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Imaging of angiogenesis in cardiology

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Abstract In the past decade, there have been major improvements in our understanding of angiogenesis at the genetic, molecular and cellular levels. Concentrated efforts in this area have led to new therapeutic approaches to ischaemic heart disease using angiogenic factors, gene therapy and progenitor cells. Despite very promising experimental results in animal studies, large clinical trials have failed to confirm the results in patients with coronary artery disease. Important questions such as selection of growth factors and donor cells, as well as the timing, dose and route of administration, have been raised and need to be answered. Molecular imaging approaches which may provide specific markers of the angiogenic process (e.g. integrin expression in endothelial cells) have been introduced and are expected to address some of these questions. Although few clinical imaging results are currently available, animal studies suggest the potential role of molecular imaging for characterisation of the angiogenetic process in vivo and for the monitoring of therapeutic effects.

Keywords Angiogenesis · Molecular imaging · PET · RGD

Introduction

Myocardial ischaemia causes reversible or irreversible myocardial injury and may lead to heart failure due to extensive myocardial necrosis, hibernation and ventricular remodelling. At the same time, ischaemia triggers the formation of collateral vessels via angiogenesis, but this endogenous compensation may not be sufficient in advanced disease [1]. Recent advances in our understanding of angiogenesis with regard to molecular, genetic and cellular mechanisms offer the potential for therapeutic angiogenesis [2, 3]. Several angiogenic strategies, including cytokine therapies, gene therapies and cell transplantation therapies, have been examined, and animal studies have indicated enhanced angiogenesis [4–7]. Based on these animal experiments, clinical studies have been initiated and the first results of large clinical trials with recombinant proteins and gene therapies have been published [8–11]. However, the results have been variable and have raised many methodological questions, including details of the therapeutic strategy (e.g. timing, dose and route) and the use of appropriate end-points [12, 13].

Non-invasive imaging techniques are promising tools to address these unsolved questions. Assessments of myocardial perfusion and left ventricular function by means of SPECT, PET, MRI or echocardiography are important validated methods to explore the therapeutic efficacy of angiogenesis [12]. Furthermore, recent developments in molecular imaging techniques may offer more specific visualisation of therapeutic targets and provide early markers of the angiogenic process [14].

Mechanism of angiogenesis in ischaemic heart disease

Three different subtypes of neovascularisation are described: angiogenesis, arteriogenesis and vasculogenesis [15–17]. Angiogenesis is a process fundamental to neovascularisation stimulated by inflammation and ischaemia. The general definition of angiogenesis is the growth and

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development of new capillary blood vessels from pre-existing vasculature involving mature endothelial cells [17]. The process consists of an early sprouting phase followed by an introspective phase. However, the new capillaries generated via angiogenesis lack a complete medial layer, which causes abnormal permeability and vasomotor functions [17].

Arteriogenesis is the maturation of capillary vessels into fully developed vessels equipped with smooth muscle cells in the tunica media [17, 18]. In contrast to angiogenesis, arteriogenesis occurs independently of hypoxia and is typically observed outside of ischaemic regions. The stimulus for arteriogenesis is thought to be increased shear stress [19]. Arteriogenesis is important for the development of large collateral vessels and represents the target of neovascularisation therapies.

Vasculogenesis represents the formation of new capillaries during embryonic development [17, 20, 21]. This process is characterised by the differentiation of endothelial cells from cellular precursors.

Recently, experimental studies have suggested the presence of circulating endothelial progenitor cells in the postnatal phase and their incorporation into newly formed vessels. This interesting concept represents the combination of angiogenesis and vasculogenesis contributing to neovascularisation of adult tissue in response to an angiogenic stimulus such as ischaemia [21, 22].

Angiogenesis involves a complex interplay of many molecules and cells, as well as the extracellular matrix. First, the basement membrane undergoes disintegration, which is facilitated by urokinase-type plasminogen activator (u-PA) and matrix metalloproteinases (MMPs) released from activated inflammatory cells; this allows endothelial cell migration and results in the liberation of growth factors [23, 24]. Second, migration and proliferation of endothelial cells are promoted by angiogenic factors such as vascular endothelial growth factor (VGEF) and basic fibroblast growth factor (bFGF), which are released from endothelial cells and inflammatory cells [25]. Tube formation of endothelial cells and the reconstruction of the basement membrane are the final steps in angiogenesis, and are mainly modulated by signalling transduction between the extracellular matrix and endothelial cells mediated by integrins, which are expressed by the endothelial cells [26].

