs were plated on blood agar using a wide bore pipette tip to determine organ infection. The limit of detection for organ infection was 10 cfu/organ. Any Staphylococcus aureus that were recovered from the organs of lysozyme-treated mice were further tested for lysozyme resistance by plating on tryptic soy agar (BD) + 10 μg/mL lysozyme as previously described.33 Staphylococcus aureus recovered from untreated mice was used as a negative control for this determination. Growth of Staphylococcus aureus on lysozyme-containing agar at 24 h was considered to be lysozyme-resistant Staphylococcus aureus. In experiments studying neutropenic mice, mice were rendered temporarily neutropenic by intraperitoneal administration of two doses of 150 μg of a rat anti-mouse anti-Gr-1 monoclonal antibody RB6-8C54 administered 24 h prior to and 24 h after bacterial challenge. Neutropenic mice were challenged with ~2 × 107 cfu of Staphylococcus aureus due to their increased susceptibility to infection. The standard challenge dose of 2 × 107 cfu of Staphylococcus aureus was rapidly fatal to neutropenic mice (data not shown). Each animal experiment was conducted at least twice. Data presented are representative or combinations of these experiments.

Materials and methods

Materials

Recombinant mature lysozyme was produced by fermentation in E. coli and purified to homogeneity by Avecia (Stanstead, UK) under contract to Biosynexus Incorporated (Gaithersburg, MD, USA). Purified lysozyme was formulated in pH 6.5, phosphate buffered saline buffer for storage at −70°C until used. Oxacillin and vancomycin were purchased from Sigma (St Louis, MO, USA).

Staphylococcus aureus strains

Two representative strains of Staphylococcus aureus were used in these studies, a capsule type 5 methicillin-susceptible strain, ATCC 49521 and a community acquired methicillin-resistant strain NRS123 (USA40028) acquired from the Network on Antimicrobial Resistance in Staphylococcus aureus (www.narsa.net). Stocks were maintained frozen at −70°C in trypticase soy broth (BD, Sparks, MD, USA) before use in the systemic infection model. MICs of lysozyme, oxacillin and vancomycin for these two staphylococcal strains were determined as per CLSI (formerly NCCLS) guidelines as modified in the work of Kusuma and Kokai-Kun31 as 0.016, 0.2 and 1 mg/L, respectively, for ATCC 49521, and 0.008, >16 and 1 mg/L, respectively, for NRS123.

Mouse systemic infection model

Challenge. All animal experiments were conducted in accordance with the guidelines of the Biosynexus Institutional Animal Care and Use Committee and the Office of Laboratory Animal Welfare, National Institutes of Health. Briefly, Staphylococcus aureus was plated on tryptic soy agar plus 5% sheep blood (blood agar, Remel, Lenexa, KS, USA) from a frozen stock. Following 24 h of incubation of the agar plate, three isolated colonies were transferred to 1 mL of trypticase soy broth. The broth culture was incubated overnight (16–20 h) at 37°C with shaking. Based on preliminary experiments to determine a challenge dose of Staphylococcus aureus that resulted in consistent systemic infection without rapidly killing the mice (data not shown), the broth-grown bacteria were diluted to ~2 × 107 cfu/mouse in phosphate buffered saline (PBS; Cambrex, Walkersville, MD, USA). Actual Staphylococcus aureus challenge titres were determined by serial 10-fold dilution and plating on blood agar. Six-week-old female CF-1 mice were used in these studies (HSD, Indianapolis, IN, USA). Mice were challenged with either strain of Staphylococcus aureus in a final volume of 200 μL of PBS by intravenous tail injection. The day of challenge was designated as day 1 of the experiment.

