Redeploying β-Lactams Against *Staphylococcus aureus*: Repurposing With a Purpose

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(See the major article by Waters et al on pages 80–7.)

Keywords. β-Lactams; MRSA; MSSA; synergy; *Staphylococcus aureus*.

β-Lactam antibiotics have been a mainstay of clinical therapeutics for approximately 70 years, especially for methicillin-susceptible *Staphylococcus aureus* (MSSA) infections. Since approximately one half of *S. aureus* bacteremias are caused by MSSA [1], the antistaphylococcal β-lactams remain key elements of therapeutic strategies for such infections. Data from a number of clinical trials have documented the therapeutic superiority of antistaphylococcal β-lactams over vancomycin for MSSA bacteremic infections, including endocarditis [2–4]. Further, the American Heart Association has consistently recommended β-lactams as the treatment of choice for MSSA endocarditis [5].

Recently, the antistaphylococcal β-lactams have emerged as an additional tool for treating recalcitrant methicillin-resistant *S. aureus* (MRSA) bacteremic infections, often in combination with daptomycin. The β-lactams that have been deployed off label in combination for this scenario include nafcillin, oxacillin, and cefaroline [6, 7]. This apparent synergy extends to both persistent MSSA infections and persistent MRSA infections, suggesting that a novel mechanism(s) is involved. Studies from several laboratories [8, 9] have identified that the key synergistic event is likely the capacity of the β-lactams of interest to block penicillin-binding protein1 (PBP1). This synergistic event with daptomycin occurs whether the PBP1 blockade is promiscuous (ie, whether, like nafcillin, it binds to PBP1–4) or occurs in a more PBP1-specific manner [10]. Of interest, this daptomycin–β-lactam synergy is also seen with other cationic peptides, including those of the innate host defense system (eg, LL-37) [10]. The 2 main theories about the mechanism(s) of this synergy between cationic peptides (eg, calcium daptomycin) and PBP1-targeting β-lactams are (1) enhanced binding of daptomycin to the divisome, its principal site of action; and/or (2) augmentation of the functional activity of daptomycin without increasing binding [6].

In the current issue of *The Journal of Infectious Diseases*, Waters et al [11] propose yet another role for β-lactams in anti-staphylococcal therapeutics—an antivirulence mechanism to enhance clinical outcomes in MRSA infections, using oxacillin as the proof-of-principle β-lactam. It should be emphasized that defining an agent’s specific antivirulence properties is difficult. This difficulty is because virulence per se potentially encompasses the sum of a complex set of pathophysiologic events and metrics: (1) in vitro effects on growth rates and/or growth yields, (2) organism transmissibility (ie, the ability to colonize and persist on biologic surfaces, such as on nasal epithelium or on damaged cardiac valves), (3) intrinsic pathogenicity at the site of infection (including toxin production and biofilm formation), and (4) the capacity to evade the innate and adaptive immune systems.

Repurposing existing compounds for influencing bacterial virulence or the outcomes of bacterial infections has become de rigueur over the past 2 decades. Examples include (1) using statins to improve outcomes in sepsis and bacterial pneumonias [12, 13]; (2) using statins to reduce the capacity of *S. aureus* to produce carotenoid pigments, thus improve the ability of the host to eliminate this organism via the oxidative limb of the innate immune system [14]; (3) using aspirin and its congeners to enhance antistaphylococcal therapeutics [15]; and (4) using azithromycin and other macrolides as immunomodulating agents in treating infections [16]. The notion of repurposing β-lactams to affect bacterial virulence independently, over and above their intrinsic bactericidal effects, is not new. More than 30 years ago, a number of experimental endocarditis investigations confirmed that subbactericidal exposures of viridans group streptococci to β-lactam agents impeded the capacity of these pathogens to adhere to and colonize cardiac vegetations [17–19]. This nonbactericidal impact of β-lactams against endocarditis-causing pathogens, confirmed experimentally, has been leveraged into the current approach to antimicrobial prophylaxis of endocarditis, as recommended by the American Heart Association [20]. Thus, β-lactam...
prophylaxis is recommended to be given as late as 2 hours after invasive dental procedures in patients with high-risk underlying valve diseases, taking advantage of the probable antiadherence plus pro-opsonophagocytic properties of these antibiotics [20].

