Proteins mediating collagen biosynthesis and accumulation in arterial repair: novel targets for anti-restenosis therapy

Azriel B. Osherov1, Lara Gotha1, Asim N. Cheema2, Beiping Qiang1, and Bradley H. Strauss1*

1Schulich Heart Program, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Room A-253, Toronto, Ontario, Canada M4N 3M5; and 2Terrence Donnelly Heart Center, St Michael’s Hospital, University of Toronto, Toronto, Ontario, Canada

Received 27 August 2010; revised 31 December 2010; accepted 10 January 2011

Abstract

Events contributing to restenosis after coronary interventions include platelet aggregation, inflammatory cell infiltration, growth factor release, and accumulation of smooth muscle cells (SMCs) and extracellular matrix (ECM). The ECM is composed of various collagen subtypes and proteoglycans and over time constitutes the major component of the mature restenotic plaque. The pathophysiology of collagen accumulation in the ECM during arterial restenosis is reviewed. Factors regulating collagen synthesis and degradation, including various cytokines and growth factors involved in the process, may be targets for therapies aimed at prevention of in-stent restenosis.

Keywords

Extracellular matrix • Balloon angioplasty and stenting • Extracellular response • Collagen synthesis and processing

1. Introduction

The arterial response to injury caused by percutaneous interventions (PCIs) involves interactions between circulating leukocytes, platelets and various cellular and extracellular components of the vessel wall.1,2 To date, most attention has been directed towards cellular events, particularly arterial smooth muscle cell (SMC) proliferation. The role of the extracellular matrix (ECM), and its predominant component, collagen, in mediating the arterial repair response has been less appreciated, despite an improved understanding of its role in affecting cellular behaviour and modulation of cellular interactions.3 Several studies have demonstrated that collagen accumulation in the ECM plays a major role in the development of restenosis, after both balloon angioplasty and stenting.4–7 In this paper, we review the critical involvement of ECM proteins, particularly collagen, in arterial repair and development of restenosis after PCI with potential therapeutic implications.

2. Cellular response after PCI

Leucocytes and platelets are present within the arterial media, adventitia and the surrounding tissue soon after arterial injury (Figure 1). These cells release cytokines and growth factors, including tumor necrosis factor α (TNF-α),9 platelet-derived growth factor (PDGF),10 and transforming growth factor β (TGF-β),11 that modulate cellular behaviour. Adventitial fibroblasts may change their phenotype and migrate towards the arterial intima,12 while medial SMC become metabolically active and proliferate.13 This early burst of cellular activity lasts only a few weeks,5,14 and is followed by a second and more prolonged phase characterized by prominent ECM deposition in the vessel wall.15,16

3. Extracellular response after PCI

It is now recognized that ECM is an active component of the vessel wall responsible for cellular interactions that play a critical role in the pathophysiology of vascular diseases.17 The ECM is composed of a variety of molecules, including collagen, elastin, glycoproteins, and proteoglycans. In a muscular coronary artery, type III collagen is the most abundant matrix protein.18

4. Collagen synthesis and processing in the ECM

The collagen molecule is first synthesized in the fibroblast and SMC as a 300 nm long procollagen molecule where the triple helix is flanked by globular N- and C-peptides.19,20 The triple helix is formed by the propagation of the interchain disulphide bonds from C- to the N-
terminus between the three procollagen chains at the endoplasmic reticulum. Successful formation of the collagen triple helix requires that any cis-peptide bonds involving proline residues are converted to trans-form by the enzyme peptidyl-prolyl cis-trans isomerase, and a number of proline residues in single-stranded procollagen chains are converted into hydroxyproline by the enzyme prolyl-4-hydroxylase (P4H).21 The procollagen molecules associate transiently with HSP47 (chaperone protein) during collagen folding and transport from the endoplasmic reticulum to the Golgi apparatus.22 The procollagen molecules are transported to the Golgi apparatus and secreted out of the cell in Golgi transfer vesicles or smooth vacuoles.