Treatment. Lysozyme treatments (1–50 mg/kg) were given at various intervals beginning 3 h after bacterial challenge. Lysozyme was administered by intravenous injection either once on the day of challenge or once per day for up to 3 days starting on the day of challenge and administered at 24 h intervals in 200 μL of PBS. Oxacillin (50 mg/kg) was administered intramuscularly in 100 μL of PBS four times per day with 3 h between doses, while vancomycin was administered intravenously at 15 mg/kg twice a day with 9 h between doses based on current dosing recommendations.32 Treatments with oxacillin or vancomycin were also initiated 3 h after bacterial challenge on day 1.

Recovery of bacteria. In some experiments, mice were bled at 24 h post-bacterial challenge to determine bacteraemia. Briefly, 100 μL samples of blood were drawn and mixed with 900 μL of PBS containing 500 U of heparin (Sigma), and 100 μL of this mixture was plated on blood agar. The limit of detection for bacteraemia was 100 cfu/mL of blood. Mice that survived until the end of the experiment were sacrificed on either day 4 or 6 of the experiment by CO2 asphyxiation, and various organs were removed to determine organ infection. The whole organ was removed and placed in 900 μL of sterile PBS and then mechanically disrupted by macerating the organ with a sterile Pasteur pipette and then vortexing vigorously. Note, preliminary experiments determined that further disruption of the organ by sonication did not lead to additional recovery of bacteria (data not shown). Mechanically disrupted organs were further diluted in PBS as needed. Aliquots (100 μL) of supernatant from disrupted organs were plated on blood agar using a wide bore pipette tip to determine organ infection. The limit of detection for organ infection was 10 cfu/organ. Any Staphylococcus aureus that were recovered from the organs of lysozyme-treated mice were further tested for lysozyme resistance by plating on tryptic soy agar (BD) + 10 μg/mL lysozyme as previously described.33 Staphylococcus aureus recovered from untreated mice was used as a negative control for this determination. Growth of Staphylococcus aureus on lysozyme-containing agar at 24 h was considered to be lysozyme-resistant Staphylococcus aureus.

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Statistical analysis of data

The statistical significance of the differences between cfu recovered from various groups was analysed using a variance technique based on the ranks (Kruskal–Wallis test). The reasons this statistical analysis was used are twofold. First, the distribution of the cfu was not normally distributed, and the non-parametric test statistic was an appropriate alternative. Second, the data were left-censored due to limits of detection, and the sample size was too small for other methods to be appropriate. By ranking the data, the values that were below the detection limit were always placed in the lowest rank thus alleviating the problems caused by data under the lower detection limit. Differences were considered significant if P < 0.05.
Results

Treatment of systemic methicillin-susceptible S. aureus infection with lysostaphin

To determine the efficacy of lysostaphin in clearing bacteraemia and organ infection, mice were challenged with methicillin-susceptible S. aureus (MSSA) and then treated with lysostaphin or oxacillin for 3 days. When control groups of mice were challenged with 2 × 10⁷ S. aureus ATCC 49521, all control animals developed bacteraemia by 24 h post-challenge (Figure 1). Four of ten mice treated with a single administration of 1 mg/kg lysostaphin had no detectable bacteraemia at 24 h, while no bacteraemia was detected at 24 h in any of the mice treated with a single administration of 5 mg/kg of lysostaphin. On day 6, surviving mice in each group were sacrificed and infections of the spleen, liver and kidneys were determined. Three animals in the control group had succumbed to infection prior to day 6 while the remaining seven animals were infected in the spleen, liver and kidneys (Figure 1). Control animals with >10⁵ cfu recovered from the liver or kidney also consistently had visible abscesses on those organs at the time of sacrifice. Treatment with 1 mg/kg lysostaphin per day for 3 days cleared the visible organ abscesses, significantly reduced the bacterial load in all three organs and cleared some organs of infection (no cfu recovered) in some animals (Figure 1). Cfu recovered from mice treated with 5 mg/kg lysostaphin once daily for 3 days were significantly lower than from mice treated with 1 mg/kg. Lysostaphin at 5 mg/kg resulted in no detectable S. aureus in livers and kidneys and clearance of infection from the spleens of 5 of 10 of the mice (Figure 1). It should be noted that in this model, significant lung and heart infections were not detected on day 6, even in control animals. As a comparison, oxacillin, a drug used to treat MSSA infections, was also effective in this model when delivered at 50 mg/kg four times per day for 3 days, clearing S. aureus infection in four of five animals leaving one animal with an infected kidney. Two treatments of oxacillin,

