Review

Collateralization and the response to obstruction of epicardial coronary arteries

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Summary

Occlusive coronary disease is an important cause of global morbidity and mortality. While mechanical revascularization is effective, some individuals are not amenable to such interventions, and have a poorer prognosis. However, collateral circulation can protect and preserve myocardium around the time of coronary occlusion, contribute to better residual myocardial contractility, and lessen symptoms. We describe the anatomy and physiology of coronary collateralization, its component parts (angiogenesis and arteriogenesis), the current methods for definition of the collateral response and how this might be manipulated. The manipulation of this process is a realistic possibility for future adjuvant treatment of coronary artery disease.

Vascular collateralization in the coronary circulation

Coronary artery disease is an important global cause of morbidity and mortality. Currently, treatment to improve anginal symptoms and coronary flow includes anti-anginal medication (β-blockers, nitrates, calcium channel antagonists), angioplasty and coronary stenting or coronary artery bypass grafting. However, patients with diffuse chronic occlusive coronary artery disease or small-calibre distal disease are often unsuitable for epicorony revascularization, and represent a major residual clinical challenge. Symptom control in this group can be poor. Clinical observation has shown there are individuals who, despite such anatomy, are able to maintain good left ventricular function and sustain a reasonable quality of life, albeit sometimes with a little exertional pain and limitation. One of the key features of these cases is often the development of a collateral coronary circulation.

In this review, we examine the processes behind this relatively under-explored but long-established aspect of cardiac biology, and consider its contemporary prospects as a new therapeutic target, rather than simply a clinical pathological observation.

Vasculogenesis, angiogenesis and arteriogenesis in relation to collateral vessel formation

Vasculogenesis describes the formation of a primitive network of new blood vessels in the embryo from undifferentiated precursor cells (angioblasts), and the differentiation of angioblasts to endothelial cells. This is the initial step to blood vessel formation de novo. It is currently thought that
vasculogenesis does not play a significant role in adult collateralization. Angiogenesis describes the process whereby newly differentiated endothelial cells migrate, differentiate and multiply to form vascular channels. These channels can be formed in three basic ways: by sprouting from an established vessel; by endothelial cells growing and dividing a ‘mother’ channel; and by intussusception, where the vessel is infiltrated by matrix followed by vessel growth.

Smooth muscle cell precursors will coat the channels and initiate smooth muscle differentiation. In the embryo, cells lining the coronary artery wall arise from the epicardial layer of the heart. These cells can differentiate into smooth muscle cells and fibroblasts, forming the vascular media and adventitia. In the heart, the capacity for angiogenesis is not confined to the embryonic period, and can continue throughout life. This can contribute in part to the generation of new collateral flow in the adult.

There is a further mechanism for collateral flow in the adult that is constitutive. Baroldi and colleagues demonstrated confluent corkscrew-shaped collateral channels 20–350 µm in diameter and 1–5 cm in length within the same coronary territory (homocoronary type), or between and two coronary territories (intercoronary type), as long ago as the 1950s. The highest density of these vessels (especially the intercoronary type) was found in the territory between two coronary arteries. This circulation is not visible angiographically at rest. It was presumed that these collateral links did not carry significant blood flow in healthy young adults. However, their definition post mortem implies some flow (or they would simply thrombose or occlude), and their contribution during exercise stress has not been defined. More recently, use of a collateral flow index (an indirect measure of the collateral circulation, see below) has demonstrated that in individuals with angiographically ‘normal’ coronary arteries, or haemodynamically insignificant coronary stenoses, 22% have measurements compatible with a mature collateral system. In those being assessed for ischaemia, 17% did not have ischaemic ECG changes, and 27% had no chest pain during balloon occlusion of flow. This study is the first to demonstrate the function of collateral circulation in the absence of occlusive or high-grade coronary artery disease.

Arteriogenesis is the mechanism responsible for growth and development of the collateral circulation from existing immature arterioles. In this mechanism, angiogenesis is important to provide the capillary network allowing these vessels to mature following an ischaemic stress. As an acute mechanism, these vessels do not have an important role. However this mechanism may be important in increasing the capacity of a poorly developed collateral circulation over time.

The development of coronary collaterals: from the non-occlusive to the occlusive state

Collateral vessels can be present in hearts with patent epicardial coronary arteries. It has been appreciated for many decades that cutting a ligated coronary artery is associated with continued bleeding as a result of collateral retrograde flow. The diameter of collateral channels can be similar to that of epicardial coronary arteries, or as small as 20–50 µm. The constitutive number and density of collateral vessels is likely to be under genetic control, with the capacity and number of channels varying between individuals. Thus there is a simple genetic basis to how well an individual’s myocardium can be protected from epicardial coronary artery occlusion. Apart from genetic factors, presumably phasic blood flow is necessary to maintain these vessels. As such, a pressure gradient is required across the collateral channel, although the direction of flow is immaterial. In the normal heart, the differential pressures during ventricular systole between the deeper and more superficial layers of the myocardium, the right and left ventricle (i.e. the anterior wall of the right ventricle close to the interventricular septum, left ventricle and apex), and the junction of the atria and ventricles provide such a gradient, and collaterals are more prominent at these sites.

