Review

Therapeutic interventions for enhancing collateral development by administration of growth factors: basic principles, early results and potential hazards

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Abstract

The importance of spontaneously developing collateral vessels to supplement perfusion of tissue rendered ischemic by vascular obstruction was recognized many years ago. However, it was not until potent angiogenesis factors were identified, purified, and produced in sufficient quantities, that the field began its rapid development. In the early 1990s it was first shown that basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) proteins could actually stimulate collateral flow. However, additional studies also demonstrated that the duration of exposure of the vessels to angiogenesis factors was critical, and that the administration of proteins, with their relatively brief half-lives, may pose important practical limitations. The demonstration that gene therapy can improve collateral function presents one of the solutions to the conundrum, since gene therapy can be considered a sophisticated form of a sustained delivery system. The results of several clinical trials have been reported. All involve administration of single angiogenesis agents, and most are Phase I trials. The two studies rising to Phase II status demonstrated no treatment effect on the primary end-point. It may therefore be relevant to consider that the molecular mechanisms responsible for angiogenesis are extraordinarily complex, and an optimal angiogenesis intervention may require a ‘multiple factor’ strategy. It is important to note that no serious side-effects ascribable to an angiogenesis agent were recognized in these trials. However, angiogenesis agents are potent molecules with multiple activities. It is therefore possible that they might occasionally cause side-effects, some serious. Among these, based on their biologic activities, are neovascularization of non-targeted tissues, expansion and induction of instability of atherogenic plaque, and growth of tumors. In summary, there is ample experimental evidence justifying an optimistic outlook relating to our eventually being successful in enhancing collateral flow to ischemic tissue in a clinical setting. However, we are not there yet, and identification of the optimal angiogenesis strategy is still unclear. Additional experimental work, in parallel with large, carefully controlled clinical trials are needed to continue the exciting advances of the last decade, and to achieve the goal of providing patients with alternative potent therapies to improve collateral flow, and thereby to alleviate their symptoms and perhaps to prolong their lives. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Angiogenesis; Collateral circulation; Coronary circulation; Growth factors

1. Introduction

The functional importance of spontaneously developing collateral vessels in supplementing perfusion of the myocardium rendered ischemic by coronary obstruction was recognized many years ago, prompting attempts, both experimental and clinical, to enhance collateral development. These initial ‘preangiogenesis era’ interventions were surgical and generally aimed at trying to establish connections between extracardiac and coronary arteries. The strategies included obliterating the pericardial sac with mechanical abrasion and the addition of asbestos powder [1], tacking omentum to ischemic hearts [2,3], removing the epicardium [4], or combining several of these with the addition of implanting the internal mammary artery into the myocardium [5]. Subsequently, pha-
macologic strategies were employed in both experimental and clinical studies in an attempt to revascularize ischemic hearts [6–8].

However, the field was severely hampered by the lack of potent angiogenesis factors. This was resolved by the identification and purification of vascular endothelial growth factor (VEGF) in the late 1970s and in the 1980s [9–13], and basic fibroblast growth factor (bFGF, or FGF2) and acidic FGF (aFGF or FGF1) in the 1980s [14–18]. The practical application of these agents to experimental studies in large animal models of ischemia had to await the development of technologies that allowed the angiogenesis proteins to be produced in sufficient quantities, and subsequently the development of DNA technology and gene delivery techniques so that gene therapy studies became possible. Once these breakthroughs were achieved, studies exploring the potential of various angiogenesis strategies to develop clinically relevant therapeutic approaches to both myocardial and leg ischemia proliferated, so that over the past half-decade the field has moved forward with great momentum.

However, in parallel with substantive scientific contributions has been a cacophony of media and ‘scientific’ hype, often making it difficult to distinguish between what actually has been accomplished relating to a proven clinical strategy, and what we would like to believe has been accomplished. And often missing is thoughtful attention to the potentially serious complications that might occur when administering such biologically potent agents to patients. Following, therefore, is a brief overview focusing on these issues.

2. Experimental and clinical data

2.1. Proteins

Several early studies were published demonstrating that VEGF, aFGF (FGF1) and bFGF (FGF2) proteins produced in vitro changes compatible with their having angiogenesis potential [19–23]. These studies were followed by in vivo work showing these factors actually do stimulate the growth of new vessels [19,24,25]. The more recent elegant genetic studies of vasculogenesis occurring during embryogenesis in the mouse add to the now unequivocal evidence documenting the critical importance of many molecules, including major contributions of VEGF and angiopoietin-1, to the development of mature, branching blood vessels [26–28].

