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Combination therapy with iron chelation and vancomycin in treating murine staphylococcemia

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Conflict of interest We declare that we have no conflict of interest.

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Abstract

Iron acquisition is a virulence factor for *Staphylococcus aureus*. We assessed the efficacy of the iron chelator, deferasirox (Def), alone or in combination with vancomycin (Van) against two methicillin-resistant *S. aureus* (MRSA) strains in vitro and in a murine bacteremia model. In vitro time–kill assays were carried out against MRSA or vancomycin-intermediate *S. aureus* (VISA) strains. The impact of Def on Van binding to the surface of *S. aureus* was measured by flow cytometry. Furthermore, we compared the efficacy of Def, Van, or both drugs in treating *S. aureus* bacteremia in a murine model. Combination therapy reduced MRSA and VISA viability in vitro versus either drug alone or untreated controls (p <0.005); this outcome was correlated with enhanced Van surface binding to *S. aureus* cells. In vivo, Def+Van combination therapy significantly reduced the bacterial burden in mice kidneys (p =0.005) and spleen (p <0.001), and reduced the severity of infection with MRSA or VISA strains compared to placebo-treated mice. Our results show that Def enhances the in vitro and in vivo capacity of Van-mediated MRSA killing via a mechanism that appears to involve increased binding of Van to the staphylococcal surface. Iron chelation is a promising, novel adjunctive therapeutic strategy for MRSA and VISA infections.

Introduction

Staphylococcus aureus, a persistent nasal colonizer in approximately one-third of the human population [1], causes infections ranging from superficial skin lesions to life-threatening syndromes such as endocarditis, osteomyelitis, toxic shock, and sepsis [2–4]. Indeed, *S. aureus* is the second leading cause of healthcare-associated bacteremia, accounting for ~13 % of such infections. Of note, the acquisition of nosocomial *S. aureus* bloodstream infections leads to extended length of hospital stay and increased antibiotic usage, costs, and mortality [5].

Recent data indicate that iron acquisition is absolutely required for multiple stages of *S. aureus* pathogenicity and virulence [6, 7]. *S. aureus* possesses several iron uptake systems, including the iron-regulated surface determinant system [8–10], siderophores such as staphyloferrin A [11], staphyloferrin B [12], and aureochelin [13], the transferrin receptor [14], and heme or hemoprotein-specific staphylococcal receptors [7]. Furthermore, patients suffering from iron overload are at increased risk of developing *S. aureus* bacteremia [15]. Our previous study showed that the iron chelator, deferasirox (Def), significantly protected mice from mucormycosis, and enhanced the efficacy of liposomal amphotericin B in treating experimental mucormycosis and aspergillosis [16–18]. Therefore, we hypothesized that using Def alone or in combination with conventional antibiotic therapy might also prove to be effective against *S. aureus* in vitro and in vivo.

Materials and methods

Bacterial strains and culture conditions

S. aureus LAC, a USA300 methicillin-resistant *S. aureus* (MRSA) clinical isolate, was provided by Dr. Frank DeLeo (NIAID/NIH), while *S. aureus* Mu50 is a vancomycin-intermediate *S. aureus* (VISA) strain obtained from the NIAID's Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA). Both strains were maintained in stock cultures containing brain heart infusion (BHI) medium+17 % glycerol (Sigma-Aldrich) and stored at −80 °C until thawed for use. Working plates were subcultured from the stock cultures by streaking a loopful on Luria-Bertani (LB) agar plates and incubating at 37 °C for 24–48 h prior to storing them at 4 °C. Overnight cultures were inoculated in BHI medium and grown at 37 °C in a shaking incubator for 12–16 h. On the day of each experiment, the bacterial cells (100 μl) were passaged into fresh BHI medium and incubated at 37 °C for 2.5–3 h to yield exponential phase cells. The bacterial cells were then harvested by centrifugation at 4,000×g for 5 min and washed twice with phosphate buffered saline (PBS), resuspended in PBS, and the inoculum determined by McFarland standard reading using a desktop portable photometer. All inocula estimates were confirmed by quantitative plate counts.