The extent of coronary collateralization may also be defined by intrauterine factors such as fetal hypoxia or maternal smoking, which may stimulate or reduce collateral development. There are no direct studies which address this area, despite the postulated importance of fetal growth on the prevalence of adult cardiovascular disease.

Collateral vessels assume greater importance following sub total or total coronary occlusion. Failure of epicardial coronary flow creates immediate tissue hypoxia, which collateral channels can compensate for fully, partially, or to a minor degree. Anecdotal case reports show that even occlusion of the left main-stem vessel can not only be survived but also associated with preserved left ventricular function through the impact of collateral flow.

The severity of the coronary stenosis plays a part in the intensity of arteriogenesis. Physically, flow is inversely proportional to resistance, and will be greater if the collateral circulation offers less
resistance in comparison to occluded or stenosed native vessels. Higher flow in turn will be associated with greater shear stress and increased collateral vessel development.

The collateral response

The collateral response may vary from complete (i.e. the collateral vessels supply the same volume of blood as the occluded or stenosed coronary artery previously did) to absent. There are a number of factors that may predict whether the collateral response of an individual will be adequate. As already discussed, the severity of stenosis and the presence of residual ischaemic stimulus is important. Other factors that may be involved include: the effects of increasing age; the impact of lipid-lowering therapy or high levels of high density (HDL) cholesterol on vascular structure; the effects of arterial vasodilators such as nitrates or calcium-channel-blocking drugs; the presence of severe triple-vessel coronary artery disease; a history of previous myocardial infarction; a long history of ischaemic symptoms without occlusive infarction; preserved left ventricular systolic function; and the presence or absence of diabetes.11–13 Diabetics generally have a poor acute collateral response, because of diffusely impaired endothelial function.14 After the acute phase (>3 months), this mechanism may be irrelevant, as there is then no significant difference between non-diabetics and diabetics.15

The importance of the collateral response is easy to see. In a study of 1164 patients undergoing primary angioplasty for acute myocardial infarction, 23% had angiographic evidence of collateralization at presentation. This group had a lower incidence of anterior myocardial infarction (assuming that all regions of the myocardium have the same potential to form collateral vessels) at 41% vs. 55% (p<0.001), cardiogenic shock (9% vs. 14%, p<0.001) and 6-month mortality (4% vs. 9%, p=0.011).16

However, the support of collateralization needs time to develop. In studies on dogs, inducing ischaemia by repetitive brief (seconds) coronary occlusion does not lead to collateral formation. Longer periods of occlusion result in a marked collateral response.17 Post-mortem studies suggest a greater degree of collateralization in patients with a longer duration of ischaemic symptoms in life.18 In 50 patients undergoing angioplasty for total chronic occlusions, collateral vessels were associated with normal left ventricular wall motion if the occlusion had occurred >3 months previously.19

Thus collateral vessels have great potential to protect muscle during infarction. While collateral channels can be apparent after first balloon inflation during coronary angioplasty, they do not provide any useful blood supply. Such channels open as a result of back-pressure where forward flow is obstructed by the balloon.20 A sustained stimulus is needed to promote the processes of developing the response. Changes in the composition of the surrounding tissue because of increased extracellular matrix turnover (mediated by ischaemia) may influence tissue resistance locally and thereby contribute to collateral expansion.

The regulatory mechanisms of collateralization

As already discussed, two related processes, angiogenesis and arteriogenesis, both play a role in collateral vessel formation. Although the two mechanisms are probably a continuum, they have different regulatory mechanisms in the formation of collaterals in coronary artery disease.

Angiogenesis

Coronary transmural flow is highly dependent on perfusion pressure distal to a stenosis.21 In angiogenesis, growth of new vessels from the donor artery has to occur distal to the occlusion or critical stenosis. Since these vessels form de novo, and are hence not connected, perfusion pressure gradients will not play a major role. Growth factors expressed in response to hypoxia or hypoperfusion can act on donor vessels and may directly stimulate angiogenesis.

