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Review

Integrin antagonists

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Abstract. Integrins are a family of cell surface glycoproteins that mediate numerous cell-cell and cell-matrix interactions and are involved in biological processes such as tissue morphogenesis, leukocyte recirculation and migration, wound healing, blood clotting and immune response. Aberrant cell adhesion has been implicated in the pathogenesis of several diseases, including a number of inflammatory disorders such as rheumatoid arthritis, inflammatory bowel disease and asthma, as well as cancer and coronary heart disease. As such integrins are seen as excellent targets for the development of therapeutic agents. This report begins with an examination of the structure of integrin molecules and their ligands and then goes on to review the current state of development of antiintegrin antagonists.

Key words. Integrins; cell adhesion; antagonists; inflammation; cancer; arthritis; therapeutics.

Introduction

Many functions of multicellular organisms involve complex interactions between cells or between cells and the extracellular matrix (ECM). Cell adhesion is therefore of fundamental importance in a diverse range of biological processes, including cell differentiation, embryonic cell migration, maintenance of tissue integrity, the immune system and blood coagulation [1–4]. Importantly, alterations or aberrations in cell adhesion, and in particular integrin-mediated cell adhesion, have been implicated in the pathogenesis of a number of diseases such as atherosclerosis, cancer and a variety of inflammatory conditions [5–7], making integrins an attractive target for the development of therapeutic agents [8, 9].

This article reviews our current knowledge on the structure of integrin molecules, their ligands and the interaction between the two. We then go on to outline the potential of targeting these interactions in several disease states and report on the development of anti-integrin therapies.

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Integrin structure

Integrins are a large family of heterodimeric cell surface adhesion receptors, composed of an α and β subunit, that bind to a wide variety of ECM and cell surface ligands [10]. To date, 17 α and 8 β subunits have been identified, which can associate in a restricted manner to form at least 23 different integrins (fig. 1). An α-subunit usually only associates with a particular β-subunit (e.g. $\alpha 5$ only binds to $\beta 1$), whereas β-subunits are more promiscuous (e.g. $\beta 2$ binds to αM, αL, αX and αD [11]). Exceptions include $\alpha 4$, which can bind to $\beta 1$ and $\beta 7$, and αv, which binds to $\beta 1$, $\beta 3$, $\beta 5$, $\beta 6$ and $\beta 8$. Each subunit is composed of a large extracellular domain (typically 1000–1150 amino acids for the α subunit and 740–780 amino acids for the β subunit), a single transmembrane domain of about 20 amino acids and a short cytoplasmic domain of 40–50 amino acids (fig. 2). An exception to this is the $\beta 4$ subunit which has an extended cytoplasmic domain of over 1000 amino acids [12].

One of the most striking features of all integrin α-subunits is that the N-terminal half of the subunit consists

of a sevenfold repeating unit of about 60 amino acids. These seven repeats have been proposed to fold into a single compact domain in which the individual repeats are arranged around a pseudosymmetry axis, rather like the blades of a propeller, to form a toruslike structure called a β-propeller [13]. A consequence of this model is that residues involved in ligand binding should be found on the upper face of the propeller. Direct evidence in support of the β-propeller model comes from the work of Irie et al. [14], who used $\alpha 4/\alpha 5$ chimeras to identify loops in $\alpha 4\beta 1$ responsible for ligand binding. Loops identified as critical for ligand binding are found in repeats 2 and 4 on the upper face of the β-propeller (fig. 2). Very recently, Mould et al. [15] have identified residues in $\alpha 5\beta 1$ that are very close to the ligand-binding site of this integrin; these amino acids are located near the apex of a putative loop on the upper face of the propeller. Ligand binding by integrins is divalent cation-dependent, although the exact role of cations has yet to be elucidated. While repeats 4–7 of the α-subunit (repeats 5–7 in some integrins) contain several putative cation binding sites of the general structure DxDxDGxxD (single letter code where x represents any amino acid), these sites are predicted to be on the lower face of the propeller [13], suggesting an indirect role of cations in ligand binding.

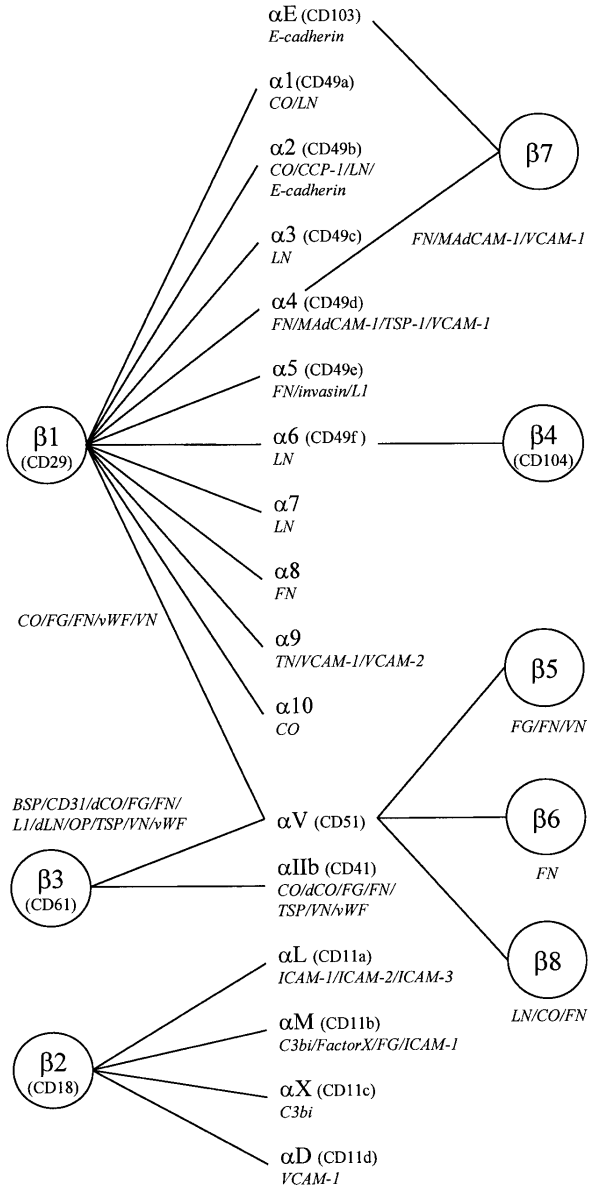


Figure 1. Integrin α/β heterodimers identified to date are shown grouped according to the broad families described in the text. The ligands bound by the heterodimer are indicated in italics either under the α-subunit, the β-subunit or adjacent to the line joining the α/β pair. Ligands are listed in alphabetical order and therefore do not indicate the major ligand of a given receptor. Ligand abbreviations: BSP, bone sialoprotein; C3bi, complement component; CD31, cluster of differentiation antigen 31; CO, collagens; dCO, denatured collagens; CCP-1, collagen C-propeptide (type 1); FG, fibrinogen; FN, fibronectin; ICAM, intercellular adhesion molecule; L1 is a recently discovered cell adhesion molecule; LN, laminins; dLN, denatured laminin; MAdCAM-1, mucosal addressin cell adhesion molecule-1; OP, osteopontin; TN, tenascin; TSP-1, thrombospondin; VCAM-1, vascular cell adhesion molecule-1; VN, vitronectin; vWF, von Willebrand factor.