Susceptibility testing and time–kill assay

The in vitro susceptibility of MRSA strain LAC300 or the vancomycin-intermediate MRSA strain (VISA) Mu50 against Def or vancomycin (Van) were determined by standard Clinical and Laboratory Standards Institute (CLSI) microdilution methods [19]. We also determined the minimum bactericidal concentrations (MBC) by spotting samples from all of the 96-well plates on LB plates and incubating at 37 °C for 24 h. The MBC was defined as the least concentration of the drug at which the organism failed to grow on the LB plate. To evaluate the potential enhanced activity of Van in combination with Def against the above two MRSA strains in vitro, the isolates were 'preconditioned' in BHI with 0.5× minimum inhibitory concentration (MIC) of Def (25 mg/L) at 37 °C overnight to reduce intracellular iron stores versus BHI alone ('non-preconditioned' control). Preconditioned or non-preconditioned bacterial cells [1×10^6 colony-forming units (CFU)/ml] were transferred into BHI medium containing 1× of MIC of Def (50 mg/L), Van (0.78 and 6.25 mg/L for MRSA LAC300 or VISA, respectively), or a combination of both drugs. Inoculated BHI medium without drugs served as controls. Aliquots were obtained at 0, 4, 6, and 24 h for CFU quantification. Data were expressed as mean $\log_{10}$ CFU/ml [± standard deviation (SD)]. All experiments were performed in triplicate.

Effect of Def treatment on Van binding to the bacterial cell surface

The impact of Def on Van binding was measured by flow cytometry. Overnight culture of bacterial cells were collected, washed, adjusted to 1×10^6 CFU/ml, and exposed to: (1) 1× MIC of fluorescently labeled Van (Bodipy®FL VAN; Invitrogen Corp., Carlsbad, CA, USA; at 0.78 and 6.25 mg/L for MRSA LAC300 and VISA Mu50, respectively) or (2) 1× MIC of Bodipy®FLVan plus Def at 1× MIC (50 mg/L) for 6 h at 37 °C, as described above for the in vitro time–kill assays. The maximum excitation and emission spectra of the Bodipy®FL VAN are 504 and 511 nm, respectively [20]. The binding of Van was assayed

by quantitative flow cytometry (FACScalibur; Becton-Dickinson) [21, 22]. For each sample, 10,000 cells were acquired and analyzed for quantitative green fluorescence, indicating the extent of Van binding. *S. aureus* cells without any antibiotic exposure were used as negative controls. Data were expressed in terms of the intensity of fluorescence (relative fluorescent units), as well as the percentage of acquired cells with positive fluorescence (set at>10 fluorescent units in the FL-1 channel) [21, 22].

Animal model

We also studied the potential for enhanced activity of Van in combination with Def in a well-characterized murine staphylococcemia model. BALB/c mice were infected via the tail vein with 6×10^7 bacterial cells. Treatment with Def (Novartis Pharmaceuticals) [15 mg/kg, administered twice daily via oral gavage in 0.5 % hydroxypropylcellulose (Klucel)] was started 4 h postinfection, while Van (2 mg/kg, administered twice daily via tail vein injection) was started 24 h postinfection. These doses were selected based on pilot studies showing limited efficacy of either drug alone, which would likely allow for the detection of enhanced drug combination activities. Additionally, a dose falling between 3.74 and 11 mg/kg/d of Van has been reported to result in 50 % survival of mice infected with an MRSA strain with a Van MIC of 1.0 mg/L [23]. Treatment continued for 2 days. Control mice were given 0.5 % Klucel. Tissue bacterial burdens (mean $\log_{10}$ CFU/g±SD) for two prototypical hematogenously seeded target organs (kidneys, spleen) served as the primary end-point and histopathological examination of kidneys stained with Gram stain served as a secondary end-point.

Statistical analysis

Differences in growth rates in vitro or tissue bacterial burdens in the infected organs were compared by the non-parametric Wilcoxon rank-sum test. *p*-values <0.05 were considered significant. All procedures involving mice were approved by our Institutional Animal Care and Use Committee (IACUC), according to the National Institutes of Health guidelines for animal housing and care.

Results

Def acts synergistically with Van in killing *S. aureus* in vitro

The Def MICs and MBCs were 50 mg/L for both strains. The MIC and MBC values for Van against MRSA LAC300 were both 0.78 mg/L, whereas they were both 6.25 mg/L for the VISA strain. In preconditioned or non-preconditioned medium, Def alone resulted in a~2–3 $\log_{10}$ CFU/ml reduction in counts at all time points tested for both strains compared to controls (p <0.005 by Wilcoxon ran- sum test). Further, Def was superior to Van at all time points against MRSA LAC300 (p =0.002) (Fig. 1a, b). Similarly, compared to Van, Def reduced VISA counts by ~1.5–2.5 logs early (4 h time point), although this effect was not durable (Fig. 1c, d). In contrast, Van had no significant effect on reducing MRSA LAC300 or VISA CFU compared to controls at all time points. These studies are in agreement with several previous reports showing a modest effect of Van killing of MRSA in vitro [24–26]. Interestingly, the combination of Def+Van significantly inhibited the growth of both strains

at both early and late time points, compared to controls as well as Def or Van alone, both in Def preconditioned or Def non-preconditioned medium (p <0.005) (Fig. 1).

