CONCISE COMMUNICATION

In Vitro Resistance to Thrombin-Induced Platelet Microbicidal Protein in Isolates of Staphylococcus aureus from Endocarditis Patients Correlates with an Intravascular Device Source

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Platelet microbicidal proteins (PMPs) are small antimicrobial peptides secreted by mammalian platelets. In vitro resistance of Staphylococcus aureus strains to PMPs correlates with more extensive disease in experimental infective endocarditis (IE). To determine whether this same relationship exists in human S. aureus IE, we evaluated the in vitro PMP susceptibility phenotype of isolates from 58 prospectively-identified patients with definite S. aureus IE. On multivariate analyses, patients with S. aureus IE complicating an infected intravascular device were significantly more likely to have IE caused by a PMP-resistant strain (P = .0193). No correlations were detected between in vitro PMP resistance among S. aureus strains and the severity of human IE. This work supports the concept that in vitro PMP resistance in clinical S. aureus strains is associated with important clinical characteristics of S. aureus endovascular infections in vivo.

A host defense role for platelets against endovascular infections has been linked to platelet microbicidal proteins (PMPs) [1–8]. PMPs are released from platelets at sites of endovascular damage or microbial colonization and kill common bloodstream pathogens, including Staphylococcus aureus, in vitro. Because the isolation of S. aureus from the bloodstream is frequent, but S. aureus endovascular infections (e.g., infective endocarditis [IE]) are uncommon, PMPs may play a role in preventing serious endovascular infections [8].

Because organisms exhibiting PMP-resistance in vitro have a distinct survival advantage for induction and/or propagation of endovascular infections [2–4], we reasoned that in vitro PMP resistance in clinical S. aureus strains might also be related to a more severe clinical course in patients with IE caused by such strains. Recent in vivo data support this hypothesis. Animal models infected with a PMP-resistant strain of S. aureus had larger valvular vegetations, higher vegetation and renal abscess densities, slower rates of bacteremia clearance, and earlier onset and greater degree of aortic regurgitation than did animal models with IE induced by an isogenic PMP-susceptible strain of S. aureus [5].

To study the relationship between the PMP susceptibility phenotypes of S. aureus and the clinical manifestations of human S. aureus IE more systematically, we designed the present study to include the following important features: (1) evaluation of a relatively large group of bacteremic isolates from prospectively identified patients with definite S. aureus IE at a single medical center; (2) utilization of well-defined and well-validated clinical criteria [9] to prospectively designate cases of IE; and (3) performance of PMP susceptibility assays by blinded investigators at a center different from that enrolling study patients.

Materials and Methods

S. aureus strains. Blood culture isolates were identified as S. aureus and stored for future use, as described elsewhere [10].

Patient selection. Patients with S. aureus bacteremia were prospectively identified between September 1994 and May 1998, evaluated for IE, and followed up for 12 weeks after the date of the initial positive blood culture, as described elsewhere [10, 11]. For the current study, only isolates from patients with clinically or pathologically definite IE were further analyzed. IE was defined according to the Duke criteria [9]. Embolic events were defined as any clinical or radiographic evidence of thromboembolic episodes...
(e.g., a cerebrovascular embolic event or a cutaneous or deep-tissue infarct). A cerebrovascular embolic event was defined as the presence of both clinical (i.e., focally abnormal neurologic examination results) and radiologic evidence of an acute thromboembolic event. Renal involvement complicating IE was defined as the presence of hematuria plus a positive urine culture for \textit{S. aureus}. Septic shock was defined according to Consensus guidelines [12].

**Echocardiography.** Specific echocardiographic findings were analyzed as individual indicators of IE, as described elsewhere [11]; 57 of the 58 study patients underwent some form of echocardiography. Nine of the 58 study patients with definite IE underwent transthoracic echocardiography (TTE) only, 4 underwent transesophageal echocardiography (TEE) only, and 44 received both TTE and TEE. All echocardiograms were read at the time of the procedure by an experienced echocardiographer. To reduce potential bias, all echocardiograms were later blindly reinterpreted by one of the authors (G.E.P.). Vegetation size was calculated in square millimeters, as described elsewhere [13]. The median value was 61.5 mm²; vegetations \(>61.5 \text{ mm}^2\) were defined as “large.”

**In vitro PMP assays.** Because all our prior data on the relationships between in vitro PMP susceptibility phenotypes in \textit{S. aureus} and in vivo infection progression involved the use of thrombin-induced PMP-1 (tPMP-1), we used this peptide in the current study. The preparation of tPMP-1, bioactivity assays of tPMP-1 preparations, and in vitro assays for tPMP-1 susceptibility phenotypes were performed as detailed elsewhere [7]. In vitro PMP assays were performed previously in 18 of the 58 isolates as part of another report [2].

