Abstract

Therapeutic neovascularization for cardiovascular ischemia is a promising avenue in spite of disappointing early clinical trial results. The concept of three different mechanisms of neovascularization has served to define potential therapeutic targets such as vascular remodeling and stem cell recruitment, but it is anticipated that this will lose significance as the pleiotropic nature of angiogenic cytokines becomes fully understood. With the rapidly growing body of data on growth factors and pro-angiogenic strategies, approaches will emerge that are more effective than the ones that have been tested clinically thus far. Combinations of growth factors, for instance to stabilize vessels, or growth factors combined with cell transplants deserve more attention but will make the design of preclinical and clinical studies increasingly complex. Recent developments suggest that when using the appropriate dose and treatment regimens, even single growth factor therapy can result in stable and functional vessels. Whether gene therapy or protein therapy will be optimal for this purpose depends mainly on technical developments in vector design and production and on progress in the engineering of slow release matrix formulations for proteins.

With the increasing complexity of therapeutic strategies, it remains imperative that these approaches are rationally based on fundamental and preclinical data.

Keywords: Ischemia; Neovascularization; Angiogenesis; Growth factors

1. Introduction

The blood vessel system, being the largest organ in our body, is present in all tissues except cornea and cartilage. Being ubiquitous, the vascular system is vulnerable to many pathological processes. In fact, the atherosclerosis-caused obstruction of large or medium sized arteries in the myocardium and brain leads to the most common and lethal human diseases in industrialized countries. Thus, prevention and treatment of tissue ischemia has become an important goal. Fortunately, blood vessels can regenerate from preexisting ones and replace the function of diseased arteries [1,2]. Indeed, experimental and early clinical studies show that stimulation of blood vessel growth (therapeutic neovascularization) is beneficial to perfusion and function of target organs [3]. Most of these studies have utilized angiogenic factors to induce new blood vessel growth in ischemic myocardium or skeletal muscle. For successful therapeutic neovascularization in ischemic tissues, several conditions apply: (1) newly formed blood vessels must be functional and supply the ischemic region with oxygenated blood; (2) the functional blood vessels must remain stable; and (3) neovascularization in ischemic tissue should be tightly regulated to attain maximum efficiency.

Current therapeutic approaches aim at delivering highly effective angiogenic factors to the ischemic region [4,5]. Preclinical and early clinical proof of concept has been...
provided and the next challenge is to translate this into an effective and safe therapy for patients. Some concern about efficacy in generating new blood vessels of adequate structure, function, and stability has been raised. On the safety side, local as well as systemic side effects of growth factors need to be minimized, even when primarily local administrations are being implemented [6]. In this review, we will discuss the rationale of novel strategies to achieve this therapeutic goal.

2. Integrated biological concept of neovascularization

Vasculogenesis, angiogenesis and arteriogenesis are theoretically distinct processes leading to neovascularization (as reviewed by Carmeliet [7]). This concept has been very useful to describe neovascularization under in vitro and specialized in vivo conditions, and it has greatly increased our insight into distinct mechanisms of vessel growth [7] and vessel expansion [8]. However, it is questionable whether the distinction is useful to describe in vivo neovascularization of ischemic ‘non-tumor’ tissue or to serve as a basis for therapeutic neovascularization.

New endothelial tubes, the basic building block of any blood vessel, can form by differentiation and morphogenesis of precursor cells, also referred to as vasculogenesis. Alternatively, they sprout from existing blood vessels or are formed by intussusception, processes that collectively fall under angiogenesis [9]. During growth of new vessels, vasculogenesis and angiogenesis occur simultaneously and they probably should not be considered as separate events. Secondary to the formation of endothelial tubes or primitive blood vessels, a process of vessel maturation starts when pericytes are recruited to the endothelial tubes [10,11]. Sometime during this stage either an arterial or a venous fate will be adopted which further determines the development. The subsequent formation of a full blown artery requires outward remodeling of the vessel, a process that is likely similar, if not identical, to collateral formation or arteriogenesis. To complicate matters even further, endothelial precursor cells participate in both angiogenesis and arteriogenesis [12,13].