Def enhances Van binding to the *S. aureus* cell surface

In an attempt to study the potential mechanisms by which Def might enhance the activity of Van, we studied the effect of Def treatment on the ability of Van to bind to the cell surface of *S. aureus* [27]. The impact of Def on Van binding is showed in Fig. 2 (panels a and b for MRSA and VISA strains, respectively). A significant increase in Van binding was observed in the combination Def and Van group as compared to Van alone, as indicated by a substantial increase in FL-1 intensity (Fig. 2; rightward curve shift). The mean FL-1 fluorescence values in the combination groups were significantly higher than the Van alone groups (185.19±12.3 for combination vs. 55.87±7.8 for Van alone using the MRSA strain [Fig. 2a, p <0.0003]; and 235.1±21.5 for combination vs. 66.5±6.3 for Van alone using the VISA strain [Fig. 2b, p <0.003]). These results suggest that Def exposure significantly enhanced cell surface Van binding in both study strains. Importantly, the increased Van binding in the combination groups correlated with their in vitro susceptibility profiles above (Fig. 1). Although the VISA strain appears to have bound less Van than the MRSA strain (63.52±10.47 % for the VISA strain vs. 78.50±5.72 % for the MRSA strain), this difference was not statistically significant (p >0.05).

Def+Van combination therapy is effective against *S. aureus* in vivo

We also studied the effect of combination Def+Van therapy in vivo using a murine staphylococcemia model of infection. Our preliminarily studies showed that the kidneys represented a major target organ in this model (Fig. 3a). Therefore, we selected this organ as one principle readout target for the in vivo efficacy of Def+Van in terms of MRSA burdens. We also evaluated the effect of drug treatments in the spleen, since this organ is an important clinical target in staphylococcemic patients [28, 29]. Moreover, the spleen is a very important lymphoid organ, and our drug treatments might have an effect on enhancing the immune response to *S. aureus* infections. As shown in Fig. 3b, c, the kidneys had an MRSA LAC300 bacterial load of >8 $\log_{10}$ CFU/g, as compared to ~7 $\log_{10}$ CFU/g for VISA-infected mice (placebo groups). Only Def+ Van combination therapy substantially reduced bacterial burdens in the kidneys of mice infected with MRSA LAC300 (Fig. 3b) (p <0.02) or VISA strains (Fig. 3c) (p <0.04) when compared to placebo (control) mice. Further, combination therapy significantly reduced kidney bacterial burden as compared to Van alone (p =0.017 or <0.04 for MRSA LAC300 [Fig. 3b] or VISA [Fig. 3c] strains, respectively) and compared to Def-treated mice infected with MRSA LAC300 (Fig. 3b) (p =0.015). Interestingly, Van treatment alone did not result in a significant decrease in the kidney bacterial burden (Fig. 3b, c). Combination therapy also significantly reduced splenic bacterial burden as compared to placebo or in mice treated with Def alone (p <0.005 [Fig. 3b]), and strongly trended to reductions in bacterial burden compared to Van alone treated mice in animals infected with the MRSA LAC300 strain (p =0.06). However, combination therapy had no effect on the spleen bacterial burden in mice infected with the VISA strain when compared to Van or Def treatment alone (Fig. 3c).

Because kidneys represented the primary target organ, we further investigated the effect of combination treatment on the histology of this organ collected from mice infected with the MRSA LAC300 strain and treated with the above treatment regimens. The histopathological examination corroborated the CFU results, since kidneys collected from combination-treated mice demonstrated fewer abscesses with less staphylococcal organisms seen on Gram stain compared to the placebo or monotherapy treatment arms (Fig. 4).

Discussion

Iron is not only a critical nutrient source for microorganism growth, but it also enhances microbial virulence and impairs host antimicrobial responses [30, 31]. Existing data suggest that iron acquisition is critical for *S. aureus* survival and its subsequent virulence [6, 7]. For example, *S. aureus* harboring mutations in a gene cluster required for the transport of heme iron were less virulent in animals, with a diminished ability to form abscesses in kidneys and liver as compared to a wild-type strain [32]. We have previously demonstrated the feasibility of using the iron chelator, Def, in combination therapy with antifungal drugs in mice infected with aspergillosis and mucormycosis [16–18]. Given the importance of iron in the virulence of *S. aureus*, we studied the potential use of Def in combination with Van against two prototypical MRSA isolates reflecting the range of current pathogenic strains. Our results showed that Def+Van combination therapy was more efficacious than single-drug therapy. This enhanced combination drug effect was very pronounced in vitro (with growth inhibition of both MRSA and VISA strains), while being rather modest in vivo.