Tissue hypoxia is a strong initiator of angiogenesis in the setting of ischaemia. As an acute local reaction, vasodilation to increase blood flow as a means to increase oxygen delivery is observed. But further compensatory processes such as angiogenesis involve changes in gene transcription [27]. The transcription factors, the heterodimeric hypoxia-inducible factors (HIFs), are known to play a key role in modulating expression of hypoxia-sensitive genes, including VGEFs, VGEF receptors, bFGFs, nitric oxide synthase (NOS) and angiopoietin genes [27–29]. In normally oxygenated tissue, HIFs are downregulated by ubiquitination and proteasomal degradation, but are rapidly activated in hypoxic conditions and bind to promoter elements, inducing transcription of hypoxia-sensitive genes. Once reoxygenation has occurred, HIFs again undergo degradation, and the transcription activation is downregulated [28] (Fig. 1).

Another important stimulus for angiogenesis appears to be the inflammatory processes which are observed in ischaemic tissues. The presence of inflammatory cells such as monocytes, macrophages, platelets and mast cells is associated with local secretion of angiogenic factors including several cytokines, growth factors and proteinases [30]. Among these factors, PR39 and interleukin-1 are reported to enhance HIF activities [31, 32].

In general, angiogenesis is well regulated by a number of stimulatory and inhibitory factors. Activators include vascular growth factors, some cytokines, extracellular matrix macromolecules, cell adhesion molecules and others (Table 1) [33]. Endothelial cells and macrophages produce most of these factors. On the other hand, other cytokines (e.g. interferon α and interleukin-12), steroids (e.g. medroxyprogesterone acetate and cortisone), heparin binding factors (e.g. protamine, platelet factor IV and VEGF receptor antagonist), protease inhibitors and antibiotic substances are reported to inhibit angiogenesis [34, 35].

Among these numerous modulators of angiogenesis, VEGF is one of the most important and potent stimulators of endothelial cell activation and proliferation [15, 36]. VEGF is a homodimeric protein belonging to the cystine

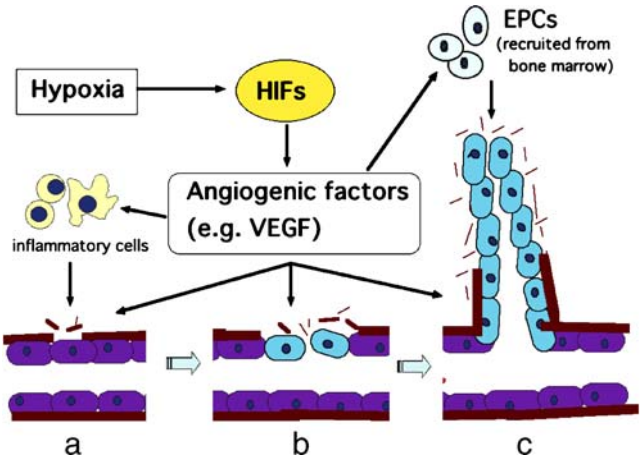

Fig. 1 Steps in angiogenesis, and a part of the mechanism of angiogenesis induced by hypoxia (see text). Angiogenesis consists of degradation of the basement membrane (**a**), activation of endothelial cells (**b**) and endothelial cell migration, and proliferation and synthesis of a new basement membrane (**c**). Hypoxia induces multiple angiogenic factors, including vascular endothelial growth factor (*VEGF*), via activation of hypoxia-inducible factors (*HIFs*). These angiogenic factors enhance the angiogenesis sequence. Inflammatory cells and endothelial progenitor cells (*EPCs*) derived from bone marrow are involved in angiogenesis

Table 1 Examples of angiogenesis activators

Class	Activator
Growth factors	Vascular endothelial growth factor
	Fibroblast growth factor
	Platelet-derived growth factor
	Epidermal growth factor
	Hepatocyte growth factor
	Transforming growth factor
	etc.
Cytokines	Tumour necrosis factor-β
	Interleukin-1
	Interleukin-8
	Interleukin-6
	etc.
Others	Angiogenin
	Angiotropin
	Human angiogenic factor
	Nitric oxide
	Matrix metalloprotease
	etc.