About one-third of integrin α-subunits contain an inserted I-domain of approximately 200 amino acids. This domain is homologous to the von Willebrand factor (vWF) A3-domain [16] and, when present, is inserted between blades 2 and 3 of the β-propeller. I-domains are of interest because when expressed as soluble recombinant proteins they mimic the ligand-binding characteristics of the intact integrin. This suggests that binding of ligand to I-domain-containing integrins is mediated primarily by the I-domain [17, 18]. In addition, I-domains are the only part of the integrin molecule for which detailed structural information is available (fig. 2), with crystal structures having being determined for the I domains from αM [19], αL [20] and $\alpha 2$ [21] subunits. The I-domain forms a Rossmann dinucleotide-type fold (fig. 2), with a central β-sheet composed of five parallel and one antiparallel strands surrounded by seven α-helices with a divalent cation coordinated by loops at the top of the module to form a so-called metal ion-dependent adhesion site (MIDAS) [22]. In the I-domain the cation is coordinated by five amino acid side chains either directly or indirectly through a water molecule. Three of the coordinating residues are contained within a sequence D × S × S located in the loop between β strand A and α helix 1, while the other two coordinating residues are a threonine and an aspartate found on the loop between α helices 3 and 4, and the loop between β strand D and α helix 5, respectively (fig. 2). The crystal structure of the αM I-domain complexed with Mg^{2+} is unusual in that the sixth coordination position of the cation is occupied by a glutamic acid

residue (E314) from a neighbouring I-domain in the crystal lattice, rather than by a water molecule [19]. This intriguing observation has led to speculation that this interaction may mimic ligand binding, since the glutamate residue resembles the aspartic acid residue found in the common recognition sequences RGD (Arg-Gly-Asp) and LDV (Leu-Asp-Val) of most integrin ligands (see below).

The crystal structure of αM complexed with Mn^{2+} has also been determined [23] and shows a number of important structural differences compared with the Mg^{2+}-occupied protein [19], suggesting that the difference between the Mg^{2+}- and Mn^{2+}-containing crystal structures may represent the active and inactive states of the integrin (in contrast, the crystal structure of the αL I-domain had no major structure rearrangements whether in the presence of Mg^{2+} or Mn^{2+} or in the absence of cation [24]).

The N-terminal region of β-subunits contain a highly conserved region of approximately 200 amino acids, which has been predicted on the basis of hydropathy analysis to have structural similarity to the I-domain subunit [25]. This domain contains the D × S × S sequence found in I-domains (see above), while the two other potential residues for cation coordination are also conserved in β1, β2, β3 and β5 subunits [26–29]. More recently, Emme et al. [30], using structure prediction methods, suggested that while the β I-domain does in fact contain a MIDAS motif, the overall structure may not be identical to the structure found in α-subunit I-domains.