There are no published data on the pharmacokinetics of Def in mice at the administered dose of 30 mg/kg/d. However, data from dogs, rats, and marmosets using a range of 10–25 mg/kg Def dose indicate rapid absorption of the drug, with maximal plasma concentrations reached within 1 h, followed by rapid decrease in the drug concentration, with an estimated half-life of ~1 h [33]. Importantly, Def demonstrates a time-dependent, rather than concentration-dependent, killing activity [16]. Thus, as a time-dependent effector drug, maintaining the concentration of the iron chelator above the MIC of the organism throughout the dosing interval most closely correlates with the antimicrobial effect. Therefore, the relatively modest efficacy of the chelator–vancomycin combination therapy in vivo may be explicable by the relatively high MIC and MBC of Def (50 mg/L), a Def level unlikely to be maintained in mice for a long period of time, given its relatively short half-life. Finally, in humans, a Def dose of 20 mg/kg yields achievable mean serum peak and trough levels of only ~38 and ~17 mg/L, whereas a dose of 40 mg/kg yields an achievable mean serum peak and trough levels of only ~86 and ~26 mg/L [34]. Hence, it is possible that Def may be ultimately more useful as a topical agent for superficial infections or as a mucosal MRSA ‘decolonizer’ (rather than for treating systemic infections).

Our data show that Def treatment significantly enhances the capacity of Van to bind to the cell surface of *S. aureus* (Fig. 2). It is not clear how a reduction in iron uptake leads to increased Van surface binding in *S. aureus*. However, it is tempting to postulate that this Def-associated augmentation of Van surface binding accounts, at least in part, for the in vitro and in vivo synergy of this drug combination against *S. aureus*. Our results provide an impetus into further studies to assess the potential of such combination therapy against *S.*

aureus infections, and into understanding the mechanism behind this possible synergistic effect between these two drugs.

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References

1. van Belkum A, Melles DC, Nouwen J, van Leeuwen WB, van Wamel W, Vos MC, Wertheim HF, Verbrugh HA. Co-evolutionary aspects of human colonisation and infection by *Staphylococcus aureus*. Infect Genet Evol. 2009; 9(1):32–47. [PubMed: 19000784]
2. Mertz PM, Cardenas TC, Snyder RV, Kinney MA, Davis SC, Plano LR. *Staphylococcus aureus* virulence factors associated with infected skin lesions: influence on the local immune response. Arch Dermatol. 2007; 143(10):1259–1263. [PubMed: 17938339]
3. Millar BC, Prendergast BD, Moore JE. Community-associated MRSA (CA-MRSA): an emerging pathogen in infective endocarditis. J Antimicrob Chemother. 2008; 61(1):1–7. [PubMed: 17962214]
4. Saavedra-Lozano J, Mejías A, Ahmad N, Peromingo E, Ardura MI, Guillen S, Syed A, Cavuoti D, Ramilo O. Changing trends in acute osteomyelitis in children: impact of methicillin-resistant *Staphylococcus aureus* infections. J Pediatr Orthop. 2008; 28(5):569–575. [PubMed: 18580375]
5. Pittet D, Wenzel RP. Nosocomial bloodstream infections. Secular trends in rates, mortality, and contribution to total hospital deaths. Arch Intern Med. 1995; 155(11):1177–1184. [PubMed: 7763123]
6. Hammer ND, Skaar EP. Molecular mechanisms of *Staphylococcus aureus* iron acquisition. Annu Rev Microbiol. 2011; 65:129–147. [PubMed: 21639791]
7. Mazmanian SK, Skaar EP, Gaspar AH, Humayun M, Gornicki P, Jelenska J, Joachmiak A, Missiakas DM, Schneewind O. Passage of heme-iron across the envelope of *Staphylococcus aureus*. Science. 2003; 299(5608):906–909. [PubMed: 12574635]
8. Mack J, Vermeiren C, Heinrichs DE, Stillman MJ. In vivo heme scavenging by *Staphylococcus aureus* IsdC and IsdE proteins. Biochem Biophys Res Commun. 2004; 320(3):781–788. [PubMed: 15240116]
9. Skaar EP, Schneewind O. Iron-regulated surface determinants (Isd) of *Staphylococcus aureus*: stealing iron from heme. Microbes Infect. 2004; 6(4):390–397. [PubMed: 15101396]
10. Tiedemann MT, Heinrichs DE, Stillman MJ. Multiprotein heme shuttle pathway in *Staphylococcus aureus*: iron-regulated surface determinant cog-wheel kinetics. J Am Chem Soc. 2012; 134(40): 16578–16585. [PubMed: 22985343]
11. Konetschny-Rapp S, Jung G, Meiwes J, Zähner H. Staphyloferrin A: a structurally new siderophore from staphylococci. Eur J Biochem. 1990; 191(1):65–74. [PubMed: 2379505]
12. Drechsel H, Freund S, Nicholson G, Haag H, Jung O, Zähner H, Jung G. Purification and chemical characterization of staphyloferrin B, a hydrophilic siderophore from staphylococci. Biometals. 1993; 6(3):185–192. [PubMed: 8400765]
13. Courcol RJ, Trivier D, Bissinger MC, Martin GR, Brown MR. Siderophore production by *Staphylococcus aureus* and identification of iron-regulated proteins. Infect Immun. 1997; 65(5): 1944–1948. [PubMed: 9125585]
14. Modun B, Williams P. The staphylococcal transferrin-binding protein is a cell wall glyceraldehyde-3-phosphate dehydrogenase. Infect Immun. 1999; 67(3):1086–1092. [PubMed: 10024547]

