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A Critical Review of Clinical Arteriogenesis Research

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In human hearts, an extensive pre-existing collateral network is present. This was shown unequivocally some 50 years ago in a series of very detailed post-mortem angiographic studies. In these studies, it was also observed that the pre-existent collateral vessels enlarge upon closure of an epicardial coronary artery, resulting in large collateral conduit arteries, in sharp contrast to earlier claims that human coronary arteries are functional end arteries. These insights still form the basis for the concept of arteriogenesis as positive remodeling of pre-existent arteriolar connections. Subsequent experimental studies disclosed the putative role of circulating cells, especially monocytes, which invade the proliferating vessel wall and secrete growth factors, degrading enzymes and survival factors that are required for the development of a mature collateral circulation. Experimental stimulation of arteriogenesis is feasible but to date a relatively low number of clinical studies, with no or limited success, have been performed. The use of intracoronary derived collateral flow index can increase the sensitivity to detect the effects of pharmacological compounds on arteriogenesis, which is important in first proof-of-principle studies. These invasive measurements also allow the detection of patients with an innate defect in their arteriogenic response to coronary obstruction. In a reversed bedside-to-bench approach, the characterization of ribonucleic acid and protein expression patterns in these patients generated new targets for therapeutic arteriogenesis. (J Am Coll Cardiol 2010;55:17–25) © 2010 by the American College of Cardiology Foundation

A little more than 50 years ago, the first of a large series of studies on the extent of the collateral circulation in the human heart was published (1). Using high-resolution post-mortem angiography, these studies delivered final proof of the presence of collateral vessels between the different vascular territories of the normal healthy human heart, refuting claims that coronary arteries are functional end arteries. These studies also showed that the diameter of these pre-existent collateral vessels increases upon coronary occlusion. This still forms the basis for the concept of arteriogenesis, which is the development of large caliber collateral arteries from a pre-existing network, in response to arterial occlusive disease.

Experimental studies showed that the increase in diameter of collateral vessels is not passive dilation, but active proliferation of endothelial as well as smooth muscle cells. This opened the field for pharmacological modulation of collateral vascular development.

Currently, several candidates for pharmacological stimulation of arteriogenesis are known, the tools to measure the effects in patients are available, and the first clinical studies have been published. The intracoronary measurements of collateral flow in combination with ribonucleic acid microarray techniques and proteomics now also allow the identification of biological pathways that are linked to insufficient collateral artery growth. This opens new ways to find arteriogenic targets in patients that subsequently can be tested in validated experimental models. The present review is dedicated to 5 decades of clinical arteriogenesis research, summarizes our current understanding of arteriogenesis from a historical perspective, and outlines future developments.

Morphology of the Coronary Collateral Circulation in Humans: Post-Mortem Analysis

Precise morphology remains the province of post-mortem angiography. Some 50 years ago, it was widely believed that the coronary arteries of humans were end arteries (2). However, when using a more precise technique of post-mortem angiography, it was convincingly demonstrated that, in fact, in all human hearts an extensive network is present, connecting the different vascular territories of the heart (3). The contrast medium employed in this technique consisted of bismuth oxychloride 20% in gelatin prepared from a filtered solution, resulting in a maximal particle size of 2.0 μm and penetration to a minimal lumen diameter of 15 μm. Another important aspect for maximal penetration is pressure control of the injection of the contrast medium and nonsimultaneous injection of the left and the right coronary arteries.

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Abbreviations and Acronyms

CFIp = pressure-derived collateral flow index

FGF = fibroblast growth factors

GM-CSF = granulocyte-macrophage colony-stimulating factor

LAD = left anterior descending artery

LCx = left circumflex artery

MCP = monocyte chemotactic protein

RCA = right coronary artery

Anatomy of pre-existing coronary anastomoses. In the normal heart, superficial and deep collateral arteries are present. Superficial collateral arteries are found mainly at the interface between arterial territories, located at the anterior wall of the right ventricle, between branches of the left anterior descending artery (LAD) and the right coronary artery (RCA), near the posterior interventricular groove between the RCA and the left circumflex artery (LCx) (varying greatly depending on the balance of RCA and LCx), near the apex between branches of the LAD and marginal branches of the RCA and LCx, and in the atrial wall. In contrast to the dog, in humans, superficial collateral arteries are relatively small in number and caliber (20 to 200 μm in the healthy human heart).