In the extracellular space, the C- and N-propeptides are removed from the procollagen molecule by procollagen metalloproteinases, N- and C-proteinase, and the triple helix of the collagen molecule is transformed into the characteristic fibrillar structure. The final step involves the covalent cross linking of collagen chains and fibrils. Lysyl oxidase enzyme23 allows formation of inter- and intra-molecular cross links between lysine and hydroxylysine residues, as well as between N- and C-peptide regions that provide strength and stability characteristic of collagen fibres.

4.1 Integrins
Integrins are the receptors that mediate cell adhesion to ECM and participate in signal transduction through the cell membrane. These interactions have been reviewed extensively.24–26 Integrins consist of one alpha and one beta subunit forming a non-covalently bound heterodimer: 18 alpha (determines integrin ligand specificity) and 8 beta (connects to the cytoskeleton and affects multiple signalling pathways) subunits that combine to form 24 different receptors. The integrin dimers can be broadly divided into three families consisting of the beta1, beta2/beta7, and beta3/alphaV integrins. Beta1 associates with 12 alpha-subunits and can be further divided into ARG-GLY-ASP (RGD), collagen, or laminin binding. Only a subset of integrins (8 out of 24) recognizes the RGD sequence in the native ligands. In some ECM molecules, such as collagen and certain laminin isoforms, the RGD sequences are exposed upon denaturation or proteolytic cleavage, allowing cells to bind these ligands by using RGD-binding receptors. For a majority of integrins, the linkage is to the actin cytoskeleton.

4.2 Integrin function
The integrins function as mechanosensors that transfer bi-directional signalling to affect cell physiology.27 Upon ligand binding, integrins undergo conformational changes leading to outside-in signalling. Integrin signalling occurs through cytoplasmic molecules, such as talin, kindlin, α-actinin, filamin, cytohesins, 14-3-3 proteins. The inside-out and outside-in activations through the integrins are regulated by phosphorylation of the β2 chain or the αL and αM integrin polypeptide.
Small G proteins, Rac1 and Cdc42, induce the formation of actin polymers, which eventually lead to cell spreading and migration.

5. Collagen breakdown

Two families of proteolytic enzymes, matrix metalloproteinases (MMPs) and cysteine proteases, are mainly responsible for collagen degradation in the arterial wall. MMPs mediate extracellular breakdown, while cathepsins, a family of papain-like lysosomal cysteine proteases, perform intracellular or lysosomal breakdown. Compared with cathepsins, MMPs are more efficient collagenolytic enzymes due to their specific binding characteristics and ability to unwind the triple helix.

5.1 MMPs

MMPs act on susceptible regions near the C-terminus of the collagen molecule. The site of MMP activity on collagen is characterized by a high amino acid content segment forming a ‘tight’ triple helix prior and a low amino acid content segment forming a ‘loose’ triple helix immediately after the cleavage site. Several mechanisms have been proposed, including simultaneous binding, followed by relative movement of the catalytic domain in relation to the haemopexin-like domain. This bending and distortion of the triple helix results in breaks and unwinding of the triple helix (Figure 2).

A variety of MMPs participate in the collagen degradation process. Interstitial collagenases (MMP1, MMP8, and MMP13) are the most prevalent MMPs that cleave fibrillar collagens, while gelatinases are active against non-fibrillar collagen components of the ECM. MMP2 is capable of cleaving collagen fibres independently or in synergism with MT1-MMP. Among the membrane-type MMPs, MMP14 and MMP16, can also degrade native collagen. The collagen breakdown products are substrates for the gelatinases (MMP2, MMP9). MMP activation is achieved by cleavage of a pro-MMP form, and is regulated by several cytokines and growth factors. MMP activators include uPA, MT-MMPs, IL-1, PDGF, and TNF-α, while TGF-β, heparin, steroids, and tissue inhibitor of metalloproteinases (TIMP 1-4) inhibit MMP activity.