15. Singh N, Sun HY. Iron overload and unique susceptibility of liver transplant recipients to disseminated disease due to opportunistic pathogens. Liver Transpl. 2008; 14(9):1249–1255. [PubMed: 18756456]
16. Ibrahim AS, Gebermariam T, Fu Y, Lin L, Husseiny MI, French SW, Schwartz J, Skory CD, Edwards JE Jr, Spellberg BJ. The iron chelator deferasirox protects mice from mucormycosis through iron starvation. J Clin Invest. 2007; 117(9):2649–2657. [PubMed: 17786247]
17. Ibrahim AS, Gebremariam T, French SW, Edwards JE Jr, Spellberg B. The iron chelator deferasirox enhances liposomal amphotericin B efficacy in treating murine invasive pulmonary aspergillosis. J Antimicrob Chemother. 2010; 65(2):289–292. [PubMed: 19942619]
18. Ibrahim AS, Gebremariam T, Luo G, Fu Y, French SW, Edwards JE Jr, Spellberg B. Combination therapy of murine mucormycosis or aspergillosis with iron chelation, polyenes, and echinocandins. Antimicrob Agents Chemother. 2011; 55(4):1768–1770. [PubMed: 21263057]
19. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. CLSI; Wayne, PA: 2009.
20. Lemaire S, Kosowska-Shick K, Julian K, Tulkens PM, Van Bambeke F, Appelbaum PC. Activities of antistaphylococcal antibiotics towards the extracellular and intraphagocytic forms of *Staphylococcus aureus* isolates from a patient with persistent bacteraemia and endocarditis. Clin Microbiol Infect. 2008; 14(8):766–777. [PubMed: 18727801]
21. Xiong YQ, Van Wamel W, Nast CC, Yeaman MR, Cheung AL, Bayer AS. Activation and transcriptional interaction between agr RNAII and RNAIII in *Staphylococcus aureus* in vitro and in an experimental endocarditis model. J Infect Dis. 2002; 186(5):668–677. [PubMed: 12195354]
22. Xiong YQ, Willard J, Yeaman MR, Cheung AL, Bayer AS. Regulation of *Staphylococcus aureus* alpha-toxin gene (*hla*) expression by *agr*, *sarA*, and *sae* in vitro and in experimental infective endocarditis. J Infect Dis. 2006; 194(9):1267–1275. [PubMed: 17041853]
23. Hilliard JJ, Fernandez J, Melton J, Macielag MJ, Goldschmidt R, Bush K, Abbanat D. In vivo activity of the pyrrolopyrazolyl-substituted oxazolidinone RWJ-416457. Antimicrob Agents Chemother. 2009; 53(5):2028–2033. [PubMed: 19273686]
24. Pistella E, Falcone M, Baiocchi P, Pompeo ME, Pierciaccante A, Penni A, Venditti M. In vitro activity of fosfomycin in combination with vancomycin or teicoplanin against *Staphylococcus aureus* isolated from device-associated infections unresponsive to glycopeptide therapy. Infez Med. 2005; 13(2):97–102. [PubMed: 16220029]
25. Shelburne SA, Musher DM, Hulten K, Ceasar H, Lu MY, Bhaila I, Hamill RJ. In vitro killing of community-associated methicillin-resistant *Staphylococcus aureus* with drug combinations. Antimicrob Agents Chemother. 2004; 48(10):4016–4019. [PubMed: 15388469]
26. Allen GP, Cha R, Rybak MJ. In vitro activities of quinupristin–dalfopristin and cefepime, alone and in combination with various antimicrobials, against multidrug-resistant staphylococci and entero-cocci in an in vitro pharmacodynamic model. Antimicrob Agents Chemother. 2002; 46(8): 2606–2612. [PubMed: 12121940]
27. Eliopoulos GM, Thauvin C, Gerson B, Moellering RC Jr. In vitro activity and mechanism of action of A21978C1, a novel cyclic lipopeptide antibiotic. Antimicrob Agents Chemother. 1985; 27(3): 357–362. [PubMed: 3994349]
28. Attaran S, Wragg A, Awad WI. Aortic valve endocarditis with splenic and brain abscesses: difficult management issues. Heart Surg Forum. 2011; 14(2):E139–E141. [PubMed: 21521679]
29. Goldwater PN. Sterile site infection at autopsy in sudden unexpected deaths in infancy. Arch Dis Child. 2009; 94(4):303–307. [PubMed: 18794179]
30. Alexander J, Limaye AP, Ko CW, Bronner MP, Kowdley KV. Association of hepatic iron overload with invasive fungal infection in liver transplant recipients. Liver Transpl. 2006; 12(12): 1799–1804. [PubMed: 16741903]
31. Weiss G, Fuchs D, Hausen A, Reibnegger G, Werner ER, Werner-Felmayer G, Wachter H. Iron modulates interferon-gamma effects in the human myelomonocytic cell line THP-1. Exp Hematol. 1992; 20(5):605–610. [PubMed: 1587306]
32. Skaar EP, Gaspar AH, Schneewind O. IsdG and IsdI, heme-degrading enzymes in the cytoplasm of *Staphylococcus aureus*. J Biol Chem. 2004; 279(1):436–443. [PubMed: 14570922]