knot growth factor family. Several isoforms exist (VEGF 121, 145, 165, 189 and 206), derived from splicing of a single gene [37]. These isoforms differ in their binding affinity to heparin and to receptors. Two distinct receptor tyrosine kinases have been identified as VEGF receptor-1 (Flt-1) and VEGF receptor-2 (KDR), expressed on the surface of endothelial cells [38]. These two receptors share approximately 44% amino acid homology, but have been suggested to have distinct biological roles [36, 39]. VEGF receptor-1 is the major mediator of endothelial cell proliferation and survival in angiogenesis. In contrast, VEGF receptor-2 does not mediate mitotic activity of endothelial cells, but is necessary for organisation of the vascular system during early embryonic development [36, 37]. As described above, VEGF and VEGF receptor gene expression is induced by hypoxia, as well as by other growth factors and cytokines, and mediated through the activation of HIF expression [40–43].

The extracellular matrix is another well-known key factor that regulates angiogenesis by controlling mobilisation of cells and storage of growth factors [44]. In addition, signal transduction between the extracellular matrix and endothelial cells is an important factor regulating cell migration, proliferation and survival. The integrin superfamily, which is characterised by a heterodimeric receptor with α and β subunits, plays an important role in endothelial cell attachment and signal transduction [45]. The presence of 18 different α subunits, nine different β subunits and 24 different combinations of α and β subunits has been reported for the integrin family [46]. Endothelial cells express eight different integrins depending on the location and activations [46]. Integrin αvβ3, αvβ5 and α2β1 are barely detectable in the quiescent state, but are upregulated in angiogenic endothelial cells [47]. α5β1, α1β1 and α3β1 are expressed at low levels in the quiescent state, and α5β1 shows increased expression in the angiogenic state [48, 49]. Integrins recognise specific extracellular matrix ligands necessary for migration and other molecules such as the arginine-glycine-asparagine (RGD) sequence [50, 51] (Table 2).

During recent years, αvβ3 integrin has been well studied. The crystal structure of αvβ3 integrin demonstrates binding of the RGD sequence with the extracellular structure of both αv and β3 subunits [52, 53]. Blockage of αvβ3 integrin by antibody or peptide antagonists has prevented blood vessel formation in a variety of animal experiments [54–56]. Anti-αvβ3 integrin antibody is reported to block bFGF-induced angiogenesis and to inhibit up to 50% of VEGF-induced angiogenesis [55]. These data clearly indicate the fundamental role of the integrins in angiogenesis. Via the RGD sequence, αvβ3 integrins seem to mediate adhesion of endothelial cells to extracellular matrix molecules and enhance endothelial cell proliferation, migration and tube formation [49]. In addition to their RGD-dependent action, αvβ3 integrins are reported to bind to VEGF, bFGF, VEGF receptors and insulin receptors, to promote angiogenesis. Inhibitors have also been identified which directly bind to αvβ3 integrin, including thrombospondin, MMPs and tumstatin [49, 57, 58]. Although the exact interactions have not yet been defined, these data suggest an important and complex role for integrin αvβ3 expression in regulating angiogenesis.

Table 2 Endothelial cell integrins, their extracellular matrix (ECM) ligands and expression

Integrin subtype	ECM ligand	Expression in quiescent vessels	Angiogenic activation
αvβ3	RGD sequence Vitronectin Fibronectin von Willebrand factors Thrombospondin, etc.	−	+
αvβ5	RGD sequence Vitronectin	−	+
α5β1	RGD sequence Fibronectin	+	+
α1β1	Collagen Laminin	+	
α2β1	Collagen	+	+
α6β1	Laminin	+	
α6β4	Laminin	+	
α3β1	Laminin Reelin Thrombospondin	+	

Therapeutic myocardial angiogenesis in ischaemic heart disease

Advances in the understanding of angiogenesis mechanisms have led to the development of new angiogenesis therapies for ischaemic heart disease. Administration of growth factors such as FGF, VEGF, hepatocyte growth factors and platelet-derived growth factors, as recombinant proteins or as the gene encoding these proteins, has been reported to increase capillary density and collateral vessels in various animal models with ischaemia [4, 5, 7, 59, 60]. Several routes—intravenous, intracoronary, intramyocardial and intrapericardial injection—have been developed to deliver the angiogenic substances to the heart. Based on successful observations in animal studies, clinical trials have been initiated. One of the first reports of clinical therapeutic angiogenesis was a randomised study of 40 patients using intramyocardial injection of recombinant FGF during bypass surgery [61]. The study showed successful enhancement of angiogenesis, as demonstrated by angiography, in the FGF-injected patients compared with control patients. In addition, continuous improvement of the left ventricular ejection fraction was observed in the FGF group at long-term (3 years) follow-up [62]. Several other phase-1 clinical studies with recombinant proteins and gene therapies for angiogenesis, mainly with FGF and VEGF, have followed and have yielded similarly promising results regarding improvements in symptoms and perfusion.