5.2 ADAMTS

A family of proteases, the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs), has been linked to atherosclerosis and possibly restenosis. These are secreted enzymes that regulate ECM turnover by degrading specific matrix components, such as versican and procollagen. ADAMTS-1, degrades versican, the primary proteoglycan component of the vascular ECM, thereby facilitating the migration of SMCs and intimal hyperplasia. ADAMTS-7 facilitates SMC migration and intimal thickening, possibly through degradation of cartilage oligomeric matrix protein (COMP). Another subgroup of ADAMTS (2, -3, and -14) are termed procollagen-n-proteinases, due to their involvement in the removal of N-terminal peptides from procollagen to form mature collagen; their role in intimal hyperplasia is yet to be determined. The tight substrate specificity of ADAMTS enzymes makes them attractive pharmaceutical targets. Inhibitors originally designed for ADAMTS-4 to reduce cartilage breakdown could be useful in reducing SMC migration.

5.3 Restenosis after PCI

Restenosis mechanisms differ between balloon angioplasty and stenting. Intimal hyperplasia accounts for all late lumen loss following stenting, but for <40% after balloon angioplasty, with the remainder due to constrictive remodelling.

5.4 Collagen accumulation and restenosis

The relationship between collagen accumulation and constrictive remodelling has been characterized by inconsistent results. Experimental angioplasty studies have shown associations between
collagen accumulation and both expansive remodelling and constrictive remodelling. In a cryotherapy treated, balloon injury rabbit model, collagen content in all the arterial wall layers was correlated with constrictive remodelling.

Stenting causes an even greater increase in collagen accumulation, in both arterial intima and media/adventitia layers compared with balloon angioplasty. This is likely due to extensive tissue damage and persistent circumferential stretching, both well-recognized stimuli for enhanced collagen synthesis.

6. Temporal pattern of collagen turnover

Collagen turnover studies after experimental angioplasty and stenting have indicated that the collagen accumulation phase is more prolonged compared with the cellular proliferation phase. Collagen degradation is also an integral aspect of arterial repair. Despite marked increases in collagen synthesis early after experimental angioplasty, collagen accumulation is delayed for weeks, a period when the rate of new collagen synthesis is in decline. These findings suggest that regulation of matrix content is also dependent on degradative processes. MMPs, MMP2, MMP9, and membrane metalloproteinases are activated early after balloon injury, and MMP2 activity in particular remains elevated for months. Arterial stenting results even more marked changes in gelatinolytic activity, particularly MMP9.

7. Significance of collagen turnover

Collagen breakdown products stimulate fibroblasts, enhance inflammation, and increase SMC motility. In addition, degraded collagen also increases localized MMP expression on SMC, further stimulating cell migration. In contrast, intact fibrillar collagen has potent inhibitory effects on SMC migration and proliferation. ECM protein synthesis and expression of specific matrix receptors. Thus the presence of fibrillar collagen may be important for maintenance of a quiescent ECM milieu. Plasma levels of the active isoforms of MMP-3 and MMP-9 were associated with coronary in-stent restenosis.

7.1 Collagen turnover as a therapeutic target

The complexities of collagen metabolism result in a number of potential targets for anti-restenosis therapy. A particular advantage is the potential for local therapy, such as drug-eluting stents or balloons, which may obviate the occurrence of deleterious effects due to systemic (and higher dose) administration. Moreover, only a relatively short duration of treatment (i.e. weeks) is needed to antagonize the fibrotic reaction, compared with other fibrotic disorders in haematology, pulmonology, and rheumatic diseases.
8. Inhibitors of collagen biosynthesis

Stabilization of collagen may facilitate restenosis following certain types of arterial injury. Lysyl oxidase forms covalent bonds across collagen molecules in the extracellular space. Lysyl oxidase expression is increased following arterial injury, and precedes collagen accumulation and intimal hyperplasia. Inhibition of lysyl oxidase prevents fibrosis in non-vascular tissue; only limited data are available following arterial injury. β-aminopropionitrile (β-APN), an irreversible inhibitor of lysyl oxidase, decreased collagen cross-linking and late lumen loss after experimental laser angioplasty, but not after balloon angioplasty. Laser angioplasty causes more extensive and deeper arterial injury than balloon angioplasty and thus may exaggerate this repair mechanism. β-APN treatment in a rabbit atherosclerotic balloon injury model resulted in favourable remodelling, including a reduction in restenosis, and neointimal collagen content. Systemic use of β-APN may cause musculoskeletal and neurological damage; hence the need to test these compounds using localized delivery systems.

