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Blockade of glycoprotein IIb/IIIa by crotavirin, a member of disintegrins, prevents platelet from activation and aggregation by *Staphylococcus aureus* bacteria

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Abstract Interaction with circulating platelets is considered an important virulent mechanism for *Staphylococcus aureus* (*S. aureus*) bacteria to induce endocarditis, a severe infectious disease with high incidence of systemic thrombosis. It therefore represents an important target for pharmacological intervention. In this study, we found that the clinical isolate *S. aureus* 30326 induced activation and aggregation of washed human platelets in a fibrinogen-dependent manner and this platelet reactivity was abrogated by crotavirin, a snake venom-derived glycoprotein (GP) IIb/IIIa antagonist, indicating that crotavirin is able to protect platelets from activation and aggregation by *S. aureus* 30326. When tested at a concentration that prevented the platelet reactivity of *S. aureus* 30326, crotavirin also interfered with the binding of bacteria to washed human platelets supplemented with fibrinogen. The fibrinogen-binding activity of *S. aureus* has been shown to be essential for *S. aureus* to trigger platelet activation and aggregation. Crotavirin failed to affect the fibrinogen binding of *S. aureus*

Abbreviations: GP, glycoprotein; PDGF, platelet-derived growth factor; FITC, fluorescein isothiocyanate; CFU, colony forming unit; BCECF-AM, 2′,7′-bis(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester; SNARF-AM, 5-(and-6)-carboxy SNARF®-1, acetoxymethyl ester.

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30326 and neither did it bind to this microbe, suggesting that the inhibitory action of crotavirin on the *S. aureus* 30326—platelet interaction resulted from the occupation of platelet GPIIb/IIIa. Taken together, these results demonstrate an important role for GPIIb/IIIa in mediating the interaction of platelets with *S. aureus* in the presence of fibrinogen and platelet GPIIb/IIIa thus appears to be a new target for the intervention of *S. aureus*—platelet interaction.

Introduction

Infectious endocarditis is a severe disease caused mostly by the infection of Gram-positive bacteria on the endocardial surface of the heart. Systemic embolization is one of the most important complications that occurred frequently in these patients [1]. Viridians streptococci is the leading cause of this disease. However, a recent clinical observation reported that the incidence of *Staphylococcus aureus* endocarditis has increased and instead becomes the leading cause in adults [2]. Despite the more advanced, improved diagnostic equipment, antibiotic therapy and surgical technique, *S. aureus* endocarditis remains a severe illness with high mortality and complication rate [1]. The emergence of antibiotics-resistant *S. aureus* also creates a serious problem [3]. Facing these therapeutic challenges, a better understanding of the pathogenic mechanisms of *S. aureus* and exploring a more efficacious therapeutic strategy become urgent.

It is currently believed that circulating platelets play an important role in the pathogenesis of bacterial endocarditis. Platelets may aid bacteria attached to endocardium and the inhabited bacteria recruit more platelets to deposit on the lesion, thus facilitating the formation of macroscopic vegetations, which may be dispersed away from the valve surface resulting in systemic embolization. Evidences for the involvement of platelets in this disease include the vegetation lesion composed largely of aggregated platelets [4,5] and the common pathogens of bacterial endocarditis stimulated platelet activation and aggregation in vitro [6,7]. Recent studies further reported that the reactivity of *S. aureus* in triggering platelet activation and aggregation correlates with its capacity to induce experimental endocarditis [8,9]. Since the interaction of microbes with platelets plays an important role in the pathogenesis of *S. aureus* endocarditis, it would be clinically significant if we can find some candidates which block *S. aureus*—platelet interaction.

S. aureus-induced platelet activation and aggregation in vitro has been recognized for a long time [6] and this platelet reactivity relies on the plasma fibrinogen [10,11]. A variety of *S. aureus* surface proteins with fibrinogen-binding activity, including clumping factors A and B, and the serine-aspartate repeat protein SdrE, have been shown to participate in the platelet reactivity of *S. aureus* [8,9,12]. However, the platelet receptor(s) involved in platelet activation and aggregation by *S. aureus* remain unclear. Although a previous study reported that platelet binding by *S. aureus* is mediated by the direct interaction of clumping factor A (ClfA) with a novel 118-kDa platelet membrane receptor [13], this result was obtained under a condition devoid of fibrinogen and the role of this platelet receptor in the response of platelets to *S. aureus* has not yet been defined. The important issue, "what is the reaction sequence for *S. aureus* to trigger platelet activation and aggregation in the presence of fibrinogen", thus remains to be answered. Previous studies have revealed that immobilized fibrinogen can interact with resting platelet glycoprotein (GP) IIb/IIIa and subsequently triggers intracellular signaling events [14—16]. Since the fibrinogen-binding proteins expressed on the surface of *S. aureus* together with plasma fibrinogen appear to be critical for the platelet reactivity of *S. aureus*, we proposed the hypothesis that *S. aureus* may interact with platelet GPIIb/IIIa via surface-immobilized fibrinogen and thereby causing platelet activation and aggregation. In this hypothesis, the fibrinogen receptor GPIIb/IIIa is critical for the binding of bacteria and the subsequent activation of platelets. To verify this hypothesis, the snake venom-derived GPIIb/IIIa antagonist, crotavirin [17], was tested on the platelet reactivity of *S. aureus* in washed human platelet supplemented with fibrinogen to see if the blockade of platelet GPIIb/IIIa prevents platelets from interaction with and activation by *S. aureus*. We used crotavirin as the research tool because the binding assay of crotavirin has been well established and its function and mechanism of action have also been well characterized [17,18].