Deep collateral arteries are more frequent than the superficial collateral arteries and are often of larger caliber (100 to 300 μm, sometimes even more). Transventricular septal collateral arteries are well recognized, connecting the LAD and the posterior descending artery, arising from RCA or LCx. Transventricular collateral arteries are sometimes exploited to open chronic total coronary occlusions in a retrograde fashion (4). Collateral arteries in the subendocardial plexus of the left ventricle form a network of intercommunicating arterial channels throughout the free wall, largely conforming to the columnae carneae. They are rather poorly represented in clinical angiography, possibly on account of intermittent filling and dilution of contrast medium. A schematic overview of the anatomic location of collateral arteries in the heart is provided in Figure 1.

Enlargement of coronary collateral arteries in obstructive coronary artery disease. Evidence from morphological studies is entirely consistent with the concept that the larger caliber collateral arteries displayed in disease result from enlargement of pre-existing anastomoses of smaller caliber in the normal heart. There is no need to postulate new arterial anastomoses in the human heart. First, there are no anatomic patterns of enlarged collateral arteries in disease that do not have their counterparts in the normal heart. Second, there are sufficient numbers of anastomoses in the normal heart to account for the numbers found in disease. The difference is not in number but in size, showing a shift to the right regarding vessel diameter (5) (Fig. 2).

Ischemic myocardial damage and the collateral circulation. It has long been observed that the extent of ischemic myocardial damage consequent on coronary artery occlusion usually falls short of the entire arterial territory. The background in human coronary disease is extremely heterogeneous with many factors involved, but the outstanding factor that limits the extent of myocardial damage following complete coronary occlusion is the degree of development of the collateral circulation at the time (6). Where there is only a small increase in diameter of collateral arteries, damage tends to be massive. Moderate enlargement greatly restricts the extent of the damage. Where the subendocardial plexus has

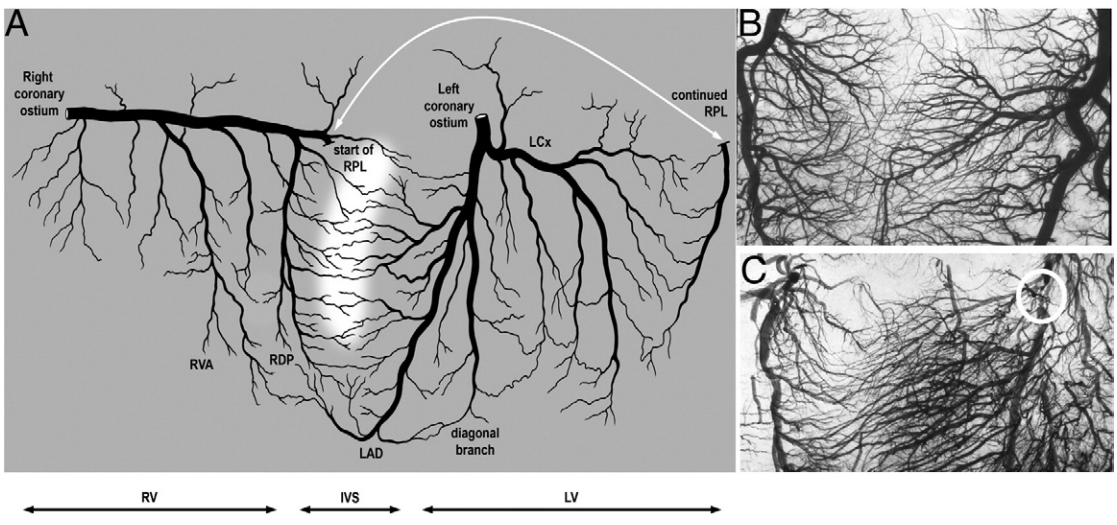

Figure 1 Coronary Collateral Circulation

(A) Anatomic distribution of pre-existing collateral vessels in the heart. The interventricular septum (IVS) **(highlighted area)** is of special interest, showing a large amount of collateral vessels. **(B)** Post-mortem angiogram showing pre-existing collateral vessels in absence of coronary artery disease. **(C)** Outgrowth of collateral vessels in presence of obstruction of the left anterior descending artery (LAD) **(white circle).** LCx = left circumflex artery; LV = left ventricle; RDP = posterior descending artery; RPL = right posterolateral artery; RV = right ventricle; RVA = right ventricular artery.

