

Therapeutic integrin inhibition: Allosteric and activation-specific inhibition strategies may surpass the initial ligand-mimetic strategies

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The heterodimeric cell adhesion molecules integrins are composed of an α - and a β -subunit, which are noncovalently linked to each other. Both subunits are type I membrane glycoproteins, and 18 different α -subunits and eight different β -subunits have so far been identified, which combine to form 24 different integrin heterodimers. These can be grouped into subfamilies according to the identity of their β subunit. Each subunit comprises a large extracellular domain followed by a single transmembrane-spanning domain and a short cytoplasmic tail (with the exception of α_4). All integrins seem to have the ability to change their conformation upon cell activation (1). Control of this conformational change is vital for cell function and needs to be tightly regulated in a rapid and coordinated manner. This is especially true for cells that circulate in blood such as leukocytes and platelets. For example, the integrin $\alpha_{IIb}\beta_3$ (GP IIb/IIIa, CD41/61) on circulating non-stimulated platelets needs to be maintained in a conformation that does not allow binding of natural ligands such as fibrinogen. However, upon vessel injury platelets become activated and the activation status of $\alpha_{IIb}\beta_3$ -integrin has to be changed within the shortest possible time frame to allow fibrinogen binding, platelet adhesion and aggregation, and thus vessel sealing. Similarly, integrins expressed on leukocytes are maintained in a low affinity state for their ligands until activated by the immune or inflammatory response. This conformational change of integrins induced upon cell activation has been termed inside-out signaling. However, integrins can also act as cell sensors, reporting engagement of integrins with ligands, resulting in outside-in signaling. The latter seems to be the reason why some integrin blocking strategies have limitations as discussed below.

The two β_3 -integrins, platelet integrin $\alpha_{IIb}\beta_3$ and integrin $\alpha_v\beta_3$ (vitronectin receptor, CD51/CD61) have been extensively characterized and serve as a paradigm for the mechanism of integrin activation. The resolution of the crystal structure of the extracellular domain of $\alpha_v\beta_3$ -integrin was a milestone towards the now generally accepted model of conformational changes

that occur during integrin activation (2). The general structure of the extracellular part of integrins can be separated into an N-terminal head domain followed by the upper and lower legs (or stalks) (3). In the middle of the stalks, a “knee” or “genu” region has been postulated at which the extracellular integrin domains appear to be bent (3). The current model sees the resting integrin in a bent conformation that opens in a switchblade-like mechanism upon integrin activation and ultimately results in the exposure of the ligand-binding site in the integrin head domain (4). Although the vast majority of the integrin heterodimer is localized extracellularly, the short intra-cytoplasmic tails are essential for the regulation of integrin function and for inside-out as well as outside-in signaling (5–8). Separation of the cytoplasmic domains by mutation or binding of the cytoskeletal protein talin or other cytoplasmic effector proteins induces separation of the α -subunit and β -subunit lower legs. This lower leg separation then leads to a destabilisation of the interface between the lower legs and headpiece. The result of this destabilisation is an extension of the headpiece and the ligand-binding pocket therein (9). Highly conserved motifs such as the GFFKR motif have been identified within the cytoplasmic domains and have been found to be crucial for integrin activity (5, 6, 10, 11).

The therapeutic blockade of integrins is highly attractive for many areas of medicine, including antithrombotic, antiinflammatory and anti-cancer therapy. Pioneering work of Dr. B. Collier established the first therapeutic integrin blockade used in humans: the monoclonal antibody 7E3 directed against $\alpha_{IIb}\beta_3$ -integrin (12). Thereafter, small-molecule ligand mimetics were developed using the receptor-binding site RGD within fibrinogen as a template: eptifibatid and tirofiban (for detailed review see [13]). Despite the initial great enthusiasm generated by the possibility to block the final common pathway of all types of platelet activation, fibrinogen binding, the clinical success of the aforementioned $\alpha_{IIb}\beta_3$ -inhibitors (all are strictly for intravenous administration) proved to demonstrate major limitations. Low-risk patients, clopidogrel-pretreated patients and patients treated