![Figure 1](http://jac.oxfordjournals.org/)

Figure 1. Colony forming units recovered from blood (24 h post-challenge) and organs (6 days post-challenge) of MSSA-challenged mice following 3 days of lysostaphin treatment. Mice (5 or 10 mice per group as indicated on the figure) were challenged with 2 × 10⁷ S. aureus ATCC 49521 and then beginning 3 h post-challenge, mice were treated with either nothing (control), lysostaphin (1 or 5 mg/kg given intravenously once per day) or oxacillin (50 mg/kg given intramuscularly four times per day) for 3 days. Twenty-four hours post-challenge (following one lysostaphin or two oxacillin treatments), mice were bled to determine bacteraemia. On the sixth day (72 h after the last treatment), the animals were sacrificed and organs were cultured for S. aureus. Note, three animals in the control group succumbed to their infections prior to day 6, so only seven symbols appear in the control group for spleen, liver and kidney. Symbols indicate cfu recovered from individual organs while horizontal lines indicate geometric means for each group. Symbols on the x-axis indicate no S. aureus were recovered from the organ. The asterisks indicate significance (P ≤ 0.05): *the result is significantly different from the control group; **the result is significantly different from the 5 mg/kg lysostaphin group.
administered 3 and 6 h post-challenge were not as effective as lysostaphin for clearance of bacteraemia however (Figure 1). In follow-up experiments, we found that lysostaphin delivered at 5 mg/kg for 2 rather than 3 days did not consistently clear \(S.\) aureus infection (data not shown).

To determine if a single, high-dose infusion of lysostaphin would suffice to eradicate systemic \(S.\) aureus infection, we challenged mice with MSSA and treated with a single bolus of lysostaphin at 25 or 50 mg/kg, 3 h post-challenge. Either dose of lysostaphin cleared 100% of bacteraemia at 24 h after a single dose (data not shown). A single dose of 50 mg/kg also cleared 100% of the kidneys, 83% of the spleens and 67% of the livers of the challenged mice upon sacrifice on day 6. Reducing the treatment to a single dose of 25 mg/kg was significantly less effective than 50 mg/kg for clearance of the liver and demonstrated a trend towards being less effective in the other organs (Figure 2).

**Treatment of systemic MRSA infection with lysostaphin**

Realizing that many difficult-to-treat \(S.\) aureus infections are due to methicillin-resistant bacteria, we sought to determine the effectiveness of lysostaphin for clearing an MRSA infection caused by one of the recently emerged community-acquired MRSA strains. When CF-1 mice were challenged with \(2 \times 10^7\) cfu of NRS123 (USA400), all control animals had infected organs on day 6 (Figure 3). During the development of the mouse challenge model for this work, several MRSA strains were evaluated (data not shown). The criterion we used to select the challenge strain was that it would cause reproducible organ infection in the mice without rapidly killing the animals. Strain NRS123 was chosen as being a relevant community-acquired MRSA strain \(^{29}\) and one which caused reproducible organ infection without causing the animals to succumb to infection prior to the day of sacrifice. None of the MRSA examined, including NRS123, caused detectable bacteraemia in control mice at challenge doses that were not rapidly lethal to the animals. When mice challenged with NRS123 were treated once-a-day for 3 days with 5 mg/kg lysostaphin, all 10 treated mice, sacrificed on day 6 after challenge, were cleared of \(S.\) aureus in their kidneys and had significantly reduced infections in their spleens and livers when compared with the control group (Figure 3). Reducing the lysostaphin dose to 1 mg/kg resulted in significantly reduced..
clearance of the spleens and kidneys of mice treated with this dose when compared with those treated with 5 mg/kg lysostaphin. Vancomycin, a drug often used to treat MRSA infections, delivered at 15 mg/kg twice a day for 3 days was also significantly less effective than 5 mg/kg lysostaphin for clearing MRSA infection of the liver and kidney (Figure 3).

