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BRIEF REPORT

Ticagrelor Increases Platelet-Mediated *Staphylococcus aureus* Killing, Resulting in Clearance of Bacteremia

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Platelets are a critical immune defense against *Staphylococcus aureus* bloodstream infections. *Staphylococcus aureus* α-toxin is a virulence factor that decreases platelet viability and accelerates platelet clearance. It has been shown that ticagrelor blocks α-toxin–mediated platelet injury and resulting thrombocytopenia, protecting mice in a lethal *S. aureus* sepsis model. We now present the use of ticagrelor as adjunctive therapy in a patient with a *S. aureus* endovascular infection and thrombocytopenia, associated with restoration of platelet count and bacteremia clearance. Ticagrelor enhanced platelet killing of the *S. aureus* bloodstream isolate from the treated patient in vitro.

Keywords. endovascular infections; *Staphylococcus aureus*; MSSA bacteremia; platelets; ticagrelor; innate immunity.

Staphylococcus aureus is a major bloodstream infection pathogen, leading to life-threatening complications secondary to endovascular seeding, metastatic foci, and disseminated intravascular coagulation. Despite aggressive antimicrobial therapy, *S. aureus* bacteremia is associated with high morbidity and mortality, underscoring the need for novel treatment strategies. There is mounting evidence to suggest that the newest antiplatelet agent, ticagrelor, may have unappreciated properties in vivo that may facilitate bacteremia clearance [1–4]. Ticagrelor is a reversible inhibitor of the platelet adenosine diphosphate P2Y12 receptor approved for the prevention of cardiovascular events in patients with acute coronary syndrome or a history of myocardial infarction [5]. A post hoc analysis of the Comparison of Ticagrelor (AZD6140) and Clopidogrel in Patients With Acute Coronary Syndrome (PLATO) trial [1], revealed that patients treated with ticagrelor had a lower risk of infection-related death than those treated with clopidogrel bisulfate [6]. In the Targeting Platelet-Leukocyte Aggregates in Pneumonia With Ticagrelor (XANTHIPPE) study, ticagrelor was associated with improved lung function in patients hospitalized for pneumonia [2]. In vitro studies have further revealed that ticagrelor, albeit at supraphysiological concentrations of 20–40 mg/L, can kill gram-positive bacteria, such as methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus*, and enterococci, including vancomycin-resistant strains [3]. Interestingly, the therapeutic effect of ticagrelor against *S. aureus* in a murine subcutaneous implant infection model was greater than predicted by in vitro activity [3]. While these effects were only documented at drug concentrations well above those observed in conventionally dosed patients treated for cardiovascular disease, the murine studies suggest an alternative explanation for ticagrelor's anti-infective properties and possible cooperative activity with host innate immune defenses, such as platelets.

Platelets play important functions beyond hemostasis through antimicrobial defense. These cells are able to detect pathogens, recruit leukocytes, and even exert direct bactericidal activity by deploying antimicrobial peptides [7]. Our group and others have found that thrombocytopenia (and not leukocyte count) is associated with increased mortality in *S. aureus* bacteremia irrespective of serum levels of the proinflammatory cytokine interleukin 1β or Acute Physiology and Chronic Health Evaluation (APACHE) score [4]. We demonstrated that the pore-forming α-toxin of *S. aureus* accelerates platelet clearance via the hepatic Ashwell–Morell receptor pathway [4], leading to thrombocytopenia and reduced innate immune clearance of infection. Ticagrelor blocked this α-toxin–mediated thrombocytopenia [4], and was protective in an MSSA-induced murine bacteremia model [4]. These data suggest that ticagrelor may provide benefits in difficult-to-treat *S. aureus* infections not through direct bactericidal activity, but rather through preservation of platelets, allowing them to perform their critical role in clearing infection.