33. Nick H, Wong A, Acklin P, Faller B, Jin Y, Lattmann R, Sergejew T, Hauffe S, Thomas H, Schnebli HP. ICL670A: preclinical profile. Adv Exp Med Biol. 2002; 509:185–203. [PubMed: 12572995]

34. Nisbet-Brown E, Olivieri NF, Giardina PJ, Grady RW, Neufeld EJ, Séchaud R, Krebs-Brown AJ, Anderson JR, Alberti D, Sizer KC, Nathan DG. Effectiveness and safety of ICL670 in iron-loaded patients with thalassaemia: a randomised, double-blind, placebo-controlled, dose-escalation trial. Lancet. 2003; 361(9369):1597–1602. [PubMed: 12747879]

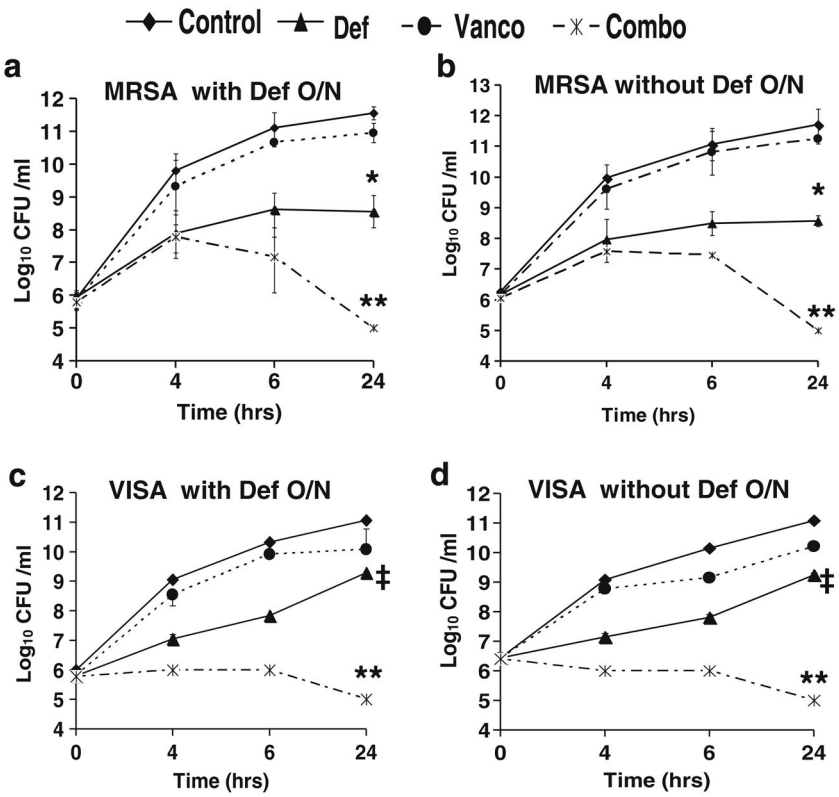

Fig. 1.