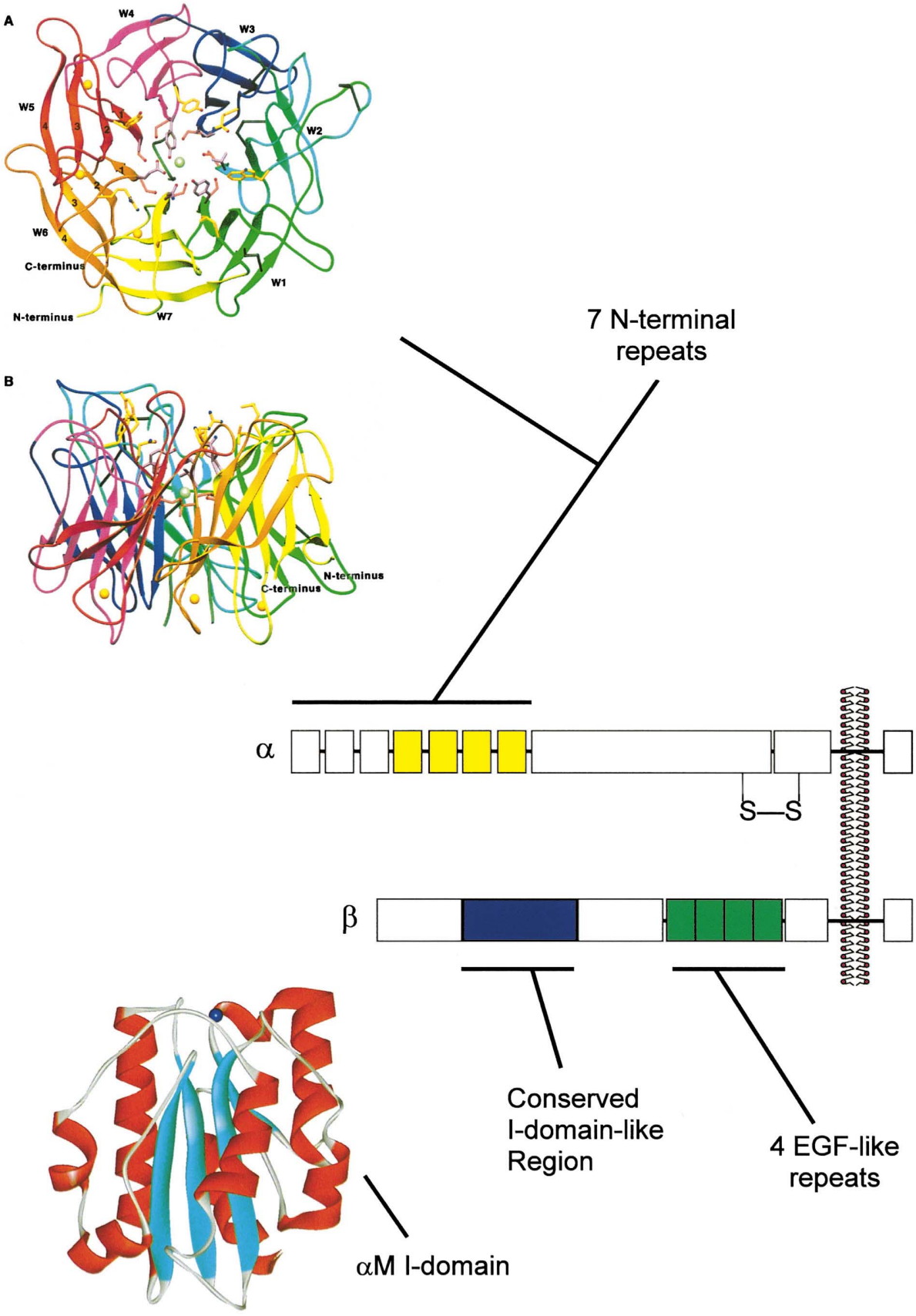
Integrin ligands

As the identification of key recognition motifs within integrin ligands was the starting point for the development of integrin antagonists, it is valuable to discuss in this section the current state of our knowledge of integrin ligands.

Integrin ligands are usually members of either the cell-surface immunoglobulin superfamily, such as the intracellular adhesion molecules (ICAM-1, -2, -3) and vascular cell adhesion molecule (VCAM-1), or are large modular ECM molecules, such as fibronectin, collagen and laminin [31]. Key sequences involved in integrin binding were identified by generating progressively smaller and smaller proteolytic fragments of the parent molecule or synthetic peptides and testing these for their retention of adhesive activity. Using this approach RGD was first identified as a key integrin recognition motif in fibronectin [32], and has since been found in several other ligands including fibrinogen and vitronectin.

Fibronectin is a large dimeric protein comprised of three prototypical modules, known as type I, II and III, which are arranged in a tandem array of about 30 repeats. Type III repeats are of particular interest as not only are they the most common of the fibronectin modules but because the 10th type III repeat contains the RGD motif (fig. 3) involved in binding to $\alpha5\beta1$, $\alpha v\beta3$ and $\alpha IIb\beta3$ integrins. In addition to the RGD sequence in the 10th repeat, there is an additional sequence (Pro-His-Ser-Arg-Asn) found in the 9th type III repeat which, although contributing relatively little binding energy directly, synergizes with the RGD sequence [33]. Recently, Leahy et al. [34] have solved the crystal structure of fibronectin type III repeats 7–10, and this reveals that the RGD sequence and 'synergy' site are on the same face of the molecule. In addition, the RGD sequence is found on a highly exposed loop, projecting away from the molecule, while the 'synergy' site is found at the junction of a loop and β-strand, placing them in an ideal position to interact simultaneously with integrin (fig. 3).

In addition to RGD, a second commonly occurring integrin recognition sequence has been identified. This sequence is loosely termed LDV, but unlike RGD which is invariant, this sequence has limited variability around a definable consensus sequence [L/I-D/E-S/T/V (single letter amino acid codes)]. The prototype LDV sequence was identified in the IIICS region of fibronectin [35] and shown to bind to $\alpha4\beta1$, but this has since been shown to occur in a number of integrin ligands including VCAM-1, mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and ICAM-1, -2 and -3. VCAM-1 contains a large extracellular region made up of seven immunoglobulin domains. While the LDV binding motif is located in domain 1 and domain 4, VCAM-1, like fibronectin, also seems to employ 'synergy' sites. Newham et al. [36] used site-directed mutagenesis to map residues in VCAM-1 that were involved in binding to $\alpha4\beta1$ and $\alpha4\beta7$ to show that in addition to residues found at and near the LDV motif, there is a pronounced requirement for certain residues located in domain 2. A deeper understanding of the molecular basis of the binding 'footprint' of VCAM-1 comes from the examination of its X-ray structure [37]. This shows that the LDV motif (IDS is the actual sequence) is on a highly solvent-exposed C-D loop, pointing away from the molecule in an ideal position to interact with integrin, while the residues in domain 2 implicated in binding (the putative 'synergy' site) are predominantly located on a unusually long C-E′ loop on the same face of the molecule as the LDV motif (fig. 3). Thus two very different integrin ligands (fibronectin and VCAM-1) may share the same 'binding topology', in that (i) both


A
W4
W3
W5
W2
W6
C-terminus
W1
N-terminus
W7
B
N-terminus
C-terminus
7 N-terminal repeats
α
S—S
β
Conserved I-domain-like Region
4 EGF-like repeats
αM I-domain

contain a dominant acidic binding site flanked by an adjacent synergy site 30–40 Å away, (ii) these active sites are found on the same face of adjacent domains and (iii) these motifs are found on surface-exposed loops. This observation raises the intriguing possibility that integrins in general may engage at least two sites on the ligands [31].

Integrins in health and disease

Platelet aggregation and αIIbβ3 receptor

Thrombosis of coronary arteries continues to be the major cause of death in heart attack and stroke. Platelets play a crucial role in this process since platelet aggregation and adherence is the first step in the development of a thrombosis which can cause blockage of blood vessels at sites of injury or atherosclerotic plaques.

Platelet aggregation is one of the best-understood cell-adhesive events, involving the interaction between the αIIbβ3 (platelet glycoprotein IIb/IIIa) receptor on the surface of the platelet and soluble fibrinogen. When platelets are stimulated by physiological molecules (e.g. thrombin, ADP, collagen) binding to membrane receptor-coupled G proteins, intracellular signalling pathways ('inside-to-outside' signalling)—mediated by several groups of molecules (including Ca^{2+}, lipids and protein kinases)—cause αIIbβ3 integrin to undergo conformational changes. These changes correlate with a switch in the receptor to a high-activity state in which it can bind to a number of ligands, including fibrinogen, vitronectin and vWF [38]. Fibrinogen is the preferred ligand by virtue of its relatively high plasma concentration. While fibrinogen contains two RGD sites (located on its α-chain) as a minimal recognition sequence, it also contains at the carboxy-terminal end of its γ-chains the unique sequence KQAGDV, which is essential for integrin binding. Indeed, isolated γ-chains support aggregation in the absence of the α-chain, while peptides containing the KQAGDV motif inhibit platelet aggregation and block the binding of fibrinogen and RGD peptides to αIIbβ3 [39, 40].

