Therapeutic arteriogenesis: from experimental observations towards clinical application [cum laude]
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STIMULATION OF ARTERIOGENESIS;
A NEW CONCEPT FOR THE TREATMENT OF ARTERIAL OCCLUSIVE DISEASE

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CHAPTER 2

Abstract
After birth two forms of vessel growth can be observed; angiogenesis and arteriogenesis. Angiogenesis refers to the formation of capillary networks. Arteriogenesis refers to the growth of preexistent collateral arterioles leading to formation of large conductance arteries that are well capable to compensate for the loss of function of occluded arteries. The process of arteriogenesis is initiated when shear stresses increase in the preexistent collateral pathways upon narrowing of a main artery. The increased shear stress leads to an upregulation of cell adhesion molecules for circulating monocytes, which accumulate subsequently around the proliferating arteries and provide the several required cytokines and growth factors. Several strategies are currently tested for their potential to stimulate the process of arteriogenesis. These strategies focus either at shear stress, at direct stimulation of endothelial and smooth muscle cell growth or at the monocytic pathway and promising results were obtained from experimental studies. However, some important questions remain to be answered before arteriogenesis can be brought from bench to bedside.
Introduction
Coronary artery disease is still the most frequent cause of death in the Western world. Outside the Western world, the number of patients with coronary artery disease or peripheral vascular disease is increasing rapidly. Current options to treat occlusive arterial disease include medical therapy or revascularization techniques such as percutaneous transluminal angioplasty (PTCA or PTA) or bypass surgery. These techniques have been developed over the last decades and can be performed nowadays with low morbidity and mortality in patients with chronic coronary artery disease. However, a large number of patients remain for whom this kind of therapy is not feasible either primarily or after non-successful PTA-PTCA or bypass-surgery, and for many patients outside the industrialized world it is unaffordable. Moreover, the increased survival of patients with acute coronary syndromes, treated medically or with revascularization techniques, leads to an increase in the number of patients with chronic arterial disease. The stimulation of collateral artery growth (arteriogenesis) and/or capillary network growth (angiogenesis) would be of potential benefit to these patients. It is estimated that diseases that may be treated with drugs, either inhibiting angiogenesis or stimulating angiogenesis and/or arteriogenesis, encompass around 500 million cases in Western nations. Therefore, a lot of effort has been put forward in recent years to unravel the mechanisms of vessel growth before and after birth.

Angiogenesis: Mechanisms
Background
Pioneering work in the field of angiogenesis, especially with regard to the vascularization of tumors has been performed by Folkman. Angiogenesis refers to the sprouting of endothelial cells leading to capillary networks. Defective oxygenation of cells, as can be observed during pathological events like cancer, stroke and ischemic vascular disease, leads to the expression and activation of the transcription factor HIF-1. HIF-1 functions as a master regulator of oxygen homeostasis and its expression leads to an increase of the transcription of several genes including those encoding for NOS 1-3 and VEGF. As a consequence, one of the first recognizable phenomena during tissue ischemia is vasodilation due to increased levels of NO and other not yet defined transmitters. Secondly, an increase in vascular leakage is observed, due to the increased levels of VEGF (also known as Vascular Permeability Factor). In fact, the occurrence of oedema is a strong predictor of the angiogenic response. Apart from oedema, VEGF also induces, albeit moderately, endothelial cell proliferation. This leads to budding, sprouting and the formation of capillary networks.

Therapeutic stimulation of angiogenesis:
Therapeutic angiogenesis has gained interest tremendously, leading to the start of the first clinical trials using FGF-1 or VEGF-A in 1994. Although initially very promising results were reported from small non-controlled studies in patients with
either peripheral arterial disease\textsuperscript{17,18} or coronary heart disease\textsuperscript{16,19-21} these results could not be confirmed in the subsequent placebo-controlled multi-center studies. One of the shortcomings of strategies designed to stimulate angiogenesis as a treatment for occlusive artery disease is the fact that capillary networks are formed instead of large conductance arteries. Flow is related to diameter in the fourth potency according to Poisseuille's law. It is clear that in order to replace a large conductance artery an enormous amount of capillaries (diameter around 10 micrometer) is required. The efficacy of capillary networks to conduct blood flow is even further reduced due to high losses of energy in these small vessels. Capillary networks are not designed by nature to conduct blood but rather to deliver locally nutrients and oxygen. These considerations might explain the disappointing results of the VIVA-trial that did not show any improvement of the primary endpoints after the intracoronary delivery of VEGF-A.