However, the results of subsequent large randomised placebo-controlled trials are inconsistent (Table 3). The VIVA (Vascular Growth Factor in Ischemia for Vascular Angiogenesis) trial was a prospective, randomised, placebo-controlled study to evaluate a single intracoronary VEGF-1 protein administration followed by three intravenous doses in 178 patients with reversible SPECT perfusion defects [9]. There were no significant improvements in exercise time and angina scores at 60 days. Although there was a significant reduction in angina symptoms in the highest dose group, no objective improvements in perfusion were observed on SPECT and angiography at up to 1 year of follow-up. FIRST (FGF-2 Initiating Revascularization Support Trial) was another study with intracoronary infusion of FGF-2 protein at three different randomised doses, as well as a placebo, in 337 patients with chronic angina [8]. There were no significant differences in exercise time and regional perfusion as assessed by SPECT after 90 days.

In the case of the gene delivery approach, the AGENT (angiogenic GENe Therapy) trial randomised 79 patients with chronic stable ischaemic heart disease to receive intracoronary injections of adenovirus vector containing the FGF-4 gene or placebo [10]. There were no significant improvements in exercise treadmill testing with FGF-4 treatment after 4 and 12 weeks, except for a subgroup of patients with baseline treadmill testing equal to or lower than 10 min [10]. In the REVAS (Randomised Evaluation of VEGF for Angiogenesis in Severe Coronary disease) trial, cardiac gene transfer was performed by intramyocardial injection via a mini-thoracotomy using adenovirus vector containing the VEGF121 gene in 67 patients with severe angina and no options for revascularisation [11]. There were significant improvements in exercise tolerance after 12 and 26 weeks. However, nuclear perfusion imaging yielded better results in the control group, although the therapeutic group achieved higher exercise workloads.

Consequently, the results from clinical trials are inconclusive, and there remain many issues that need to be addressed, including appropriate selection of patients,

Table 3 Summary of large clinical trials of therapeutic angiogenesis

Study	No. of pts.	Patient's status	Protein or gene	Route	Results of imaging	Report
VIVA	178	Chronic angina, unsuitable for revascularisation	VEGF165 protein	Intracoronary + intravenous	No significant changes in myocardial perfusion imaging at 60 days between treated and placebo groups	Henry et al. 2003 [9]
FIRST	337	Chronic angina, unsuitable for revascularisation	FGF-2 protein	Intracoronary	No significant changes on myocardial perfusion imaging at 90 and 180 days between treated and placebo groups	Simons et al. 2002 [8]
AGENT	79	Chronic angina	FGF-4 gene, adenoviral vector	Intracoronary	No significant changes in stress-induced wall motion on echocardiography between baseline vs 4 or 12 weeks	Grines et al. 2002 [10]
REVAS	67	Chronic angina, unsuitable for revascularisation	VEGF121 gene, adenoviral vector	Intramyocardial	Myocardial perfusion imaging favoured the control group	Stewart et al. 2006 [11]

choice of angiogenic factors, optimal dose of therapeutic agents and the timing and route of the application delivery method.

Cell-based therapy is another therapeutic strategy in ischaemic heart disease. In post-natal angiogenesis, endothelial progenitor cells are thought to be recruited from bone marrow via peripheral blood to ischaemic lesions and then incorporated into new vessel formation. Based on this hypothesis, endothelial progenitor cells are employed as potential donor cells in therapeutic angiogenesis. Endothelial progenitor cells, which have the potential to proliferate and differentiate into mature endothelial cells, are characterised by surface markers such as VEGFR-2, CD34 and CD133, and can be isolated from peripheral blood, bone marrow and umbilical cord blood [63]. However, recent studies have demonstrated that haematopoietic stem cells and mesenchymal stem cells without expression of CD34 are also progenitor cells for angiogenesis [64]. Thus, the exact lineage and phenotype of endothelial progenitor cells still needs to be clarified [65].

