Inhibition of vascular smooth muscle cell adhesion and migration by c7E3 Fab (abciximab): a possible mechanism for influencing restenosis

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Abstract

Objectives: Brief intravenous administration of chimeric antibody c7E3 Fab during coronary angioplasty has been shown in some studies to provide long term protection against coronary events. Smooth muscle cell (SMC) adhesion and migration are key initial steps in the development of restenosis. The purpose of this study was to investigate the effect of c7E3 Fab on adhesion and migration of SMC to the extracellular matrix (ECM) proteins osteopontin (Opn) and vitronectin (Vn). Methods: Adhesion of human vascular SMCs to ECM proteins was quantified using a CyQUANT assay kit. Migration of SMCs to Vn, Opn and PDGF was studied using a modified Boyden’s chamber migration assay. Integrin expression was determined by immunoprecipitation. Results: c7E3 Fab reduced SMC adhesion on Vn and Opn to 69.2 ± 3.3% (P < 0.001) and 52.5 ± 4.8% (P < 0.001) respectively, compared to adhesion without antibody present. This reduction was the same as that for anti-αβ3 integrin antibody LM609 (P < 0.5). The combination of anti-αβ3 integrin antibody and c7E3 Fab had a greater effect than either antibody alone (P < 0.001). c7E3 Fab reduced SMC migration to Vn and Opn to 51.6 ± 8.9% (P < 0.001) and 20.3 ± 6.1% (P < 0.001) respectively, compared to migration in the absence of antibodies. Again, similar results were seen with LM609. PDGF-induced SMC migration was also inhibited by c7E3 Fab (P = 0.004) and LM609 (P = 0.001), but to much less an extent. The migration SMCs from a culture found not to express the integrin was unaffected by these antibodies, strengthening the argument that c7E3 Fab inhibits SMC function via this integrin. Conclusions: c7E3 Fab inhibits the adhesion and migration of SMCs via the integrin. The inhibition, however, is partial, and varied depending on type of ECM protein and αβ3 integrin expression. Some of the clinical benefits of c7E3 Fab may be due to its effect on SMCs. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Extracellular matrix; Monoclonal antibodies; Receptors; Restenosis; Smooth muscle

1. Introduction

Administration of c7E3 Fab (abciximab, Reo Pro) to prevent thrombotic complications following percutaneous coronary interventions has been associated with long-term reductions in subsequent coronary events or need for revascularisation in some trials [1]. This observation led to speculation that this antibody may also influence restenosis.

Although the antibody c7E3 Fab was developed to be specific for the glycoprotein IIb/IIIa integrin expressed on the surface of activated platelets [2,3], it has recently been confirmed that it also binds the αβ3 integrin [4]. This receptor is found on the surface of several cells, including human vascular smooth muscle cells (SMCs) [5,6]. It is upregulated following vascular injury [7,8], and is involved in SMC adhesion and migration [9–14], both of which are important initial steps in the development of the neointimal tissue that is responsible for vessel restenosis [5]. Animal studies have demonstrated that blockade of the αβ3 integrin after angioplasty results in reduction of neointimal formation [14–16].

The parent IgG of c7E3 Fab, m7E3, has been shown to reduce thrombospondin- and thrombin-induced SMC proliferation in vitro, but has no effect on PDGF- or serum-
induced proliferation [7]. c7E3 Fab has been shown to reduce adhesion of α3β1-expressing M21 melanoma cells in vivo [4]. Its effect on human vascular SMC adhesion and migration, however, has not been reported. In this study, the influence of c7E3 Fab on adhesion and migration to SMC substrates vitronectin (Vn) and osteopontin (Opn) was studied. Both these extracellular matrix proteins are present in increased quantities at the site of vascular injury [16–18], and are implicated in adhesion and migration processes mostly mediated via the α3β1 integrin [9–12,16,17]. Platelet derived growth factor (PDGF) was included in the migration studies as it is an important chemotactic agent following vascular injury [19]. Migration studies using cells not expressing the α3β1 integrin were used as a negative control. In all experiments, abciximab was studied in parallel with LM609, which is known to block the binding of α3β1, and inhibit cell adhesion to Vn.