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a potent and highly specific stimulator of mitosis in endothelial cells, and five isoforms have been identified. VEGF binds to two receptors, flt-1 and KDR (flk-1).22 These are located on endothelial cells, and are part of the tyrosine kinase receptor super-family. The expression of VEGF is quickly induced by hypoxia.23 In cell culture, hypoxia stabilizes VEGF mRNA and increases its half-life from 43 min to 106 min. This is thought to be due to the effect of proteins induced by hypoxia, in particular HuR (a 36 kDa RNA-binding protein involved in the post transcriptional control of VEGF gene expression), to specific sequences in the VEGF mRNA 3'-UTR region.24 HuR is the main induced protein stabilizing VEGF.25
Levels of VEGF in coronary sinus samples from patients with total coronary occlusion (a potent stimulus for collateral vessel formation) are considerably higher than in patients with stenosed but patent coronary arteries (400.2 vs. 103.3 pg/ml, respectively), and VEGF is expressed in atherosclerotic coronary arteries. Unstable coronary atherosclerosis is associated with thrombus formation, and the thrombin mediating this pathology can enhance the effect of VEGF. Thus VEGF may be a key factor in defining the extent of collateral vessel formation in ischaemic heart disease, and could mediate its effects by the following mechanisms: increasing vascular permeability to promote a milieu into which endothelial cells will grow and migrate, acting as a growth stimulus to endothelial cells, inducing serine proteases to break down extracellular matrix, allowing endothelial cell growth, preventing endothelial cell apoptosis by inducing the expression of Bcl-2 and A1 (anti-apoptotic proteins), acting as a chemotactic for monocytes and macrophages, and directly stimulating the production of local nitric oxide (NO).

**Nitric oxide**
The basic role of NO is vasodilatation, which will in turn augment plasma protein leak. Like VEGF, it prolongs endothelial cell life and enhances the proliferation and migration of the endothelium. It up-regulates the expression of αvβ3 integrin, a trans-membrane receptor that has a role in the activation of matrix metalloproteinases, and cell migration by attaching to fibronectin. Further, NO can block angiostatin, an inhibitor of angiogenesis.

**Angiopoietin**
The Tie2 receptor is an endothelial tyrosine kinase receptor that shares homology with the VEGF receptors. In cell culture experiments using human coronary endothelial cells, hypoxia leads to a time-dependent increase in expression of the Tie2 receptor, for which angiopoietins 1 and 2 are important ligands. The release of angiopoietin-1 may be mediated by VEGF. This compound prevents vascular leak, and is also involved in the maturation of newly formed endothelium, vascular myogenesis and arteriogenesis, where it has roles in vessel remodelling (for example the promotion of endothelial sprouting), maturation and stabilisation (i.e. apposing vascular myocytes towards endothelium). Angiopoietin-2 is also induced by VEGF, and in contrast to angiopoietin-1, is thought to be involved in neovascularization.

**Matrix metalloproteinases and their inhibitors**
In order for cells to migrate, the extracellular matrix needs to be broken down. This requires the production and activation of matrix metalloproteinases (MMP) and their inhibitors. Matrix metalloproteinase 2, membrane-bound metalloproteinase-1 (MT-1 MMP), the integrin αvβ3 and tissue inhibitor of metalloproteinase-2 (TIMP) initiate extracellular matrix degradation, and promote endothelial cell mobilisation. In a dog model of total coronary artery occlusion, MMP-1 expression is increased, and TIMP-1 expression decreased in collateral vessels after occlusion of the left circumflex vessel.

In another study using a dog model of coronary collateralization, MMP-2 and -9 and TIMP-1 showed a temporal association with collateral vessel formation (i.e. the enzymes and inhibitor rose during arteriogenesis, and were subsequently down-regulated when the vessels matured). Connective tissue growth factor (CTGF) may be pro-angiogenic, and is also induced by hypoxia. This factor can increase MMP-2 expression.

The extracellular matrix is integral to angiogenesis and collateral vessel maturation, as it provides the substrate within which these processes take place. While the role of the matrix has been disregarded in the past, there is now growing evidence that MMPs and TIMPs are vital to the initiation and development of this vascular response.

**Arteriogenesis**
Shear stress is the most important stimulator of arteriogenesis. Under normal circumstances, where most blood flow is through epicardial coronary arteries, small collateral channels will be vestigial, at least at rest. The relative occlusion of low-resistance epicardial vessels will divert flow through collateral channels, accompanied by proportionately greater shear forces. This will lead to endothelial cell activation, and an increase in size of the collaterals in an attempt to normalize shear forces. Ischaemia does not appear to be important in this process, although evidence is derived from work in dog and pig models. There are no human observations that explain why, despite severe ischaemia and high shear stress, some patients develop little or no collateral response.

The mechanisms by which shear forces activate endothelial cells are not fully integrated. Wall stress can affect cellular depolarization and repolarization by affecting the flux of calcium and potassium in and out of the endothelial cell. It is plausible that via altered contraction these
changes then lead to the expression of growth factors and mediators such as MCP-1, TGFβ, FGF or TNFα.

