Platelet Activation by an Extracellular Adherence Protein From *Staphylococcus aureus* Acting via Modulation of Sulfhydryl Groups on Platelets

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In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, there is an article by Bertling et al., which describes platelet activation by a staphylococcal extracellular adherence protein. Eap is a protein that is produced exclusively by *Staphylococcus aureus*. The authors demonstrate adherence of Eap to platelets followed by activation of the important glycoprotein complex glycoprotein (GP) IIb/IIIa (the α<sub>IIb</sub>β<sub>3</sub> integrin), enhanced binding of fibrinogen and other adhesive proteins, platelet aggregation, and granule secretion. They also provide convincing evidence that modulation of sulfhydryl groups on platelets by Eap is involved in the platelet activation that occurs.

See accompanying article on page 1979

The authors speculate that Eap, through its effect on platelet activation, may contribute to the many vascular complications that can follow on from *S. aureus* infections, complications such as infective endocarditis, disseminated intravascular coagulation, thrombocytopenia, arteriosclerosis, myocardial infarction, and stroke.

Further, they speculate that molecules like Eap produced by other bacteria and microorganisms may also activate platelets, and this may be a general mechanism through which vascular complications occur after infection.

Eap has already been shown to bind to several cellular and plasma proteins, thereby promoting or modulating various cellular interactions and processes, but until now, no effect on platelets has been demonstrated.

The purpose of this editorial is to summarize what has been discovered. For ease of understanding, in the Figure, I have tried to produce a schematic overview of the effects of Eap on platelets, as described by Bertling et al.

Eap consists of 4 to 6 tandem repeat domains, and it is only the oligomer that results in platelet aggregation; a single Eap unit is ineffective.

The authors present evidence that Eap, in the form of an oligomer, binds to platelets and specifically to glycosaminoglycans on the platelet surface. Shedding of glycosaminoglycans using chondroitinase ABC dramatically reduced the binding. Also, the measured effects of Eap on platelets were completely blocked by added glycosaminoglycans.

It turned out that various forms of heparin were also effective in inhibiting Eap-induced platelet activation, whereas the synthetic pentasaccharide fondaparinux and the direct thrombin inhibitor hirudin had no effect. Heparin is a highly negatively charged molecule and probably binds to Eap and thereby disrupts the interaction between Eap and the glycosaminoglycans on the platelet surface.

Binding of Eap and subsequent platelet activation were blocked by the hydrophobic probe bis-ANS. This suggests that hydrophobic regions that are known to be present within the Eap oligomer are involved in the binding process. In this respect, and also because Eap has the ability to self-aggregate and to bind to so many other proteins, Eap may be an example of the so-called misfolded proteins such as amyloids, which are already known to activate platelets. The fact that many microorganisms are coated with amyloid-like proteins gives credence to the idea that bacteria and microorganisms other than *S. aureus* may produce products that interact with platelets in the same way as Eap.

Having discovered that Eap activates platelets, the main focus of the article by Bertling et al is the involvement of surface-located sulfhydryls in the activation process.

The authors demonstrate a diminution by Eap in the number of free sulfhydryls on platelets by Eap, together with an enhancement of platelet thiol reductase activity. Enzymes that engage in thiol reductase activity on platelets include protein disulfide isomerase, ERP57, and ERP72, and Eap was also demonstrated to enhance directly the activity of recombinant forms of these enzymes. Further, enhancement by Eap of platelet thiol reductase was partially inhibited by an antiprotein disulfide isomerase antibody and completely inhibited by the cell-impermeable thiol isomerase inhibitor bacitracin.

Eap, in its oligomeric form, also induced the binding of fibrinogen and other adhesive proteins to platelets. It also produced activation of GPIIb/IIIa as judged by enhanced PAC-1 binding. Platelet aggregation and granule secretion also occurred with the appearance of secreted CD62P, CD63, and CD40L on the platelet surface. There was clear involvement of sulfhydryls in all of these activities in that they were inhibited by several thiol reactive agents and also by the antiprotein disulfide isomerase antibody and by bacitracin.

At the start of these investigations, it was already known that enzymatically catalyzed disulfide exchange is involved in the activation of GPIIb/IIIa by more conventional platelet agonists such as adenosine diphosphate and thrombin and that this leads to fibrinogen binding and subsequent platelet aggregation.

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1751
Here, Bertling et al have demonstrated that Eap was also able to induce fibrinogen binding through GPIIb/IIIa activation by showing that the GPIIb/IIIa antagonists abciximab and RGDS inhibit the binding. However, Eap also induced some fibrinogen binding even in the presence of these GPIIb/IIIa antagonists. Also, the signaling inhibitor iloprost, which increases cAMP in platelet through an effect at the IP receptor, only partially inhibited Eap-induced fibrinogen binding. These observations are consistent with fibrinogen binding in response to Eap occurring in 2 separate ways as indicated in the Figure, a mechanism involving GPIIb/IIIa and another signaling-independent mechanism perhaps involving direct binding of oligomeric Eap-fibrinogen complexes to the platelet surface.

Granule secretion was shown to be totally independent of GPIIb/IIIa activation but was blocked by iloprost and also by EDTA, showing granule secretion to be totally dependent on platelet signaling. So this appears to be another mechanism through which Eap activates platelets.

All in all, the article by Bertling et al produces new information that seems to be of some interest. If Eap turns out to be a major player in platelet activation induced by S. aureus, and in turn is relevant to the vascular complications that follow on from S. aureus infections, and, indeed, other bacteria and microorganisms produce similar molecules that act in a similar way to Eap, it will be important to identify pharmacological routes to limit the platelet activation that occurs. The authors have already identified heparin as one such route.

Disclosures

None.

References


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