2. Methods

2.1. Materials

The rat osteopontin (Opn) was kindly donated by Smithkline–Beecham, USA. Human vitronectin (Vn) was purchased from Life Technologies. A monoclonal antibody against smooth muscle cell α-actin (A4A) was purchased from Sigma Bioscience. The c7E3 Fab was kindly donated by Centocor, USA. Mouse monoclonal antibodies against human α3β1 integrin (MAB1976, clone LM609), α3β5 integrin (MAB1961) and vitronectin (MAB1945) were purchased from Chemicon International, FITC-conjugated F(ab’)2 fragment of rabbit anti-mouse immunoglobulin (P0313) was purchased from Dako. Mouse IgGκ antibody against trinitrophenol, (03000D, now-03191D) was used as a control irrelevant antibody in both the adhesion and migration studies. It is marketed as a control antibody by Pharmingen, and was used instead of pooled IgG, which may have contained antibodies that interacted with SMCs. It is referred to as control IgG. Mouse anti-human integrin α5 monoclonal antibody (33220D) was also purchased from Pharmingen, and was used in the adhesion assays as a second control as c7E3 Fab is not thought to interact with this integrin sub-unit.

2.2. Cell culture

Human vascular SMCs were obtained from primary explant culture of fragments of saphenous vein or aortic punch biopsy obtained from patients undergoing surgery as described previously [20]. The investigation conforms with the principles outlined in the declaration of Helsinki (Cardiovascular Research 1997;35:2–3). Briefly, the endothelium was removed by gentle scraping, and the adventitia was dissected away. The remaining tissue was cut into small pieces, and incubated in DMEM with 20% foetal calf serum (FCS), pyruvate (0.11 g/l) and gentamicin at 37°C, 5% CO2. SMCs growing out of explant were sequentially cultured in 10% FCS-containing medium. SMCs of two–six passages were used in the study. They were characterised by their typical hill and valley morphology at confluence, and by their staining with antibodies against SMC α-actin.

2.3. Adhesion assays

The adhesion experiments were carried out using cells derived from the saphenous vein from one subject. These studies were performed twice, using four wells per set of conditions. Ninety-six-well plates were pre-coated with 50 μl (100 nmol/l) of Vn or Opn at 4°C overnight, blocked with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 1 h at 37°C and rinsed with PBS. Uniform coating with Vn was confirmed by indirect immunofluorescence using mouse antibodies against Vn and FITC-conjugated F(ab’)2 fragment of rabbit anti-mouse immunoglobulin. Adhesion assays were conducted as described previously with some modifications [12,20]. Confluent cells were resuspended in serum-free DMEM with 0.25% BSA. Cells in suspension (104 cells in 100 μl) were allowed to adhere to pretreated 96-well plates for 1 h at 37°C (5% CO2). When antibodies were used, the cells were preincubated with antibodies for 30 min at 37°C and allowed to adhere in their presence. Antibodies were used at a concentration of 10 μg/ml, except for the varied dose studies, where concentrations were from 0.5 to 25 μg/ml. After adhesion the medium was removed by gentle suction, and non-adherent cells were removed by washing twice with PBS. The adhesion was quantified using a CyQUANT™ nucleic acid fluorescence assay kit (Molecular Probes). Calibration curves showed the fluorescence reading to be proportional to the cell number.

In further studies, the experiments performed with cells derived from saphenous vein were repeated using aortic SMCs in order to confirm the results with arterial cells.

2.4. Migration assays

The initial results of the adhesion experiments led us to investigate SMC migration, this time focusing the effects of c7E3 Fab and LM609 on migration to Vn and Opn. PDGF was also included as it is one of the important chemotactic growth factors present in vivo, and we wished to find out whether c7E3 Fab was able to influence migration towards it.