Monocyte chemotactic protein-1 (MCP-1) release is induced by shear stress, secondary to up-regulation and transcription of MCP-1 mRNA. MCP-1 is a powerful chemo-attractant for monocytes and macrophages and this effect of MCP-1 expression is shear-force-dependent. MCP-1 also induces the proliferation of vascular smooth muscle cells, and thereby may play a role in the maturation of the collateral channels.

Transforming growth factor β1, like MCP-1, is involved in recruiting monocytes, and has a role in vascular smooth muscle cell differentiation. Interestingly, when administered exogenously to rabbits following femoral artery ligation, it leads to an increase in the number of visible collaterals and increased collateral vessel conductance.

The involvement of activated monocytes and macrophages in collateral vessel growth creates a direct link to the biology of the ‘inflammatory response’ familiar in the pathology of CAD. In rabbit models of arteriogenesis, monocyte accumulation occurs around and within collateral vessels three days after experimental ligation with the release of inflammatory angiogenic factors, basic fibroblast growth factor (bFGF) and tumour necrosis factor α (TNF-α). These cytokines amplify the recruitment of even more monocytes and macrophages, and in a mouse model, increase arteriogenesis by signalling through its p55 receptor. Basic fibroblast growth factor has mitogenic and angiogenic properties, and in a dog model, improves collateral circulation to infarcted myocardium. In contrast, in a small study of 45 patients with coronary artery disease, bFGF was highest in individuals with minimal coronary artery disease, and the authors therefore concluded that bFGF, may be pro-atherogenic rather than exert a positive effect on collateral development. However, in 76 patients with stenotic but not occlusive coronary artery disease, there was a weak to modest positive correlation between CFI and VEGF and bFGF.

The ‘increased flow’ theory to collateral formation is probably too simplistic. The development of ipsilateral collateralization (where the restriction of flow is in the same artery and collaterals occur from branches) and contra-lateral collateralization (where the vessel originates from an open artery and the channels lead to an occluded territory) may be explained by the differential pressure gradients (the pressure in the post-stenotic segment of an occluded epicardial artery will be lower than that of an open ‘feeding’ vessel). Occlusion is not a prerequisite for this, as immature collateral channels develop between territories with different perfusion pressures. For example, collaterals between the distal left anterior descending and the distal posterior descending arteries may form if the pressures of the distal left anterior descending exceeds those of the posterior descending and vice versa.

An attractive alternative to explain arteriogenesis, is through change in the extracellular matrix surrounding the immature collateral vessel. Under normal conditions, matrix tissue surrounding the collateral channel would exert high external ‘stiffness’, keeping the channel closed. The release of growth factors and cytokines, which spread over the myocardium from ischaemic cardiac tissue under pathological conditions, may induce matrix metalloproteinases, which break down surrounding extracellular matrix. The breakdown of matrix surrounding collateral channels would reduce ‘stiffness’, and allow the expansion of these vessels. This process would then increase blood flow, and in effect induce shear forces which would sustain and develop arteriogenesis.

The collateral response of an individual patient is a complex mixture of two linked processes, angiogenesis and arteriogenesis (Figure 1). There are a vast number of cytokines and growth factors involved in mediating both these responses. Much of the experimental work has been done in vitro and in animal models, and the role of these factors in humans has not been integrated. Nonetheless, hypoxia, shear forces, VEGF, and the remodelling of extracellular matrix seem to be critical to the response, which can demonstrably have an important clinical role in preserving myocardium before and after epicardial coronary occlusion.

## Detecting collateralization

### Pathological studies post mortem

There are only a few reports of coronary collateral response post mortem. Although useful, these studies must be interpreted carefully, as they may undermine the importance of the collateral circulation in life following post-mortem change. These vessels are often less well-formed, and are likely to be more susceptible to post-mortem degradation. The early formation of collaterals in rat hearts can be picked up by periodic acid Schiff staining following ligation of the distal left anterior descending artery. Fifteen minutes after coronary occlusion, tracer was detectable in the myocardium, which progressed to all the layers of the myocardium in three hours, suggesting that time is a factor in the recruitment and dilatation of collateral channels.
Post-mortem retrograde angiography has been used in human hearts. In a study of 131 hearts injected post mortem with radio-opaque dye in gelatin, older patients tended to have more collaterals, and coronary occlusions or stenosis were associated with marked collateralization in 70% of the hearts. Regions of myocardial scarring were also associated with a larger number of collateral vessels in the peri-infarct territory. Baroldi et al., using a corrosion method to generate coronary casts, was able to define vessels between 20 and 350 μm in diameter. These eloquently demonstrated collaterals in normal hearts, and showed that in patients who had long-standing occlusive coronary artery disease, the diameter of collateral vessels had increased.