Time–kill activity of deferasirox (Def), vancomycin (Van), and combinations versus methicillin-resistant *Staphylococcus aureus* LAC300 or vancomycin-intermediate *S. aureus* (VISA) Mu50. *S. aureus* strains were 'preconditioned' (**a** and **c**) with 0.5× minimum inhibitory concentration (MIC) of Def [25 mg/L of brain heart infusion (BHI)] at 37 °C overnight to reduce intracellular iron stores versus BHI alone ('non-preconditioned' control, **b** and **d**). Preconditioned or non-preconditioned MRSA LAC300 or VISA cells [1×10^6 colony-forming units (CFU)/ml] were transferred into BHI medium containing 1× of MIC of Def (50 mg/L), Van (0.78 mg/L for the MRSA strain and 6.25 mg/L for the VISA strain), or a combination of both drugs. Inoculated BHI medium without drugs served as controls. Aliquots were obtained at the indicated time points for CFU quantification. *p <0.005 vs. control or Van, **p <0.005 vs. control or either drug alone, ‡p < 0.005 vs. control by the Wilcoxon rank-sum test

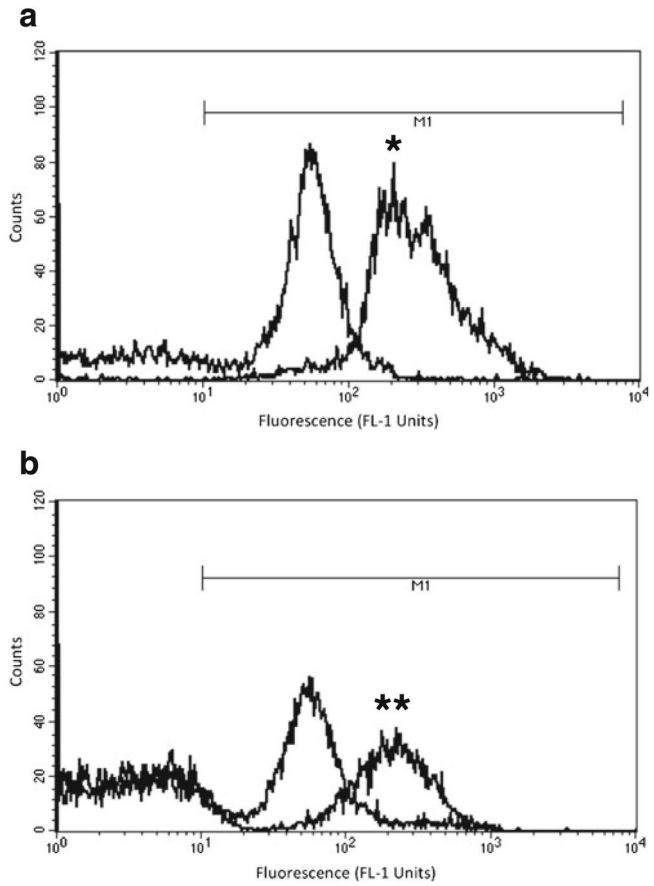

Fig. 2.

Def exposure significantly enhanced Van binding on *S. aureus* MRSA or VISA strains. Van binding to the study *S. aureus* strains was determined by measuring the fluorescence activity of Bodipy®FL Van using flow cytometry. **a**, **b** show the fluorescence units versus the number of MRSA (**a**) and VISA (**b**) cells treated with Van alone (*left curves*) and Van+Def (*right curves*). A shift in the cell population to the right indicates an increased Van binding to *S. aureus* strains. **p* <0.0003, while ***p* <0.003. *M1* represents the Van-bound population