In vitro and in vivo synergy between lysostaphin and β-lactam antibiotics has been reported. To determine whether such an effect could be demonstrated in our model, challenged mice were treated with 1 mg/kg lysostaphin with the addition of either oxacillin or vancomycin treatment. As shown in Figure 3, while 1 mg/kg lysostaphin significantly reduced the number of S. aureus recovered from all of the organs of the treated animals when compared with control animals, addition of 50 mg/kg oxacillin administered four times a day over the 3 days to 1 mg/kg lysostaphin treatment resulted in a significant improvement in the clearance of infection from all kidneys, spleens and livers when compared with 1 mg/kg lysostaphin alone; thus supporting in vivo synergy of β-lactams and lysostaphin even for MRSA. Oxacillin treatment alone had no effect on the course of the MRSA infection, as would be expected (data not shown). Treatment of challenged mice with a combination of 1 mg/kg lysostaphin and vancomycin also demonstrated a significantly enhanced clearance over either treatment alone for the spleens and livers, but this effect was not as dramatic as that for lysostaphin plus oxacillin (Figure 3). No lysostaphin-resistant variants were identified in experiments conducted with MRSA challenge and lysostaphin treatment.

**Kinetics of lysostaphin clearance of S. aureus infection**

To determine the time course of clearance of infection after lysostaphin treatment, we sacrificed animals 24 h after the final lysostaphin treatment (Figure 4) rather than 72 h after the final treatment as was done in the above experiments. All five mice treated with 5 mg/kg lysostaphin for 3 days and sacrificed 24 h later were heavily infected in the spleens and livers but did demonstrate a significant reduction in infection when compared...
with control animals. The recovered cfu were considerably higher than those from animals treated with 5 mg/kg lysostaphin for 3 days and sacrificed 72 h after the last treatment (Figure 1). Increasing the daily lysostaphin dose to 40 mg/kg given as a single dose or divided into two daily doses (data not shown) for 3 days did not have a significant impact on organ infection when compared with 5 mg/kg lysostaphin when the mice were sacrificed 24 h after the last treatment (Figure 4). Oxacillin or vancomycin treatments for 3 days were similar in their reduced effectiveness for clearance of the liver of animals that were sacrificed on day 4 when compared with 5 or 40 mg/kg lysostaphin, and significantly reduced in effectiveness for clearance of the spleen when compared with either dose of lysostaphin. Oxacillin, however, was significantly better than 5 mg/kg lysostaphin for clearance of the kidneys while vancomycin was significantly worse than 40 mg/kg lysostaphin for clearance of the kidneys (Figure 4).

We considered the possibility that this finding of organ infection in lysostaphin-treated mice sacrificed 24 h after the final lysostaphin treatment may have represented the inability of lysostaphin to access bacteria that were sequestered in neutrophils. To investigate this, the effects of lysostaphin in neutrophil-depleted, S. aureus infected mice sacrificed 24 h after lysostaphin treatment were examined. Neutropenic mice were more susceptible to S. aureus challenge than normal mice (data not shown), thus a lower challenge dose was required to achieve consistent infection in these mice without inducing rapid mortality. A significantly different result was seen in mice made neutropenic, challenged with S. aureus, treated with 5 mg/kg lysostaphin for 3 days and then sacrificed on day 4 (Figure 4, neutropenic mice). Unlike normal mice, neutropenic mice that were treated with lysostaphin for 3 days and then sacrificed 24 h later showed no detectable S. aureus in the kidneys of all treated animals and significantly reduced liver and spleen infections.