**Arteriogenesis: Mechanisms**

*Background*

The second form of vessel growth after birth, arteriogenesis, is now recognized as a mechanism distinct from angiogenesis that constitutes a potentially novel therapeutic option\textsuperscript{22-24}.

Arteriogenesis refers to the growth of preexistent collateral arterioles into functional collateral arteries. These preexistent arterioles are present in both the coronary and the peripheral circulation. In fact the presence of these preexistent collateral connections was first reported from Oxford University in 1669. There, the English anatomist Richard Lower observed the following; "Coronary vessels describe a circular course to ensure a better general distribution, and encircle and surround the base of the heart. From such an origin they are able to go off respectively to opposite regions of the heart, yet around the extremities they come together again and here and there communicate by anastomoses. As a result fluid injected into one of them spreads at one and the same time through both. There is everywhere an equally great need of vital heat and nourishment, so deficiency of these is very fully guarded against by such anastomoses"\textsuperscript{25}. Thus, this English researcher not only observed very precisely the presence of preexistent collateral connections between different vascular regions, but actually already recognized their function as alternative pathways for blood flow in case of flow deficiency. In subsequent centuries, these observations were challenged repetitively and around 1900 it was the common assumption that coronary arteries were endarteries. However, in a series of studies between 1956 and 1965, Fulton elegantly demonstrated that collateral connections between coronary arteries are abundantly present in the human heart, irrespective of the presence of coronary artery disease\textsuperscript{26}.

In contrast to the preexistent nature of these collateral vessels, their presence in pathological conditions of obstructive arterial disease was never disputed. In 1971 it was shown for the first time that preexistent collateral arterioles develop into large collateral arteries via proliferation of endothelial and smooth muscle cells and that collateral vessel growth is not simple vasodilatation\textsuperscript{27}. Moreover, the dispute about
the functionality of collateral arteries was ended by a series of studies relating the extent of their development to outcome after myocardial infarction. In these studies it was definitely shown that "collateral arteries save tissue and life".

*Arteriogenesis is mediated via increased shear stress and circulating monocytes*

The process of arteriogenesis is mediated mechanically via an increase in shear stresses. It has been described by several authors that arterial diameter increases upon an increase in wall shear stress, finally resulting in a normalization of wall shear stress. Collateral arteries are recruited after the occurrence of a haemodynamically relevant stenosis of a main feeding artery. Due to the decrease in arterial pressure behind the stenosis, blood flow is redistributed via the pre-existent arterioles that now connect a high-pressure with a low-pressure region. This leads to an increased flow velocity and hence increased shear stress in the preexistent collateral arteries. This causes a marked activation of the endothelium with increases in the expression of MCP-1 and of endothelial surface receptors involved in monocyte tethering, rolling and migration. The upregulation of cell adhesion molecules in the proliferating collateral arterioles under conditions of elevated shear stress was recently confirmed. The subsequent increased adherence of monocytes and their transformation into macrophages are obligatory for the growth of these vessels since these cells produce numerous cytokines and growth factors involved in arteriogenesis. Among these factors are MCP-1 which induces the attraction of more monocytes to the sites of proliferation, TNF-α which provides the inflammatory environment in which collateral vessels develop, b-FGF which is a mitogen for both endothelial and smooth muscle cells and MMP’s that remodel the old arterial structure and create the space that is needed for the expansion of the collateral arteries.

