In recent years, platelet receptors have been successfully targeted by small-molecule antithrombotic drugs, with the integrin α<sub>IIbβ<sub>3 and the G-protein–coupled receptor P2Y<sub>12 being prime examples. Of the two, P2Y<sub>12 is a much more traditional small-molecule target, with drugs such as clopidogrel and prasugrel interfering with the ability of another small molecule, adenosine diphosphate, to signal to induce platelet aggregation. A priori, targeting the interaction of α<sub>IIbβ<sub>3 with either fibrinogen or von Willebrand factor (VWF) would seem much more problematic, the small molecule having to disrupt the interaction of 2 very large proteins. In this case, however, the problem is simplified by the fact that short, continuous stretches of amino acids (Arg-Gly-Asp-Ser, RGDS, in both fibrinogen and VWF, and the related HHLGGAKQAGDV chain sequence in fibrinogen) make up the primary binding sites for α<sub>IIbβ<sub>3 on these ligands; this formed the basis of strategies to target the receptor. Eptifibatide, an α<sub>IIbβ<sub>3 antagonist that is based on a sequence from the pit viper protein barbourin, contains a (KGD) sequence with greater specificity for α<sub>IIbβ<sub>3 than the canonical RGD sequence. However, only a relatively small percentage of protein–protein interactions have such small interaction “hot spots” that contribute so much to the total binding energy of the interaction. The interaction between glycoprotein (GP) Ibα and VWF, which mediates the first step of platelet adhesion to sites of vessel injury, seems a particularly difficult small-molecule target, given that the interactive surface between this receptor–ligand pair covers 2600 Å, an enormous area for a small molecule to cover. Within these 2 large proteins the interaction sites reside in smaller domains, in GPIbα in a 290–amino acid sequence at the polypeptide’s N-terminus, and in VWF entirely within the 186–amino acid A1 domain. In this region of GPIbα, important sequences include an N-terminal disulfide loop known as the β-finger, and a C-terminal disulfide loop called the β-switch or regulatory (R)-loop (see figure).
In the complex of the 2 proteins, the R-loop forms a β-hairpin that contributes 2 strands to an 8-stranded β-sheet containing sequences from both molecules. The R-loop is also the site of gain-of-function mutations that produce the rare bleeding disorder platelet-type von Willebrand disease, where they produce mutant GPIbα molecules capable of interacting with VWF in the absence of the usual requirements of shear stress or modulators such as ristocetin.

Despite the many obstacles to developing small-molecule inhibitors of the GPIbα–VWF interaction, Benard et al were able to identify several that inhibited the interaction under static conditions and flow by screening a phage-display library of 11-αmino acid cystine-constrained peptides that bind GPIbα.

One of the optimized peptides, OS-1, bound GPIbα with very high affinity, the $K_D$ being 0.74 nM.

A clue that the OS-1 peptide might disrupt the GPIbα–VWF interaction by interfering with the R-loop was provided by the finding that the peptide’s affinity for GPIbα was reduced markedly in the presence of platelet-type VWD mutations.

Now, the riddle of OS-1’s mechanism of action has been solved, and the solution appears in this issue of Blood. McEwan et al determined the crystal structure of OS-1 in complex with GPIbα and found that, unlike the situation with RGD- or KGDBased inhibitors of αIIbβ3, OS-1 does not mimic the binding of VWF to GPIbα. Instead, the cyclic peptide inserts within a pocket formed by the lower part of a concave β-sheet formed by leucine-rich repeats 3 to 7 of GPIbα and the R-loop (see figure). In engaging the R-loop, OS-1 induces a tight helical structure that presumably prevents formation of the β-hairpin and the bimolecular β-sheet observed in the GPIbα–VWF complex. Thus, the mechanism of inhibition appears to have a large allosteric component, a conclusion that would have been very difficult to make without the structure. Many questions remain to be answered before OS-1 or its derivatives can be applied as antithrombotics, but the elegant structural work of McEwan et al suggests a very interesting, heretofore unrecognized, mechanism for targeting GPIbα with small molecules.

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REFERENCES


Comment on Psaila et al, page 4777

Childhood ITP: knowing when to worry?

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In this issue of Blood, Psaila and colleagues describe a large cohort of patients with ITP and intracranial hemorrhage.

Immune thrombocytopenia (ITP) is a fairly rare, generally benign illness in the pediatric population, affecting between 5 to 13 children per 100,000. Intracranial hemorrhage (ICH) is considered a rare complication occurring in a small subset of patients (< 1% in multiple studies). Because ICH is a rare but significant complication of ITP in childhood, when to consider treatment remains an issue. In this issue of Blood, Psaila et al describe a cohort of patients with ITP and ICH, examining risk factors for hemorrhage and attempting to identify an algorithm to stratify patients according to risk and allow for rational treatment decisions.

In this study, the authors examined medical information obtained through a survey that combined a retrospective and a prospective multi-institutional analyses. The calculated incidence of ICH was 0.19% to 0.78% of pediatric patients with ITP, depending on the estimate of the percentage of all bleeding episodes in this population that were captured by the survey. Controls were the next 2 patients presenting with ITP of similar duration and severity from the reporting institution and were supplemented with cases from Cornell/Weill if they were not locally available. As reported in other studies, the majority of children had platelet counts less than $20 \times 10^9$/mcL at the time of ICH, but 10% had counts higher than $20 \times 10^9$/mcL. The authors also report that almost half (45%) of the episodes of ICH occurred in patients within the first week of diagnosis, whereas 30% occurred in patients with ITP of more than 6 months’ duration, supporting older studies that the frequency of ICH is highest in the first week after diagnosis.

Outcomes for these patients are also reported and whereas 8 of 10 patients whose ICH was a presenting feature of their ITP survived without sequelae, 50% of the patients who presented within the first week of diagnosis died and 38% had neurologic sequelae. Whether this good outcome for patients with ICH on presentation is a peculiarity of this study or has some biological meaning will require further studies with more detailed clinical information on treatment in relation to ICH and greater detail on the ICH.

Next, the authors examined risk factors for ICH to identify a population of patients who might be at higher risk of bleeding and therefore might benefit from more aggressive therapy. The patients who presented with ICH as the first manifestation of the ITP were excluded from treatment/cost analysis. As in previous studies, head trauma and hemorrhagic manifestations (beyond mild cutaneous) were significant risk factors for ICH and, in the case of more bleeding manifestations, increased mortality. In contrast to other studies, the authors found no relationship between concurrent nonsteroidal anti-inflammatory drug use, wet purpura, and gastrointestinal or vaginal bleeding, but this may be due in part to the limitations of retrospective reporting in a survey study. The patients with ICH were also noted to have a lower incidence of petechiae.

The most interesting part of this paper was the presentation of algorithms stratifying patients with ITP according to risk for ICH. The algorithms were used to analyze the cost-effectiveness of therapy (excluding in this analysis those patients who presented with ICH as the initial manifestation of ITP). According to this analysis, if all patients with any bleeding other than petechiae and ecchymosis and/or head
The proof is in the crystal

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