9. Cytokine and growth factor inhibitors

Several cytokines and growth factors are known to stimulate collagen synthesis.

9.1 Inhibition of TGF-β activity

TGF-β is one of the most potent pro-fibrotic cytokines that are up-regulated after arterial injury. Additional important pleiotrophic effects on wound healing, angiogenesis, immunoregulation, and cancer may also contribute to vascular repair. TGF-β1, the predominant isoform, binds to TGF-β-type II/III receptor to phosphorylate TGF-β-type I receptor (ALK5) and increases gene expression of collagen and connective tissue growth factor (CTGF). The role of TGF-β in ECM deposition may be more complex than initially appreciated. Inhibition of TGF-β1 biological activity by ribozyme oligonucleotides or antisense oligonucleotides, overexpression of soluble TGF-β-type II receptor, and overexpression of TGF-β1 biological activity by ribozyme oligonucleotides have successfully prevented intimal hyperplasia and constrictive remodelling after experimental angioplasty. Monoclonal TGF-β neutralizing antibodies have shown variable results in preclinical and clinical studies in cancer and connective tissue disease, such as systemic sclerosis. Potential restenosis applications of larger peptide or oligonucleotides molecules require testing in appropriate models. Recently, a synthetic pyrrole-imidazole polyamide molecule targeted to the TGF-β1 promoter as a gene silencer was developed.

9.1.1 Agents with multiple effects, including TGF-β inhibition

Although not a specific TGF-β inhibitor, Tranilast [N-(3,4-dimethoxycinnamoyl)-anthranilic acid] inhibits TGF-β expression through increasing p21waf1 levels. Tranilast also influences other pathways, such as inhibition of MAP kinase activation by PDGF.
Tranilast prevented intimal hyperplasia and constrictive remodelling in experimental angioplasty studies, although study doses were 10-fold greater than usual patient dose. In a prospective, randomized study of 11,484 patients, oral tranilast did not lower restenosis rates and caused a higher rate of liver enzyme abnormalities. Pioglitazone, a peroxisome proliferator gamma-activated receptor (PPAR-γ) agonist used in diabetic patients, suppressed experimental in-stent intimal hyperplasia by attenuating TGF-β levels. Pioglitazone may also enhance apoptosis due to TGF-β1 release and selective phospho-Smad2 nuclear recruitment. These seemingly contrasting results may be due to different concentrations of pioglitazone. In cultured human dermal fibroblasts, activation of PPAR-gamma blocked Smad-mediated transcriptional responses by preventing p300 recruitment, a downstream coactivator and histone acetyltransferase involved in collagen gene expression.

CTGF, a matricellular regulatory protein, is a profibrotic factor that modifies adhesive signalling in general and mediates some TGF-β responses and may be important in myocardial fibrosis. Several non-cardiac models have studied CTGF as anti-fibrotic therapy. Hepatocellular CTGF synthesis can be inhibited by small interfering RNA (siRNA) and paraxanthine. In systemic sclerosis fibroblasts, siRNA targeting CTGF inhibited the expression of CTGF and type I and III collagen. In a hepatic stellate cell model, CTGF inhibition using hammerhead ribozyme cDNA decreased basal and secreted CTGF, and collagen I mRNA and protein levels. Anti-CTGF monoclonal antibody was safely administered to patients with diabetes and microalbuminuria. Further experiments are required to assess CTGF in restenosis models.

Imatinib mesylate is a specific inhibitor of three tyrosine kinase receptors: platelet-derived growth factor receptor (PDGFR), c-Kit, and c-Abl. A complex interaction exists between TGF-β and c-Abl; TGF-β through various kinases activates c-Abl, which activates three different pathways (smad1/4, EGR and PKCζ). Imatinib can block all three pathways. TGF-β has an additional signalling pathway to the nucleus through smad2/3, which is not affected by imatinib mesylate. Studies are needed to determine whether the c-Abl pathway is important in SMCs after vascular injury. The non-canonical pathway involving p38MAPK is also involved in multiple pleiotropic functions, ranging from cellular proliferation and survival, to cell cycle arrest. Inhibition of p38MAPK decreased human vascular SMC proliferation. Inhibitors of these TGF-β signalling pathways require further testing in appropriate restenosis models.