Figure 4. S. aureus recovered from organs of mice 4 days after MSSA-challenge following 3 days of lysostaphin treatment. Mice (5 mice per group, either normal or mice rendered neutropenic by treatment with an anti-neutrophil MAb, bottom right graph) were challenged with either 2 × 10⁷ S. aureus or 2 × 10⁵ S. aureus (neutropenic mice only) ATCC 49521, and then beginning 3 h post-challenge mice were treated with either nothing (control), lysostaphin (5 or 40 mg/kg given intravenously once per day), oxacillin (50 mg/kg given intramuscularly four times per day) or vancomycin (15 mg/kg given intravenously twice per day) for 3 days as indicated on the figure. On the fourth day (24 h after the last treatment), the animals were sacrificed and organs were cultured for S. aureus. Symbols indicate cfu recovered from individual organs. Horizontal lines indicate geometric means for each group. Symbols on the x-axis indicate no S. aureus recovered from the organ. The asterisks indicate significance (P ≤ 0.05): *the result is significantly different from the control group; **the result is significantly different from the 5 mg/kg lysostaphin group; ***the result is significantly different from the 40 mg/kg lysostaphin group. For the neutropenic mice (lower right graph): *the result is significantly different from the control group; **the result is significantly different from the 5 mg/kg lysostaphin group for the same organ in non-neutropenic mice.
when compared with normal lysostaphin-treated mice sacrificed on day 4. This demonstrated that in the absence of neutrophils, lysostaphin is able to clear the infection more quickly than in normal mice. No lysostaphin-resistant variants were identified in experiments conducted with MSSA challenge and lysostaphin treatment.

**Discussion**

This study demonstrated that three doses of lysostaphin administered once a day at 5 mg/kg for 3 days cleared *S. aureus* kidney infection by MSSA and MRSA and significantly reduced spleen and liver infections in a mouse model of systemic infection (Figures 1 and 3). Lysostaphin treatment was also successful in clearing MSSA bacteraemia following a single dose of 5 mg/kg (Figure 1). At the selected challenge dose, the MRSA strain used in these studies did not cause detectable bacteraemia. These results support lysostaphin’s capacity to penetrate tissue when administered intravenously inasmuch as lysostaphin treatment significantly cleared infections in various organs. Furthermore, the relatively short serum half-life of lysostaphin (previously determined to be <1 h) did not appear to affect the capacity of lysostaphin to clear systemic *S. aureus* infection.

A single intravenous injection of 50 mg/kg lysostaphin resulted in serum concentrations of >50 ng/mL lysostaphin for at least 5 h post-injection. This concentration of lysostaphin is well above the MIC of lysostaphin for both *S. aureus* ATCC49521 and NRS123. Nonetheless, a single bolus of 25 or 50 mg/kg lysostaphin (Figure 2) appeared to be similar to or even less effective than three injections of 5 mg/kg of lysostaphin administered over 3 days (15 mg/kg total dosing) (Figure 1). We speculate that this finding suggests *S. aureus* in some animals was transiently sequestered from lysostaphin by 3 h post-challenge (the time of the single treatment), and thus repeated dosing over subsequent days was required to consistently clear infection as these bacteria emerged from sequestration. An alternative possibility is that only a fraction of the administered lysostaphin penetrated the infected tissue before being cleared and thus repeated dosing over 3 days may be required to achieve sufficient tissue concentrations of lysostaphin necessary to clear infection in organs of the challenged animals. Either way, these findings appear to support the use of repeated administration of lower doses of lysostaphin (5 mg/kg) over a short treatment course (3 days) rather than the use of a single large bolus treatment with lysostaphin (50 mg/kg) for treatment of *S. aureus* infection.