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with fibrinolytic reagents do not seem to benefit from treatment with $\alpha_{IIb}\beta_3$ -inhibitors (14). The initial enthusiasm about $\alpha_{IIb}\beta_3$ -inhibitors also drove rapid development of orally applicable $\alpha_{IIb}\beta_3$ -inhibitors. However, four large phase III clinical trials with oral $\alpha_{IIb}\beta_3$ -inhibitors showed a significant increase in mortality in patients treated with these reagents (14, 15). One potential explanation for this extraordinary and costly failure in drug development is the finding that the current clinically trialed $\alpha_{IIb}\beta_3$ -inhibitors are all ligand-mimetics. However, since integrins also function as cell sensors, ligand-binding causes a conformational change of $\alpha_{IIb}\beta_3$ -integrin which can then cause outside-in signaling and subsequent cell activation (16). Thus, although therapeutic targeting of integrins such as $\alpha_{IIb}\beta_3$ is highly attractive, the mimicking of ligands may result in an intrinsic paradoxical integrin receptor activation and may therefore not be the ideal strategy for integrin inhibition (16).

A recent publication demonstrated that high-throughput screening can identify $\alpha_{IIb}\beta_3$ inhibitors that bind to the ligand binding site but do not act as ligand-mimetics (17). However, a low affinity of these reagents for binding to the ligand-binding site may prevent further drug development in this direction (17). Nevertheless, there are two alternative strategies that might be used to therapeutically block integrins without the intrinsic paradoxical activating effects of ligand-mimetic strategies: allosteric inhibition and activation-specific inhibition. Indeed recent publications present promising data supporting these strategies:

i) Allosteric inhibition of ligand binding to integrins may overcome the obstacles that are associated with ligand-mimetic integrin blockers. A peptide sequence within the β_3 -subunit of the platelet integrin $\alpha_{IIb}\beta_3$ that may serve as a target for allosteric inhibition of this adhesion molecule is reported by Haas in this issue of *Thrombosis and Haemostasis* (18). The reported sequence β_3 (95–105) is postulated to serve as a fulcrum for the

conformational changes that occur during integrin activation. The author elegantly demonstrated that a polyclonal antibody raised against β_3 -subunit and affinity-purified with a peptide corresponding to β_3 (95–105) efficiently blocked fibrinogen binding and platelet aggregation based on an allosteric mechanism without binding to the fibrinogen binding site in $\alpha_{IIb}\beta_3$ -integrin. Allosteric inhibition of integrins also seems to be possible with small-molecule inhibitors that target the α -subunit I domain of LFA-1 ($\alpha_L\beta_2$, CD11a/CD18) and Mac-1 ($\alpha_M\beta_2$, CD11b/CD18) (4, 19). In both cases, targeting the α -subunit as well as the β -subunit, the binding of inhibitors seems to stabilize the bent, low affinity conformation of the targeted integrin.

ii) A new strategy of therapeutic blockade of integrin receptors takes advantage of the unique situation that specific epitopes are expressed only on the extended, high affinity conformation of integrins. This allows a novel strategy, which succeeds to specifically block the activated integrins and thereby targets only activated cells (20, 21). Activation-specific blockade of $\alpha_{IIb}\beta_3$ -integrin prevents the induction of conformational changes and subsequent outside-in signalling (20). Furthermore, the specific inhibition of activated platelets prevents the prolongation of bleeding time and has the potential for strong anti-platelet effects combined with a reduction of bleeding complications (20).

In conclusion, major progress in the understanding of integrin structures and particularly of conformational changes upon integrin activation have provided the basis for an improved understanding of why the ligand-mimetic strategy of integrin inhibition comes with intrinsic paradoxical activating effects. This has also encouraged the development of additional modalities such as allosteric and activation-specific integrin inhibition. These novel strategies hold major promises for drug development in the many areas of human disease, where integrins play an essential pathomechanistic role.

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