Landmark studies on collateralization were conducted by W.F. Fulton in the 1960s. Using a novel injection technique and medium (bismuth oxychloride and gelatine), meticulous and careful dissection and stereoradiography, arteries ranging upwards from 12 μm diameter could be defined in diseased and normal human hearts post mortem. Fulton demonstrated that the augmentation of the collateral circulation arose from an enlargement of pre-existing arterial anastomoses. He also introduced the concept of the anastomotic index and score to quantify the degree of collateralization, and allow comparison between different hearts. The subendocardial plexus was studied in detail using these techniques, and shown to play a very important role in supplying blood to the myocardium during epicardial artery occlusion. He put forward the very convincing argument that the subendocardial plexus constitutes a reservoir of blood which empties in systole. In the normal heart, this is probably of little consequence, but when an epicardial artery occludes, the greater pressure in these vessels will force blood through collateral channels into the distal segment of the occluded epicardial artery (facilitating the dilatation of these vessels). This phenomenon is generally under-recognized, probably because these vessels are not demonstrated during coronary arteriography. Previous studies had failed to demonstrate collaterals, and coronary arteries were largely and incorrectly regarded as end arteries.

Animal studies complement some of these findings. However, the major drawback is that the collateral response mounted by different species is not the same (vide supra). Also, few animals naturally develop coronary atherosclerosis, stenosis or occlusive infarction. The stereoradiography

Figure 1. Angiogenesis and arteriogenesis in the context of collateral vessel development (see main text for explanation).
described by Fulton is probably the nearest to a human gold standard (albeit only achieved post mortem), since without the use of such techniques, a major part of the collateral circulation would be under-represented.71

**Increased threshold to pain on repeated exercise**

Exercise tolerance, and symptom onset is delayed when coronary patients train regularly. Mechanisms behind this response include ischaemic preconditioning,72 induction of collateralization, and the impact of exercise on endothelial function, with potential regression of atherosclerosis and improved conductance in the microcirculation.73

Collateral vessel growth could play a role, particularly in the mid- and long-term response to exercise training. In dogs, the myocardium is protected when coronary occlusion occurs over a longer period of time, as opposed to sudden occlusion. In the former, collateral circulation is able to compensate.74 Collateralization varies between species, and is maximal in guinea pigs, intermediate in dogs and variable in humans.75 The collateral flow index (vide infra) will decrease following successful epicardial coronary angioplasty,76 or following recanalization of chronic total occlusions.77 This effect could be due to the reduction of collateral channels, but the collateral flow index often remains raised, suggesting that some collateral channels remain patent. Furthermore, flow in the ‘feeding’ artery post angioplasty is greater than before, as a result of blood flowing from the previously stenosed artery through the persistent collateral vessels.78 In a human study to determine the collateral response to exercise, 23 patients underwent coronary angiography to determine their coronary anatomy, and baseline collateral density. These patients had stable coronary artery disease post myocardial infarction (>6 months), and reduced left ventricular ejection fraction. Of this group, 12 underwent exercise training for eight weeks; all subjects had repeat coronary angiography. Collaterals were simply graded from 0 to 3, i.e. no visible collaterals to abundant. There was an increase in the mean collateral score in the exercise group only, and those patients who had demonstrated a degree of collateralization prior to exercise had the greatest post-exercise response.79 Another study randomized 113 patients with chronic stable angina and left ventricular ejection fraction >35% into two groups (intervention with exercise and control). Follow-up at one year showed a slowing down of the progression of atherosclerosis, but no continued increase in collateral formation.80 This finding is in contrast to the previous study, and may be explained by the fact that disease did not progress, and in some patients there was recanalization of occluded coronary arteries. In addition, angiographically ‘invisible’ but functionally important collateral vessels may be involved.

Thus despite some controversy, it seems likely that exercise may have a beneficial effect in increasing the number of collaterals, and this may be an explanation for the symptomatic benefit of exercise programmes.

**The collateral response and prognosis**

As has been discussed above, the presence of collaterals defined at coronary angiography has prognostic significance. In the 1980s, a small prospective study of 52 patients with ischaemic heart disease showed that patients who had a poor collateral response had more complications at the time of myocardial infarction, and higher mortality.81 This is entirely analogous to the impact on outcome of early patency of an infarct-related artery in myocardial infarction, yet in contrast to the latter; collateralization has received little coherent study. Also, Hansen and colleagues later showed that patients with a good collateral circulation had significantly improved 10-year survival.82