9.2 Inhibition of PDGF activity

PDGF is released after arterial injury and promotes SMC migration, proliferation and collagen accumulation. Furthermore, collagen-type I, which is upregulated after arterial injury, acts synergistically with PDGF on SMC proliferation. Inhibition of PDGF activity, whether by direct targeting of PDGF or by the PDGFβ receptor, inhibits experimental intimal hyperplasia. The tyrosine kinase inhibitor, BMS-354825 (dasatinib), inhibited PDGF-stimulated activation of PDGFR, in rat and human SMC. A specific tyrosine kinase inhibitor reduced in-stent hyperplasia, although an adventitial antiangiogenic effect was also a potential explanation. Currumin inhibited PDGF-stimulated SMC migration, proliferation and collagen synthesis, as well as PDGF/H2O2-stimulated phosphorylation of PDGFβ receptor and downstream Akt activation. Imatinib, a PDGF tyrosine kinase inhibitor, inhibited proliferation and migration of SMC in vitro and suppressed vein graft neointima formation ex vivo. The method of delivery and the clinical context may be very important factors. A single infusion of a monoclonal antibody against PDGFβ receptor failed to prevent in-stent restenosis in humans, suggesting more prolonged exposure may be required. Moreover, systemic delivery of a neutralizing anti-PDGFβ receptor antibody in mice undergoing reperfused infarction, resulted in decreased collagen content in the healing infarct, possibly predisposing to myocardial rupture. Thus, local arterial wall delivery could more specifically and safely target the restenosis process (Table 2).

9.3 Decorin

Decorin is a small proteoglycan that regulates ECM composition through direct interactions with collagen. In addition, decorin indirectly sequesters, and inhibits TGF-β, inhibits PDGF-stimulated PDGFR phosphorylation and MMP expression. In a human study of in-stent restenosis, higher levels of decorin were associated with reduced neointimal formation. In a rabbit angioplasty model, decorin overexpression reduced arterial collagen content and intimal area. Decorin gene delivery by a recombinant adeno-associated viral vector significantly reduced collagen content and cardiomyocyte hypertrophy in spontaneously hypertensive rats. Decorin also reduced neointimal hyperplasia formation in a model of human saphenous vein segments. Decorin effects are not specific for restenosis. Decorin inhibited the progression of prostate cancer in an animal model by suppressing epidermal growth factor receptor phosphorylation and its downstream signalling, PI3K/Akt, and by triggering apoptosis. Local treatment with decorin should be feasible and evaluated in pre-clinical restenosis models.

9.4 Endothelin receptor antagonist

Endothelin 1 (ET-1) is the predominant form of endothelin secreted by arterial endothelium in response to injury and exerts its effects through ET A and ET B receptors. ET A is predominantly found in SMC and induces vasoconstriction, cell proliferation, and collagen synthesis. ET B is mainly present in endothelial cells and mediates vasodilatation by release of nitric oxide and prostacyclin. Exogenous administration of ET-1 in a rabbit angioplasty model increased both collagen synthesis and accumulation with only modest effects on cell proliferation rates. These collagen effects are either mediated directly by increase in collagen gene, and ET-1 receptor expression, or indirectly via expression of TGF-β. TAK-044, a non-selective ETA/ETB receptor antagonist, significantly suppressed both MMP-2 and MMP-9 activities and proMMP-2 levels in a rabbit angioplasty model (Table 3).

Although administration of ET A receptor antagonist inhibits collagen synthesis in vitro and decreases intimal hyperplasia after balloon angioplasty, the results of non-selective ET-1 inhibition (both ET A and ET B) after arterial injury have been inconsistent. Inhibition of the ET B receptor system caused a worsening of neointimal hyperplasia. These results suggest that selective ET A receptor antagonist may be more effective in reducing neointimal hyperplasia restenosis than agents having mixed ET A and ET B activity.