Migration studies were conducted as described previously [11,20] with some modifications. Confluent cells were resuspended in serum-free DMEM with 0.25% BSA. Cells in suspension (5×104 cells per 100 μl) were added to the upper compartment of a Transwell plate (Costar, 8-μm polycarbonate membrane). The lower compartment con-
tained 600 μl of DMEM+0.25% BSA, with Vn (20 μg/ml), Opn (20 μg/ml), or PDGF-BB (10 ng/ml), added when appropriate. The concentrations of Vn, Opn and PDGF-BB used were determined in preliminary experiments of SMC migration in the absence of antibodies, where all three were found to induce migration in a dose-dependent manner with a plateau being achieved at higher concentrations. The concentrations selected were within the plateau range. The plates were incubated at 37°C, 5% CO₂ for 6 h. The upper compartment of the chamber insert was rinsed with PBS, and the cells were fixed in 4% paraformaldehyde at room temperature for 20 min. Cells on the upper surface of the membrane were removed by gentle wiping. The inserts were incubated with 20 μg/ml of propidium iodide (Molecular Probes) in the presence of ribonuclease A (0.2 mg/ml) for 30 min at 37°C to stain cell nuclei. The membranes were mounted on glass slides and the number of migrating cells was averaged over five ×200 power fields per membrane. All experiments were performed at least twice, using three inserts for each set of conditions.

2.4.1. Preliminary migration studies and immunoprecipitation

The early observation that the cells from one particular saphenous vein did not migrate significantly to Opn led to experiments being performed to characterise the integrin expression of the cells. Immunoprecipitation was carried out on these cells, and compared to immunoprecipitation of SMCs from a saphenous vein culture that did migrate to Opn.

SMCs were grown overnight in methionine-lacking MEM supplemented with 10% FCS containing 20 μCi/ml of [35S]-methionine (Amersham). Cells were washed three times with PBS, and dissolved and scraped in RIPA buffer, containing 10 mM Tris–Cl, pH 7.0, 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% NP40, 1% sodium deoxycholate, protease inhibitors cocktail (Sigma), followed by centrifugation to remove cellular debris. Protein extracts containing equal amounts of [35S]-labelled proteins (10⁷ cpm) were diluted up to 400 μl with RIPA buffer and incubated with 5 μg of antibodies against integrins β₁, α₅, α₁β₅, and α₂β₁. Immune complexes were precipitated with 75 μl of 50% suspension of protein G-beads on ice for 1 h. The beads were preincubated with 1% BSA to reduce non-specific binding. Immune complexes were resolved by 8% SDS–PAGE and detected by autoradiography on X-OMAT film (Kodak) according to standard procedures [21].

Cells derived from these two saphenous vein sources were also compared for their ability to migrate to Vn and PDGF.

2.4.2. The influence of antibodies on SMC migration

The effect of c7E3 Fab, LM609 and control IgG on the migration of cells from each of these two saphenous vein sources was compared. The cells were preincubated with antibodies for 30 min at 37°C and allowed to migrate in their presence. Antibodies were used at a concentration of 10 μg/ml, except for the studies evaluating antibody dose curves, where concentrations were selected between 0.5 and 25 μg/ml.

Finally, the antibody experiments were repeated using cells from an aortic source that were similar in behaviour and integrin expression to those saphenous cells able to migrate to Opn.

2.5. Statistical analysis

All data are represented as mean±standard deviation. Comparison of the effect of antibodies within a treatment group was performed using one-way ANOVA. Comparison of the effect of c7E3 Fab (versus no antibody) in different experiments was performed using two-way ANOVA. All analyses were performed using spss version 9. P-values were adjusted for multiple testing, but statistical significance was interpreted with caution.

3. Results

3.1. SMC adhesion

The experiments set out to determine the effect of c7E3 Fab on SMC adhesion. Several commercial antibodies inhibiting cell adhesion were included in this study in parallel with c7E3 Fab. The antibody LM609, known to inhibit cell adhesion to Vn by blocking α₁β₁ integrin, was used a positive control of inhibition of cell adhesion. The antibody against α₁β₁ integrin is known to inhibit cell adhesion to Vn. The two antibodies (IgG and anti-α₁) were included as negative controls.

The adhesion experiments described in Sections 3.1.1 and 3.1.2 (below) were carried out using SMCs derived from saphenous vein from one subject (Figs. 1 and 2).

3.1.1. The adhesion of smooth muscle cells to vitronectin and osteopontin is inhibited by c7E3 Fab and LM609 (anti-α₁β₁) antibodies

Antibodies LM609 and c7E3 Fab inhibited SMC adhesion to Vn and Opn in a dose-dependent manner (Fig. 1A and B). The maximal level of inhibition of SMC adhesion to both Vn and Opn was observed in the range of antibody concentrations from 2 to 25 μg/ml, therefore we used antibody concentration 10 μg/ml in further experiments. c7E3 Fab decreased SMC adhesion to Opn to a greater extent than to Vn (Figs. 1 and 2) (P=0.002). Neither antibody reduced adhesion to the levels seen on untreated plastic wells (which was approximately 25–30% of adhesion seen on wells treated with ECM proteins). Neither c7E3 Fab or LM609 reduced SMC adhesion to untreated plastic (not shown). Neither control IgG nor anti-α₁β₁ antibodies reduced adhesion significantly (P=0.5 and 0.3
respectively for Vn, and $P=0.75$ and 0.08 respectively for Opn).