For therapeutic purposes, the distinction between angiogenesis, arteriogenesis and vasculogenesis is not clear either. The currently known growth factors are not selective for either mechanism of neovascularization (Table 1). Classic angiogenic growth factors such as VEGF-A and FGF-2 induce tube-like structures in endothelial cell cultures and are therefore considered angiogenic [14]. Hepatocyte Growth Factor/Scatter Factor (HGF/SF) [15], PDGF-BB [16], IL-6 [17] and MCP-1 [18] share this effect. These growth factors induce angiogenesis without exception [18] for instance in an in vivo Matrigel plug assay in the mouse (Fig. 1). Some of these factors also support vasculogenesis during adult life, as they recruit endothelial precursor cells from bone marrow [19–21] and enhance homing of these cells in or around nascent vessels. Finally, angiogenic factors induce collateral remodeling in classic models of arteriogenesis such as the ischemic hindlimb of a rat, mouse or rabbit (for review see Ref. [22]). The aim of therapeutic neovascularization is to enhance perfusion and function of end-organ tissue and the question may arise which mode of neovascularization serves this goal best. Schaper et al. have argued that large collaterals contribute more to blood flow in end organs than small angiogenic networks [23] and therefore the goal should be to enhance arteriogenesis rather than angiogenesis or vasculogenesis. In contrast, circulating precursor cells contribute less than 5% of endothelial cells in de novo vascular networks in adult tissues, which is quantitatively of little importance. However, at this point we have insufficient understanding of the relationships between angiogenesis and arteriogenesis and of the influence of sparsely recruited endothelial precursors on the development of vascular networks to judge which goal to pursue.

Table 1

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Angiogenesis</th>
<th>Arteriogenesis</th>
<th>Vasculogenesis</th>
</tr>
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<tbody>
<tr>
<td>VEGF-A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FGF-2</td>
<td>+</td>
<td>+</td>
<td>+ [101]</td>
</tr>
<tr>
<td>HGF</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MCP-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>+</td>
<td>+</td>
<td>+ [102]</td>
</tr>
<tr>
<td>TGF-β</td>
<td>+</td>
<td>+</td>
<td>+ [102]</td>
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</table>

Growth factors and cytokines have been tested in a number of models, some of which emphasize angiogenesis, some arteriogenesis and some vasculogenesis. Although many gaps still need to be filled in, especially with regard to ability to recruit precursor cells, most factors are pleiotropic in regard to mode of neovascularization. Therefore, it is questionable if the concept of three distinct forms of neovascularization remains tenable. For references other than indicated, see text.
Thus, in vivo, vasculogenesis, angiogenesis and arteriogenesis are tightly linked at physiological and biochemical levels. Some experimental models, for instance the in vivo mouse Matrigel plug and tumor models, emphasize angiogenesis, others such as hindlimb ischemia models likely represent arteriogenesis. None of these models however are completely selective and in fact the terms vasculogenesis, angiogenesis and arteriogenesis are often being confused in the description of these models.

Although the initiation of neovasculature is well understood, subsequent processes leading to an efficient arteriovenous network are still largely unexplored. For instance, the study of molecular and hemodynamic mechanisms guiding organization of the neovasculature into arterial and venous structures is still in its infancy. Insight into arterial-venous specification is mostly derived from work in the zebrafish. VEGF, Notch-Jagged and Ephrins seem to determine arterial-venous specification even before circulation of blood has commenced (for review see Ref. [24]).