9.5 Aldosterone antagonism

Systemic and locally produced aldosterone modulates arterial structure through interactions with vascular aldosterone receptors. Aldosterone increases collagen synthesis in vitro and promotes collagen accumulation and fibrosis in the arterial wall. Non-selective (e.g. spironolactone) and selective aldosterone receptor antagonists (e.g. eplerenone) inhibit restenosis after experimental angioplasty by preventing collagen accumulation.
10. Inhibitors of integrin function

Inhibition of the vitronectin receptor (integrin αVβ3) by a specific RGD antagonist prevented SMC migration and neointimal hyperplasia in a rabbit angioplasty model. In a swine model, a selective potent antagonism of integrin αVβ3 using a small molecule cyclic RGD peptidomimetic also inhibited neointimal hyperplasia. A novel class of biphenyl vitronectin receptor antagonists with nanomolar affinity have been developed, although in vivo restenosis studies have not yet been reported.

Integrins also interact with MMP-2. Vitronectin receptor blockade interferes with binding of this integrin to MMP-2, thereby reducing...

---

**Table 2** Studies *in vivo/in vitro* that shows the effect of PDGF receptor antagonism on SMC migration proliferation and collagen synthesis

<table>
<thead>
<tr>
<th>Reference</th>
<th>PDGF receptor antagonism</th>
<th>Species</th>
<th>In <em>vivo</em></th>
<th>Model and intervention (angioplasty, stenting, else)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferns et al. 97</td>
<td>Polyclonal antibody to PDGF</td>
<td>Rats</td>
<td><em>In vivo</em></td>
<td>Carotid artery of athymic nude rats induced by intra-arterial balloon catheter de-endothelialization</td>
<td>Inhibit SMC migration</td>
</tr>
<tr>
<td>Yamasaki et al. 98</td>
<td>SU9518 A synthetic indoline—one selectively inhibits the PDGF receptor kinase</td>
<td>Rats</td>
<td>Both <em>In vivo</em></td>
<td>Rat balloon arterial injury</td>
<td>Reduced intimal thickening inhibits migration and proliferation of SMC</td>
</tr>
<tr>
<td>Hart et al. 99</td>
<td>Anti-platelet-derived growth factor-β receptor antibody (+ heparin)</td>
<td>Baboon</td>
<td><em>In vivo</em></td>
<td>Saphenous artery of the baboon after balloon angioplasty</td>
<td>Decrease intimal hyperplasia</td>
</tr>
<tr>
<td>Serrys et al. 106</td>
<td>Single infusion PDGF-β receptor antibody</td>
<td>Human</td>
<td><em>In vivo</em></td>
<td>Coronary stenting</td>
<td>No change in restenosis compared with placebo</td>
</tr>
<tr>
<td>Banai et al. 100</td>
<td>Tyrophostin AGL-2043 inhibitor of PDGF β-receptor tyrosine kinase</td>
<td>Rats</td>
<td><em>In vivo</em></td>
<td>Eluting coronary stent</td>
<td>Reduces neointima formation</td>
</tr>
<tr>
<td>Chen et al. 101</td>
<td>Tyrosine kinase inhibitor, BMS-354825 dasatinib</td>
<td>Human</td>
<td><em>In vitro</em></td>
<td>Cultures of human aortic smooth muscle cells</td>
<td>Reduced migration and proliferation of SMC</td>
</tr>
<tr>
<td>Liu et al. 103</td>
<td>Dehydrozingerone</td>
<td>Rats</td>
<td><em>In vitro</em></td>
<td>VSMCs isolated from adult rats</td>
<td>Decreased SMC, migration, proliferation, and collagen synthesis</td>
</tr>
<tr>
<td>Kimura et al. 105</td>
<td>Imatinib mesylate: STI571 delivered with bio-absorbable polymeric nanoparticles (NP) formulated from the polymer polyethylene-glycol (PEG)-modified poly(ε-lactide-coglycolide) (PLGA)</td>
<td>Rabbit</td>
<td>Ex <em>vivo</em> treatment</td>
<td>Rabbit jugular vein grafted on the carotid</td>
<td>Inhibited cell proliferation and phosphorylation of the PDGF-β R</td>
</tr>
</tbody>
</table>