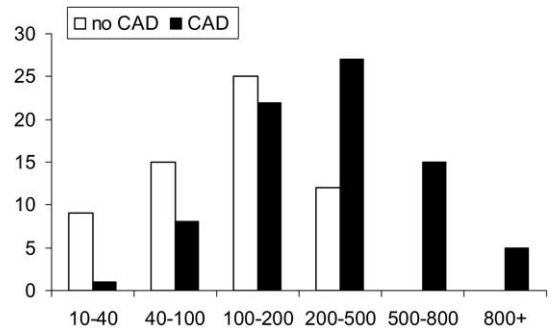

Figure 2 **Numerical Distribution of Collateral Arteries in Patients Either With or Without Obstructive CAD**

A shift to the right is recognized in patients with coronary artery disease (CAD), whereby the absolute number of collateral arteries does not increase but rather the mean diameter of these vessels increases. Adapted, with permission, from Fulton (3).

become a network of wide-bore channels throughout the inner zone, diffuse subendocardial infarction is characteristic. If total inflow to the coronary system has been curtailed by multivessel obstruction, the deep network cannot relieve ischemia but it can achieve an equitable distribution of impoverished blood supply. Thus, further epicardial artery occlusion does not result in regional infarction but rather subendocardial ischemia and diffuse subendocardial focal necrosis. Also, similar damage commonly occurs under stress of extracoronary factors, such as tachycardia, without sudden occlusion in the severely obstructed main arteries. Mitigation of ischemia by augmented collateral blood flow persists only as long as the foster arteries remain patent. When acute occlusion of the foster artery occurs, infarction may not be restricted to its own ipsiregional territory but may be extended to the territory of the artery it had been sustaining hitherto. This phenomenon is called pararegional infarction (5).

Functionality of the Coronary Collateral Circulation in Humans: Patient Studies

Angiographic studies. The introduction of coronary angiography enabled evaluation of the coronary collateral circulation in patients. The first clinical studies were based on angiographic documentation of spontaneously visible collateral arteries. A landmark study was performed by Rentrop et al. (7) that demonstrated elegantly that the visualization of collateral arteries markedly depends on the pressure gradient exerted upon the collateral vasculature, similar to post-mortem angiographic studies described earlier. In the majority of patients, collateral arteries, which are absent during baseline conditions, become apparent during balloon coronary occlusion of the recipient artery. These so-called recruitable collateral arteries are visualized by using a second arterial catheter for contrast injection in the foster artery during balloon coronary occlusion. The findings of Rentrop et al. (7) showed that previous clinical studies incorrectly classified the development of collateral arteries. Spontaneously visible collateral arteries can be considered as "a tip of the iceberg" that leaves a major part of the collateral vascular network unexplored. Unfortunately, several recently published studies use the Rentrop score for evaluation of angiograms but without the above-mentioned contrast injection in the foster artery and the complete balloon occlusion of the recipient artery.

The presence of recruitable collateral arteries protects against myocardial ischemia and left ventricular dysfunction during brief coronary collateral occlusion, indicating functional significance of recruitable collateral arteries (7). Also, survival after myocardial infarction is related to the extent of the collateral circulation (8). Angiographic studies performed for evaluation of thrombolytic therapy in acute myocardial infarction showed that collateral arteries that are initially absent become apparent within 10 to 14 days following sustained coronary occlusion (9). A study by Werner et al. (10) indicates that coronary angiography can be used to assess the functional capacity of collateral arteries in total coronary occlusions, showing that angiographic grading of collateral connections is related to the preservation of regional left ventricular function and invasively assessed parameters of collateral hemodynamics. The advantage of coronary angiography is the wide availability; however, its accuracy to quantify the capacity of the collateral circulation is limited.