CASE REPORT

We encountered a male patient in his 60s with complicated MSSA bacteremia who by transesophageal echocardiography (TEE) had a complex (>4 mm) atherosclerotic plaque of the descending aorta with an overlying protruding thrombus (Figure 1A). This complex endovascular infection resulted in multiple hematogeous infectious foci, including septic pulmonary

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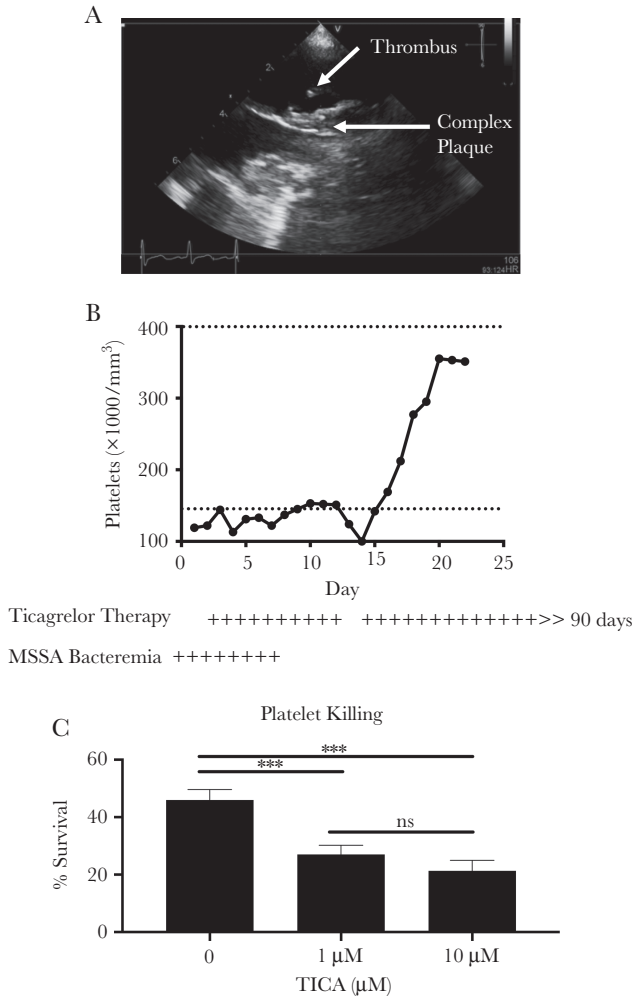

Figure 1. *A, Staphylococcus aureus* bacteremia secondary to a septic aortic thrombus (>4 mm) with multiple secondary pyogenic foci refractory to standard cefazolin and subsequent salvage therapy with cefazolin plus ertapenem for 5 days, rapidly cleared within 24 hours after the addition of ticagrelor. *B,* Thrombocytopenia resolved concurrently. Discontinuation of ticagrelor on day 12 led to rebound thrombocytopenia, and ticagrelor was restarted, once again resulting in resolution of thrombocytopenia. *C,* Addition of a physiological achievable concentration (1 μM) of ticagrelor dramatically enhanced human platelet killing of *S. aureus* ($P < .001$) in vitro. ***$P < .001$. Abbreviations: MSSA, methicillin-resistant *Staphylococcus aureus*; ns, not significant; TICA, ticagrelor.

emboli, right shoulder septic arthritis, cervical and lumbar spine osteomyelitis, and diskitis with lumbar epidural phlegmon. He also developed complex abscesses in the right gluteal muscles extending into the proximal right thigh, and femoral head and neck, leading to osteomyelitis of the right acetabulum and right lateral sacrum, the latter adjacent to hardware from a remote lumbar spine fusion.

Surgical source control was not possible and despite appropriate antimicrobial therapy, the patient remained bacteremic by daily blood cultures for 5 days. Therapy included vancomycin plus ceftriaxone on day 1, cefazolin on day 2 (due to MSSA bacteremia and penicillin allergy), and cefazolin plus ertapenem from day 3 onward based on recent use of this regimen to enhance cefazolin activity [8, 9]. Given the complex thrombus noted on TEE and accompanying thrombocytopenia, ticagrelor 90 mg every 12 hours was initiated to provide anti-platelet activity on day 5. Blood cultures cleared the next day and an increase in platelet count into the low normal range was noted (Figure 1B). Ticagrelor was stopped after 6 days given its uncertain therapeutic role in this unconventional setting, but its discontinuation was followed by an abrupt drop in platelet count (Figure 1B), prompting reinitiation of tigacrelor and a correction in thrombocytopenia. Ticagrelor was continued for a total of 3 months, followed by aspirin therapy. Antibiotics were continued intravenously for 6 weeks, followed by oral minocycline therapy planned for 12 months. The patient left the hospital for rehabilitation after 35 days and was discharged home after an additonal 3 weeks. At 90 days post presentation, the patient was able to ambulate without assistance at home and required a cane only over longer distances. At >18 months after the infection, the patient was alive and well and free of infection.