Using 10 $\mu$g/ml of antibody, cell adhesion on vitronectin was reduced to 69.2±3.3% ($P<0.001$) by c7E3 Fab, and 71.1±7.6% ($P<0.001$) by LM609, when compared to adhesion without antibody inhibition (Figs. 1 and 2). There was no significant difference between the effect of c7E3 Fab and LM609 ($P=0.5$).

Adhesion on osteopontin was reduced to 52.5±4.8% ($P<0.001$) by c7E3 Fab, and 50.1±4.8% ($P<0.001$) by LM609 (Fig. 2). Again, there was no significant difference between the effect of c7E3 Fab and LM609 ($P=0.4$).

Combining c7E3 Fab with LM609 in the same experiment (10 $\mu$g/ml of each) did not inhibit adhesion to a greater extent than either antibody alone ($P<0.5$ for Vn and 0.4 for Opn).

3.1.2. The adhesion of smooth muscle cells to vitronectin and osteopontin is further inhibited by anti-$\alpha_\beta$ antibodies

Anti-$\alpha_\beta$, integrin antibody reduced adhesion on both Vn and Opn to a similar degree to c7E3 Fab or LM609 (Fig. 2). However, whereas the addition of LM609 to c7E3 Fab showed no further reduction in SMC adhesion, the combination of anti-$\alpha_\beta$, integrin antibody with c7E3 Fab had an additive effect on SMC adhesion to Vn and Opn ($P<0.001$ compared with c7E3 Fab alone for Opn and Vn). c7E3 Fab and anti-$\alpha_\beta$, integrin antibody combined reduced cell adhesion almost to the levels seen on plastic wells that had not been treated with ECM proteins, suggesting that these antibodies act via different integrins.

3.1.3. Adhesion of SMCs derived from an arterial source is also reduced by abciximab and LM609

We also compared the effect of LM609 and c7E3 Fab on SMCs derived from aorta (from another patient) with the results obtained with the saphenous vein cells (Table 1). A similar degree of inhibition of aortic SMC adhesion was seen with the antibodies ($P<0.001$ for c7E3 Fab and LM609, compared with adhesion with no antibody present). No difference was seen between the influence of LM609 and c7E3 Fab ($P=0.7$ for Vn and 0.5 for Opn).

3.2. SMC migration

3.2.1. Immunoprecipitation demonstrated varied expression of the $\alpha_\beta$ integrin

The results of the Western Blot from the immunoprecipitation experiments can be seen in Fig. 3. A faint band can be seen corresponding to $\beta_3$ at approximately 108 Kda in the Western blot of cells derived from the saphenous vein culture that migrated in response to Opn (B), but not in the other (A). All the cells were grown under identical conditions. For the purposes of our experiments, the cells not expressing the $\alpha_\beta$, integrin acted as a
Table 1
Comparison of the effect of antibodies on adhesion of saphenous vein and aortic SMCs

<table>
<thead>
<tr>
<th></th>
<th>Vitronectin</th>
<th>Osteopontin</th>
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<tbody>
<tr>
<td></td>
<td>Saphenous</td>
<td>Aorta</td>
</tr>
<tr>
<td>Control IgG</td>
<td>97.5±7.1</td>
<td>101.2±5.2</td>
</tr>
<tr>
<td>c7E3 Fab</td>
<td>69.2±3.3(^b)</td>
<td>68.2±6.8(^b)</td>
</tr>
<tr>
<td>LM609</td>
<td>71.1±7.6(^b)</td>
<td>69.0±3.7(^b)</td>
</tr>
<tr>
<td>Adhesion on uncoated wells</td>
<td>27.5±3.6(^b)</td>
<td>21.8±3.8(^b)</td>
</tr>
</tbody>
</table>

\(^{a}\) All antibodies were used at a concentration of 10 \(\mu g/ml\). The data is presented as a percentage of cell adhesion without antibody present (mean±S.D.).