Kawamoto et al. [6] showed that human endothelial progenitor cells decrease infarct size, increase capillary density and inhibit left ventricular remodelling when intravenously injected in the ischaemic rat model [6]. Furthermore, these transplanted cells are incorporated into neovascularisation of ischaemic areas, as documented by fluorescence microscopy and immunohistochemical staining. In subsequent experiments, the same authors also demonstrated successful neovascularisation by endothelial progenitor cells in a porcine ischaemia model. Assmus et al. [66] treated 20 acute myocardial infarction patients with intracoronary infusion of progenitor cells from either the bone marrow or peripheral blood and found improved ventricular function at 4 weeks' follow-up. The results seem very promising, but further large prospective, randomised trials are required to define the efficacy and safety of cell-based angiogenic therapies.

Imaging modalities for perfusion and function in therapeutic angiogenesis

Non-invasive imaging to assess myocardial perfusion and ventricular function in therapeutic angiogenesis can be used to investigate whether blood flow and functional recovery correlate with the observed symptomatic benefit in clinical trials. For this purpose, imaging modalities include the nuclear techniques of single-photon emission computed tomography (SPECT) and positron emission tomography (PET), magnetic resonance imaging (MRI) and echocardiography. However, none of these modalities fulfils all the requirements for monitoring angiogenesis. Each method has its strengths and weaknesses concerning sensitivity, availability, reproducibility and feasibility of quantitative measurements.

SPECT perfusion imaging

Myocardial perfusion imaging with SPECT (MPI) using ^{201}Tl chloride or ^{99m}Tc-based perfusion agents is the most widely used and well-established method for diagnosis, risk stratification and viability assessment in ischaemic heart disease [67]. Some of the phase I trials of therapeutic angiogenesis have demonstrated improved myocardial perfusion using SPECT [68, 69]. However, subsequent large trials have failed to show significant improvement in perfusion recovery in the therapy group [8, 9, 11] (Table 3). The feasibility of using MPI to assess perfusion changes induced by therapeutic angiogenesis remains to be established. It is well known that SPECT imaging can monitor the improvement in perfusion after bypass surgery or percutaneous transluminal coronary angioplasty in ischaemic heart disease [70]. However, the changes in perfusion induced by angiogenic therapy may be relatively small and occur over a longer time frame than the changes observed with conventional interventional therapies. In addition, collateral vessels induced by therapeutic angiogenesis may be functionally abnormal and may cause myocardial perfusion steal [71, 72].

Further studies in animal models are required to define the value of MPI for quantitative assessment of the success of angiogenesis.

PET perfusion imaging

There are no clinical data yet, but PET perfusion imaging has some advantages over SPECT methods in the assessment of perfusion during angiogenesis therapy. Superior spatial and temporal resolution, better sensitivity and the feasibility of attenuation correlation are the major technical advantages of PET imaging [73]. These advantages make it possible to measure absolute regional blood flow with PET using ^{15}O-water or ^{13}N-ammonia perfusion tracers [74]. The concentrations of these tracers in blood and myocardial tissue and their changes over time can be quantified, and tracer kinetic models can be applied to obtain flow (per minute per tissue volume). While it seems difficult to use PET for large clinical trials because of the limited current availability, the expanding use of PET systems is making the technology available for future angiogenesis trials [75].

MRI

Because of its higher spatial resolution compared with nuclear imaging techniques and its ability to delineate soft tissue without contrast agents or ionising radiation, MRI is

well suited to assess cardiac morphology and ventricular function for ischaemic heart disease. Moreover, dynamic first-pass imaging methods using the widely available contrast agent, gadolinium diethyltriamine penta-acetic acid (Gd-DTPA), are available for the assessment of myocardial perfusion [76, 77]. Studies with the pig model of ischaemia have demonstrated the feasibility of assessing improvement in global and regional function and in myocardial blood flow as a result of therapeutic angiogenesis [4, 78, 79]. In a clinical phase I study, Laham et al. [80] used MRI with gadolinium-DTPA to assess the effects of intracoronary FGF-2 administration in 51 patients with myocardial ischaemia. In this study, MRI demonstrated improvement of regional wall thickening and a reduction in the delayed enhancement area. These preliminary data demonstrate the potential feasibility of using MRI to assess the efficacy of therapeutic angiogenesis.