Based on the profound beneficial microbiological and clinical effects of adjunctive ticagrelor, additional in vitro studies were performed on the *S. aureus* strain isolated from the patient's blood on admission.

METHODS

Bacteria and In Vitro Assays

All studies were conducted using the MSSA clinical isolate obtained from the patient described above. Bacteria were grown overnight in Luria–Bertani (LB) broth, and isolates were stored with 50% glycerol at −80°C. Fresh colonies were streaked onto LB plates each week for all experiments. Broth microdilution antimicrobial susceptibility testing to cefazolin, ertapenem, and ticagrelor (Sigma) were conducted as per Clinical and Laboratory Standards Institute guidelines under standard (10^5 colony-forming units [CFU]/mL) and high-inoculum (10^7 CFU/mL) conditions using standard cation-adjusted Mueller–Hinton broth (CA-MHB) bacteriological media and Roswell Park Memorial Institute (RPMI) physiological cell culture media supplemented with 5% LB broth. Checkerboard assays were also performed using standard and high inoculums in CA-MHB. Antibiotics were purchased from the Sharp Memorial Hospital pharmacy (San Diego, California), supplied as vials available for clinical use and administration to patients.

Platelet Studies

Venous blood specimens were obtained from healthy human subjects under a protocol approved by the local ethics committee. Platelets were isolated from blood specimens that were anticoagulated with acid-citrate-dextrose buffer (Sigma). After centrifugation of whole-blood specimens (at 200*g* for 15 minutes, without a break), the upper two-thirds of the platelet-rich plasma (PRP) was transferred to a nonsiliconized

Eppendorf tube. Human PRP was incubated with 1 μM or 10 μM ticagrelor or vehicle control at 37°C in 5% carbon dioxide (CO_2) (with rotation) for 20 minutes. After centrifugation (at 450*g* for 10 minutes), the platelet-poor plasma was removed. The remaining pellet was resuspended in RPMI prior to seeding a 96-well plate with 1×10^7 platelets/well for MSSA killing assays. *Staphylococcus aureus* was added at a multiplicity of infection of 0.01 prior to incubating at 37°C in 5% CO_2 for 90 minutes. Each well was subsequently sonicated, and samples were serially diluted for CFU enumeration.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism software, version 7.0d. *P* values <.05 were considered significant.

RESULTS

Management of persistent MSSA bacteremia is grounded in surgical source control (which was not possible in our patient) and early initiation of β-lactam therapy with penicillinase-resistant anti-staphylococcal penicillins (eg, oxacillin, nafcillin) or cefazolin. Ertapenem was used in addition to cefazolin in our patient based on emerging data showing successful use of this regimen in salvaging persistent MSSA bacteremia [8, 9]. Failure of cefazolin monotherapy for deep-seated MSSA infections with high inoculums (such as infective endocarditis) have been well-documented [10–12]. Therefore, antimicrobial susceptibility testing for cefazolin, ertapenem, and ticagrelor was performed in CA-MHB under standard (10^5 CFU/mL) and high-inoculum (10^7 CFU/mL) conditions. Susceptibility testing was also conducted in RPMI media supplemented with 5% LB, a physiologically relevant tissue culture–based medium that has been shown to be more reflective of antibiotic activity in vivo [13, 14].

In vitro assessment of cefazolin, ertapenem, and ticagrelor activity is shown in Table 1. There was a 4-fold increase in the minimum inhibitory concentration (MIC) of cefazolin under high-inoculum (10^7 CFU/mL) vs standard inoculum (10^5 CFU/mL) in CA-MHB, an effect that was abrogated in RPMI + 5% LB media or in the presence of ticagrelor (Table 1). Ticagrelor, even at supraphysiological doses, had no bactericidal activity irrespective of the inoculum or media (Table 1). As previously described [8], a much higher ertapenem MIC was seen under standard and high-inoculum testing in RPMI + 5% LB media (Table 1). Checkerboard testing revealed general additivity between ertapenem and cefazolin based on fractional inhibitory concentration index calculations, which was unchanged in the presence of ticagrelor (Table 1). Despite having no direct bactericidal activity, ticagrelor enhanced human platelet killing of MSSA at physiologically attainable concentrations, 1 μM (Figure 1C).