\(^{b}\) \(P<0.05\) compared with adhesion in the absence of antibody.

useful negative control, and will be described as \(\alpha_5\beta_3\). Those expressing \(\alpha_5\beta_3\) are described as \(\alpha_5\beta_3\).

3.2.2. Vn-, Opn- and PDGF-induced SMC migration was dependent on the expression of the \(\alpha_5\beta_3\) integrin

Migration of \(\alpha_5\beta_3\) and \(\alpha_5\beta_3\) cells to Opn, Vn and PDGF was assessed (Fig. 4.). The migration of \(\alpha_5\beta_3\) SMCs was increased in the presence of all three agents, although Vn and PDGF induced \(\alpha_5\beta_3\) cell migration to a greater extent than Opn. SMC migration was influenced by \(\alpha_5\beta_3\) integrin expression, with all three agents inducing migration of \(\alpha_5\beta_3\) SMCs to a higher degree than the level of migration of \(\alpha_5\beta_3\) SMCs. Opn did not significantly induce migration of \(\alpha_5\beta_3\) SMCs, compared to background migration levels without Opn present (\(P=0.3\)). Expression of the \(\alpha_5\beta_3\) integrin influenced migration towards ECM proteins much more than PDGF-induced migration.

3.2.3. c7E3 Fab and LM609 (anti-\(\alpha_5\beta_3\)) antibodies inhibited migration of \(\alpha_5\beta_3\) smooth muscle cells to Opn, Vn, and PDGF. Inhibition was dose dependent

As observed in the SMC adhesion experiments, inhibition of migration of \(\alpha_5\beta_3\) SMCs by c7E3 Fab and LM609 was dose dependent, with maximal inhibition being
migrating. Migration in the absence of Opn (shown). Control IgG did not significantly affect SMC but this remained significantly greater than non-induced PDGF, Opn and Vn (Fig. 5). The inhibitory effect on SMC 4. Discussion reduction via the Vn or PDGF. The vertical axis represents a percentage of SMC migration. The present study has shown that LM609 and c7E3 Fab and LM609 signifcantly reduced SMC migration to Opn was most marked, with c7E3 Fab reducing migration to just 20.3±6.1% of migration levels without antibody present (P<0.001). c7E3 Fab reduced Vn-induced migration to 51.6±8.3% of migration in the absence of antibodies (P<0.001). The inhibitory effect of achieved concentrations above 5 µg/ml (data not shown). Control IgG did not significantly affect SMC migration.

At a concentration of 10 µg/ml, both antibodies c7E3 Fab and LM609 significantly reduced SMC migration to PDGF, Opn and Vn (Fig. 5). The inhibitory effect on SMC migration to Opn was most marked, with c7E3 Fab reducing migration to just 20.3±6.1% of migration levels without antibody present (P<0.001). c7E3 Fab reduced Vn-induced migration to 51.6±8.3% of migration in the absence of antibodies (P<0.001). The inhibitory effect of c7E3 Fab on migration to PDGF was less than the effect on Vn- and Opn-induced SMC migration, but remained significant (P=0.004). In each experiment, there was no significant difference seen between the effects of LM609 or c7E3 Fab (P=0.06 for Opn, P=0.08 for Vn, P=0.6 for PDGF).

3.2.4. Migration of cells that did not express αvβ3 was not influenced by c7E3 Fab

Migration of αvβ3+ cells to Vn and PDGF was not inhibited by c7E3 Fab (P=0.8 and P=0.9 respectively), in keeping with the hypothesis that c7E3 Fab inhibits SMC migration via this integrin alone.

3.2.5. Migration of αvβ3+ SMCs derived from an arterial source behaved in a similar manner to αvβ3 saphenous vein SMCs

SMCs derived from aorta were also assessed. They were found to behave similarly to αvβ3 saphenous vein-derived cells (Table 2). Immunoprecipitation (not shown) confirmed their expression of the αvβ3 integrin. c7E3 Fab and LM609 both significantly reduced their migration to Opn, Vn and PDGF, with the greatest effect being seen on Opn-induced migration, and the least on PDGF-induced migration. c7E3 Fab reduced Opn-induced migration to just 25.5±5.2% of migration in the absence of antibody, but this remained significantly greater than non-induced migration in the absence of Opn (P<0.001).