Given this complexity of tasks, it is not clear if a single growth factor is sufficient to initiate the entire cascade of events leading to a mature, functional and stable vascular network in vivo. Conversely, it is equally unclear if a cocktail of growth factors should be used and if the composition of such a cocktail should be precisely orchestrated over time. The poor architecture and function of tumor vessels has been attributed to the uncoordinated production of angiogenic factors and imbalanced proportions [25]. For instance, vascular endothelial growth factor (VEGF) is constitutively expressed at high levels in most tumors, inducing premature and leaky vessels [26,27]. In contrast to tumors, healthy tissues express several angiogenic factors that have coordinative roles in remodeling newly formed vessels. Indeed, recent studies demonstrate that combinations of several angiogenic factors induce well-defined and stable vessels [28,29]. On the other hand, in vivo single growth factor therapy in a precisely timed release, e.g. with VEGF-A or PDGF-BB, can lead to induction of mature, persistent and functional vessels as well [30–32]. Likewise, an intervention as simple as controlling the level of VEGF in the microenvironment may convert pathologic into therapeutic angiogenesis [33].

Why would a single growth factor be effective if the entire process is so complex? The answer might be that in vivo most growth factors secondarily induce other factors that are necessary to complete specialized tasks, such as pericyte recruitment or reducing hyperpermeability. Among the many examples of growth factor induced upregulation of other growth factors or receptors are VEGFs [34–36], FGFs [28,37,38], HGF [39] and PDGF-BB [40] as primary factors and VEGF [37,39,40], VEGFR-2 [34], FGFR-2 [40], PDGF-BB [36], PDGFRβ [28], HGF [38] and angiopoietin-2 [35] as secondarily induced components of the cascade.

3. Rational basis of therapeutic neovascularization

3.1. Is there a rationale for current therapeutic strategies?

The rationale for therapeutic application of angiogenic growth factors in ischemic diseases should come from either:

1. a (relative) shortage of an angiogenic factor
2. enhancement of response by administration of supra-physiological dosages
3. an overriding inhibitory mechanism needs to be counteracted

To date, there is little information on the endogenous regulation of growth factors during conditions that favor neovascularization. In fact, most growth factors are upregulated in ischemic tissue. The best described tissue hypoxia response system is the oxygen tension sensitive degradation of the transcription factor hypoxia inducible factor-1 alpha (HIF-1α) (Fig. 2). HIF-1α induces transcription of angiogenic target genes, which include erythropoietin (EPO), VEGF-A and the VEGFR-1 receptor and about 40 other known genes (for review see Ref. [41]). Whether the HIF-1α response is rate limiting is unknown, but monocytes retrieved from patients with a poor arteriogenic response to coronary artery disease have a reduced VEGF upregulation during hypoxia [42]. Likewise, in hindlimbs of aged [43] or diabetic rabbits, hypoxic upregulation of VEGF is reduced as a consequence of a deficient HIF-1α response.

HGF/SF may also be deficient during increased demand for neovascularization. Under certain circumstances HGF is downregulated during hypoxia [44]. The same is true for its

Fig. 2. Tissue responses to ischemia. (NO, nitric oxide; HIF-1α, hypoxia inducible factor; EPO, erythropoietin; VEGF, vascular endothelial growth factor; RBC, red blood cells).
c-met receptor [45]. Interestingly, the downregulation of HGF during hypoxia seems to depend on levels of FGF-2 [38] and TGF-β [46]. In contrast, in patients with myocardial infarct, HGF was found to be significantly augmented [47].

Finally, a reduced angiogenic response in transplanted hearts in aged recipient mice seems to be related to reduced PDGF-AB recruitment [48], suggesting a potential benefit of PDGF-B or A chain supplementation.

Given the paucity of data on growth factor and growth factor receptor expression in ischemic animal models with or without risk factors or in human diseased tissue, it is fair to say that supplementation of a presumed deficiency has not been the major rationale for current therapeutic strategies.