Binding of fibrinogen to the activated integrin causes clustering of the integrin-ligand complexes, and results in the formation of adhesive 'patches' on the surface of the platelets. Since fibrinogen molecules are bivalent, they can form an integrin-fibrinogen-integrin bridge between adjacent platelets, thus mediating aggregation. The importance of αIIbβ3 in normal hemostasis is illustrated by the inherited bleeding disorder, Glanzmann's thrombasthenia, which is characterized by uncontrolled bleeding and is caused by a lack of, or mutations in, this integrin. In contrast, as noted above, uncontrolled or aberrant platelet aggregation is a primary cause of arterial thrombosis, and as such this integrin has been the target for therapeutic intervention [41]. Other conditions which could benefit from blockade of αIIbβ3 activity include unstable angina, myocardial infarction and stroke.

The validity of targeting αIIbβ3 has been vindicated from the results of several trials involving a variety of agents, including peptide and nonpeptide mimetics, and recombinant anti-αIIbβ3 antibodies [41] (fig. 4). Of particular interest is the potential of c7E3 (abciximab; REOPRO), a mouse/human chimera derived from a mouse anti-αIIbβ3 monoclonal antibody [42]. This antibody contains the variable regions of the mouse anti-αIIbβ3 monoclonal antibody, 7E3, ligated to the constant domains of human immunoglobulin G (IgG), and has been shown to inhibit platelet aggregation both in vitro and in vivo. Interestingly, it has been suggested that c7E3, in addition to its direct effect on platelet aggregation, may also function by inhibiting thrombin formation [43]. This emphasizes the close relationship between platelet activation and thrombin generation in blood coagulation. Similar results have been observed using low molecular weight inhibitors [44], raising the possibility that this dual function may be a feature of αIIbβ3 inhibitors. The first large-scale trial of c7E3 [Evaluation of c7E3 in Preventing Ischemic Complications (EPIC)] investigated the protective effect of the antibody on 2099 patients undergoing coronary intervention [45]. In summary, the trial demonstrated a 35% reduction in the primary end-point (defined in this study as death, myocardial infarction or urgent revascularization) compared with the placebo group after 30 days, while a 26% reduction was still seen after 6 months [46]. This promising result has led to the approval of this compound in the United States and Europe for use in patients deemed to be at risk of suffering ischemic complications during percutaneous

Figure 2. Schematic diagram of a generic integrin α/β heterodimer. The seven α-subunit N-terminal repeats are shown, the last four of which contain EF-handlike divalent cation binding sites (shown in yellow). These seven repeats are believed to fold to form a compact β-propeller structure (the β sheets are labelled W1–W7). The putative I-domain-like-structure found in integrin β-subunits is shown in blue. This domain is believed to fold in a manner similar to the I-domains found in approximately one-third of α-subunits. The crystal structure of αM I-domain is shown and illustrates the typical Rossmann fold adopted by I-domains. Downstream from the putative β I-domain-like motif are four cysteine-rich epidermal growth factor-like repeats (shown in green). Structures were drawn using Web Lab Viewer.

intervention. Injection of a bolus of 250 μg/kg of c7E3 led to $>90\%$ blockage of $\alpha IIb\beta 3$ and $>80\%$ inhibition of adenosine diphosphate (ADP)-induced platelet aggregation. This was maintained by a 12-hour infusion of antibody at 10 μg/kg. It is surprising that c7E3 has such a prolonged clinical effect given that its initial half-life in the plasma is only ~ 30 min. However, a substantial portion (over two-thirds) of the injected antibody remains bound to platelets, where it can redistribute to new platelets entering the circulation, thus accounting for the persistence of platelet-associated c7E3 for several weeks. One possible drawback of c7E3 treatment raised by the EPIC trial was an increase in bleeding, leading to a twofold increase in the need for red blood cell and platelet transfusion [47]. A more recent trial of 1500 patients [Evaluation of PTCA to Improve Long-term Outcome by c7E3 GPIIB/IIIA Receptor Blockade; (EPILOG)] aimed at further evaluating the efficacy and safety of c7E3 [48–50]. To improve the safety profile, a reduced dose of heparin was employed, and the enrollment was expanded to include both high- and low-risk patients. Reducing the heparin dose did not reduce the efficacy of the treatment, but did reduce the bleeding to levels seen with the placebo [48, 49]. A potential problem inherent in the use of antibodies as therapeutic agents is the possibility of adverse immune reactions. Although c7E3 was engineered to minimize this possibility, approximately 7% of individuals treated with c7E3 did develop antibodies to the murine part of the molecule [45, 50].

As well as c7E3, several peptide and nonpeptide mimetics have been the subject of clinical examination. The RGD motif has usually been the starting point for the development of these compounds, and they range from peptides retaining the RGD sequence, through RGD mimetics and molecules containing unnatural amino acids to small organic molecules that have little obvious structural similarity to RGD [51].

One such molecule that has been the subject of an extensive investigation is the peptide INTEGRILIN [52, 53], a cyclic heptapeptide, which includes the sequence KGD. This molecule acts as a competitive inhibitor

