 

# *Perioperative Platelet Activation and the Inhibitory Effect of Milrinone*

SURGERY activates the circulating pool of blood platelets. This process is most intense at sites of vascular injury, where platelets adhere and aggregate to plug vascular holes and provide the phospholipid surface necessary for coagulation proteins to assemble and generate fibrin. However, activated platelets can also be detected in blood remote from sites of surgical trauma. These less intensively activated circulating platelets may contribute to the postsurgical prothrombotic state by responding more vigorously to subsequent activating stimuli or by inducing procoagulant behavior in other cells, primarily leukocytes and endothelial cells. In this issue of ANESTHESIOLOGY, Beppu *et al.*[1] extend previous observations by characterizing perioperative activation of platelet intracellular activation pathways, monocyte tissue factor expression, and the ability of milrinone to inhibit these prothrombotic activation events.

Platelets circulate in the blood in a quiescent state and can be rapidly activated by a variety of chemical (*e.g.*, collagen, thrombin, adenosine diphosphate, epinephrine) and physical (shear stress) stimuli. Recognition of these extracellular stimuli by specific platelet surface receptors leads to activation of intracellular activation pathways, which in turn increases the surface expression of platelet adhesive molecules, such as the fibrinogen receptor (integrin $\alpha_{IIb}\beta_3$) and P-selectin. The expression of a conformationally active fibrinogen receptor transforms platelets from a quiescent to an active state capable of binding soluble fibrinogen and aggregating. This activation step is critical to clinical hemostasis and is the target of glycoprotein IIb–IIIa antagonists in current therapeutic use for prevention of coronary thrombosis. The expression of P-selectin allows platelets to bind to a constitutively expressed receptor on monocytes and neutrophils termed *P-selectin glycoprotein ligand 1*.[2] The clinical relevance of these platelet–leukocyte interactions is not entirely clear; however, greater numbers of circulating platelet–leukocyte aggregates are associated with increased risk of recurrent myocardial ischemia in patients with acute coronary syndromes.[3] In laboratory models, the interaction between platelet P-selectin and leukocyte P-selectin glycoprotein ligand 1 promotes tissue factor expression on monocytes and formation of circulating tissue factor–bearing microparticles, which contribute to thrombus formation *in vivo*.[4,5]

In their report, Beppu *et al.* confirm previous observations by demonstrating increased platelet aggregability and expression of conformationally active fibrinogen receptors (identified by PAC1 binding) on circulating platelets remote from the site of surgery. They demonstrate an increase in platelet P-selectin expression and platelet–leukocyte aggregate formation in the perioperative period, consistent with *in vivo* platelet activation. The authors also observe increased activation (phosphorylation) of several intracellular platelet pathways. Specifically, they note postoperative increases in phosphorylation of the mitogen-activated protein kinases (MAPKs), p38 MAPK and extracellular signal–regulated kinase 1/2, and of Akt, which is dependent on phosphotidylinositol 3-kinase for activation. The reported observations on MAPK pathways are of particular interest because the role of these kinases in platelet function is incompletely understood and has not been previously characterized in surgical patients. Also of great interest is the authors' observation that monocyte tissue factor expression increases postoperatively, paralleling results of platelet–leukocyte aggregate formation. The reported increase in plasma fibrin monomer is consistent with activation of platelets and monocytes, although these events are not definitively linked by the studies performed.

The authors examined the effect of milrinone infusion on platelet and monocyte function and fibrin formation *in vivo* in a randomized clinical trial of knee arthroplasty patients. Compared with placebo, a 24-h infusion of milrinone reduced the postoperative increase in platelet aggregability, PAC1 and P-selectin expression, phosphorylation of platelet p38 MAPK, extracellular signal–regulated kinase 1/2 and Akt, platelet–leukocyte aggregate formation, monocyte tissue factor expression, and fibrin monomer formation. Milrinone is a phosphodiesterase inhibitor with relative specificity for the phosphodiesterase 3 (PDE3) isoform, and milrinone infusion increases cyclic adenosine monophosphate levels in platelets and other PDE3-expressing cells.[6] Cyclic adenosine monophosphate is a second messenger regulating platelet calcium metabolism, which is critical to the activation state of platelets. An increase in cyclic adenosine monophosphate reduces platelet calcium concentration, suppressing platelet activation caused by a variety of stimuli. Although milrinone infusion is approved by the U.S. Food and Drug Administration for use in congestive heart failure, its antiplatelet effects are well known, and, indeed, another PDE3 inhibitor, cilostazol, is approved for treatment of intermittent claudication (in the United

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States) and for prevention of stroke (in Japan).[6] The reported reduction in measures of platelet activation are expected, given the ability of milrinone to inhibit platelet activation; however, its ability to suppress monocyte tissue factor expression and fibrin formation is noteworthy and suggests a link between platelet activation, tissue factor expression, and fibrin formation *in vivo*.

Beppu *et al.* follow their clinical studies with supportive *in vitro* experiments using blood obtained from normal volunteers. Incubation of samples *in vitro* with a therapeutic concentration of milrinone (1 $\mu$M) inhibited collagen– and adenosine diphosphate–stimulated platelet activation using the same metrics examined in clinical blood samples. Milrinone also inhibited collagen-stimulated tissue factor expression on monocytes, and, importantly, this effect was dependent on activated platelets because the inhibitory effect was only observed when monocytes were examined in the presence of activated platelets. Experiments with pharmacologic inhibitors of p38 MAPK, extracellular signal–regulated kinase 1/2, and phosphotidylinositol 3-kinase demonstrated the distinct sequela of platelet activation through these pathways: p38 MAPK activation seems to contribute to platelet P-selectin expression, platelet–monocyte aggregate formation, and monocyte tissue factor expression, whereas extracellular signal–regulated kinase 1/2 and phosphotidylinositol 3-kinase/Akt activation seems to contribute to fibrinogen receptor activation (PAC1 binding) and platelet aggregation.

The clinical implications of the authors' work are unclear. Milrinone is not and should not be used as an antithrombotic agent. Effective antiplatelet (*e.g.*, aspirin, clopidogrel) and anticoagulant (*e.g.*, heparin) agents, which have undergone the rigors of randomized controlled trials, are available for this purpose. It is interesting that the authors did not see a difference in blood loss or transfusion in the milrinone treated group, despite inhibition of platelet function. Similar findings have been reported in a small randomized clinical trial of milrinone in cardiac surgery patients.[7] One possible explanation is that the ability of milrinone to inhibit platelet activation, monocyte tissue factor expression, and fibrin formation is too modest to be clinically relevant. An alternative explanation is that a modest, but clinically relevant, bleeding effect could not be detected with the small sample sizes examined in these studies. A third intriguing possibility is that milrinone, and potentially other PDE3 inhibitors, do possess a clinically relevant antithrombotic effect but do not significantly alter normal hemostasis or promote bleeding. Consistent with this possibility is the observation that cilostazol does not prolong bleeding time or increase clinical bleeding events, even though it effectively inhibits platelet aggregation *in vitro* and reduces restenosis after percutaneous peripheral arterial revascularization.[8,9] Additional investigation is warranted to examine the effects of PDE3 inhibition on bleeding and thrombotic outcomes *in vivo* in animal and human studies.

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