Does exogenous supplementation of growth factors above and beyond the augmented endogenous expression perhaps leading to supra-physiological levels further enhance neovascularization? Numerous preclinical studies indeed have shown that VEGF and FGF, when appropriately administered, enhance neovascularization [3]. However, exogenous supply of growth factors leading to supra-normal levels of, for instance, VEGF and FGF may lead to pathologic vessel formation [33,49], and the therapeutic window may be narrow.

Finally, therapeutic interventions that focus on the inhibition of natural anti-vascularization mechanisms should be considered. For example, sleep-induced hypoxia of the cornea does not initiate angiogenesis, probably because of a naturally occurring dominant negative mutant HIF-1α that is almost exclusively expressed in corneal tissue [50]. Interestingly, this mutant is also regulated by hypoxia. Antagonists of this mutant HIF-1α restore the hypoxia-induced angiogenic responses of the cornea. Other natural inhibitors of neovascularization, such as endostatin [47,51] and thrombospondins [52], are upregulated during tissue ischemia. Interestingly, recent evidence shows that HGF induces angiogenesis in certain tumors in part through downregulation of Thrombospondin-1 [53], indeed suggesting that counteracting of anti-angiogenic factors may contribute to therapeutic efficacy.

In summary, we are just beginning to understand the intricate regulation of angiogenic growth factors, their receptors and innate anti-angiogenic agents in ischemic tissues. This is true for young and normal animals, but is even more pertinent to elderly patients with comorbidities that affect spontaneous neovascularization, such as diabetes, hypercholesterolemia, advanced atherosclerosis or malnutrition.

3.2. Growth factors for neovascularization

Many growth factors and associated peptides are at our disposal for therapeutic intervention, alone or in combination. Few have followed the entire evolution from in vitro to preclinical and clinical phase II studies. These factors include FGF-2, VEGF-A<sub>165</sub> and FGF-4. Others, such as VEGF-A<sub>121</sub>, HGF/SF, and the transcription factor HIF-1α are either in phase I or phase II trials. Although a number of potent angiogenic agents can be successfully employed to enhance neovascularization in animal models, significant challenges prevent easy translation into clinical efficacy.

Thus, clinical trials have been less successful in demonstrating benefits of therapeutic neovascularization. While the initial series of open label studies testing VEGF<sub>165</sub>, FGF-1 and FGF-2 all showed significant improvement in myocardial perfusion and function, a similar improvement was seen in placebo groups in larger randomized double blind trials [54,55]. Various explanations have been forwarded, including choice and formulation of growth factor, short exposure, route of administration, and selection of patients [4]. Based on these hypotheses, new strategies are being developed and tested in preclinical models that can be categorized as:

1. New, unexplored, growth factors
2. A combination of growth factors
3. Targeting a common pathway
4. Alternative modes of administration, including sustained delivery
5. Cell based therapy with or without growth factor transfection or growth factor ‘priming’.

3.3. New growth factors, old families

The VEGF family includes five structurally related members, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PIGF). The prototypic VEGF-A induces angiogenesis and vascular permeability primarily through activation of the VEGFR-2 receptor [56], whereas VEGF-B and PIGF only bind and activate VEGFR-1. VEGFR-1 mediated biological functions remain undetermined although early reports suggest that VEGF-B [57] and PIGF [58] may induce angiogenesis, arteriogenesis and vasculogenesis. VEGF-C and VEGF-D bind to both VEGFR-2 and VEGFR-3 that mediate angiogenic and lymphatic neovascularization signals, respectively [59]. Novel strategies using this family will likely be tested in the near future.

Platelet derived growth factors are members of a family that consists of PDGF-A thru -D, and are structurally related to the VEGFs [60]. It is evident from CAM assays, and from recent studies in the ischemic porcine myocardium [30], that PDGF-BB is the most promising pro- neovascularization candidate, although PDGF-CC appears to be angiogenic as well [61]. From a mechanistic point of view, PDGF-BB is an attractive candidate since it recruits pericytes [62], which presumably lead to increased stability of neovascularure. In addition, there is some evidence that PDGF-BB induces collateralization [63]. In preclinical models of myocardial ischemia, PDGF-BB improved perfusion as well as function [30].
In vivo information on the angiogenic potency of insulin like growth factor (IGF-1) and developmental endothelial locus-1 (Del-1) is much more limited. IGF-1 is both cardioprotective against ischemia and angiogenic [64], but concerns about aggravation of diabetic retinopathy and coronary artery disease [65] have forestalled further clinical development.

