Move along, nothing to see here: Btk inhibitors stop platelets sticking to plaques

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NEIGHBORHOOD WATCH

Move along, nothing to see here: Btk inhibitors stop platelets sticking to plaques

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Platelets have evolved an intricate array of overlapping mechanisms to respond effectively to vessel damage by forming a clot to minimize blood loss. Unfortunately, these same responses may also be triggered by atherosclerotic lesions in diseased arteries. Vessels can become occluded when platelets form a thrombus at the site of a ruptured plaque, causing myocardial infarction (MI) or ischemic stroke, depending on where the thrombus forms or where a resulting embolus becomes lodged.

Current therapy following MI or ischemic stroke involves treatment with antiplatelet drugs to reduce the risk of recurrent events by inhibiting one or more of the mechanisms that drive the formation of a platelet thrombus. However, the overlap between the mechanisms that drive pathological thrombus formation and those that facilitate hemostasis means that patients receiving antiplatelet medication are at higher risk of hemorrhage, including intracranial hemorrhage and gastrointestinal bleeding. The benefits of current antiplatelet therapy outweigh the risks for patients, but there is still a need for safer antiplatelet therapies that do not compromise hemostasis to better serve this patient population, which is large and growing globally.

In order to develop new antiplatelet drugs, researchers must consider the similarities and differences between the pathological process of thrombosis and the physiological process of hemostasis. Platelets skim over the surfaces of vascular endothelial cells, scanning for collagen and other exposed subendothelial matrix proteins that serve as environmental cues indicating that vessel damage has occurred. The initial interaction with exposed collagen occurs via the plasma protein von Willebrand factor, which binds to collagen and undergoes conformational change at high shear rates (present in arteries), enabling the platelet protein glycoprotein (GP) Ibα to bind to it. Collagen is also recognized directly by the platelet GPVI receptor and integrin $\alpha_2\beta_1$, which, together, mediate adhesion to collagen and trigger a cascade of intracellular signaling events that ultimately help the aggregate to grow and become stable. Atherosclerotic plaques are rich in collagen and provide a strong environmental cue to platelets to adhere and form an aggregate within the lumen of the vessel. The intracellular signaling processes stimulated in both scenarios are similar, and result in the platelets synthesizing thromboxane A_2 and secreting ADP, which, via paracrine and autocrine mechanisms, act as secondary mediators to recruit platelets to the growing aggregate and stabilize it. The antiplatelet drugs aspirin and P2Y12 inhibitors such as clopidogrel or ticagrelor target these positive feedback mechanisms to limit thrombus growth and prevent vessel occlusion. The success of these drugs in providing relatively safe platelet inhibition can be understood in the context of the 'core' and 'shell' model of thrombus formation, whereby a core of platelets is strongly activated by direct contact with collagen, and is surrounded by an outer shell of weakly activated platelets stimulated by secondary mediators. In this model, the core is required for hemostasis, whereas the shell is partially redundant but serves a pathological role in thrombosis by causing vessel occlusion. However, both aspirin and P2Y12 inhibitors are associated with increased rates of hemorrhage, suggesting that either these drugs are not sufficiently specific in targeting the shell, or the shell is also important for hemostasis.

Although the environment of an atherosclerotic plaque and that of a damaged blood vessel have similarities, some *in vitro* studies have identified differences that could be exploited pharmacologically to provide platelet inhibition specifically in the context of atherothrombosis while sparing normal hemostasis. One such difference concerns the morphologically distinct forms of type I and type III collagen present in atherosclerotic plaques, which trigger

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thrombus formation via GPVI but not via integrin $\alpha_2\beta_1$ [1]. The traditional model of the interaction of platelets with collagen involves complementary roles for integrin $\alpha_2\beta_1$ in mediating adhesion, and GPVI in stimulating intracellular signaling and full platelet activation. However, patients who are genetically deficient in platelet GPVI do not suffer from severe bleeding, suggesting that the role of GPVI is not as critical for hemostasis as its role as the primary mediator of collagen-evoked platelet activation might suggest. The observations that GPVI is critical for thrombus formation on atherosclerotic plaques but partially redundant for normal hemostasis prompted the development of GPVI inhibitors for antiplatelet therapy, which are currently undergoing clinical trials (e.g. clinicaltrials.gov NCT03312855). Meanwhile, the first clinical Bruton's tyrosine kinase (Btk) inhibitor, ibrutinib, has been approved for the treatment of chronic lymphocytic leukemia and mantle cell lymphoma, and has been found to cause GPVI-specific platelet dysfunction in patients. Btk is targeted for the treatment of B-cell lymphomas because of its central role in the signaling pathway that promotes the development of B cells and contributes towards malignancy. The role of Btk in platelets had already been established in studies of mice deficient in Btk and in humans with X-linked gammaglobulinemia who lack functional Btk; these studies reported that GPVI signaling was at least partially dependent on the presence of functional Btk. Btk and its family member Tec are known to couple the GPVI receptor to phospholipase Cγ activation and the release of Ca^{2+} from intracellular stores into the cytosol, which is a critical step in collagen-evoked platelet activation. Like GPVI inhibitors, ibrutinib does not abolish adhesion to collagen under flow, suggesting that other receptors and pathways are able to compensate for the loss of Btk activity downstream of GPVI. However, unlike the absence of severe bleeding in patients with GPVI or Btk deficiencies, ibrutinib does increase the risk of major bleeding, suggesting that the effects of ibrutinib on platelet function cannot be fully attributable to loss of Btk or GPVI signaling. In support of this, ibrutinib was found to inhibit platelet function downstream of other adhesion receptors, including GPIb and integrin $\alpha_{IIb}\beta_3$, and to inhibit other platelet kinases, most importantly Tec and Src family kinases [2,3]. Trials of the more specific, second-generation Btk inhibitor acalabrutinib showed no incidents of major bleeding, and, although the drug was found to inhibit GPVI-mediated aggregation, it did not inhibit thrombus formation on native type I collagen [4].

