Antimicrobial Effects Promoting Biofilm Formation and Persistent Disease: The Role of a DNA-Binding Regulator, SarA, in Staphylococcal Endocarditis

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(See the major article by Abdelhady et al on pages 1231–40.)

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Vancomycin, the archetypical glycopeptide introduced into clinical use as early as 1958, has been the mainstay of antiinfective therapy directed against gram-positive organisms resistant to β-lactams, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), ampicillin-resistant enterococci, and penicillin-resistant *Streptococcus pneumoniae*. In the early years after introduction into clinical use, numerous untoward effects were ascribed primarily to impurities (ie, the notorious “Mississippi mud,” as early preparations of vancomycin were termed, because of its impurity-tainted color), while more recent preparations have become more acceptable because of improved manufacturing. Vancomycin’s antibacterial mode of action is well characterized and consists of an inhibition in cell wall synthesis due to tight binding of the compound to peptidoglycan monomers containing D-alanyl-D-alanine at their free carboxyl ends, resulting in steric hindrance of transglycosylase, transpeptidase, and carboxypeptidase activity and, ultimately, in cell wall synthesis interruption. As a result, the rationale for clinical use of vancomycin appears now well-defined, both with respect to mode of action and untoward side effects, yet the emergence of reduced vancomycin susceptibility remains a major clinical issue.

Besides the so-called classical mode of action (target of bacterial resistance mechanisms), vancomycin exerts additional activities on bacteria, such as alteration of cell membrane permeability and inhibition of RNA synthesis [1]. Some of these effects can be ascribed to the autolysis induced by subinhibitory vancomycin concentrations affecting cell subpopulations, and leading to release of extracellular DNA [2], which, in turn, has been associated with formation and stabilization of biofilms [3]. Moreover, exposure of staphylococci to subinhibitory levels of vancomycin may modify the expression of staphylococcal response regulators, such as the alternative transcription factor σ^B^, and of σ^B^ -dependent genes, such as *hla* and *fnbA* [4]. Moreover, a concept emerges of vancomycin effects in addition to and/or promoted by its classical mode of action, in which there is a complex adaptation of the entire bacterial cell upon vancomycin exposure, resulting in increased cell wall turnover and reduced functionality of the staphylococcal accessory gene regulator (*agr*), which affects expression of multiple genes [5]. Interestingly, such vancomycin-induced phenomena appear to be observable in vancomycin-susceptible *S. aureus* (VSSA) and *S. aureus* with heterogeneous or intermediate susceptibility to vancomycin [5]. Moreover, intermediate vancomycin susceptibility is associated with impaired acetate catabolism; this phenomenon has been implicated in bacterial growth, antibiotic tolerance, and entry into the bacterial death phase, as well as in the expression and synthesis of the major biofilm component, the polysaccharide intercellular adhesin [6]. Although such an effect has not yet been tested as a function of direct vancomycin exposure, it can be speculated that it is not restricted to isolates of reduced vancomycin susceptibility but may even be observable in VSSA strains exposed to vancomycin. From a clinical standpoint, we have to consider that use of vancomycin (and, probably, related compounds of the glycopeptide/lipopeptide family) may not only fail to kill the offending bug (owing to poor killing activity and elevated minimum inhibitory concentrations [MICs]) but may also generate other untoward, ill-defined but clinically important effects on the
bacterium and its physiology that promote a metabolically adapted, biofilm-embedded, and difficult-to-treat bacterial consortium.

It is exactly this topic that is addressed by the contribution of Abdelhady et al in this issue of the Journal [7]. The authors investigated the effects of the exposure of healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) to subinhibitory concentrations of vancomycin, both in vitro and in vivo. Although for obvious reasons, in their article they first present the in vitro results, I will comment initially on the in vivo results. In the well-established rabbit endocarditis model, upon exposure of rabbits to clinically relevant doses of vancomycin (15 mg/kg and 7.5 mg/kg for HA-MRSA and CA-MRSA, respectively, twice daily for 3 days), the authors observed only a limited effect of vancomycin in all but 1 of the 4 isolates tested. In fact, for both the HA-MRSA isolates and 1 CA-MRSA isolate, the bacterial load in the vegetation was not altered (<1 log difference) by vancomycin treatment; only a modest reduction was achieved in either kidney or spleen bacterial loads (one strain, JE2, responded in a more pronounced manner, with a 2–4-log reduced recovery in the vegetations and organs). This experimental observation corresponds to clinical experience with vancomycin failures frequently reported in S. aureus infective endocarditis [8], particularly when treating isolates with relatively high vancomycin MICs.

An exciting observation, however, was made as isogenic mutants deficient in the sarA locus were used. The sarA locus confers control on multiple secondary regulator and target genes and has been associated with reduced virulence in various animal models. The prior observation that upon vancomycin exposure, staphylococcal biofilm formation can be enhanced [2], in conjunction with the well-documented effect of SarA as a regulator of biofilm development, prompted the authors to analyze these mutants. The efficacy of vancomycin treatment was restored in the sarA mutants, with a highly significant and important (>4-log) reduction in bacterial loads for all strains over all vegetation and tissue samples analyzed.

The mechanisms contributing to these in vivo results are suggested by the findings of significantly elevated sarA transcript levels upon exposure to 0.5 times the MIC of vancomycin in vitro and of higher sarA expression profiles in cardiac vegetation specimen from infected animals. Accordingly, biofilm formation was promoted by vancomycin, as was fibrinectin binding, whereas protease and nuclease activity were unaltered.

The importance of SarA for target tissue persistence demonstrated here, particularly under vancomycin treatment, allows us to extend further our hypotheses on regulator-dependent mechanisms of persistent staphylococcal disease. For example, Johnson et al showed that under low-iron conditions, molecules of the SERAM family of adhesive secreted staphylococcal molecules (ie, Emp and Eap) are important for biofilm formation and that, in turn, their regulation depends on iron-responsive regulators, such as Fur, as well as on global regulators, such as Sae, Agr, and SarA [9]. Our own group showed that Eap (and Emp) expression critically depends on Sae [10] and, more recently, that hemin represses (and the iron chelator bipyridine restores) both hemolytic activity and Eap expression in a Sae-dependent manner [11]. In conjunction with the results obtained by Abdelhady et al, these observations allow for further interesting hypotheses about the regulation of biofilm formation, persistence, and vancomycin efficacy, particularly under low-iron conditions, as occur in the endovascular milieu.

What perspectives can be drawn from these findings? The results obtained by Abdelhady et al suggest that a combination approach of a bactericidal treatment by a glycopenidate in conjunction with modulation of global staphylococcal regulator (SarA) activity might be desirable. Currently, no small molecular SarA inhibitors have been reported, but, as indicated in a recent review by Gordon et al [12], the crystal structure of SarA in conjunction with mutagenesis studies provide insights for potential structure-based pharmacological designs [13]. Noteworthy, another member of the SarA family of regulators, Mgra, is already a successful target for inhibitor molecules (eg, 5,5-methylene disalicylic acid), which were shown to cause effector gene alterations and virulence attenuation [14]. At times of epidemic antimicrobial resistance and lagging development of new antimicrobials, it is important to exploit new compounds for attenuation of the course of infection. Yet, in line with the principle in medicine of “do no harm”, the importance of identifying strategies for alleviation of antimicrobial-induced side effects on persistent microbes may even be more compelling. The novel information provided by Abdelhady et al may now open new approaches toward both needs.

Notes

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References


