been developed by computer modelling. To date, there are several compounds such as Aggrastat (Merck), and Integrelin (COR Therapeutics) which are commercially available and used clinically in preventing restenosis of coronary vessels after PTCA.[20,21] In contrast to the first antiadhesive agent, chimeric 7E3 Fab (Abciximab or ReoPro), a monoclonal antibody raised against platelet $\alpha_{IIb}\beta_3$, they are classified as small-molecule antithrombotic agents.

Probe for Platelet Membrane $\alpha_{IIb}\beta_3$

$\alpha_{IIb}\beta_3$ is the most abundant integrin expressed on platelet surfaces (approximately 50,000 copies per platelet). It undergoes conformational changes when platelets are activated, and subsequently associates with plasma fibrinogen leading to platelet aggregation.[36,37] Unlike fibrinogen, disintegrins bind to $\alpha_{IIb}\beta_3$ of both resting and activated platelets in a divalent-cation-dependent manner.[15] Activation of platelets by ADP markedly enhances the binding affinity of some disintegrins (e.g., trigramin, halysin, and arietin) towards platelet $\alpha_{IIb}\beta_3$.[15,38] Therefore, these disintegrins may serve as probes either for determining $\alpha_{IIb}\beta_3$ content or for distiguishing the resting state from the activated state of $\alpha_{IIb}\beta_3$.[39] We first conjugated disintegrin with a flurorescent dye, fluorescain isothiocyanate (FITC), and performed a binding study using flow cytometry. The specific binding of FITC-disintegrin to platelets is saturable, and divalent-cation dependent. Activation of platelets with ADP also markedly increased the binding of FITC-disintegrin (e.g., trigramin, halysin, and arietin), but not that of rhodostomin, consistent with the data obtained from ^{125}I-disintegrin binding studies.[15,38] Using this technique, we determined the platelet $\alpha_{IIb}\beta_3$ levels of 3 Glanzmann's thrombasthenia patients and found that less than 5% of normal platelets were detected. Therefore, these patients belong to type I thrombasthenia. Owing to the simplicity and speed of this assay, FITC-disintegrins are ideal probes for determing the binding capacity of platelet $\alpha_{IIb}\beta_3$.

$\alpha_{IIb}\beta_3$ antagonists, including ReoPro, RGD, or KGD derivatives, have been used clinically as antithrombotic agents. Owing to the unique site of action in blocking the common step of platelet aggregation, i.e., the fibrinogen binding to $\alpha_{IIb}\beta_3$ of activated platelets, they are considered to be the ideal drug for preventing platelet aggregation caused by a variety of different stimuli. Although ReoPro is effective in reducing ischemic complications in patients, an increased risk of bleeding is expected especially if an overdosage is given.[40] Based on our previous observation that the binding sites of disintegrins appear to overlap with those of 7E3 and 7E3Fab,[15,18] we further proved a good correlation between inhibition of platelet aggregation and blockade of FITC-disintegrin (e.g., crotavirin and arietin) binding to platelets by 7E3.[41] The percentage inhibition of FITC-disintegrin binding at a saturated dose reflects the extent of $\alpha_{IIb}\beta_3$ occupation by 7E3 or ReoPro. Based on the characteristics of single cell recording with flow cytometry, this method provides a feasible monitoring method using 5 μl of blood sample for detecting the degree of platelet $\alpha_{IIb}\beta_3$ blockade by c7E3, allowing us to find an optimal dosage. However, this method can not be applied to the monitoring of Integrelin, a small-molecule $\alpha_{IIb}\beta_3$ antagonist, probably due to different binding epitopes or binding kinetics (unpublished data).

Disintegrin Interaction with Cells Other than Platelets

Knusen et al. first showed that trigramin inhibited adhesion and the spread of human melanoma cells on both immobilized fibrinogen and fibronectin.[18] Then disintegrins were found to inhibit adhesion of human umbilical vein endothelial cells (HUVECs) to vitronectin and fibrin through integrin $\alpha_V\beta_3$.[42] Subsequently disintegrins were reported to block the adhesion of B16F10 mouse melanoma cells to fibronectin and vitronectin.[43] Triflavin has been further demonstrated to inhibit lung colonization of B16F10 melanoma cells in an experimental metastasis model.[44] Similarly, the adhesion between tumor cell lines (e.g., hepatoma, cervical carcinoma, human prostate carcinoma, breast and colon adenocarcinomas) and extracellular matrices was blocked by disintegrins. Scarborough et al. have investigated the effects of various disintegrins on the binding of fibronectin and vitronectin to their respective