Flow and pressure measurements in the collateral circulation. Intracoronary flow and pressure measurements enable more accurate measurements of the collateral circulation than angiography. Initial clinical studies were based upon measurements of coronary wedge pressure through the fluid-filled balloon catheter (11). The introduction of ultrathin guidewires equipped with Doppler crystal and pressure sensors facilitated assessment of collateral hemodynamics directly in the epicardial segment of coronary arteries. The first studies were performed with a 0.014-inch Doppler wire in an angioplasty model in the recipient coronary artery (12). Collateral flow was assessed as an antegrade, retrograde, or bidirectional flow velocity signal, depending upon the position of the guidewire tip (12–14). However, collateral flow can also be assessed in the foster artery as a transient flow increase during balloon coronary occlusion that corresponds to collateral flow detected in the donor artery (15). Collateral flow assessed by blood flow velocity or wedge pressure measurement in the recipient artery appeared to be a better predictor for the occurrence of transient myocardial ischemia than angiographic grading of collateral vessels (13). Blood flow velocity during balloon coronary occlusion divided by blood flow velocity after successful angioplasty provides the velocity-derived collateral flow index. In a similar fashion, the development of the collateral circulation can be assessed with a pressure guidewire to assess the pressure-derived collateral flow index (CFIp) (Fig. 2). CFIp correlates well with velocity-derived

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collateral flow index (14). CFIp is easier to apply and has a better reproducibility because the signal is not critically dependent upon the position of the guidewire tip in the epicardial segment. In a study by Pijls et al. (16), it was demonstrated that a low CFIp (<0.23) was associated with more ischemic events during 1-year follow-up as compared with patients with a high CFIp. Recently, a 10-year follow-up study on a large cohort of patients showed low CFIp as an independent prognostic factor for cardiovascular mortality (17).

Using both flow velocity and pressure measurements in a large cohort of patients, Seiler et al. (14) found coronary lesion severity to be the only independent determinant of collateral vascular growth. However, the correlation between lesion severity and CFIp is very weak and virtually nonexistent when leaving out patients with a total coronary occlusion (18). About two-thirds of patients do not have sufficient collateral flow to prevent myocardial ischemia during coronary occlusion, and this is the group of patients that would benefit from a proarteriogenic therapy. On the other hand, in patients without significant lesions or even absence of visible lesions there is still a large proportion of patients with a high CFIp (19), suggesting that innate factors are also responsible for the development of the collateral circulation.

Aarnoudse et al. (20) measured real volumetric coronary flow in patients by using thermodilution. Theoretically, this allows measurement of absolute collateral flow and resistance rather than flow velocity (20). Future studies have to determine the feasibility of this technique for quantification of collateral flow.

Influence of vasodilatory substances on collateral flow. Mature collateral arteries respond to administration of nitroglycerin and adenosine (21,22). Following intravenous administration of adenosine, coronary collateral steal occurs in 10% of the patients with nontotal coronary obstructions and in one-third of the patients with a chronic total coronary occlusion (23,24). These studies demonstrate the feasibility of studying the dynamic behavior of the collateral circulation following pharmacological intervention and using this technique as a method of choice in clinical studies on stimulation of collateral artery growth.

Some Insights From Experimental Studies

Degree of adaptation. The time for the development of a true collateral circulation capable of delivering a sufficient blood supply is about 3 days in the healthy dog heart. However, there is a wide variation within different species and even within different strains of the same species (25,26). The important question is if collateral vascular supply is sufficient to avoid ischemia under exercise conditions. This is difficult to study in experimental animals. A generally accepted substitute for exercise is the pharmacological induction of vasodilation and the calculation of maximal conductance, which is the reciprocal value of resistance. In a series of animal studies with chronic progressive coronary artery occlusions and acute femoral artery occlusion, we could show that about 30% to 40% of the maximal conductance of a normal artery is restored by collateral artery growth (27). This deficit remains constant for the entire lifetime of the animals, and in exercise-trained mice, this leads to a significant reduction in their ability to run on a treadmill (25).