*Morphological changes during arteriogenesis*

Several morphological changes can be observed in the proliferating arteries. Endothelial cells are activated and transform into a synthetical phenotype with an increase in the endoplasmatic reticulum, the number of mitochondria and the size of the Golgi apparatus. They also lose volume control, swell and upregulate adhesion molecules. The lamina elastica interna is degraded, facilitating monocyte and smooth muscle cell trafficking within the vessel wall, but within the next few weeks new elastin is synthesized by smooth muscle cells and a new internal elastic lamina is formed. Numerous mitotic cell divisions of both endothelial- and smooth muscle cells can be observed and the arterioles are remodeled into collateral arteries, expanding their original diameter up to a 20-fold increase in the canine model. This increase in diameter is about 10-fold in the rabbit hind limb model and 2-fold in the mouse hind limb model. A characteristic of coronary collateral arteries in the canine (but also in the hindlimb collaterals of rabbits) is the formation of a cell-rich intima, which can assume a large fraction of the new arterial mass. It is believed that the reduction of the number of collaterals as a function of time after coronary occlusion (to the advantage of the few large remaining vessels) is caused by the obliteration of
the lumen by excessive intimal proliferation. Thus, the increase in diameter is not simple dilation of the preexistent collateral vessels, but a morphogenic adaptation to their new physiological role with an increase in the number of smooth muscle cell layers.

**Angiogenesis versus Arteriogenesis**

*The human heart*

Previous animal experiments had shown that the adaptation to chronic experimental coronary occlusion can either proceed via the arteriogenic pathway (canine model) or via a predominantly angiogenic pathway (pig model). The latter is characterized by low pressures in the post-occlusive arterial system (peripheral coronary pressure), which stems from the fact that capillary connections (with low intravascular pressures) between adjacent vascular territories were the initial substrates of the collateral circulation. On the basis of these experiments it was possible to predict whether arteriogenesis or angiogenesis was the prevailing pathway of adaptation. The principle of these earlier animal experiments can now be repeated in human patients thanks to the miniaturization of pressure and flow catheters. Results by Piek and others have shown that single vessel occlusions are characterized by relatively high peripheral (post-occlusive) pressures indicative for arteriogenesis like in the canine model. A minor difference with results obtained in the dog coronary system was that injection of vasodilators like adenosine or nitroglycerine into the coronary system of patients did cause increases of collateral blood flow, but no fall of post-occlusive pressure, as always observed with adenosine (but not with nitroglycerine) in the dog. This can be explained by the dilatation of both the collateral vessels as well as of the resistance vessels. Since a decrease of collateral resistance can only be imagined in the presence of smooth muscle, a predominantly arteriogenic mechanism must be assumed to exist in man. Multiple vessel occlusion in man leads to low post-occlusive pressures in contrast to the dog model where the occlusion of the left circumflex- plus the right coronary artery did not lead to reduced peripheral coronary pressures. We may thus conclude that multiple occlusions in the human heart may give rise to a mixed arteriogenic/angiogenic type of adaptation. The reason for this is not well known but may reside in the reduced ability to recruit smooth muscle to the enlarging capillary collateral vessel, the basic defect in the heart of the domestic pig.

**Different mechanisms of induction**

As outlined above, angiogenesis and arteriogenesis are two distinct processes. First of all, the driving mechanisms differ. While angiogenesis is induced by hypoxia, arteriogenesis is induced by an increase in shear stress. It was shown that collateral arteries develop in non-ischemic areas. This can also be observed in patients with distal peripheral arterial disease in whom the collateral arteries origin from areas as proximal as the thigh region, far remote from the ischemic vascular territories. A retrograde signaling substance, traveling against the arterial blood flow over such
large distances is unlikely and, in contrast to angiogenesis, ischemia is most probably not a major determinant for arteriogenesis.

Different chemokines and growth factors
The factors involved in both processes also differ. In figure 1 it is outlined which factors were shown to be angiogenic, which factors were shown to be arteriogenic and which factors stimulate both processes. Factors inducing angiogenesis induce proliferation of endothelial cells, whereas factors stimulating arteriogenesis induce also proliferation of smooth muscle cells.

Different involvement of circulating cells
Further differences between angiogenesis and arteriogenesis exist in the role played by circulating cells. While monocytes play a crucial role during arteriogenesis, angiogenesis is partially depending on lymphocytes. It was shown in nude mice, lacking T lymphocytes, that angiogenesis is inhibited. This defective angiogenesis was normalized after the application of VEGF\(^{55}\). However, arteriogenesis, as measured with both X-ray angiograms and flow measurements, is unaffected in these animals (own data).

Vasculogenesis: ongoing process after birth?
Vasculogenesis refers to the formation of a primitive network of blood vessels during embryogenesis. Angioblasts differentiate into endothelial cells forming a vascular network\(^{56}\).