In animal experiments, the collateral derived myocardial blood flow to the muscle (at the time of myocardial infarction) predicts the size of the infarct. In dogs, the absence of a collateral blood flow at 6 h results in most of the at-risk myocardium undergoing necrosis. However, if the collaterals contributed to >25% of the blood flow, then the at-risk myocardium remains viable for longer.83 Pressure-derived fractional collateral flow (PDCF) (see below) has been used to estimate the impact of collateralization in 70 patients with acute myocardial infarction undergoing primary angioplasty. The value for PDCF >24% was used to indicate adequate collateralization,84 and the patients were divided into two groups (<24% and >24%). The main finding was that patients who had a higher PDCF index suffered less myocardial damage, and in these patients the time to reperfusion was not a critical factor for determining subsequent myocardial function. In other words, their collateral circulation protected the ‘late presenters’.85 Also, assessment of the collateral circulation using fractional blood flow in a prospective study, showed that individuals with a high fractional blood flow index had fewer subsequent ischaemic events.84 Moreover, CFI has also been used to show the
important prognostic role of the collateral circulation in chronic stable angina. Patients \((n=403)\) underwent coronary angiography and measurement of the CFI using intracoronary pressure or Doppler, and were followed up for a mean of 94 weeks. Although patients with a higher CFI suffered more angina, they had fewer major cardiac events (i.e., cardiac death, or acute coronary syndrome); 2.2% vs. 9%, suggesting a protective role of the collateral circulation, and supporting earlier evidence.

Quantification of collateralization during life

One of the main problems with the detection of collateral vessels in routine clinical practice is their variable but generally small calibre. Many of the controversies over the importance of the coronary collateral circulation are undoubtedly because we fail to ‘see’ them with current technology, and therefore consistently underestimate their presence and importance to the affected patient.

Comparisons of techniques

Coronary angiography

Coronary angiography is now a well-established routine. While the quality of imaging is constantly improving, and allows better definition of coronary anatomy and analysis of smaller-calibre vessels, there is still a major issue of resolution where collateral vessels are the target.

Gibson et al. \(^{88}\) have recently proposed a new protocol for documenting collaterals. In order to obtain good images of the collateral circulation, the 7-inch magnification mode has been recommended. The optimum gantry angle and skew should be selected for accurate collateral definition, and to reduce image foreshortening. The contrast injections and imaging should be prolonged to define late filling, which is very common with collateral flow. ‘Film’ speed data acquisition is recommended, at a rate of 30 frames/s. These criteria are summarized in Table 1. A similar classification by Rockstroh et al. \(^{89}\) included the calculation of collateral network flow capacity, which approximates the total collateral blood flow. Their protocol used magnified images (by a factor of five), and used precision steel wire to accommodate error due to phantom measurements (Table 1). They also suggested that measurements be made, and images stored, in diastole. Recently, Werner et al. have devised a novel method of scoring collaterals during diagnostic angiography in patients with total chronic occlusions, based on the size of the collateral connection (Table 1), which is much more closely associated with invasive parameters of collateral flow than the latter described methods. \(^{90}\) Furthermore, higher scores are associated with less left ventricular wall motion abnormality.

Collateral flow index \(^{11,91}\)

This technique involves the introduction of an intracoronary pressure tipped guide wire at the time of coronary angioplasty (PTCA). The pressure derived collateral flow index (CFIp) is determined by the simultaneous measurement of mean aortic pressure (Pa) and distal coronary pressure (occluding pressure (OP) or coronary wedge pressure (CWP) (only if left ventricular end diastolic pressure < 15 mmHg)) at the end of 1 min of balloon occlusion. The following equation \(^{92}\) is used to calculate the CFIp:

\[
\text{CFIp} = \frac{(\text{OP or CWP} - \text{CVP})}{\text{Pa} - \text{CVP}}
\]

where CVP is the central venous pressure.

As its name suggests, the CFI is a derived variable. The assumption of this method is that following total coronary occlusion; any residual flow can only be via collateral channels. It does not give any information about where the collaterals are located anatomically, but represents the ‘total blood flow diversion’. The CFI can then be correlated with the findings at coronary angiography, to define the volume of blood flowing through the collateral channels for a specified arterial territory.

If the intracoronary pressure sensor is replaced with an intracoronary Doppler guide wire with a 12 MHz piezoelectric crystal at its tip, the velocity-derived CFI (CFIv) can be determined by use of the following equation:

\[
\text{CFIv} = \frac{\text{VTId}}{\text{VTIb}}
\]

Where VTId is the ratio of the flow velocity-time integral distal to the occluded stenosis and VTIb is the baseline flow velocity-time integral obtained at the same site after PTCA.