Echocardiography

Myocardial echocardiography is inexpensive, widely available and non-invasive. Therefore it is an attractive technique for evaluation of ventricular function and blood flow. Its temporal and spatial resolution is superior to that of nuclear imaging techniques. Measurement of myocardial perfusion with echocardiography involves intravenous injection of gas-filled microbubbles as red cell tracers, which are acoustic scatterers, to delineate regional microvascular perfusion [81]. Mills et al. [82] showed the potential of this technique in the evaluation of the natural history of collateral development following coronary occlusion in the dog heart. Recently, new approaches to quantify myocardial blood flow with constant venous infusion of microbubbles have been proposed, and Villanueva et al. demonstrated improved collateral flow and reserve with VEGF therapeutic angiogenesis, using the technique in a dog model of myocardial infarction [83, 84]. One of the major limitations of this method is the dependence on experienced operators. Nuclear imaging and MRI are superior to echocardiography in this respect. However, its low cost and non-invasiveness still make it attractive for the assessment of therapeutic angiogenesis strategies in future clinical routine use.

Imaging of molecular markers of angiogenesis

Changes in myocardial perfusion and ventricular function are important as surrogate markers of clinical outcome, allowing assessment of therapeutic angiogenesis strategies. However, molecularly targetted imaging of angiogenic signalling pathways promises to be a further step forwards in the evaluation of specific and early aspects of angiogenesis mechanisms. As discussed above, angiogenesis is a multi-step, highly regulated process involving numerous growth factors and interactions between a number of cell types. Therefore, in order to induce sufficient angiogenesis with therapeutic strategies in ischaemic heart disease, optimised interventions at several steps of the angiogenesis pathway with multiple factors or progenitor cells seems to be required. In vivo monitoring of specific key steps of angiogenesis over time, which is uniquely achieved with molecular imaging techniques, is expected to solve the demanding task of optimising the therapeutic strategies. Recently, some initial animal studies have been performed on molecular targeted imaging for angiogenesis in ischaemic disease.

αvβ3 integrin imaging

As has been previously remarked, αvβ3 integrin expression on vessels is generally rare in adults, but it is seen on endothelial cells during angiogenesis in response to angiogenic growth factors such as bFGF [85]. Furthermore, αvβ3 integrin expression is fundamental for endothelial cell proliferation, adhesion and survival. Therefore, it is a good candidate for targeted delivery of imaging agents.

Some radiolabelled tracers targeting αvβ3 integrin expression were recently introduced for PET and SPECT imaging including ^{18}F-galacto-RGD [86, 87], ^{111}In-RP747 [88] and ^{99m}Tc-NC100692 [89, 90]. These tracers have demonstrated favourable characteristics for imaging of integrin expression in tumour models.

Meoli et al. [91] reported, for the first time, non-invasive nuclear imaging of myocardial angiogenesis in an experimental animal model of myocardial infarction using ^{111}In-RP747. Eleven rats were studied 2 weeks after myocardial occlusion (45 min) and reperfusion using ^{111}In-RP747 or a non-specific control agent. Histological examination revealed neovascularisation and over-expression of αv and β3 integrin subunits in the infarcted region. ^{111}In-RP747 retention was seen (twofold increase) in the infarcted regions, but no regional retention was observed with the non-specific control compound, indicating specific ^{111}In-RP747 uptake for αvβ3 integrin expression on angiogenic endothelial cells. Further studies in transient coronary occlusion canine models were performed with SPECT. Serial SPECT imaging was performed 10 h, 1 week and 3 weeks after reperfusion. Images demonstrated focal tracer accumulation, with the maximum increase in uptake occurring at 1 week.

Another report on angiogenesis imaging targeting αvβ3 integrin in ischaemic tissue was published by Hua et al. [90]. They studied mice with femoral artery occlusion at days 1, 3, 7 and 14 using a ^{99m}Tc-NC100692 SPECT tracer. Tracer uptake was assessed non-invasively with a pinhole

planar imaging system. Increased uptake was observed at day 3 after femoral occlusion; it peaked at day 7 and decreased by 14 days. Additional experiments with fluorescent NC100692 analogue confirmed the localisation of the tracer in endothelial cells on tissue sections.