**Table 3** Studies *in vivo/in vitro* that shows the effect of endothelin receptor agonism and antagonism on SMC migration proliferation and collagen synthesis

<table>
<thead>
<tr>
<th>Reference</th>
<th>Endothelin receptor agonist and antagonist</th>
<th>Species</th>
<th>In <em>vivo</em></th>
<th>Model and intervention (angioplasty, stenting, else)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rozvi et al. 115</td>
<td>ETA-specific antagonist BQ123</td>
<td>Pig</td>
<td><em>In vitro</em></td>
<td>Porcine SMC’s from coronary and aorta</td>
<td>The ETA-specific antagonist BQ123 significantly inhibited the stimulatory effects of ET-1. (Decrease in collagen-type I synthesis)</td>
</tr>
<tr>
<td>Barolet et al. 116</td>
<td>Endothelin-1 s.c.</td>
<td>Rabbts</td>
<td><em>In vivo</em></td>
<td>Iliac artery angioplasty</td>
<td>Significant increases in intimal thickness, collagen synthesis, ETA and ETB receptor expression</td>
</tr>
<tr>
<td>Reel et al. 118</td>
<td>A non-selective ETA/ETB receptor antagonist, TAK-044</td>
<td>Rabbts</td>
<td><em>In vivo</em></td>
<td>A soft silicon collar around the left carotid artery in rabbits</td>
<td>Significantly suppressed both gelatinase MMP-2,9 activities and proMMP-2 levels</td>
</tr>
<tr>
<td>Kitada et al. 121</td>
<td>A-192621, a selective ETB receptor antagonist, ABT-627, a selective ETA receptor antagonist, and J-104132, an ETA/ETB dual receptor antagonist</td>
<td>Rat</td>
<td><em>In vivo</em></td>
<td>Balloon-injured rat carotid arteries</td>
<td>Selective ETB receptor antagonist: significantly increased neointima/media ratio. selective ETA receptor and dual ETA/ETB receptor antagonist: decreased neointima/media ratio</td>
</tr>
</tbody>
</table>
cell invasion. Integrin alpha V inhibition abrogates tyrosine phosphorylation at focal adhesion sites by blocking vitronectin-initiated stimulation of the FAK and diminishes MMP-2 secretion, leading to reduced migration and invasion of human coronary artery SMC.

Cytoskeleton and focal adhesion site rearrangements are modulated by the decanoyl-RVKR-chloromethylketone (dec-CMK) inhibitor of furin-dependent integrin cleavage (Furin-dependent integrin processing). This has been shown to decrease vitronectin-induced (outside-in) FAK- and paxillin-phosphorylation, causing reduced inside-out dependent migration. Blockade of integrin processing via the inhibition of furin may provide a novel approach to modulate integrin signalling and motility in vascular SMCs.

### 11. Inhibitors of collagen proteolysis

It may be expected that a proteolytic inhibitor would alter the balance between synthesis and degradation to favour matrix protein synthesis and promote collagen accumulation; however the experimental data have suggested otherwise. In balloon angioplasty models, MMP inhibitors reduce neointima formation and prevent constrictive arterial remodelling by reducing collagen accumulation in the vessel wall. MMP inhibition also significantly reduced in-stent hyperplasia in a rabbit model, although other MMP inhibitors have not prevented neointimal hyperplasia in an atherosclerotic primate stent model.

Another potential MMP target is the macrophage MT-MMP-driven proteolytic cascade of pro-MMP-2 activation. Activation of membrane-bound MT1-MMP, the major activator of pro-MMP-2, requires intracellular endoproteolytic cleavage of its precursor protein. The activation of MT1-MMP requires proprotein convertases, such as furin, which belong to a family of proteins with similar function (PC1, PC2, PACE4, PC4, furin, PCSA and PCSB, and PC7). Inhibition of furin convertase by furin-specific siRNA, or by using the specific pharmacological furin-like PC inhibitor dec-CMK, dramatically reduced pro-MMP-2 activation. Inhibition of integrin pro-alpha v processing via furin-inhibition potently reduced pro-MMP-2 binding to the cell surface, and vitronectin-induced vascular SMC motility.