Coronary angiography and myocardial contrast echocardiography \(^{93}\)

Some groups have explored combinations of angiographic and echocardiographic techniques. At the time of coronary angiography, sonicated echocardiographic contrast is injected selectively into the donor or contralateral coronary artery. Short-axis echocardiographic images can then assess
myocardial perfusion by the collateral channel by scoring the contrast pattern of the collateral dependent myocardial segments (0 = none, 0.5 = patchy or epicardial, 1 = homogenous). Using CFI as a reliable surrogate of the collateral circulation, there is a strong correlation between CFI and collateral flow by myocardial contrast echocardiography.94

**Table 1** Grading of collateral vessels

<table>
<thead>
<tr>
<th>Definition of collateral vessels</th>
<th>Collateral frame count</th>
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<tbody>
<tr>
<td>These are vessels that:</td>
<td>Number of cine frames required for contrast media to reach recipient vessel.</td>
</tr>
<tr>
<td>1. Anastomose with the distal segment of the same artery, or another collateral.</td>
<td></td>
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<tr>
<td>2. Have a mean diameter of &lt;0.7 mm.</td>
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<tr>
<td>3. Extend beyond one half of the distance between the epicardial artery segment that they originate from and any adjacent epicardial artery segment.</td>
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<tr>
<td>4. Arise at a branch angle of &lt;135° degrees from the upstream vessel.</td>
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<tr>
<td>5. Are excessively tortuous.</td>
<td></td>
</tr>
<tr>
<td>6. Have newly developed, and were not present on previous studies.</td>
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**Segmental coronary anatomy classification**

Labelling of individual coronary artery and branches. For example left main stem is LM or CASS 11 based on Coronary Artery Surgery Study.

**Type of collaterals**

| SE | Septal |
| AT | Atrial |
| BR | Branch-branch in ventricular free wall |
| BL | Bridging across lesions |

**Collateral flow grade**

| Grade 0 | No flow |
| Grade 1 | Barely apparent. Dye not visible throughout cardiac cycle but in at least 3 consecutive frames. |
| Grade 2 | Moderately opaque. Dye present >75% of cycle. |
| Grade 3 | Well opacified. Clear antegrade dye motion |
| Grade 4 | Well opacified, fills antegrade, very large >0.7 mm. |

**Collateral network flow capacity**

\[ R = 0.5 \times \mu \times \frac{L}{d^4} \]

\[ R = \text{flow resistance in } \text{mmHg/ml per min} \]

\[ \mu = \text{blood viscosity (0.03 } \text{g/cm/sec)} \]

\[ L = \text{estimated collateral length (mm)} \]

\[ d = \text{collateral diameter (mm)} \]

\[ G_T = \frac{1}{R_T} = \sum \frac{1}{R_i} \]

\[ G_T = \text{collateral perfusion capacity} \]

\[ R_i = \text{individual resistance estimate} \]

**Collateral connection (CC) diameter**

| CC0 | no continuous connection* |
| CC1 | continuous threadlike connection* |
| CC2 | continuous small side branch-like connection* |

**Non-invasive methods for quantifying the collateral circulation**

Currently, there are significant developments in computed tomography and magnetic resonance imaging in coronary artery disease. In animal experiments, MRI has shown some promise in...
the detection of collateral vessels. Recently, Plein and colleagues devised a protocol for magnetic resonance coronary angiography (MRCA). The resolution of current MRCA cannot demonstrate angiographically-visible collateral vessels, but will show retrograde filling of occluded arteries, giving indirect evidence of a collateral circulation.

The use of radioisotopes to detect collaterals is also being developed as a research tool in animals. Technetium-99m sestamibi and 18-fluoro-2-deoxyglucose have been used as to quantify collateral channels indirectly, by studying the metabolic demands of hibernating and ischaemic myocardium.

Sensitivity and specificity

One of the main problems with assessing the collateral circulation using coronary angiography is that vessels < 100 μm will not be detected. This will considerably underestimate the presence and impact of a collateral response with clinical significance. Interobserver variability has been quoted to be <5% for the detection of visible collaterals using coronary angiography, when the investigators are two experienced cardiologists. The average variance in the assessment of collateral diameter is of the order of 0.101 mm.

There is a correlation between visual assessment of the collateral circulation and the CFI (r = 0.33, p < 0.05), but the correlation is poor when angiographic collateral grade is <1. The CFI is meant to be a more sensitive assessor of the collateral circulation, because it accounts for angiographically invisible collaterals. This makes a number of assumptions that may not be valid, but for the practicing clinician performing routine diagnostic coronary angiography, it is often the only way of assessing the collateral circulation, and although less sensitive than invasive assessment (which will give no information on anatomy), is still a useful tool.

Myocardial contrast echocardiography (MCE) is superior to coronary angiography in detecting collateral flow. Following successful coronary angioplasty in 20 patients, repeat angiography was associated with apparently complete disappearance of the collateral circulation. MCE however showed that 35% of the patients still had a demonstrable collateral flow. Cost and image quality are major limitations to the routine use of MCE.