Our previous in vitro analyses of common bloodstream pathogens for tPMP-1 susceptibility profiles revealed little overlap of strains from patients with well-defined IE versus strains from patients without any evidence of IE, at a breakpoint of \(\geq 70\%\) survival in the 2 h in vitro assay [2, 6]. For this reason, we categorized all strains exhibiting \(\geq 70\%\) survival following a 2 h exposure to tPMP-1 as “resistant” for the current study. All assays and data analyses of tPMP-1 in vitro studies were performed by 2 investigators (A.S.B. and M.R.Y.), who were blinded as to the clinical details of the patients.

**Statistical analyses.** Bivariate analyses were conducted between in vitro PMP-1 susceptibility profiles on individual patient isolates (i.e., resistant vs. susceptible) and clinical outcome variables in these patients with IE, using logistic regression. Odds ratios (ORs) were computed, and the Wald \(\chi^2\) test was used to determine significance; inferences were confirmed using exact methods [14]. Relationships were considered significant when the 2-sided \(P\) value was \(<0.05\) (table 1). The OR for relapse compared with all other outcome variables was not estimable, because none of the patients with IE caused by a tPMP-1-resistant \textit{S. aureus} isolate experienced

### Table 1. Relationships between resistance to thrombin-induced platelet microbicidal protein (tPMP-1), clinical and echocardiographic characteristics, and clinical outcome in 58 patients with \textit{Staphylococcus aureus} infective endocarditis (IE).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>tPMP-1 susceptible ((n = 19 [33%]))</th>
<th>tPMP-1 resistant ((n = 39 [67%]))</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical characteristics</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fever</td>
<td>9 (47)</td>
<td>16 (41)</td>
<td>0.773</td>
<td>0.254-2.355</td>
<td>647</td>
</tr>
<tr>
<td>Septic shock</td>
<td>8 (42)</td>
<td>17 (44)</td>
<td>1.063</td>
<td>0.551-2.097</td>
<td>915</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>4 (21)</td>
<td>5 (13)</td>
<td>0.551</td>
<td>0.128-2.497</td>
<td>421</td>
</tr>
<tr>
<td>Hemodialysis dependency</td>
<td>3 (13)</td>
<td>18 (46)</td>
<td>4.571</td>
<td>1.269-16.960</td>
<td>031</td>
</tr>
<tr>
<td>Injection drug use</td>
<td>7 (37)</td>
<td>1 (3)</td>
<td>0.045</td>
<td>0.002-0.288</td>
<td>0056</td>
</tr>
<tr>
<td>Embolic events, any(^a)</td>
<td>10 (53)</td>
<td>13 (33)</td>
<td>0.450</td>
<td>0.144-1.374</td>
<td>1623</td>
</tr>
<tr>
<td>Cerebrovascular event(^b)</td>
<td>3 (16)</td>
<td>5 (13)</td>
<td>0.784</td>
<td>0.170-4.200</td>
<td>7587</td>
</tr>
<tr>
<td>Renal involvement(^c)</td>
<td>1 (5)</td>
<td>5 (13)</td>
<td>2.647</td>
<td>0.387-52.754</td>
<td>391</td>
</tr>
<tr>
<td>Intravascular device source(^d)</td>
<td>3 (16)</td>
<td>25 (64)</td>
<td>9.524</td>
<td>2.626-46.306</td>
<td>002</td>
</tr>
<tr>
<td>Methicillin-resistant \textit{S. aureus}</td>
<td>4 (21)</td>
<td>16 (41)</td>
<td>2.609</td>
<td>0.77-10.47</td>
<td>155</td>
</tr>
<tr>
<td>Nosocomial/nursing home acquired</td>
<td>7 (37)</td>
<td>23 (59)</td>
<td>2.464</td>
<td>0.812-7.95</td>
<td>163</td>
</tr>
<tr>
<td>Echocardiographic characteristics</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Large vegetations</td>
<td>25 (68)</td>
<td>14 (67)</td>
<td>0.960</td>
<td>0.310-3.096</td>
<td>944</td>
</tr>
<tr>
<td>Multiple vegetations(^e)</td>
<td>4 (21)</td>
<td>10 (26)</td>
<td>0.773</td>
<td>0.187-2.756</td>
<td>7019</td>
</tr>
<tr>
<td>Valve thickening</td>
<td>10 (53)</td>
<td>24 (62)</td>
<td>1.376</td>
<td>0.471-4.404</td>
<td>5188</td>
</tr>
<tr>
<td>Periannular destruction(^f)</td>
<td>3 (16)</td>
<td>8 (21)</td>
<td>1.440</td>
<td>0.343-6.936</td>
<td>6675</td>
</tr>
<tr>
<td>Clinical outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death due to IE</td>
<td>6 (32)</td>
<td>9 (23)</td>
<td>0.650</td>
<td>0.192-2.284</td>
<td>0489</td>
</tr>
<tr>
<td>Relapse</td>
<td>4 (21)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complicated endocarditis(^d)</td>
<td>3 (16)</td>
<td>11 (28)</td>
<td>2.095</td>
<td>0.555-10.274</td>
<td>3062</td>
</tr>
</tbody>
</table>

\(^a\) Includes cerebrovascular embolic events (8 patients), deep-tissue emboli (6 patients), and peripheral/cutaneous emboli (9 patients).