Del-1 is an extracellular matrix protein that induces arteriogenesis through binding to endothelial integrin $\alpha_v\beta_3$ leading to induction of the transcription factor Hox D3 and integrin $\alpha_v\beta_3$ [66]. Although successful transfection with Del-1 has been carried out with retroinfusion in the heart [67], efficacy has not been reported yet.

HGF/SF was originally described as a hepatocyte mitogen, but is also a particularly effective endothelial cell mitogen [68]. Most actions of HGF are mediated via the c-met receptor, which is upregulated in ischemic tissues [45]. There is a growing body of evidence that protein and gene therapy with HGF improves blood flow in various hindlimb [44] and cardiac models [69] of ischemia. HGF/SF is currently in phase I trials in patients with peripheral artery disease (PAD).

In addition to these potent angiogenic factors, several other factors, including nerve growth factor (NGF) and NPY, have been found to induce new blood vessel growth in ischemic tissues [70,71]. Extensive preclinical testing of these growth factors is still ongoing and may yield novel targets for therapeutic neovascularization.

### 3.4. Combination of growth factors

Combinations of growth factors add to the complexity of the therapeutic strategy. The number of questions regarding for instance relative dosages, timing of different components and modes of delivery for each component, simply multiplies. However, rational strategies for combination therapy may be based on either synergism between therapeutic effects of growth factors or complementary functions of growth factors, such as initiation and maturation of blood vessels.

Synergism of growth factors has been shown extensively in vitro between for instance FGF-2 and VEGF-A and VEGF-C [72]. In various animal models, FGF-2 and PDGF-BB were also synergistic due to upregulation of PDGF receptors by FGF-2 [28]. Synergism is only helpful when it is limited to the therapeutic effect and when growth factor specific side effects, such as the transforming effect of FGFs or the hyperpermeability by VEGF-A, can be prevented by reducing the dose of either without losing efficacy. None of these combinations has yet been tested in a clinical setting.

Another rational approach is to combine factors that are pro-angiogenic, for example, VEGF or FGF-2, with pro-maturation factors such as angiopoietin-1 or PDGF-BB that primarily mediate recruitment of pericytes. The presence of mural cells (pericytes) prevents hyperpermeability but also stabilizes the nascent vasculature [11,73]. Effective complementary functionality may be specific for sets of growth factors for example angiopoietin-1 with VEGF-A [74] and PDGF-BB with FGF-2 [28].

### 3.5. Targeting a common pathway

Among the common pathways of growth factor transduction, the generation of nitric oxide (NO) stands out as a potential target for drug therapy. Several lines of evidence, mainly from in vitro studies, suggest that NO is involved in the angiogenic effect of VEGF, FGF and TGF$\beta$ (for review see Ref. [75]). However, in vivo experience with NOS inhibitors or eNOS and iNOS knockout mice is controversial. The most convincing in vivo evidence is derived from the study by Murohara et al. [76], in which L-arginine promoted neovascularization in a rabbit ischemic hindlimb model. Accordingly, neovascularization in ischemic hindlimbs of eNOS deficient mice is disturbed. In contrast, in a nonsurgical mesenteric window model of angiogenesis, NO seems to be a negative regulator angiogenesis [77]. In our own experience with an in vivo Matrigel pellet model in wild-type mice fed L-NAME and in eNOS and iNOS deficient mice nitric oxide was not involved in FGF-2 and VEGF-A induced angiogenesis (unpublished observations). Likewise, in a tumor model of angiogenesis and in the in vivo Matrigel model, NO donors appear to be anti-angiogenic [78]. These observations are contrasted by several reports on NO as a mediator of angiogenesis induced by various growth factors including VEGF, FGF and angiopoietin-1 [79,80]. It is therefore fair to say that considerable controversy on the role of NO in angiogenesis and arteriogenesis still exists and that NO donors or NO synthase inhibitors have not been clinically tested for their efficacy in stimulating or inhibiting angiogenesis, respectively.