The growing body of evidence surrounding Btk inhibitors and their effects on platelets begs the question of their potential efficacy in the prevention of atherothrombosis. The recent study by Busygina *et al.* sought to address this idea directly by studying the effects of Btk inhibitors *in vitro* and *ex vivo* on the interactions of platelets with atherosclerotic plaque tissue derived from carotid endarterectomy specimens [5]. Thrombus formation on homogenized plaque or tissue sections under flow was abolished by treatment of whole blood with the Btk inhibitors ibrutinib, acalabrutinib, and ONO/GS-4059 (a new-generation Btk inhibitor, also referred to as tirabrutinib). However, thrombi forming on native type I collagen were unaffected by acalabrutinib or ONO/GS-4059, and only partially inhibited by higher concentrations of ibrutinib. The finding that ibrutinib abolished thrombus formation on plaque tissue, but only partially inhibited thrombus formation on type I collagen, was recapitulated with blood samples from patients receiving ibrutinib orally (420 mg daily). The authors argue that the use of plaque tissue and the use of native type I collagen represent the pathological process of atherothrombosis and the physiological process of hemostasis, respectively, and therefore that Btk inhibitors preferentially target the pathological processes while sparing hemostasis. If this model is correct, Btk inhibitors could represent an entirely new approach to antiplatelet therapy, whereby the interaction of platelets with the culprit lesion that triggers vascular events is selectively prevented, while the ability of platelets to trigger clotting in response to damaged, non-diseased tissue is spared. This view requires acceptance of the simplified models of hemostasis and thrombosis used in the study, which cannot fully incorporate rheological considerations, the contribution of coagulation or extracellular Ca^{2+} entry, and numerous other factors that contribute to these processes. However, short of utilizing a more complex animal model in which thrombosis is triggered by atherosclerotic plaque rupture or erosion, it would be difficult to improve on the approach taken by Busygina *et al.*

Busygina *et al.* also theorized that the irreversible mode of action of ibrutinib could be exploited to achieve inhibition with low or intermittent dosing, owing to the lack of protein turnover in the anucleate platelet. Antiplatelet therapy with the irreversible cyclooxygenase 1 inhibitor aspirin exploits the lack of platelet protein turnover to provide effective platelet inhibition at low doses while minimizing off-target effects. Utilizing this approach, the authors were able to selectively block thrombus formation on plaque material by orally dosing healthy volunteers with 120 mg of ibrutinib every day or every other day, and achieve similar levels of inhibition as observed in patients receiving 420 mg daily. The use of low-dose ibrutinib for antiplatelet therapy could reduce adverse events and avoid the off-target inhibition of Src family kinases that is likely to underpin severe platelet dysfunction and bleeding caused by higher concentrations of ibrutinib [6]. This strategy could be even more effective with the more specific Btk inhibitor acalabrutinib, which was only utilized in the *in vitro* part of the study, but may be associated with fewer adverse events [4].

Overall, the study provides a compelling basis for thinking of Btk inhibitors as potential antiplatelet drugs.

No other kinase inhibitor has been approved for antiplatelet therapy, and the mode of action of ibrutinib and other Btk inhibitors in targeting signaling evoked by adhesion receptors rather than the conventional approach of preventing interactions of platelet receptors with their ligands is highly novel. Although existing approved Btk inhibitors may have potential as antiplatelet drugs, clinical trials to compare their efficacy with that of current therapies could still be an expensive and risky undertaking for the pharmaceutical industry. The authors suggest a highly targeted approach for the therapeutic use of Btk inhibitors as antiplatelet drugs by incorporating them into prophylactic antithrombotic treatment prior to elective percutaneous coronary interventions, which can expose plaque material within the vascular system. Clinical evidence that the interaction between platelets and plaque material can be pharmacologically targeted would be a very important development, not only in the context of Btk inhibitors, but also for antiplatelet drug development more generally.

Addendum

A. P. Bye and J. M. Gibbins prepared the manuscript.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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