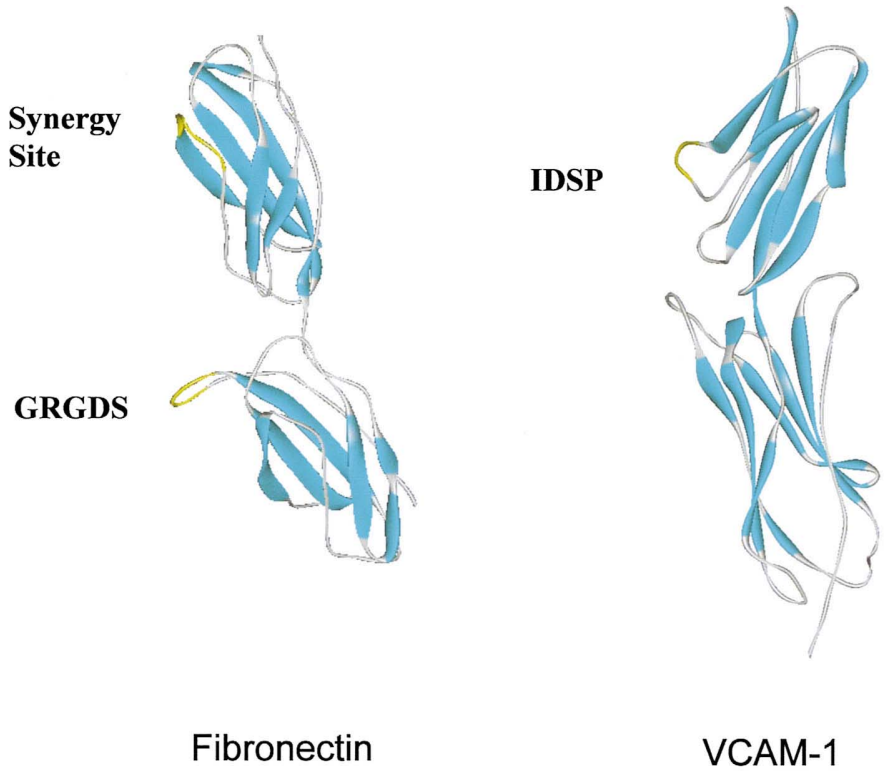


Figure 3. Crystal structures of type III repeats 9 and 10 of human fibronectin (left) and domains 1 and 2 of human VCAM-1 (right). In fibronectin, the RGD recognition motif is found on a highly solvent exposed loop (yellow), with the synergy site (yellow) on the same face of the molecule 3–4 nm away. Similarly, in VCAM-1 the IDSP motif (yellow) is found on an exposed loop in an ideal position to interact with integrin. Structures were drawn with Web Lab Viewer.

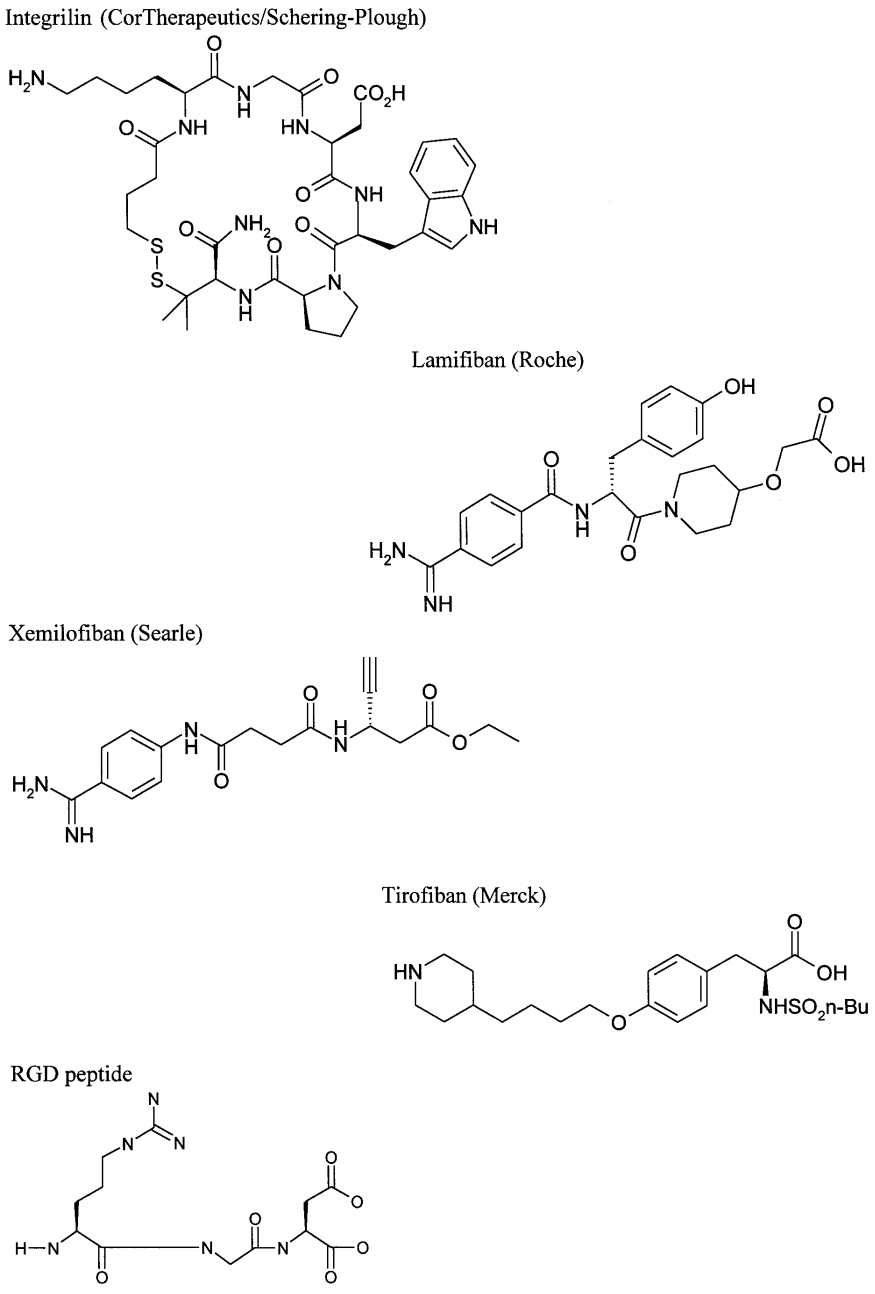


Figure 4. Chemical structures of selected αIIbβ3 (GP IIb/IIIa) receptor antagonists that are currently being developed by the pharmaceutical industry. The RGD peptide, the 'natural' ligand of αIIbβ3, is depicted to illustrate the differences between the potential therapeutic agents and the original peptide sequence.

with a short half-life of about 2 h; when evaluated in a phase III study [Integrilin to Minimize Platelet Aggregation and Prevent Coronary Thrombosis II (IMPACT-II)], the compound showed a 30–35% reduction in primary end-point at 24 h and a 13–19% reduction at 30 days. Importantly, it did not cause any increase in bleeding [52, 53].