Structure and ultrastructure. The first sign in the development of collateral vessels is the activation of the endothelium (28). Endothelial cells lose osmotic control and swell. Their ultrastructural phenotype changes toward a proliferative appearance and eventually the cells divide as can be demonstrated by proliferation markers such as KI-67. At about 12 h after endothelial activation, monocytes adhere to and penetrate the intima and start to digest the internal elastic lamina by secreting proteases such as matrix metalloproteinases. This is an important step in the outward remodeling of collateral arteries, and mitosis of the smooth muscle cells follows (29) (Fig. 3). Often, a subintimal proliferation and accumulation of cells can be observed that can reach proportions as to near occlusion of the collateral vessel. We explain this as a pruning mechanism. Many pre-existing collateral vessels participate but only a few reach mature dimensions; the others degenerate. This makes sense from a physiological standpoint: minimal resistance to flow is reached with few large vessels according to Poiseuille's law (30).

Shear stress as initiator of arteriogenesis. The initiator of arteriogenesis is increased fluid shear stress. Upon arterial occlusion, the pressure gradient induces unidirectional flow from the high-pressure to the low-pressure region along pre-existing collateral vessels (31). The increased flow leads to increased fluid shear stress, which is proportional to the velocity of blood flow. Shear stress acts directly upon gene expression involved in the endothelial cell cycle (32). Shear stress also permits the attraction and adhesion of monocytes via endothelial expression of the chemokine monocyte chemotactic protein (MCP)-1 and the adhesion molecules intercellular adhesion molecule-1 and vascular cell adhesion molecule (33).

Invading monocytes produce the necessary growth factors, which are probably a complex cocktail of factors. The crucial role of the monocytes is exemplified by the observations that genetic targeting of the *MCP*-1 gene and of the gene of the MCP-1 receptor (*CCR2*) leads to a defective collateral growth (34,35). Acute reduction of the number of monocytes in the peripheral blood by bone marrow toxins, such as 5-fluorouracil, inhibits arteriogenesis; however, when the occlusion is performed during the rebound phase of monocyte recovery, arteriogenesis is stimulated (36).

Because it is difficult to alter pharmacologically the intensity of fluid shear stress, we designed an experiment to maximize fluid shear stress with a microsurgical operation

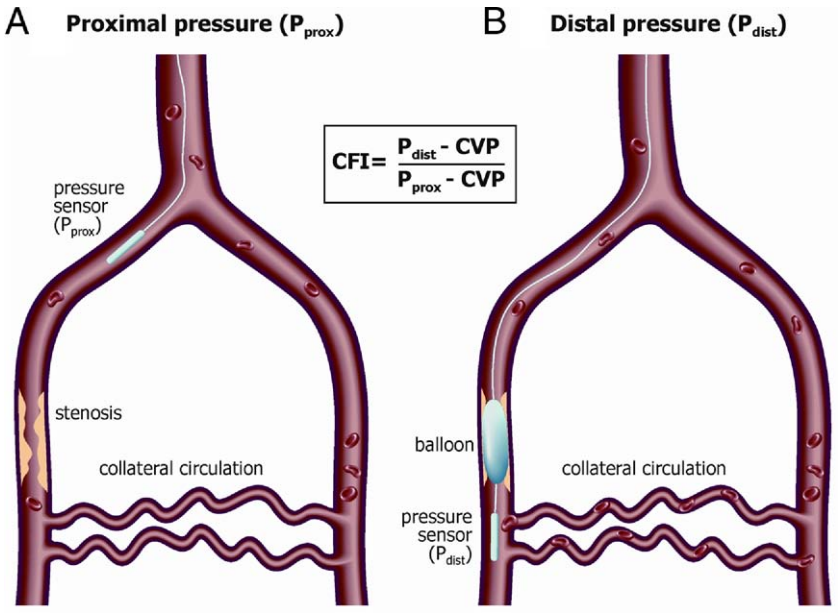

Figure 3 Measurement of CFI

Coronary pressure is first measured proximal to the stenosis **(A)** and then distal to the stenosis during complete balloon occlusion **(B).** Collateral flow index (CFI) is then calculated according to the formula in the **box**. Central venous pressure (CVP) is normally around 5 mm Hg. The better the development of the collateral circulation, the higher the distal pressure during balloon inflation and the closer the value of CFI gets to 1. P_{dist} = distal pressure; P_{prox} = proximal pressure.