### 3.6. Alternative modes of administration, sustained delivery

An important issue in development of effective therapeutic neovascularization is duration and location of delivery. How long should exposure to angiogenic factors last? As most angiogenic factors also act as survival factors for endothelial cells, exposure to a single growth factor probably needs to be sustained to prevent early apoptosis of target cells [81]. In a recent study with a combination of growth factors, the decision on blood vessel stability was made early during neovascularization [82], suggesting that combination therapy may be of short duration.

Meantime, several matrices have been designed to deliver growth factors over longer time. We used sepharose heparin beads for PDGF-BB delivery and achieved excellent first order release kinetics over 10 days, resulting in improvement of flow and function in ischemic myocardium [30]. Design of more sophisticated polymers has already evolved into effective dual release characteristics for two simultaneously delivered growth factors [29]. This field will
likely gain momentum after the successes with biocompatible slow release polymers on coated stents. An alternative to sustained protein delivery is gene therapy with plasmid, replication deficient adenovirus, adeno-associated virus (AAV) or lentivirus (for review see Gruchala et al. [83]). The choice between sustained delivery of protein and gene therapy will eventually be based on pharmacokinetic, safety and cost considerations.

The site of delivery needs to be optimized for most formulations and growth factors (for review see Simons and Post [84]). Extensive work has been done on biodistribution of growth factor—mainly FGF-2—delivery, resulting in preferences for intramyocardial (or intramuscular for PAD) and pericardial delivery over intravenous or intracoronary administration [84]. Intramyocardial injections into ischemic areas provide significant local concentration of the growth factor [3] and this local advantage is even more pronounced with protein-encoding adenoviruses or plasmids [85]. Although it is reasonable to assume that delivery should be directed to the ischemic zone, border zone and watershed areas, comparative efficacy studies are sparse. For integral delivery, sustained intra-arterial delivery is probably superior as has been shown by numerous ischemic hindlimb studies [8]. This however may be technically difficult in the coronary system. An alternative mode of delivery is retroinfusion into the coronary veins. Single infusion of FGF-2 [86] and gene transfer [87] were successful with this technique, but final feasibility and efficacy await clinical testing.

3.7. Cell based therapy with or without growth factor transfection

Bone marrow derived stem cells have been found to play a role in the contribution to neovascularization in ischemic tissues [13,88]. The pluripotent hematopoietic stem cells can differentiate into endothelial cells that participate in the process of neovascularization [89]. Differentiation of stem cells to endothelial cells is regulated by several growth factors. Among these growth factors, VEGF selectively induces endothelial cell differentiation through activation of VEGFR-2. Thus, delivery of VEGF to an ischemic tissue could activate neovascularization through angiogenesis and vasculogenesis. Alternatively, injection of stem cells into the circulation or the ischemic region may accelerate the process of neovascularization [90]. It needs to be emphasized that the mechanism of these effects is largely unknown. Direct incorporation of these cells in newly formed vessels seems to be a relatively rare phenomenon, ranging from <1% to a maximum of 5%. An intriguing recent observation suggests that HGF may favorably affect grafting of transplanted myocytes into infarcted cardiac tissues [91], thus potentially opening the way for combined cell and angiogenic therapy. Finally, transplanted cells can be used as carriers for target genes that need to be delivered [92].