Several nonpeptide αIIbβ3 inhibitors have also been the subject of clinical trials (fig. 4), including the compound Tirofiban (AGGRASTAT) [54, 55], which show a

degree of protection similar to the peptide INTEGRILIN. Although Tirofiban bears no obvious structural similarity to RGD, it is believed to mimic the geometric, stereotactic and charge characteristics of RGD [56]. A phase III investigation of Tirofiban [Randomized Efficacy Study of Tirofiban for Outcomes and Restenosis (RESTORE) Trial] in patients with an increased risk of arterial closure (e.g. unstable angina, recent myocardial infarction) showed a 38% reduction in the clinical composite end-point at day 2 and a 27% reduction at day 7. However, by day 30 there was no significant difference between placebo and Tirofiban [57]. A further follow-up trial carried out to investigate the effect of administering Tirofiban in the absence of concomitant intravenous heparin treatment [Platelet Receptor Inhibitor for Ischemic Syndrome Management (PRISM)] revealed a significantly lower death rate on day 30 (2.3% versus 3.6%) in Tirofiban (+ aspirin)-treated patients compared with those who received heparin (+ aspirin) [58]. While the above molecules show promise, the focus is now on the development of orally active antagonists, several of which are undergoing clinical investigation. One such molecule, xemilofiban [59, 60], is an orally active fibrinogen receptor antagonist. It is an ester prodrug with an (aminobenzamidino)succinyl structure based upon the RGD sequence of fibrinogen. In a pilot study Xemilofiban was administered to 23 patients with unstable angina undergoing percutaneous transluminal coronary angioplasty [59, 60]. ADP-induced aggregation was found to be inhibited by $92.5 +/- 6.1\%$ by Xemilofiban in comparison to a placebo effect of $3.9 +/- 16.1\%$ at 6 h after the first drug application. The inhibitory effect decreased to 52.1% after 2 and to 29.9% after 4 weeks on the drug—the latter not being significantly different from the baseline. Recently, however, its development, along with that of another orally active fibrinogen inhibitor, Orbofiban, was halted by Searle when extended clinical trials showed them to be ineffective in the prevention of blood clot formation in coronary arteries [61].

sibrafiban, a double-prodrug form of Ro 44-3888 [62, 63], and Lefradafiban [64], a prodrug of Fradafiban are just two other examples of orally active αIIbβ3 antagonists which are now undergoing clinical trials for the prevention/therapy of acute coronary syndrome [65]. In contrast to the oral antiplatelet drugs currently available (e.g. aspirin), this new generation of orally active αIIbβ3 antagonists shows a superior anti-aggregatory profile by blocking the final steps of platelet activation. In spite of the encouraging results obtained up to now, there is still a need for more data before these promising drugs become routinely used for clinical applications.

Leukocyte-endothelial interactions

Leukocytes play a crucial beneficial role in normal host defence against both bacterial and viral infections as well as against tumours by migrating from the bloodstream to the sites of injury (a process known as extravasation). However, since uncontrolled or excessive leukocyte migration has been implicated in a number of inflammatory disorders [e.g. rheumatoid arthritis, asthma, multiple sclerosis, inflammatory bowel disease (IBD) and reperfusion injury following stroke or myocardial infarction], leukocyte-endothelial interactions have been the focus of much attention by the pharmaceutical industry [9, 66].

Leukocyte extravasation is a complex, multistep process involving several different families of cell-adhesion molecules. The initial phase is known as rolling and involves weak transient interactions between the selectins, but the actual transmigration event is mediated by interactions between αLβ2 and ICAM-1, -2 [11, 67] and α4β1 and VCAM-1 [68, 69] or in the case of the gut, α4β7 and MAdCAM-1. While there is a large body of evidence implicating all three integrins in inflammatory diseases [70], most effort has concentrated on the α4 integrins rather than the αLβ2 because of concern that blocking the β2 integrin may severely compromise immune responses by blocking neutrophil migration at sites of infection (which functions as a frontline defense against most microbial pathogens). The importance of this integrin in immune function is demonstrated by the problems in clearing infections experienced by people who lack β2 integrins and suffer from leukocyte adhesion deficiency [71].

Several reports from animal models of inflammatory diseases have indicated that the α4β1:VCAM-1 adhesion pathway may be important in mediating the migration of leukocytes from the blood to tissues at sites of inflammation (reviewed in [70]) and that anti-α4β1 antibodies can be effective in reducing migration and thus prevent disease. For example, the anti-α4 antibody HP1/2 blocked eosinophil recruitment in guinea pig skin by 50–80% [72], while Abraham et al. blocked leukocyte infiltration into the lungs of sheep in a model of allergic asthma [73, 74] with the same antibody.

The most advanced α4β1 antagonist is a humanized antibody called ANTEGREN [75] (table 1). An initial indication of the potential of this reagent comes from the work of Yednock et al. [76] who used this antibody to prevent inflammatory lesions in the brain in an experimental model of autoimmune encephalomyelitis in rodents. In a phase I clinical study the antibody was well tolerated and proven to be safe. Encouraging results arose from phase II studies with patients suffering from multiple sclerosis or IBD. Phase III studies are planned [http://www.athenaneuro.com/pressrelease012298.html, 77].

Several companies have focused on developing small molecule antagonists to $\alpha4\beta1$ [77] (fig. 5). Most of these have taken the LDV motif of VCAM-1 as a starting point. Probably the most advanced of these compounds is the Biogen compound, BIO1211, a capped LDV derivative [77]. This molecule demonstrated a high degree of efficacy and potency in a sheep model of allergic bronchoconstriction when administered as a single daily dose of 0.1 mg/kg in aerosol form. BIO1211 has just passed phase I clinical trials successfully. The other $\alpha4$ integrin involved in inflammation is $\alpha4\beta7$, whose usual ligand is MAdCAM-1. This integrin acts as a homing receptor for lymphocytes to Peyer's patches in the gastrointestinal (GI) tract [78]. Inhibition of the $\alpha4\beta7$:MAdCAM-1 interaction either with antibodies or small molecular antagonists could reduce the recruitment of inflammatory lymphocytes to the GI tract. Moreover, cytokine levels might also be reduced, leading to a selective attenuation of chronic inflammation within the GI tract without interfering with the peripheral lymphoid compartment [79]. Potential diseases that might benefit from such therapies include IBD (i.e. ulcerative colitis and Crohn's disease); a treatment for ulcerative colitis is particularly desirable since this disorder greatly increases the risk of colon cancer [80].

In addition, $\alpha4\beta7$ antagonists could be useful for the treatment of insulin-dependent diabetes mellitus. This autoimmune disease is caused by the destruction of insulin-producing cells in the β-islets of the pancreas. A prominent role during this destructive process is played by activated lymphocytes migrating via $\alpha4\beta7$:MAdCAM-1 interaction [81, 82].