DISCUSSION

We describe the successful use of ticagrelor as adjunctive therapy in a patient with disseminated staphylococcal disease without the option of surgical source control and refractory to antibiotic therapy, resulting in clearance at 24 hours of persistently positive blood cultures concomitant with an increase in platelet count. MIC testing showed that the therapeutic benefit of ticagrelor was not the result of direct bactericidal activity,

Table 1. Antibiotic Susceptibility of a Bloodstream Methicillin-Susceptible *Staphylococcus aureus* Isolate Tested by Minimum Inhibitory Concentration and Checkerboard Assays at Standard (10^5 Colony-Forming Units [CFU]/mL) or High (10^7 CFU/mL) Inocula Using Nafcillin, Cefazolin, Ertapenem, and Ticagrelor, and Corresponding Combinational Therapy Assessed by Checkerboard Assays

MIC, mg/L								
	NAF		CZ		ETP		TICA	
	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7
CA-MHB	0.50	1	1	4	0.50	1	64 μM	64 μM
RPMI	0.50	1	0.50	1	16	32	64 μ M	64 μ M

MIC, mg/L, in CA-MHB				
	CZ		ETP	
TICA	10^5	10^7	10^5	10^7
0	1	4	0.50	1
+16 μM	0.50	1	0.50	0.50

Checkerboard (CA-MHB)			
TICA	10^5	FICI	Interpretation
0	CZ + ETP	0.75	Additivity
+16 μM	CZ + ETP	0.75	Additivity

FICIs were interpreted as follows: synergy, FICI of ≤0.50; additivity, FICI of >0.50 to ≤1.0; no interaction, FICI of >1 to ≤4; antagonism, FICI of >4. Ticagrelor is expressed in μM.

Abbreviations: CA-MHB, cation-adjusted Mueller–Hinton broth; CZ, cefazolin; ETP, ertapenem; FICI, fractional inhibitory concentration index; NAF, nafcillin; RPMI + 5% LB, Roswell Park Memorial Institute 1640 plus 5% Luria-Bertani broth; TICA, ticagrelor.

even at supraphysiological concentrations. Rather, ticagrelor boosted the bactericidal activity of platelets at systemic concentrations (1 μM or 0.52 μg/mL) found in patients receiving standard dosages for the treatment of cardiovascular disease (maximum concentration = 1.2 μg/mL after one 180-mg loading dose and 0.75 μg/mL at 90 mg twice-daily steady state). These findings provide the first bedside-to-bench example in real time of the pleiotropic effects of ticagrelor that support noncoronary indications for ticagrelor. These findings also shed light on several well-accepted clinical observations [2, 6]. First, the in vivo bactericidal activity of ticagrelor requires the presence of platelets, explaining the discrepancy in the lack of direct in vitro antibacterial activity of ticagrelor at physiologic concentrations observed by us and others [5]. Second, the use of ticagrelor in patients receiving coronary artery stenting may offer unintended antimicrobial therapy, perhaps contributing to the exceedingly low rates of infection of these implanted biomedical devices [15].

Disseminated staphylococcal infections can be life-threatening despite targeted directed therapy secondary to this bacteria's impressive repertoire of virulence factors that enable it to cause systemic illness, from tissue-based or vascular-based infection. High-inoculum infections of endovascular-based infection further concentrate these virulence factors and pose challenges to antimicrobial therapy. Here we show that ticagrelor, a commonly prescribed agent in cardiovascular disease used to mitigate the thrombotic effects of platelets, can potentially be repurposed as an adjunctive therapy to harness the innate immune function of platelets to treat endovascular *S. aureus* infection. Clinical trials to further examine the benefit of ticagrelor in *S. aureus* endocarditis and other endovascular infections are needed.

Notes

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All 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.

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