Recently some reports were published claiming that so-called endothelial progenitor cells can induce vessel growth after birth\(^{57,58}\). However, these interesting findings raise unresolved questions. Of specific concern are the cellular markers used to identify the so-called endothelial progenitor cells. It has been shown in vitro that a subset of CD34\(^+\) hematopoietic stem cells can differentiate into endothelial cells\(^{59-61}\). However, the CD34\(^+\) population is very heterogeneous and the origin of the endothelial progenitor cells remains unclear.

The only way to differentiate endothelial progenitor cells from other cell types is the co-expression of CD34, VEGFR2 and ACC 133. Such cell type classification has until now only been performed in one single study\(^{62}\). This study did not relate the endothelial progenitor cells to angiogenesis. Of particular interest are the recent findings that CD34\(^+/\)CD14\(^+\) monocytes express the same surface cell markers under angiogenic stimulation. A strong expression was observed of the endothelial markers von Willebrand Factor, VE-cadherin and ec-NOS (data from Schmeisser, submitted to the current issue). Furthermore, it has been claimed that endothelial cells are unique in their feature to form tubular structures when taken into culture. However many other cells, including monocytes, can display the same structural changes. Therefore it might well be that the results obtained from in-vivo experiments stimulating the differentiation and mobilization of endothelial progenitor cells were not mediated by endothelial progenitor cells but rather by the common progenitor cell, the haemotopoetic progenitor cell or even mature monocytes. In this context it
is noteworthy that it was claimed that GM-CSF, known to induce the release of monocytic progenitor cells, can induce vasculogenesis via “endothelial progenitor cells” \(^6\).

Thus, although it is an interesting concept to treat obstructive arterial disease via recapitulation of vasculogenesis, many uncertainties will have to be unraveled before this potential therapeutic pathway can be applied in the clinical setting.

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**Figure 1.** Three forms of vessel growth, angiogenesis, arteriogenesis and vasculogenesis, are depicted. It shows the differences in underlying mechanisms of induction, as well as the involved mediators and the role of growth factors that are mentioned in this review. Furthermore, it emphasizes the overlap between angiogenesis, arteriogenesis and vasculogenesis. It is currently unknown whether vasculogenesis is operative in the process of vessel growth in response to obstructive arterial disease (abbreviations in text).
Strategies to stimulate arteriogenesis via circulating monocytes

The role of MCP-1

The role of monocytes during arteriogenesis can be utilized to stimulate arteriogenesis. It was shown that the increased attraction of monocytes to the sites of collateral artery development with the use of MCP-1 significantly increased the number of visible collateral arteries (> 50 micrometer) and the conductance capacity of the collateral circulation\(^4,64\). Human MCP-1, also called human macrophage/monocyte chemotactic and activating factor (MCAF), is an 8.6 kDa protein containing 76 amino acid residues. It is a strong chemoattractant for monocytes and binds to the monocyte via the CCR-2 receptor. MCP-1 is not only chemoattractive for monocytes in the standard Boyden chamber assay but also increases transendothelial migration of monocytes\(^65\). The blockage of the endothelial binding sites for monocytes with the use of ICAM antibodies abolishes completely the arteriogenic effect of MCP-1, showing that this effect is indeed monocyte-mediated (data submitted for publication in the Journal of Leukocyte Biology, I. Hoefer).

The role of GM-CSF

Apart from the increased attraction of monocytes a second mechanism can be utilized for the stimulation of arteriogenesis. Granulocyte-monocyte colony-stimulating factor (GM-CSF) prolongs the life-span of monocytes/macrophages via the inhibition of apoptosis of these cells both in vitro and in vivo\(^66,7\). It was shown that the continuous infusion of GM-CSF after femoral artery occlusion in the rabbit hindlimb also stimulated the development of collateral arteries\(^68\). Moreover, the combination of both MCP-1 and GM-CSF had a synergistic effect leading to a 40 % flow restoration one week after femoral artery ligation up to a 75 % flow restoration four weeks after ligation. This combination therapy is the most successful strategy of reperfusion via collateral growth thus far reported from experimental studies.