Practicality

Coronary angiography is a widely available and simple investigation, which can define collateralization that may be important in clinical decision-making. More modern equipment will have improved accuracy, resolution and magnification, and help detect and quantify smaller vessels more accurately. In order to define CFI, the operator must be familiar with pressure wire technology, and have access to more expensive equipment. Therefore, these studies will probably be restricted to specialized tertiary referral units. While the use of non-invasive methods (such as myocardial contrast echocardiography) is more appealing for patients and potentially more accurate for investigators, this is still at an early stage of development.

New techniques for the manipulation of collateral vessel growth

What is the value of further research into collateralization, given that the concept has been recognized for fifty years? There has been much interest in the manipulation of the factors that control angiogenesis and arteriogenesis. Manipulation of vessel growth could allow better preservation of myocardial function following infarction over and above reopening of native vessel occlusions at percutaneous coronary intervention, and could be enacted at the time of these mechanical treatments to further improve their effectiveness on damaged but viable muscle.

The use of VEGF in promoting angiogenesis in humans has been a topic of many extensive reviews, and only a brief summary is presented here. Three small clinical trials administering VEGF systemically have shown benefit in reducing anginal symptoms in the short term. The introduction of VEGF DNA using plasmid vectors directly into human hearts has also been encouraging, with improvement in symptoms. This has been complemented by improved angiographic appearances and reduction in ischaemia. While the number of patients involved in these trials has been small, the safety of exogenous VEGF in humans still needs to be addressed more fully. The pro-angiogenic interaction of VEGF with occult malignancy is unknown. Moreover recent data suggest that VEGF may enhance the atherosclerotic process by attracting and activating macrophages, and thus could be counter-productive.

The pro-angiogenic effect of FGF has been well documented in animal experiments, and this led to a trial involving 20 patients with severe triple-vessel coronary artery disease with normal left ventricular function undergoing surgical revascularization. At the time of surgery, FGF-1 was directly injected close to the anastomosis of the left internal...
mammary artery and proximal LAD. At 12 weeks follow-up, a dense capillary network was noted to emerge from the proximal LAD and grow towards ischemic myocardium, supplying tissue that surgery had not been able to revascularize. Another small trial of eight patients conducted in a similar way with basic FGF showed similar results and was found to be relatively safe in the short term (3 month follow-up). Three-year follow up data on the use of FGF have shown continuous improvement of the left ventricular ejection fraction and symptoms. The authors suggest the combination of FGF and surgical revascularization could be a standard adjuvant treatment of the future.

Vascular endothelial growth factor, FGF and granulocyte monocyte colony-stimulating factor (GM-CSF) are the only growth factors to be tested in humans. Many other growth factors are being tested in the laboratory. In a rat hind-limb ischaemia model, human macrophages and platelets were transplanted intramuscularly into the ischaemic thighs. At 4 weeks, histological examination of muscle tissue showed increased capillary growth in the group that received the macrophage and platelet transplant. Furthermore, increased expression of VEGF was identified from these cells. GM-CSF and MCP-1 infusion into surgically created femoral artery stumps significantly increased collateral circulation formation by activating and attracting macrophages towards ischaemic muscle, and by increasing monocyte synthesis by the bone marrow. The combination treatment via a continuous infusion resulted in a collateral circulation that was able to restore flow to 80% of normal, and was assumed to be due to the effect of GM-CSF. Recently, the administration of intracoronary followed by subcutaneous GM-CSF to adults with coronary artery disease who were not candidates for revascularization improved CFI, indicating a potential role for this growth factor in modifying the collateral response. In Apo-E-deficient mice, local MCP-1 infusion to ligated femoral arteries enhanced the collateral vessel formation, but increased systemic atherosclerosis. This clinically significant side-effect needs to be defined better in humans before using growth factors that may have deleterious effects.

Targeting both angiogenesis and arteriogenesis is therefore important, but which one should be the primary focus of intervention? As discussed above, a lot of emphasis is being placed on angiogenesis, which is important because these techniques can be used to feed areas of ischemic myocardium directly. However, what about the main feeding vessels? Angiogenesis takes time, and vessels may have to grow large distances. On the other hand, we already have preformed collaterals, and we must concentrate now on how to exploit the potential of these vessels, and stimulate them to provide for the shortfall in myocardial blood flow. The answer is probably to use both mechanisms and apply these during conventional mechanical revascularization.

In summary, the collateralization response to ischaemia and infarction is a complex process that is regulated at many levels. Unravelling the mechanisms and growth factors involved could lead to the development of new ways to treat ischaemic heart disease, and alleviate much human suffering, over and above the already substantial impact of contemporary percutaneous epicardial coronary interventions.

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