4. Side effects

We may distinguish growth factor specific side effects such as hyperpermeability by VEGF and side effects that are directly related to neovascularization, such as tumor development, aggravation of diabetic retinopathy and, possibly, atherosclerosis [6]. VEGF/VPF (vascular permeability factor) displays 50,000-fold greater vascular permeability activity than histamine [93]. This effect is probably mediated through a mechanism of induction of endothelial fenestrations, although other mechanisms have not been ruled out [82].

Numerous studies have shown that tumor growth and diabetic retinopathy can be dependent on angiogenesis and angiogenic growth factors (for review see Refs. [94,95]). Little evidence exists however, that exogenous growth factors actually stimulate tumor growth and aggravate retinopathy. Although overt tumors and diabetic retinopathy have been exclusion criteria in clinical trials, in over more than 1000 patients now treated with either angiogenic proteins or gene therapy, no complications with regard to these two diseases have been noted.

The pro-atherogenic effect of especially VEGF has been tested in preclinical models of atherosclerosis. VEGF enhanced plaque formation when exogenously administered [96] whereas flt-1 blockade reduced atherogenesis [97]. In accordance, general angiogenesis inhibitors such as TNP40 or angiostatin reduce experimental atherosclerosis [98]. However, in a recent clinical trial with VEGF gene therapy this pro-atherogenic effect could not be corroborated [99]. Likewise, in a large animal model of atherosclerosis, FGF2 did not have an effect on restenosis after coronary artery stenting [100]. With these controversial results, a definitive pro-atherogenic property of angiogenic growth factors can neither be excluded nor be qualified as clinically relevant. Therefore, in future clinical trials, progression of atherosclerosis needs to be on the forefront of attention.

5. Summary

From current preclinical and clinical experience some directions can be extracted for future preclinical and clinical studies. Exposure to growth factors needs to be long in order to initiate vessel growth but also to avoid early regression of nascent blood vessels. The choice between gene or protein therapy depends mainly on technical developments in vector design and production on one hand and the engineering of slow release matrix formulations for proteins on the other. Combinations of growth factors, for instance to stabilize vessels, deserve more attention but will make the design of
preclinical and clinical studies increasingly complex. Recent developments suggest that with the appropriate dose and treatment regimens, even single growth factor therapy can result in stable and functional vessels.

The concept of three different mechanisms of neo-vascularization has served to define potential therapeutic targets, such as vascular remodeling and stem cell recruitment, but it is anticipated that this concept will lose significance as the pleiotropic nature of the angiogenic cytokines becomes fully understood. In measuring outcome in clinical and especially basic studies, measures of angiogenesis, arteriogenesis and vasculogenesis need to be integrated in the assessment of efficacy.

With the vast and rapidly growing body of data that has been obtained on growth factors and pro-angiogenic strategies, approaches will emerge that are more effective than the ones that have been tested clinically up until now. It remains imperative however, that these approaches are rationally based on fundamental and preclinical data.

6. Summary box

Key issues of angiogenic therapy in the treatment of ischemic diseases

<table>
<thead>
<tr>
<th>Issues</th>
<th>Solutions</th>
</tr>
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<tbody>
<tr>
<td>Choice of angiogenic factors</td>
<td>Single vs. combinations angiogenic/arteriogenic</td>
</tr>
<tr>
<td>Functional arterial vessels</td>
<td>Vascular remodeling</td>
</tr>
<tr>
<td>Stability of blood vessels</td>
<td>Combinations of angiogenic and arteriogenic vessels</td>
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<td>Prevention of vascular leakage</td>
<td>Arteriogenic factors</td>
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<td>Delivery of angiogenic factors</td>
<td>Sustained release by slow-release polymers, protein therapy vs. gene therapy</td>
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<tr>
<td>Angiogenic factor exposure</td>
<td>Short term vs. long term</td>
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<tr>
<td>Prevention of undesirable effects</td>
<td>Dormant cancer growth, arteriosclerotic plaque growth, and hemangioma</td>
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</tbody>
</table>

References

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