^{18}F-galacto-RGD is a PET tracer for αvβ3 integrin expression developed at our institution [86, 87]. Specific tracer uptake was confirmed by dose-dependent blocking effects with pre-treatment using a non-radiolabelled cyclic RGD sequence containing peptide and using tumour cells which express αvβ3 integrin on their surface [92]. The feasibility of determination of αvβ3 integrin expression on endothelial cells was studied in A431 tumour cells which induce extensive angiogenesis and do not express integrin [93]. Using the PET tracer, we studied tracer accumulation in ischaemic–reperfusion model rat hearts [94]. Rats were subjected to 20 min of coronary occlusion and subsequent reperfusion. Following 3 weeks of recovery, 37 MBq of ^{18}F-galacto-RGD was injected via the tail vein, and evaluation of tracer uptake in the myocardium with in vivo PET imaging and ex vivo autoradiography was performed. Homogeneous tracer distribution was observed in normal control rats; however, focal increased tracer uptake was shown with autoradiographic analysis in ischaemic rat hearts. In vivo small animal PET imaging successfully visualised the focal tracer uptake in areas with a corresponding reduction in ^{13}N-ammonia perfusion (Fig. 2). In this study, the potential feasibility of non-invasive visualisation of integrin expression in ischaemic myocardium with PET was demonstrated. Already, ^{18}F-galacto-RGD has been applied in clinical settings to evaluate tumour expression of αvβ3 [95]. A sufficient tumour to background ratio was obtained with PET imaging in this clinical study. Furthermore, there is a favourable tracer biodistribution because of predominantly renal tracer elimination, and the effective dose is similar to that of ^{18}F-FDG [96]. Future clinical application in the assessment of therapeutic myocardial angiogenesis is expected using ^{18}F-galacto-RGD.

Targeted contrast ultrasound molecular imaging of integrin expression has been reported recently. Lipid microbubbles were targeted for integrin expression by surface conjugation with either monoclonal antibody against the αv integrin subunit or RGD-containing peptide [97, 98]. Leong-Poi et al. [98] assessed the antigenic response to ischaemia and FGF-2 using a hindlimb ischaemia rat model. Targeted bubble signals from the ischaemic limb were increased at 4 and 7 days after ligation of the iliac artery, and FGF-2 enhanced the signal increase. Unlike nuclear tracers, microbubbles circulate exclusively within the intravascular space, and therefore the retention reflects only the signal from endothelial cells that are located in accessible neovasculature [99]. An interesting feature of microbubbles is the potential for targeted local delivery of drugs, genes or cells by acoustic destruction of the microbubbles [100–103]. This is still in the early stages