The effects of antibodies against $\alpha4$ or $\alpha4\beta7$ have been investigated in a colitis model with cottontop tamarins. These monkeys experience a spontaneous acute and chronic colitis similar to ulcerative colitis. While anti-E-

Table 1. Overview of selected, available and upcoming anti-integrin therapeutics.

Target integrin	Indication	Developer	Antagonist	Application	Current status
αIIb$\beta3$	Myocardial infarction	Centocor/Eli Lilly	REOPRO (c7E3)	i.v.	on the market
	Coronary thrombosis	CorTherapeutics/ Schering-Plough	INTEGRILIN (Eptifibatide)	i.v.	on the market
	Adjunct to angioplasty; unstable angina	Merck & Co.	Tirofiban (AGGRASTAT)	i.v.	on the market
	Myocardial infarction	Boehringer Ingelheim	Lefradafiban	p.o.	phase III
	Acute coronary syndrome	Roche/Genentech	Sibrafiban (Ro483657)	p.o.	phase III
	Restenosis after PTCA	Searle	Xemilofiban	p.o.	phase III stopped
	Unstable angina	Searle	Orbofiban	p.o.	phase III stopped
$\alpha4\beta1$	MS, IBD	Athena Neurosciences (Elan)/ Cytel	ANTEGREN (AN-100226, humanized mAb)	i.v.	phase II
	Asthma, IBD	Biogen/Merck & Co.	BIO1211 (peptidomimetic)	aerosol, p.o.	phase I preclinical
	Chronic inflammatory diseases	Cytel	CY9652 (peptidomimetic, CS-1 derivative)	p.o.	preclinical
	Asthma, RA	Hoechst Marion Roussel	peptidomimetic	aerosol, p.o.	preclinical
	Asthma, RA	Texas Biotechnology	TBC772 (cyclic peptide), TBC3342 (peptidomimetic)	aerosol, p.o.	preclinical
	MS, RA	Zeneca	ZD-7349 (modified tetrapeptide), peptidomimetics	i.v., p.o.	preclinical
$\alpha4\beta7$	IBD	LeukoSite/Genentech	LDP-02 (humanized mAb)	i.v.	phase I
$\alpha v\beta3$	Cancer, metastasis	IXSYS	LM609 (mAb, Vitaxin)	i.v.	phase I
	Cancer, thrombosis	E. Merck KGaA	cyclic peptide, peptidomimetic	i.v./p.o.	preclinical
	Restenosis, osteoporosis	Merck & Co.	peptidomimetic	i.v.	preclinical
	Cancer	Searle	peptidomimetic	i.v.	preclinical
	Osteoporosis	Hoechst Marion Roussel	peptidomimetic	p.o.	preclinical
	Restenosis, osteoporosis	Smith Kline Beecham	peptidomimetic	p.o.	preclinical

IBD, inflammatory bowel disease; MS, multiple sclerosis; PTCA, percutaneous transluminal coronary angioplasty; RA, rheumatoid arthritis; i.v., intravenous; p.o., per os.

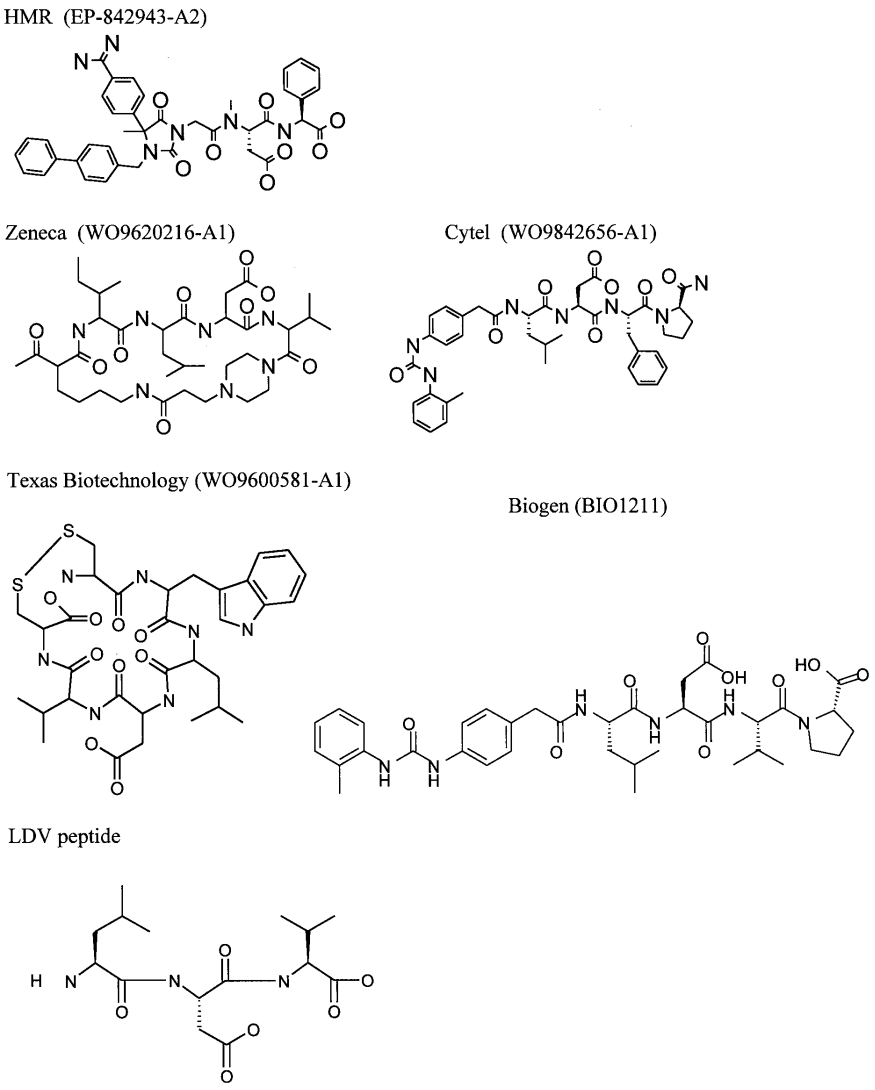


Figure 5. Chemical structures of selected VLA-4 ($\alpha 4\beta 1$) antagonists that are currently being developed by the pharmaceutical industry. The LDV peptide, one of the 'natural' ligands of VLA-4, is depicted to illustrate the differences between the potential therapeutic agents and the original peptide sequence.

selectin monoclonal antibodies (mAbs) showed no significant amelioration of the disease, treatment with the anti-human $\alpha 4$ mAb HP1/2 led to an attenuation of acute colitis [83]. In a second study, an antibody to $\alpha 4\beta 7$ was effective in the chronic phase of the disease [84]. While a first antagonist of $\alpha 4\beta 7$, a humanized mAb (LDP-02), has undergone a phase I clinical trial and was well tolerated, several possible inhibitory peptides and peptidomimetics are under development. These have been based on several regions found in both the first and second Ig-like domain of MAdCAM-1, which have been shown to play an important role in binding $\alpha 4\beta 7$ [36, 85]. Chemically modified peptide sequences which mimic the conserved amino acid motif LDTSL of MAdCAM-1 are currently being investigated [86].