4. Discussion

Restenosis following successful angioplasty remains a major limitation to the long term success of the procedure. The introduction of stenting has reduced the vessel wall remodelling and elastic recoil that contribute to the process [22], but restenosis still occurs due to neointimal formation, and in-stent restenosis is particularly difficult to treat [23]. It is known that restenosis involves the migration of SMCs across the basement membrane to the intima, where they proliferate [5]. The migration process involves degradation of old ECM, cell detachment, migration, and formation of new cell adhesions and deposition of new ECM. Interestingly, ECM in atherosclerotic vessels contains proteins such as Vn, Opn and thomboxipondin, which are not detectable in normal blood vessels [16–18,24]. Vascular damage exposes SMCs to serum containing high concentrations of Vn (0.25–0.4 mg/ml). Opn and especially Vn have been shown to induce SMC migration [9–12,16,17].

The present study has shown that LM609 and c7E3 Fab both act via the αvβ3 integrin to inhibit SMC adhesion and migration to Vn and Opn. The concentration of c7E3 Fab used in the experiments was 10 µg/ml, which is similar to the therapeutic plasma concentration of c7E3 Fab, of around 6 µg/ml [25].
The inhibitory effect of c7E3 Fab on SMC migration was much higher than on SMC adhesion to both Vn and Opn, which would be expected, as cell migration occurs through multiple cycles of cell attachments and detachments. It is also consistent with previously published data demonstrating that the \( \alpha_\beta_3 \) integrin contributes to SMC migration to a greater extent than adhesion [12]. The inhibitory effect of c7E3 Fab and LM609 was greater for Opn than Vn, suggesting that the relative role of \( \alpha_\beta_3 \) integrin in the mediation of these cell functions varies depending on ECM substrate.

It has frequently been observed that expression of the \( \alpha_\beta_3 \) integrin varies in human vascular SMCs in culture and in vivo [12,26,27]. The chance identification of a culture of saphenous vein derived SMCs that did not express the \( \alpha_\beta_3 \) integrin (\( \alpha_\beta_{3v} \)) enabled further confirmation of the role of this integrin and c7E3 Fab in SMC migration. While these \( \alpha_\beta_3 \) SMCs acted as a useful negative control, they probably play little role in the pathology following vessel wall injury. The \( \alpha_\beta_3 \) integrin is up-regulated following vascular injury and plays a major role in SMC migration in vivo in postangioplasty events [7,8,16]. It is therefore expected that SMCs following vascular injury in vivo would behave in a similar way to the \( \alpha_\beta_3 \) cells in our experiments. Opn did not induce migration of \( \alpha_\beta_3 \) SMCs significantly, although Vn and PDGF did still induce some migration. From this, we can conclude that \( \alpha_\beta_3 \) integrin is the major integrin responsible for migration to Opn, although anti-\( \alpha_\beta_3 \) integrin antibody does not abolish Opn-induced migration. Vn- and PDGF-induced migration of SMCs appears to involve not only anti-\( \alpha_\beta_3 \) integrin, but other cellular receptors as well. Vn and PDGF-induced migration of \( \alpha_\beta_3 \) SMCs was not inhibited by c7E3 Fab, consistent with the hypothesis that c7E3 Fab acts via this integrin.

It is interesting to note that SMC migration to PDGF was only slightly reduced by LM609 and c7E3 Fab. The published data on the role of \( \alpha_\beta_3 \) integrin on PDGF-induced SMC migration are inconsistent. Itoh et al. [28] showed that both anti-\( \beta_1 \) integrin and anti-\( \alpha_\beta_3 \) integrin antibodies inhibited SMC migration to PDGF, whereas other studies found that the inhibitory effect of anti-\( \beta_1 \) integrin antibodies on SMC migration is small or negligible compared to the effect of anti-\( \alpha_\beta_3 \) integrin antibody LM609 [13,14]. It is feasible that the inhibitory effect of anti-integrin antibodies would vary according to the expression of different integrins in studied cell lines.