(37). The femoral artery of rabbits was occluded and the distal stump was connected side-to-side with the accompanying vein, thereby creating an arteriovenous shunt where most of the collateral flow is drained directly into the venous system. The increase in blood flow gives rise to an increase in collateral growth and with it collateral diameter and number, which increased in turn the velocity of flow in a positive feedback loop. This experiment showed that the conductance values 1 week after shunt closure were almost identical to the conductance values of the nonoccluded contralateral artery. Four weeks of shunting increased the maximal conductance to twice the normal value of the nonoccluded artery, defining the goal of future drug- or growth-factor studies as to reach normal maximal conductance.

The role of ischemia. Arteriogenesis occurs almost always in the presence of ischemia but arteriogenesis is not caused by ischemia. This is not always realized because angiogenesis, with which arteriogenesis is often confounded, is driven by ischemia. This is easily observed in hind-leg ischemia: after femoral artery occlusion, collateral arteries develop in the upper leg, surrounded by normally perfused skeletal muscle, but ischemia and necrosis develop in the foot. Furthermore, collateral arteries are perfused with arterial blood, and the vascular tissue is sufficiently oxygenized as shown in experimental as well as clinical studies (38,39). That ischemia is not a prerequisite to arteriogenesis was recently shown in an elegant approach in a zebra fish embryo model (40). In the zebra fish embryo, the tissue oxygenation is maintained even in the absence of blood circulation due to diffusion. Gray et al. (40) also showed that in this model, arterial occlusion leads to formation of collateral vessels in the absence of ischemia.

Placebo-Controlled Clinical Studies on Stimulation of Arteriogenesis

Experimental studies have shown the potential of numerous exogenously applied growth factors to stimulate collateral artery growth. During the last 10 years, these experimental studies have been followed up by initial patient studies aiming at modification of collateral artery growth.

Granulocyte-macrophage colony-stimulating factor (GM-CSF). To date, only 1 study (41) specifically sought to determine the effects of a pro-arteriogenic factor in coronary artery disease patients using intracoronary measurements on the capacity of the collateral circulation. In this study, 21 patients were randomized to treatment with GM-CSF or placebo. GM-CSF was earlier shown to be pro-arteriogenic in a rabbit hind limb model of collateral artery growth (42) (Fig. 4). Patients were treated for a period of 14 days with subcutaneously injected GM-CSF in addition to a bolus injection at the first examination. CFIp was measured at days 0 and 14. The increase in CFIp was significantly larger in the treatment group than in the placebo group. It should be noted, however, that the effects depended in part on a single outlier in the treatment group, showing a particularly strong increase in CFIp. Nevertheless, this was the first and so far only placebo-controlled

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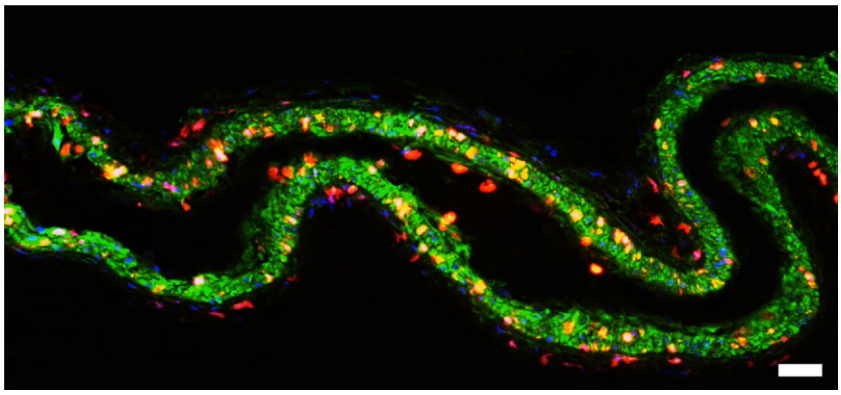