One concern of targeting $\alpha 4$ integrins is a possible deleterious effect on physiological functions such as normal immune and inflammatory responses, infections, hematopoesis, neural development, wound healing, placenta formation and muscle development [70, 87]. Although acute neutrophil-dependent clearance of infectious agents should not be affected by anti-$\alpha 4$ antagonists (see above), rats treated with the anti-$\alpha 4$

mAb TA-2 were unable to effectively resolve intestinal nematode infections, underlining the important role of $\alpha 4$ integrins in lymphocyte-dependent intestinal immunity [88], making it clear that potential side effects of anti-$\alpha 4\beta 1$ antagonists need to be investigated further.

Adhesion molecules as targets for cancer therapy

Alterations in the adhesive properties of tumour cells have been implicated in the metastatic capacity of many cancers. Inhibiting the function of molecules which are involved in pathological processes such as tumour neovascularization and metastasis could therefore be of significant therapeutic benefit.

One of the most promising therapeutic targets for cancer therapy is the integrin $\alpha v\beta 3$, which can bind to a number of ECM components such as vitronectin, fibronectin, fibrinogen and thrombospondin [89, 90] and is expressed on a limited set of cell types and tissues and highly upregulated on activated endothelial cells. The fact that $\alpha v\beta 3$ is constitutively expressed on several malignant tumour cells makes it an attractive target for therapeutic intervention. Several studies suggest a corre-

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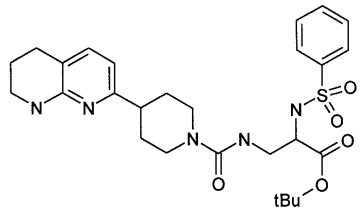

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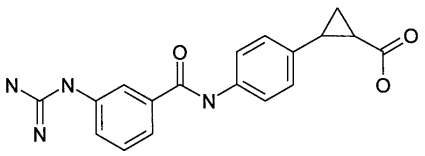

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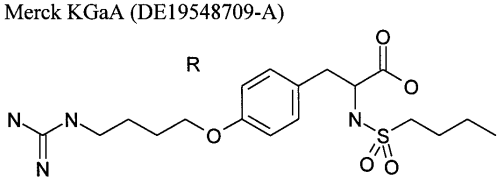

RGD peptide

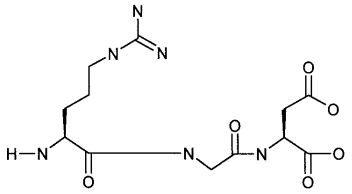

Figure 6. Chemical structures of selected $\alpha v\beta 3$ (vitronectin receptor) antagonists that are currently being developed by the pharmaceutical industry. The RGD peptide, a 'natural' ligand of $\alpha v\beta 3$, is depicted to illustrate the differences between the potential therapeutic agents and the original peptide sequence.

lation between highly expressed $\alpha v\beta 3$ and an increase in invasive ability of tumours, including liver cancer and melanomas [91–94]. For example, in contrast to noninvasive horizontally growing lesions, invasive lesions of melanomas growing vertically show high levels of $\alpha v\beta 3$ expression. This dissemination of tumour cells has been shown to be blocked by monoclonal antibodies and peptide antagonists of $\alpha v\beta 3$ in several model systems [92, 95, 96]. In addition, the preferential expression of $\alpha v\beta 3$ on newly forming blood vessels adds to its promise as an antitumour target, since tumour proliferation and metastasis are highly dependent on neovascularization. Antagonists of $\alpha v\beta 3$ have been shown to block tumour-associated angiogenesis by causing apoptosis of the new blood vessel cells [95]. As neovascularization is also involved in other pathologic conditions such as rheumatoid arthritis and diabetic retinopathy, $\alpha v\beta 3$ antagonists might also be applied for these indications, as recently suggested [97, 98].

The pharmaceutical industry is actively developing several anti-$\alpha v\beta 3$ antagonists (Table 1, fig. 6). One such group of inhibitors currently under investigation is synthetic cyclic RGD-peptides and peptidomimetic derivatives. Crucial to the development of agents selective for $\alpha v\beta 3$ (as opposed to the related $\alpha IIb\beta 3$ integrin) is the three-dimensional structure and the regions flanking the RGD-motif.

In terms of chemical synthesis, this goal can be achieved using compounds with a shorter distance between carboxyl and guanidino groups and inserting a hydrophobic residue next to the RGD sequence [99], suggesting, based on previous experience, that potent small molecule inhibitors of $\alpha v\beta 3$ might soon enter clinical trials.

Further issues to address

Since in addition to mediating cell adhesion directly, integrins are intimately involved in the generation of intracellular signals. Binding of ligand to integrins may lead to so-called outside-to-inside signalling resulting in cytoskeletal reorganization, modulation of differentiation and induction of gene expression [100, 101].

One concern therefore is that ligand mimetics, such as peptides or nonpeptidic antagonists may trigger the same pathways with unforeseen consequences. However, to date there have been no definitive reports in the literature addressing this issue.

Conclusion

Due to the significant increase in our understanding of integrins and their diverse functions in physiological and pathological conditions, these molecules have become very interesting targets for a variety of therapeutic areas. Based on this knowledge, researchers from both academic and industrial laboratories are focusing on the development of antagonists of integrins. Modern techniques such as combinatorial chemistry and parallel synthesis have opened up new possibilities for the design and development of specific, small-molecule integrin antagonists. Based on encouraging data from in vitro studies, animal models of diseases and, most important, from clinical trials, it is ever more likely that anti-integrin therapies will soon become a routine part of regular clinical practice (table 1).

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