Waters et al [11] substantially advance our understanding of the antivirulence properties of the β-lactams both in vitro and in vivo. In vitro, these investigators show that β-lactams repress toxin production in MRSA via an agr-inhibitory mechanism. They provide several cogent lines of evidence in this regard.

Using indirect evidence of cytolytic toxin repression by β-lactams (in human neutrophil lysis assays), they demonstrated generally reduced lysis with oxacillin exposures. Unfortunately, this effect was not studied with murine neutrophils (which is more pertinent to their experimental in vivo model). Murine neutrophils are relatively resistant to the effects of important staphylococcal toxins, including Panton-Valentine leukocidins [21]. Moreover, the outcomes of toxin suppression and neutrophil lysis were medium dependent, with oxacillin actually increasing lysis in CCY medium. This increase in lysis brings to mind the study by Kernodle et al [22], in which naftillin increased the capacity of 37 S. aureus strains (both MSSA and MRSA) to induce α toxin production by both agr and non-agr mechanisms. Moreover, in that latter investigation, culture supernatants from naftillin-induced S. aureus strains actually increased murine virulence in an intraperitoneal infection model. We are left with the conclusion that the antivirulence effects of β-lactams, as documented by Waters et al [11], may well be strain dependent.

By RNA sequencing (comparing oxacillin-exposed vs control cells), the authors show evidence of agr repression. Moreover, agr knockouts of the study strains demonstrated a reduced capacity to lyse neutrophils.

Of note, oxacillin exposures led to substantial effects on the cell wall machinery of S. aureus, including activation of wall teichoic acid production, which translated into augmentation of C3b complement deposition and enhanced opsonophagocytosis. Again, the readouts of these latter functional assays were performed using human phagocytes; the linkage with outcomes in their murine infection models remains unproven.

Finally, in their murine in vivo studies, Waters et al used 2 distinct models, bacteremia and pneumonia. As with most in vivo investigations, the devil is in the details. For example, at 28 hours after infection in the bacteremia model, there was little bacterial load difference in kidneys and only modest differences in spleens of animals treated with 2 oxacillin doses (7.5 and 75 mg/kg). At 7 days after infection, there was significantly reduced virulence in both kidneys and spleens, although in kidneys, there was a heterogeneous outcome from animal to animal, featured by overlapping of untreated control and oxacillin-treated animal bacterial loads. Given that oxacillin exposures will increase opsonophagocytic killing in vitro, it makes sense that the spleen would exhibit the largest readouts. In contrast, in the pneumonia model, there was a significant impact on both blood culture clearances and in vivo survival in the 2 oxacillin dose regimens. Curiously, no lung bacterial load data were presented.

The investigations by Waters et al [11] are well done, clearly presented, and hypothesis generating. Further investigations with more animal models and additional MRSA strains should be done. Moreover, advanced randomized clinical trials addressing the effects of β-lactams in MRSA infections need to be performed. It is encouraging that a recent, small (60 patient) open-label and randomized trial in Australia of vancomycin, with or without the β-lactam fluoxacillin, demonstrated reduced duration of bacteremia by approximately 1 day in the combination therapy group [23]. This bacteremia duration difference, however, was not statistically significant (P = .06), and there was no discernible salutary effect of the combination regimen on either 28-day or 90-day mortalities or on apparent metastatic infection frequencies.

There are substantial notes of caution in metric outcomes and data interpretations in the article by Waters et al. In addition, there are limited clinical data published to date on β-lactam enhancement of MRSA treatment efficacy. Thus, the recommendation by Waters et al that “β-lactam antibiotics should be included in the treatment regimen for patients with MRSA infections” [11] seems somewhat premature. This issue, albeit very important for practitioners, awaits the outcomes of large, randomized controlled clinical trials for definitive adjudication.

Note

Potential conflict of interest. A. S. B. reports grants and other supported activities from ContraFect Pharmaceuticals, grants from Debiopharma International, and grants from Trellis Bioscience, all outside of the submitted work. Y. Q. X. reports research grants from Trellis Bioscience, ContraFect Pharmaceuticals, and Debiopharma International, all outside of the submitted work. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

6. Dhand A, Bayer AS, Pogliano J, et al. Use of antistaphylococcal beta-lactams to increase daptomycin activity in eradicating persistent bacteria due to...