Previous studies have demonstrated that there is a synergistic *in vitro* and *in vivo* effect between lysostaphin and β-lactam antibiotics, even for MRSA, and indeed, we saw results consistent with synergy in this study (Figure 3). Addition of oxacillin to lysostaphin treatment significantly improved the effectiveness of lysostaphin allowing the effective therapeutic dose of lysostaphin to be reduced from 5 to 1 mg/kg for treatment of an MRSA infection. In addition to having the benefit of allowing a lower dose of lysostaphin to be used for treatment, adding β-lactam antibiotics would have the added benefit of preventing the possible emergence of lysostaphin-resistant *S. aureus* during treatment as it has been demonstrated that lysostaphin resistance and β-lactam resistance are mutually exclusive. While no synergy has been demonstrated between vancomycin and lysostaphin, there also appeared to be a significant additive effect between lysostaphin and vancomycin treatment in this study (Figure 3).

When mice were sacrificed 24 h after the conclusion of treatment rather than 72 h after the conclusion of treatment, substantial amounts of *S. aureus* were recovered from the spleens and livers of lysostaphin-treated mice when compared with the *S. aureus* recovered from the spleens and livers of lysostaphin-treated mice sacrificed 72 h after the last lysostaphin injection (Figure 1). This was the case even though mice in both experiments were treated identically for the first 3 days of the experiment. Untreated control animals were heavily infected in all organs on both days of sacrifice. Since the serum half-life of lysostaphin is <1 h, it is unlikely that this difference in recovered bacteria represents the continued antibacterial activity of lysostaphin in serum during the 48 h difference in sacrifice times. It is possible, however, that the tissue half-life of lysostaphin may be longer and thus tissue-bound lysostaphin could continue to effectively kill staphylococci during the 48 h interval between a day 4 and a day 6 sacrifice after infection; this remains to be determined. Another likely possibility is that *S. aureus* can survive within phagocytic cells like neutrophils, but lysostaphin is unable to enter these cells. Thus bacteria recovered from the spleens and livers of lysostaphin-treated mice sacrificed on day 4 of the experiment may represent *S. aureus* recovered from within phagocytic cells that were protected from lysostaphin activity. When lysostaphin-treated mice were sacrificed on day 6 (Figure 1), the *S. aureus* sequestered within the neutrophils may have already been killed by these cells thus resulting in a greater reduction of infection in all organs. Consistent with this possibility are the data presented in Figure 4, demonstrating that mice rendered neutropenic for the entire course of the experiment and then lysostaphin-treated were cleared of *S. aureus* infection significantly more than non-neutropenic animals sacrificed on day 4. In these neutropenic mice, there were no neutrophils to shield the *S. aureus* from lysostaphin.

The lysostaphin dosing used in these experiments is consistent with the dosing of recombinant lysostaphin used in more recent studies for the treatment of rabbit endocarditis in which lysostaphin dosage ranged from 2 to 30 mg/kg per day, and suckling rats which received 1 mg/kg lysostaphin intraperitoneally. This dosing level is in contrast, however, to earlier studies using lysostaphin purified from its natural host. In those early animal studies, doses as high as ~125 mg/kg lysostaphin were used to treat infected mice, and multiple doses of >30 mg/kg lysostaphin were most effective in a dog model of endocarditis, although no animals were sterilized in that study. This difference perhaps reflects the higher specific activity of recombinant lysostaphin. To date, there is only one documented case of lysostaphin being used to treat a systemic *S. aureus* infection in a human. The present study supports the use of a short course of lysostaphin, up to 3 days, for treatment of systemic *S. aureus* infection. It appears that smaller doses spread over several days may be more effective in terms of total drug dose than a single larger dose of lysostaphin, and addition of oxacillin and/or vancomycin can further reduce the lysostaphin dose needed to clear infection. Determination of the actual dosing of lysostaphin in humans, however, will have to await clinical trials.
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Transparency declarations

All authors are, or formerly were, employees and stock holders of Biosynexus Incorporated, and Biosynexus is in the process of developing lysostaphin for commercial use.

References

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