\(^b\) Defined as presence of clinical (e.g., focally abnormal neurologic examination results) and radiologic evidence of an acute thromboembolic event.

\(^c\) Defined as hematuria plus a pure growth of \textit{S. aureus} from a urine culture specimen.

\(^d\) Intravascular catheter or synthetic hemodialysis graft; greater than the median vegetation size (61.5 mm²).

\(^e\) At least 1 vegetation identified on echocardiography.

\(^f\) Includes paravalvular abscess, valve avulsion, valve dehiscence, or valve perforation.

\(^g\) Death due to \textit{S. aureus} IE, presence of valvular perforation, paravalvular abscess, ruptured chordae, or need for valve replacement.
a clinical or microbiologic relapse of their infection. Therefore, relapse was not considered in further statistical evaluation models.

To identify any independent associations between in vitro tPMP-1 resistance and specific clinical parameters of the IE study population, multivariate logistic regression analysis was performed. Clinical variables found to be significant in the bivariate analyses were considered as candidates for inclusion in the multivariate analysis model. Thus, we modeled hemodialysis dependence, injection drug use, and intravascular device source in a multivariate logistic regression. Hemodialysis dependence did not independently contribute significantly in the multivariate model, owing to a correlation between hemodialysis dependence and an intravascular device source (Kendall’s $\tau = 0.64$). Therefore, our final multivariate model contained injection drug use and intravascular device source as potential independent correlates of patient outcomes.

Results

Patient selection, categorization, and clinical findings. Sixty-five patients had definite S. aureus IE [9]. Of these 65 patients, blood culture isolates from 58 patients were available for tPMP-1 susceptibility profiling. Among these 58 patients with definite IE, 54 were clinically confirmed and 4 were histopathologically confirmed. Data from these 58 patient-isolate pairs formed the basis for the further analyses in this investigation. Twenty (34%) of the 58 patients were infected with methicillin-resistant S. aureus. Twenty-eight patients (48%) had community-acquired IE, whereas 30 patients (52%) had hospital-acquired or nursing home-acquired IE. Injection drug use was the presumed route of infection in 8 patients, all of whom developed community-acquired S. aureus IE. Four of these 8 patients had right-sided IE, 3 had left-sided IE, and 1 patient had both left- and right-sided IE.

Embolic events ($n = 23$) were confirmed by computed tomography in 11 patients (cerebrovascular and/or deep-tissue embolic events), by chest radiograph in 3 patients (septic pulmonary emboli), and by clinical examination in 9 patients with cutaneous emboli.

Relationship of clinical and echocardiographic features of IE with tPMP-1 susceptibility profiles. Isolates were susceptible to tPMP-1 in 19 patients (33%) and resistant to tPMP-1 in 39 isolates (67%; table 1). Patients with tPMP-1-resistant strains were significantly more likely to have developed IE as a consequence of an infected intravascular device (OR, 9.524; 95% confidence interval [CI], 2.628–46.306; $P = .002$). This association between tPMP-1 resistance and the presence of an intravascular device persisted when multivariate logistic regression analysis was employed (OR, 5.77; 95% CI, 1.445–29.543; $P = .0193$). These patients with tPMP-1–resistant strains were also more likely to be hemodialysis dependent than were patients with IE due to tPMP-1-susceptible strains.

Several clinical and echocardiographic parameters had been predicted a priori to be associated with tPMP-1 resistance in vitro (table 1). No statistically significant association was detected between tPMP-1 resistance in vitro and any of these clinical or echocardiographic parameters.

The relationships of various study patient characteristics were evaluated. The presence of an intravascular device was strongly correlated with dependence on hemodialysis (Kendall’s $\tau = 0.63623$; $P = .001$) but was negatively correlated with injection drug use (Kendall’s $\tau = -0.38644$; $P = .0035$). Thus, the correlation noted above between tPMP-1 resistance in vitro and hemodialysis dependence was based in the intravascular device infection source, rather than in any parameters specific for hemodialysis. Injection drug use was strongly correlated with community acquisition (Kendall’s $\tau = 0.41$; $P = .0018$).