The role of TGF-β

The expression of TGF-β by different cell types increases under conditions of increased shear stress\(^69-72\). Moreover an increase in TGF-β has been found around growing collateral vessels in both experimental settings\(^73\) as well as in humans\(^74\). Recently it was found in our laboratory that TGF-β exerts arteriogenic properties. TGF-β markedly increased the capacity of the collateral vasculature as compared to the control animals when infused directly into the collateral circulation of the rabbit hindlimb\(^75\). TGF-β is a well-known chemoattractant for monocytes. Moreover, it stimulates the expression of IL-1, TNF-α, b-FGF and PDGF by these cells\(^76,77\). Therefore, most probably the arteriogenic potency of TGF-β is also monocyte-mediated.

Other strategies to stimulate arteriogenesis

Exercise

In a recently published meta-analysis, it was shown that exercise is the most effective treatment, as compared to medical treatment and smoking cessation, to
alleviate symptoms of claudicatio intermittens and to increase walking distance in patients with peripheral arterial disease Fontaine stage II. Exercise improves the utilization and extraction of oxygen from erythrocytes, influences blood viscosity, raises the pain threshold and inhibits the progression of atherosclerosis. In addition, it is believed that exercise improves collateral flow. However, the exact influence of exercise on arteriogenesis in the peripheral circulation is unknown.

Exercise was also reported by some authors to stimulate collateral flow in the coronary circulation. It was shown that an 8-weeks training program increased the contractile response to low-dose dobutamine in patients with chronic coronary artery disease and a left ventricular ejection fraction below 40%. Moreover thallium uptake as well as coronary collateral score improved after the exercise training. Exercise leads to an increased cardiac output, an increase in coronary perfusion and an increase in shear stress along the arterial branches of the coronary circulation. In the presence of a high-grade stenosis, flow will be redirected partially through the preëxistent collateral connections and thus exercise might lead to an increase in shear stress in these vessels and, hence, to an induction of arteriogenesis. However, the study by Belardinelli, using thallium scintigraphy, differs from a large number of clinical studies showing no evidence at all for improved collateralization after various exercise programs. Earlier rigorous studies in experimental animals with chronic coronary artery occlusions (littermates served as controls) also showed no improvement of collateral blood flow after months of training that raised heart rate to over 200 bpm. Furthermore, it is uncertain whether a decrease in shear stress after cessation of the exercise program will lead to a regression of the collateral circulation. Finally, this therapy is not feasible in patients with end-stage obstructive arterial disease who are unable to perform exercise training.

Heparin

It was shown by Fujita et al. that heparin induces collateral formation upon repetitive occlusion of the left circumflex in dogs. These results initiated several clinical studies. Patients with stable angina were treated with either heparin combined with an exercise program or an exercise program alone. Angiographic collateral score increased in patients treated with the combination therapy as compared to patients treated with exercise therapy alone. In addition the rate-pressure product at onset of angina and ST depression increased. A subsequent randomized study showed comparable effects of enoxaparin. Other authors confirmed the beneficial effects of heparin treatment, both in patients with stable coronary artery disease or on cardiac rehabilitation, although no direct evidence for collateral artery growth was provided. The mechanism through which heparin influences arteriogenesis remains largely unclear, although it has been suggested that heparin increases collateral vessel growth via the induction of bFGF release.
bFGF

bFGF is a mitogen for both endothelial cells as well as smooth muscle cells and increases both angiogenesis and arteriogenesis in experimental in vivo models. It was shown in the rat hindlimb circulation as well as in the dog coronary circulation that an intra-arterial infusion of bFGF significantly increases flow restoration upon arterial occlusion. These positive experimental results could not be reproduced in the placebo controlled FIRST trial. In this trial 337 patients, ineligible for CABG or PTCA, were treated with one intracoronary bolus of bFGF in a dose of 0.3, 3 or 30 microgram/kilogram. This treatment resulted in a reduction of anginal symptoms, while the primary endpoint, exercise duration, remained unchanged. It can be speculated that single bolus delivery is not sufficient for the stimulation of arteriogenesis. Furthermore, it can be anticipated that more factors are needed at different time points. Another possibility is the fact that for arteriogenesis most probably more factors are needed at different time points. Therefore, mono-therapy with bFGF may not be sufficient to potentiate arteriogenesis and, hence, induce symptomatic improvement.