There may also be effects through TGF-β1 pathways since furin inhibition significantly decreased TGF-β1-induced MT1-MMP/MMP-2 activation and TGF-β1-enhanced fibroblast motility. Inhibition of furin-like PCs, by adenosiv overexpression of α1- PDX, blocked proTGF-β activation and Smad phosphorylation, and resulted in a reduction in adventitial and intimal areas (Table 4).

#### 11.1 Why proteolytic inhibitors may work

Heightened collagen turnover (synthesis and degradation) resulting from increased MMP activity is characteristic of a range of arterial pathological states with remodelling changes, such as arterial injury, cessation of flow, and augmentation of flow. Collagen degradation products are potent stimulants for monocytes and fibroblasts, both of which contribute to collagen accumulation after arterial injury. Fibrillar collagen, on the other hand, inhibits SMC migration and proliferation, ECM protein synthesis, and expression of matrix receptors, thereby maintaining a quiescent ECM milieu. MMPs also play a critical role in the maladaptive arterial response after injury, including facilitation of cellular migration, activation and release of pro-fibrotic growth factors, induction of apoptosis, inflammation and activation of ET-1. By preventing collagen degradation and interrupting the positive feed-back loop of collagen breakdown products on collagen synthesis, proteolytic inhibitors could result in decreased total collagen accumulation, which is an appealing target for anti-restenosis therapy.

### 12. Conclusion

Although the promising results of current drug-eluting stents have heightened the focus on cell cycle regulating compounds, collagen biosynthesis and accumulation remain important therapeutic targets to consider for the prevention of restenosis. Appreciation of the role of collagen in the development of intimal hyperplasia and constrictive remodelling has allowed for the identification of novel therapeutic agents showing promise in various animal models. Further research, including randomized trials, is needed to assess the safety and efficacy of these compounds in the human setting. To date, no studies have been reported of sustained release by drug-eluting stents of anti-fibrotic strategies alone or in combination with inhibitors of cellular proliferation. In particular, temporal release of agents that would initially have an early anti-proliferative effect followed by an anti-fibrotic effect is intriguing, since it mimics the natural healing processes within the vessel wall. Current stent designs are now available that can deliver more than one agent
according to different release kinetics. This type of strategy could limit the toxicity of current regimens being used that provide sustained and potent anti-proliferative effects that interfere with endothelialization and vessel wall reactivity, and predispose to late stent thrombosis. While there are a myriad of options available for these targets, our favoured approach to inhibit restenosis by preventing collagen accretion would be small molecule inhibitors against TGF-β, which can be reliably eluted from a stent platform directly into the damaged vessel wall. The alternative targets would be PDGF receptor blocker or MMP inhibitors, or combinations of these targets.

Acknowledgement
Ms Aarti Inamdar for preparing the extraordinary images and making the cellular pathways perceptible.

Conflict of interest: none declared.

References


Role of collagen in restenosis

122. Lombes M, Oblin ME, Gasc JM, Baulieu EE, Farman N, Bonvalet JP. Immunohisto-


121. Kitada K, Yui N, Matsumoto C, Mori T, Ohkita M, Matsumura Y. Inhibition of

et al

100. Banai S, Gertz SD, Gavish L, Chorny M, Perez LS, Lazarovichi G


et al


97. Ferns GA, Raines EW, Sprugel KH, Motani AS, Reidy MA, Ross R. Inhibition of

2006; Cardiol

2006; Mol Pharmacol

nib).

Circ Res

2008; Int J Cardiovasc Intervent

2007; Circulation: Cardiovasc Radiat Med


2005; Circulation 2001;104:467–472.


2006; Am J Pathol

2003; J Pharmacol Exp Ther


2008; J Pharmacol Exp Ther


2001; Circulation 2001;104:45–62.


2005; Circulation 2001;104:45–62.


2001; Circulation 2001;104:45–62.