Figure 4 **Proliferating Collateral Artery in the Rabbit Hind Limb**

The collateral vessel was fortuitously cut along the longitudinal axis over a relatively long trajectory. Ki-67 **(yellow)** was used as a proliferation marker, smooth muscle cells were detected with an alpha-smooth muscle cell actin antibody **(green)**, and nuclear staining was performed with Hoechst 33342 **(blue).** The **white bar** represents 20 μ.

study showing a direct treatment effect on CFIp. A subsequent study, which was planned to include a larger group of patients, showed similar results but was abrogated after the occurrence of 2 early stent thromboses in the treatment group (42,43). In a placebo-controlled trial in patients with intermittent claudication, we found no effect of GM-CSF on walking distance (44). A specific problem with studies on GM-CSF is the fact that blinding is very difficult because side effects are numerous and easily recognized by patients as well as by physicians/researchers.

Fibroblast growth factors (FGFs). Several FGFs have been used in clinical trials on collateral artery growth. Schumacher et al. (45) published the first patient trial on the effects of a growth factor on myocardial vascular growth. They performed a randomized placebo-controlled study in patients undergoing coronary artery bypass grafting of the LAD. All patients had remaining stenoses of the distal LAD, and FGF-1 was injected in close vicinity to the vessel wall. Treated patients showed more contrast accumulation around the distal LAD, suggestive of increased angiogenesis. At the same time, treated patients also showed more retrograde filling of LAD segments distal to the stenosis, suggesting increased arteriogenesis. Surprisingly, there has never been a follow-up study on the effects of FGF-1 in coronary artery disease patients.

FGF-2 is the most widely studied fibroblast growth factor, and it has an effect on both angiogenesis and arteriogenesis (46,47). Only 2 placebo-controlled trials have been published on the effects of FGF-2. In a small placebo-controlled trial, Laham et al. (48) implanted FGF-2 loaded pellets in nonrevascularizable territories as an adjuvant to standard coronary artery bypass graft. Three months after implantation, they observed a decrease in size of the perfusion defect as assessed by rest thallium/dipyridamole sestamibi imaging in the high-dose FGF-2 group. A large subsequent trial studied the effects of a single intracoronary infusion of FGF-2 (49). Only at day 90 was there improvement in the treatment group with regard to symptoms of angina. These differences were no longer detectable at day 180. Also, no differences were found between the placebo and the treatment groups with regard to exercise treadmill time or single-photon emission computed tomography imaging.

The proarteriogenic effects of FGF-4 are much less well documented. In fact, it was only in 2003 that it was shown by Rissanen et al. (50) that in a rabbit hind limb model the infusion of FGF-4 leads to increased collateral flow mainly via an increase in diameter of pre-existing collateral channels, that is, arteriogenesis. In patients with stable coronary artery disease, a serotype 5 adenovirus encoding for the FGF-4 gene was administered intracoronary with the intention to achieve vessel wall transfection and prolonged FGF-4 release into the coronary system (51). The initial results showed a tendency to improved exercise endurance on a treadmill in patients with poor performance at baseline. Unfortunately, no follow-up angiography or perfusion imaging was performed. Also, the effectiveness of the transfection of the vascular wall was unclear. Even in experimental settings, the transfection of endothelium appears to be extremely cumbersome. A follow-up trial in a larger group of patients was abrogated prematurely.

Vascular endothelial growth factor. The most widely studied growth factor with regard to vascular growth is vascular endothelial growth factor. This factor is a strong promoter of angiogenesis (52). Some evidence is also available for effects on arteriogenesis, although this is still debated. Positive small patient studies led to the design of a large-scale clinical study, the VIVA (Vascular Endothelial Growth Factor in Ischemia for Vascular Angiogenesis) trial, which was also designed from a concept of stimulation of angiogenesis. This study failed to show differences between the treatment and placebo groups for its primary end point, walking time (53).