Arteriogenesis: from bench to bedside

The aforementioned experimental studies on stimulation of arteriogenesis offer promising new treatment-options for clinical application. However, several issues will still have to be addressed before therapeutic arteriogenesis will become a clinical reality.

Can arteriogenesis be stimulated when the collateral circulation has already matured?

Potential candidates for arteriogenic therapy are patients at a progressive stage of their disease. Therefore, unlike the experimental models, their collateral circulation has been remodeled already for a long time period. Nevertheless, these patients remain symptomatic in spite of maximal growth of the collateral circulation. Whether such mature vessels remain responsive to arteriogenic therapy remains unknown. In this context, it is interesting that the further growth of collateral arteries three weeks after ligation of the femoral artery in young and otherwise healthy rabbits cannot be stimulated with MCP-1 alone. However, a combination of MCP-1 and GM-CSF can still increase the capacity of a matured collateral circulation.

Arteriogenesis and atherosclerosis- a difficult match

The hypothesis that angiogenesis stimulates the atherosclerotic process was first proposed in 1984 by Barger et al. It was observed that microvascular networks arising from native vasa vasorum were more abundantly present in atherosclerotic vessels. Moreover, proliferation rates of endothelial cells of up to 43% were found in these plaques, whereas normal endothelial cells are quiescent and undergo mitosis only occasionally. The hypothesis of Barger was recently confirmed in a paper published by the group of Folkman. They showed that the inhibition of angiogenesis via TNP-470 or Endostatin caused a reduction in atherosclerotic plaque
growth in ApoE deficient mice, suggesting a direct role of angiogenesis in the progression of atherosclerotic plaques. Such direct involvement in formation of atherosclerotic plaques does not apply to arteriogenesis. However, when studying the morphology of coronary collateral arteries, it became clear that several morphological aspects of arteriosclerosis can also be recognized during arteriogenesis. The invasion of monocytes, the inflammatory environment, the elastolysis, the migration and proliferation of smooth muscle cells, the upregulation of adhesion molecules, the tortuosity of inflected arteries are characteristic of both arteriogenesis and atherogenesis. Moreover, most angiogenic growth factors are pro-thrombotic. The major difference is, however, that the arteriogenic process leads to positive arterial remodeling while atherosclerosis leads to negative remodeling. On the molecular level the difference is the substrate (a small arteriole in arteriogenesis) which can be positively remodeled by monocytes whereas the large artery in arteriosclerosis cannot.

Although the process of arteriogenesis itself is not involved in atherosclerotic plaque development, it is not known if the stimulation of arteriogenesis will aggravate plaque formation. It involves many features like neo-intima formation and monocyte invasion that are also part of the atherosclerotic process. The first described arteriogenic substance, MCP-1, has been associated with the development of atherosclerosis, although no causal relationship is proven. Interestingly, the second described arteriogenic factor, GM-CSF, was shown repetitively to be lipid-lowering and anti-atherosclerotic both in animal models as well as in patients.

It is known that the angiogenic response to ischemia is reduced under conditions of hyperlipidemia in Watanabe heritable hypercholesterolemic rabbits and in the Apo-E deficient mice. However, it is unknown whether arteriogenesis is equally reduced under hyperlipidemic conditions and whether hyperlipidemic patients are responsive to arteriogenic therapy. A report published by Abace showed that arteriogenesis is reduced in diabetic patients with coronary artery disease suggesting that this process may be influenced by systemic disease. On the other hand, a former report showed no reduction in arteriogenic response in hyperlipidemic dogs that became atherosclerotic (with development of coronary occlusions) after induction of hypothyroidism and a high cholesterol diet.

Is body mass a determinant of therapeutic success?