Patients who were injection drug users were more likely to be infected with tPMP-1–susceptible strains (7 of 8 patients). This association was present in both bivariate (OR, 0.045; 95% CI, 0.002–0.288; $P = .0056$) and multivariate (OR, 0.099; 95% CI, 0.005–0.692; $P = .045$) logistic regression analyses.

Discussion

The present study demonstrates that patients with S. aureus IE complicating an infected intravascular device were ~10-fold more likely to have tPMP-1–resistant blood culture isolates than were patients with IE arising from either an apparent primary focus or a noncatheter source. This finding is consistent with previous results [2]. The association between intravascular device infection and tPMP-1–resistant staphylococci is also consistent with the clinical observation that an indwelling catheter is, overall, an important risk factor for invasive staphylococcal infections [11, 15].

Hemodialysis dependence was significantly associated with IE caused by tPMP-1–resistant strains in bivariate analyses. This finding is consistent with previous observations that hemodialysis-dependent patients are at increased risk for staphylococcal infection [10, 15] and may be due to the strong correlation between hemodialysis dependence and the presence of an intravascular device, rather than any intrinsic characteristics of the hemodialysis population. However, other features of this patient subset might also contribute to the propensity of their tPMP-1–resistant staphylococcal strains to induce IE (e.g., platelet dysfunction related to renal failure [16]).

When S. aureus colonization occurs at vascular catheter sites, platelets accrue at such sites and likely release PMPs. Thus, the survival of colonizing S. aureus strains at these sites would depend, at least in part, on their intrinsic tPMP-1 susceptibility profile [1]. It follows, then, that intravascular infections caused by tPMP-1–resistant strains of S. aureus may be more likely to disseminate and induce IE than are tPMP-1–susceptible counterpart strains. More than two-thirds of the bacteremic S. aureus isolates from this patient cohort with IE were resistant to tPMP-1 in vitro, paralleling our prior observations that tPMP-1–resistant strains are associated with a more progressive form of experimental IE than that seen with isogenic tPMP-1–susceptible
strains [4, 5]. These differences in clinical severity between tPMP-1–resistant and tPMP-1–susceptible strains in experimental IE have been ascribed to an enhanced capacity of the resistant bacteria to survive at, and disseminate from, the infected vegetation site, in the presence of locally secreted tPMP-1 [4].

Clinical and echocardiographic features associated with tPMP-1 resistance in animal IE models [5] were not found to be significantly associated with tPMP-1 resistance in the clinical IE strains evaluated in this study. There are several possible explanations for these latter disparities. First, the duration of clinical IE varied widely, providing a potentially confounding variable. For example, the reported duration of symptoms among the study patients ranged from 1 to 14 days. This wide range in disease duration in patients with clinical IE provided an inconstant interval for the development of clinical complications and/or larger valvular vegetations, regardless of the tPMP-1 susceptibility profile of their infecting strains. By contrast, the duration of experimental IE in the animal studies was equivalent in animals infected by either the tPMP-1–susceptible or tPMP-1–resistant strains. Secondly, experimental IE is induced by a large intravenous bacterial inoculum given as a bolus challenge (10^6–10^7 colony-forming units) [3–5], thus magnifying the chances of developing complications of IE at the site of catheter-induced valvular trauma. Last, our relatively small sample size may have hindered detection of clinical or echocardiographic parameters that are associated with in vitro tPMP-1 resistance.

Of the 8 injection drug users in our study population, 7 (88%) were infected with tPMP-1–susceptible isolates. This association may be due in part to colonization of these injection drug–using patients by a clonally related staphylococcal strain [17]. Outbreaks of infection due to a single bacterial strain among drug users sharing injection paraphernalia commonly occur [18]. Similarly, it is possible that tPMP-1 resistance may be associated with clonally related S. aureus strains among the patients in this current report. Although we cannot exclude this possibility, pulsed-field gel electrophoresis (PFGE) data obtained from a subset of study isolates suggest that multiple strains were present. None of the 5 isolates in the present study that were previously evaluated by PFGE [10] shared identical PFGE banding patterns.

In summary, the present study further supports the concept that, among bacteremic S. aureus strains, resistance to tPMP-1 is associated with specific clinical characteristics of S. aureus endovascular infections in vivo. The presence of an intravascular device in a patient with S. aureus IE is strongly associated with resistance to tPMP-1 in the infecting strain. Moreover, these data underscore the concept that PMPs play an important role in limiting IE in the setting of vascular device infections caused by susceptible strains; in contrast, it appears that resistant strains can circumvent PMP activities at sites of vascular catheter infections and have a survival advantage in terms of inducing IE. Future studies will be necessary to identify whether tPMP-1 resistance in a pathogen infecting an intravascular device also increases the likelihood of local or metastatic complications in addition to IE. Moreover, the mechanism by which tPMP-1 resistance develops in such strains remains to be elucidated.

References