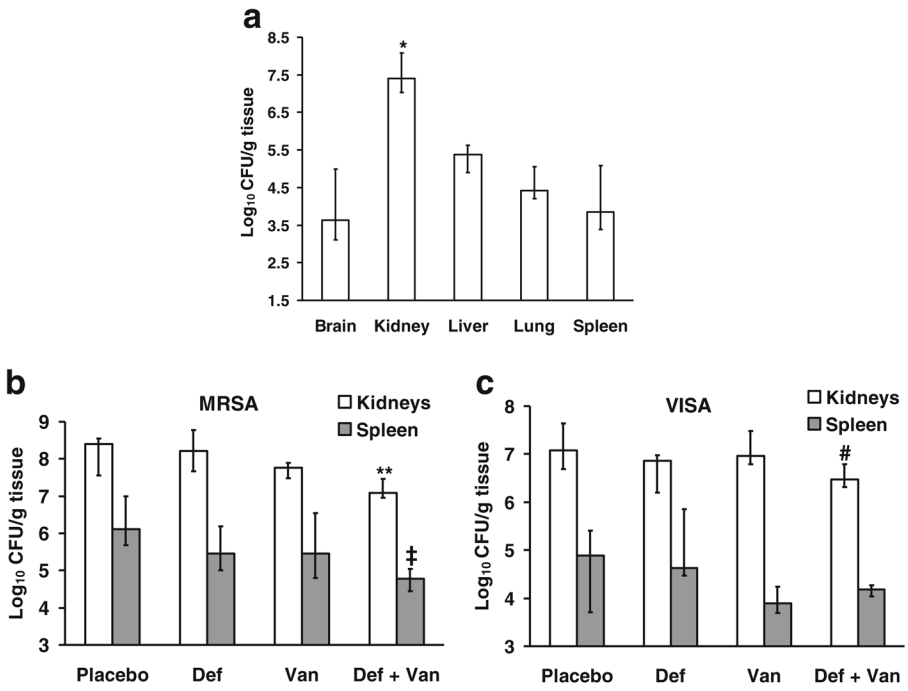

Fig. 3.

Efficacy of Def and Van combination therapy in mice infected with the MRSA LAC300 or the VISA strain. Bacterial tissue tropism was quantitatively determined after intravenous inoculation of mice with the MRSA LAC300 strain (1×10^7 cells) (**a**). For the treatment efficacy studies, mice were infected with the MRSA LAC300 (**b**) or the MRSA VISA strain (**c**) via tail vein injection with 6×10^7 cells. Mice were treated with Def (15 mg/kg/d, n =16 for the MRSA LAC300 strain and n =9 for the VISA strain), Van (2 mg/kg/bid, n =15 for the MRSA LAC300 strain and n =8 for the VISA strain), Def+Van (n =14 for the MRSA LAC300 strain and n =10 for VISA strain), or placebo (n =17 for the MRSA LAC300 strain and n =9 for the VISA strain). Combination treatment significantly reduced bacterial burden in the kidneys of mice infected with either strain (**b**, **c**) and in spleens collected from mice infected with the MRSA LAC300 strain (**b**), but not from mice infected with the MRSA VISA strain (**c**). Data are displayed as medians±interquartile ranges. *p <0.0002 vs. all other organs; **p <0.02 vs. all other treatments; ‡p <0.005 vs. placebo or Def treatments; and #p <0.04 vs. placebo or Van treatments

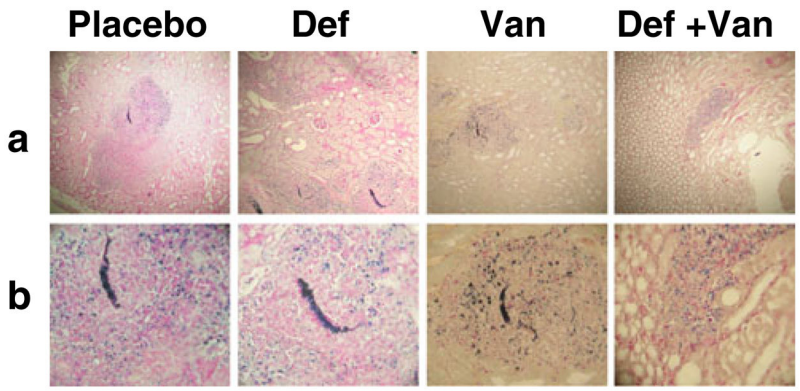

Fig. 4.

Def and Van combination therapy reduced disease severity in mice. Representative histopathological sections from kidneys are shown. All mice were infected with the MRSA LAC300 strain via tail injection with 6×10^7 cells. Although abscesses were seen in kidneys from all treatments, sections from the combination therapy arm had much less bacteria compared to all other treatments. Magnification=40× (**a**) or 400× (**b**). Sections were stained by Gram stain to show *S. aureus* as dark purple clusters