Another unsolved question is the responsiveness of collateral vessels in bigger species. The outgrowth of arterioles into functional collateral arteries involves several cycles of cell division. The pre-existing arteriolar connections, from which the collateral arteries develop, are about the same size in different species (~30-50 µm). However, mouse collateral arteries only double their size, in the rabbit they reach up to 200-300 µm, whereas in man they can reach diameters of about 2 mm. Thus, the number of required cell divisions (and therefore the required period of time, since the cell cycle is constant among the different species) depends mainly on the species' size. It can be anticipated that both the dose and the duration of treatment may be different in humans.
How can arteriogenic substances be delivered?
Another issue that has to be addressed is the choice of a delivery strategy. Arteriogenic growth factors, like angiogenic growth factors, were shown to be most effective when administered continuously and intra-arterially. Such treatment for a prolonged time period of several days is an option in the peripheral circulation. However, such an approach is not feasible in the coronary circulation. This implies that different strategies have to be developed. Gene transfer to vascular wall cells is a promising option. A stable transfection will result in a continuous intraarterial delivery without any exogenous instrumentation (except for the transfection catheter). Other options are the use of coated slow-releasing stents or pericardial delivery. A very interesting concept is the use of the monocytes themselves as substance carriers to the proliferating arteries. The topic of delivery strategies was recently reviewed by Kornowski.

Which endpoints should we use?
Finally, clear endpoints need to be defined in order to evaluate future arteriogenic therapies. Options for these endpoints in the peripheral circulation are contrast angiograms and repeated hemodynamic measurements to document collateral vessel growth. Such endpoints are more difficult to establish in the coronary circulation. Current clinical trials on stimulation of angiogenesis/arteriogenesis often make use of parameters like NYHA classification and the need for medication. Several non-invasive techniques like perfusion scintigraphy, positron emission tomography (PET) and myocardial contrast echocardiography (MCE) allow assessment of myocardial perfusion in the region of interest. Recently, Hendel et al. reported a beneficial effect of rhVEGF administration as documented by a reduction of perfusion defects using SPECT-imaging. Nevertheless, these aforementioned non-invasive diagnostic techniques have not been validated for evaluation of collateral flow to jeopardized vascular territories. Moreover these techniques are, at present, unable to assess flow in subendocardial regions, known to be preferential regions for collateral vascular growth in humans. Therefore more reliable endpoints are a prerequisite for establishment of arteriogenic therapy. Since the first trials are focused at the “proof of the concept” of stimulation of collateral artery growth, these endpoints should also include visualization of the collateral arteries and, even more importantly, evaluate their hemodynamic properties. Repeated coronary angiography allows serial visualization of collateral arteries. Collateral vessel growth can be quantified according to the Rentrop classification that corresponds to haemodynamic measurements of collateral vascular supply as demonstrated in an angioplasty model.

Technical refinements in the field of interventional cardiology have led to the introduction of guidewires, equipped with miniaturized sensors for intracoronary pressure or blood flow velocity measurements. These guidewires were introduced for evaluation of coronary lesion severity by assessment of fractional or coronary flow velocity reserve distal to a coronary narrowing. Moreover, these guidewires were also suitable to assess collateral flow in the donor or recipient
coronary artery\textsuperscript{116}. In particular, the combined measurement of collateral flow and pressure in the recipient coronary artery allows documentation of the collateral vascular resistance \textsuperscript{52,53}. Several studies have demonstrated the feasibility of these techniques for evaluation of collateral vascular resistance as well as the documentation of the pharmacological responsiveness of the collateral vascular bed after administration of vasodilators \textsuperscript{51}. These intracoronary diagnostic techniques are promising tools for assessment of collateral vascular resistance, the ultimate endpoint for arteriogenic therapy.

A shortcoming however of the currently available intra-coronary diagnostic techniques is that they depend on the measurement of flow and pressure distal to the site of the lesion. In particular in the first stages of clinical trials on arteriogenesis, patients will be selected for whom revascularization is not an option. This implies that the peripheral measurements are not feasible. It therefore will be a major challenge to develop reliable new strategies to evaluate the functionality and the haemodynamic changes of the collateral circulation in this group of patients.

Conclusion
Stimulation of arteriogenesis, i.e. growth of preexisting collateral arterioles, is a very tantalizing concept although it is clear that many questions have to be answered before this novel approach has finalized its development from bench to bedside. If successful, therapeutic arteriogenesis will lead to large functional "natural bypasses". These vessels will be capable to serve as conduit arteries that can compensate for the functional loss of the affected arteries. However, it appears unlikely that this will be reached by application of a single growth factor. It is conceivable that several, not yet defined, growth factors, either in combination or at successive time intervals are required for the positive remodeling of preexistent collateral vessels.
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CHAPTER 2


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