A Reversed Bedside-to-Bench Approach

The growth factors mentioned previously were originally identified in experimental models of collateral artery growth and then subsequently tested in patient trials in a classic bench-to-bedside approach. The reversal into a bedside-to-bench approach is an appealing alternative. The failure of the clinical trials on arteriogenesis probably relates to the fact that unidentified factors determine the extent of the outgrowth of collateral arteries in patients. Therefore, we first have to identify the biological pathways that drive human arteriogenesis before designing new clinical trials. Unfortunately, in sharp contrast to the wealth of experimental data, the molecular background of arteriogenesis in humans is largely unexplored. This can be attributed to the fact that collateral arteries are not accessible in patients for biopsy, and post-mortem, the identification of collateral arteries is cumbersome. Monocytes, the circulating cells that are key players in arteriogenesis, however, are easily obtainable. Previously, we found that patients with a CFIp below 0.25 showed decreased monocytic expression of the pro-arteriogenic factor CD44 (54). This study showed that the direct comparison of monocytic expression patterns of patients with either a poor or a well-developed collateral circulation can lead to the identification of new targets for stimulation of arteriogenesis. We recently elaborated on these early findings and performed CFIp measurements in 50 patients with single-vessel coronary artery disease. Transcriptome analysis of circulating monocytes provided 246 differentially regulated genes between good and bad arteriogenic responders. These differences were detected in monocytes activated by lipopolysaccharide. Resting monocytes did not reveal large differences between the 2 groups. Thus, activation of monocytes, or cellular stress testing as we refer to it, can reveal differences between good and bad arteriogenic responders. Pathway analysis showed that especially genes related to interferon-beta, a type I interferon, were up-regulated in bad arteriogenic responders, potentially hampering effective arteriogenesis in these patients. In a murine hind limb model of arteriogenesis, we indeed found an antiarteriogenic effect of exogenously applied interferon-beta, thereby completing the bedside-to-bench approach (55).

Other groups have also exploited the differences in arteriogenic response in patients to identify new targets after comparative transcriptomics. In these studies, only resting cells were studied (56) or capacity of the collateral circulation was not measured invasively but estimated from angiograms (57), which probably explains the relatively low differential expression found in these studies.

Conclusions

Insights from studies that were initiated 50 years ago have taught us that healthy human hearts have an extensive network of pre-existing collateral connections. In the case of obstructive coronary artery disease, there is an increase in diameter of these connections and no increase in number or change in anatomical distribution. These observations strongly support the concept of arteriogenesis as outward remodeling of a pre-existing collateral network.

Substantial progress has been made regarding our insight into the dynamic behavior of the collateral circulation in obstructive coronary artery disease and the potential of intracoronary hemodynamic diagnostic techniques to assess the effect of future treatment modalities aiming to improve collateral flow.

The clinical trials on stimulation of arteriogenesis performed so far did not show benefit. Several factors have contributed to the lack of proof for feasibility of therapeutic arteriogenesis. First, there is a large gap in knowledge on arteriogenesis between the clinical and experimental settings. Several of the molecular mechanisms underlying the process of arteriogenesis have been unraveled in experimental studies but were never corroborated in clinical studies. Second, only a very limited number of placebo-controlled studies has been performed, which prohibits the acceptance or the refusal of the concept of therapeutic arteriogenesis at this time. Third, the clinical studies were initiated at a time when it was still widely believed that stimulation of angiogenesis, which results in increased formation of capillary networks, should be the goal. However, in patients with obstructive coronary artery disease this is most likely not the case as the formation of large caliber conductance arteries, as seen during the process of arteriogenesis, is far more efficient in restoring interrupted blood flow (58). Finally, the previously mentioned intracoronary techniques for the measurement of the extent of the collateral circulation were never employed, with the exception of a single study (41).

Thus, future clinical trials in patients with obstructive coronary artery disease should be designed, focusing on arteriogenesis and arteriogenic factors, and efficacy in these first trials should be measured using intracoronary measurements of flow and pressure. Preceding future clinical trials, or at least in parallel with these trials, we have to increase our knowledge on the specific molecular mechanisms of arteriogenesis in humans. The availability of high-throughput genomic or proteomic analysis systems will propel this kind of research. The integration of this knowledge in the design of future clinical trials will hopefully lead to the often heralded, but still not witnessed, clinical implementation of therapeutic arteriogenesis.

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