Our data on SMC migration to Vn also indicate other receptors may be involved. Recently, urokinase-type plasminogen activator receptor uPAR and plasminogen activator inhibitor PAI-1 were identified as Vn-binding proteins in several cell types [29,30]. These proteins regulate the same site on Vn as integrins and, therefore, compete with integrins for Vn binding. Both uPAR and PAI-1 are involved in SMC migration [30–32]. Furthermore, an involvement of the plasminogen activator system in SMC migration after coronary angioplasty may have important implications for the outcome of post-angioplasty events and needs further investigation. c7E3 Fab may exert beneficial effects via pathways other than those demonstrated in these experiments. Its role in platelet inhibition reduces the thrombus load, potentially limiting platelet degranulation and release of growth factors and chemotactants, such as PDGF. Recent studies have implicated \( \alpha_\beta_3 \) integrin in SMC apoptosis. VanderZee et al. [33] reported that anti-\( \alpha_\beta_3 \) integrin antibody LM609 adminis-

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**Table 2**

Comparison of the effect of antibodies on migration of \( \alpha_\beta_3 \) saphenous vein and aortic SMCs

<table>
<thead>
<tr>
<th></th>
<th>PDGF Saphenous vein</th>
<th>PDGF Aorta</th>
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<th>Vitronectin Aorta</th>
<th>Osteopontin Saphenous vein</th>
<th>Osteopontin Aorta</th>
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<tr>
<td>Control IgG</td>
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<td>102.4±8.9</td>
<td>98.4±14.4</td>
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<tr>
<td>c7E3 Fab</td>
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<td>87.4±13.4b</td>
<td>51.7±8.9b</td>
<td>53.4±5.6b</td>
<td>20.5±6.1b</td>
<td>23.5±5.2b</td>
</tr>
<tr>
<td>LM609</td>
<td>90.3±11.7b</td>
<td>90.0±10.6b</td>
<td>55.4±7.0b</td>
<td>57.2±8.5b</td>
<td>16.8±8.1b</td>
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<tr>
<td>Non-induced migration</td>
<td>5.1±2.8b</td>
<td>8.3±3.5b</td>
<td>5.6±2.5b</td>
<td>4.6±1.9b</td>
<td>8.4±4.1b</td>
<td>6.7±2.8b</td>
</tr>
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</table>

*All antibodies were used at a concentration of 10 μg/ml. The data is presented as a percentage of cell migration without antibody present (mean±S.D.).

*P<0.05 compared with migration in the absence of antibody.

*Non-induced migration is migration without ECM/PDGF in the lower compartment of the migration chamber.
tered after angioplasty reduced intimal thickening in rabbit model by inducing apoptosis in SMCs. Since both anti-αβ3 integrin antibodies c7E3 Fab and LM609 had very similar effect on SMCs, it would be expected that c7E3 Fab is able to induce apoptosis in SMCs by blocking αβ3 integrin.

Overall, the inhibitory effect of c7E3 Fab on SMC adhesion and migration (present study), on SMC proliferation [7], and an involvement of αβ3 integrin-mediated apoptosis in restenosis demonstrate a convincing role for c7E3 Fab in the inhibition of SMC functions implicated in the restenotic process. However, other pathways are involved. The diversity of in vivo mechanisms may ensure that the effects in vitro are not translated into therapeutic benefit. Although the EPIC trial [34] and some provisional 6-month angiographic results for the EPISTENT trial [35] suggested a role in reducing restenosis, the ERASE-R study, designed to investigate prospectively the effect on in-stent restenosis by c7E3 Fab, failed to show a reduction in intimal hyperplasia at 6 months [36]. Recently published primate experiments have shown that a high systemic dose of c7E3 Fab, which would not be tolerated in man, can reduce neointimal formation [37]. It is feasible that adjustments in the dose and mode of administration of c7E3 Fab may be required in order to maximise clinical potential.

Integrin inhibition may be required for up to 2 weeks, as this is the period during which the αβ3 integrin is upregulated after vascular injury [7,8,16]. Local drug delivery of c7E3 Fab, perhaps loaded onto a stent, may be the way forward to ensure prolonged administration and to achieve higher local concentrations. Our research [38] demonstrates that c7E3 Fab can be adsorbed onto polymer-coated stents. The antibody elutes slowly in vitro, penetrating underlying SM layers, and significantly inhibits platelet deposition. The use of such loaded stents could influence SMC activity within them.

Acknowledgements

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References