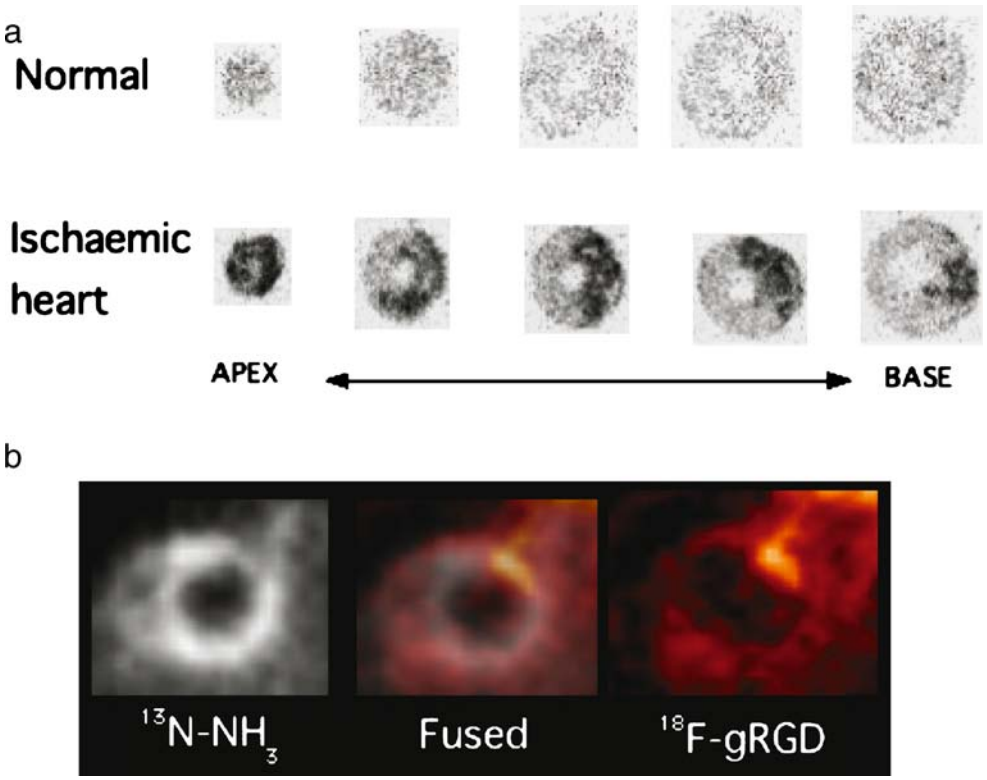

Fig. 2 Imaging of αvβ3 integrin expression in a rat model of coronary occlusion (20 min)/reperfusion using ^{18}F-galacto-RGD. **a** Autoradiographic images of a non-operated normal heart and an ischaemic heart with occlusion/reperfusion at 1 week. Focal tracer accumulation is seen in the ischaemic heart. **b** In vivo short axis PET images demonstrating focal uptake of ^{18}F-galacto-RGD in the corresponding area, with a decrease in perfusion on ^{13}N-ammonia PET, 1 week after the ischaemic event

of animal studies, but echocardiography with molecular targeted microbubbles is a very promising technique for optimising angiogenesis therapies, as well as molecular angiogenesis imaging.

VEGF receptor expression imaging

VEGF is a most important and potent angiogenic factor in the regulation of endothelial cell proliferation and survival, as we have described above. Expression of the VEGF gene and VEGF receptors is strongly enhanced by hypoxia in the angiogenic process. Therefore, VEGF receptors are good candidates for imaging angiogenesis in ischaemic tissues.

Using ^{111}In-labelled recombinant human VEGF121 in a rabbit model of unilateral hindlimb ischaemia, Lu et al. [104] tested the imaging of VEGF receptor expression in ischaemic tissues. Ten days after femoral artery occlusion, tracer uptake was assessed by postmortem gamma counting studies and planar scintigraphic imaging. Tracer uptake in ischaemic muscle was significant increased over contralateral and sham-operated normal perfused muscle. The increased uptake was also detected by scintigraphic imaging, but this finding was subtle on the planar scintigraphic image. Further SPECT studies may be required over time and from an early assessment time point.

Matrix metalloproteinase (MMP) activity imaging

MMPs are proteolytic enzymes that cause extracellular protein degradation. In the early stage of angiogenesis, they are responsible for degradation of vascular basement membranes, resulting in endothelial cell migration and growth factor liberation [23, 24]. These proteinases are also responsible for left ventricular remodelling by degrading extracellular matrix in the heart after myocardial infarction [105]. The recent finding that pharmacological inhibition of MMPs attenuates left ventricular dilatation in the infarcted mouse heart has led to the proposal that MMP inhibitors have the potential to be used therapeutically after myocardial infarction [105]. However, there remain several questions, including the optimal timing for, the spectrum and the specificity of the blocking therapy, and its effects on angiogenesis [105, 106].

Recently, Su et al. [107] demonstrated the feasibility of MMP activation imaging in a murine model of myocardial infarction. Regional uptake of ^{111}In-labelled MMP- targeted radiolabelled tracer (^{111}In-RP782) in the infarcted area was confirmed by autoradiography 1 week after myocardial infarction. Using a similar ^{99m}Tc-labelled tracer, non-invasive visualisation of tracer uptake was shown 1 and 3 weeks after myocardial infarction in regions of decreased ^{201}Tl perfusion. While further confirmatory animal studies are required, this non-invasive technique holds promise for the provision of insights into the role of MMPs not only in ventricular remodelling but also in angiogenesis in ischaemic heart disease.

Summary

Changes in myocardial perfusion and ventricular function imaged non-invasively by means of SPECT, PET, MRI or echocardiography are important as surrogate markers of clinical outcome in therapeutic angiogenesis. New molecular imaging strategies which target specific aspects of the angiogenic process, such as integrin overexpression, VEGF receptor expression and MMP activation, have been proposed recently. These new approaches are expected to answer numerous questions that have arisen in recent large clinical trials of therapeutic angiogenesis (e.g. regarding the selection of growth factors and progenitor cells, timing, dose and route of administration). The results of early animal studies of these new molecular imaging strategies are very promising, but further efforts need to be made to establish their sensitivity, specificity and quantitative capability in monitoring the specific molecular events of angiogenesis for clinical use.

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