That bFGF and VEGF proteins could actually stimulate the development of collaterals to tissues supplied by an obstructed artery, and in the process augment tissue blood flow, was first demonstrated in the early 1990s. In the experiments on myocardial ischemia, a portion of the left ventricle of dogs was made ischemic by gradual occlusion of the circumflex coronary artery. The intracoronary or left atrial administration of bFGF, or intracoronary administration of VEGF proteins daily for 28 days, significantly increased collateral flow [29–31]. Likewise, studies in the rabbit ischemic hind limb model demonstrated that intramuscular administration of bFGF protein daily for 2 weeks significantly improved limb perfusion [32].

Although such studies demonstrated proof of concept, additional studies also raised issues that still have not been resolved. For example, in attempts to determine a clinically feasible strategy for delivery of protein to enhance collateral flow, different durations of intra-arterial protein administration were studied in the canine myocardial ischemia model. While 28 days of administering boluses of VEGF into the left atrium improved collateral flow, 7 days of administration did not [33]; and while 7, and as little as 2 days of administering bFGF intracoronary improved collateral flow, a single bolus injection did not [34]. These results demonstrated, at least in this model of myocardial ischemia, that the duration of exposure of the vessels supplying the ischemic tissue to angiogenesis factors was critical for a therapeutically relevant effect.

Additional studies employing 125I-labeled bFGF demonstrated that route of administration was another critical factor in determining local tissue uptake [35], and potentially, therapeutic response. Thus, whereas 3–5% of an i.c.-administered dose of bFGF was recovered in the myocardium, only 0.5% of an i.v.-administered dose was recovered. The most plausible explanation for these findings derives from the fact that myocardial uptake is dependent on peak serum concentration; because bFGF has a heparin binding domain, considerable first pass uptake in the lungs will occur following i.v. administration (the lungs contain large amounts of heparan sulphates) resulting in a blunted peak serum concentration presented to the myocardium when compared to the very high concentrations presented to the myocardium with bolus injection directly into the coronary artery.

The biologic consequences of these differences were demonstrated in angiogenesis studies of the same canine ischemia model. Collateral flow improved with i.c. administration of bFGF, but did not increase when the drug was given i.v., despite its being given for 1 week [34]. Although similar uptake studies have not been performed with VEGF, its 165 isoform (VEGF165) also has a heparin-binding domain (whereas VEGF121 does not), suggesting that similar results would obtain.

Animal studies that appear to be at variance with these results have also been reported. Thus, Lopez et al. [36] delivered VEGF165 to a porcine model of myocardial ischemia (ameroid occlusion of the circumflex coronary artery) by three different local intracoronary delivery systems (via an InfusaSleeve catheter, via intracoronary bolus infusion, and by epicardial implantation of an osmotic delivery system). VEGF was administered 3 weeks after ameroid placement, and indices of collateral function were assessed at that time (baseline) and 3 weeks later.
Whereas there was no significant improvement in circumflex territory perfusion in control pigs, improved circumflex perfusion was demonstrable within each VEGF-treated group, using paired t-tests to compare pre- and post-treatment perfusion values. Though these data are suggestive of a VEGF treatment effect, they are not convincing. First, ongoing collateral development has been observed in pigs throughout the 6-week period following circumflex ameroid placement [37]. In the Lopez study, however, the control group did not exhibit the expected increase in circumflex territory perfusion during that interval. Second, direct comparisons between individual VEGF treatment groups and the control group were not statistically significant. Only when all three VEGF treatment groups were combined in a post-hoc analysis was a statistically significant difference demonstrable between VEGF groups and the control group. Third, there were three deaths in VEGF-treated animals during the investigation. Elimination of three animals in a small study such as this could importantly affect the results through selection bias. Thus, while suggestive, the data from this experiment do not unequivocally demonstrate that a single bolus i.c. injection of VEGF is capable of increasing collateral flow to a greater extent than that which occurs in the absence of therapy.

Hariawala et al. [38] also reported improved flow in a similar model. However, this study is flawed by the fact that intracoronary bolus administration of VEGF (2 mg) caused severe hypotension that led to the acute death of four of eight animals in the treated group; hence, the surviving animals, which were found to have greater collateral flow than the untreated controls, may have survived only because they had greater intrinsic collateral flow. These investigators also demonstrated in the rabbit hind limb model of ischemia that a single dose of intrafemoral administration of bFGF or VEGF<sub>165</sub> improves collateral flow and, surprisingly, that a single i.v. dose of VEGF<sub>165</sub> also improves flow [39,40]. There are thus conflicting results reported in the literature relating to whether a single intra-arterial bolus injection of VEGF or bFGF proteins improves collateral flow, and whether improvement occurs following iv administration, at least for heparin-binding agents.

2.2. Genes

Gene therapy presents one of the solutions to the possible dosing conundrum, since gene therapy can be considered a sophisticated form of a sustained delivery system. Once transfected, the target cell expresses gene product for days, weeks, or longer, depending on the specific tissue transfected and on the specific vector used.

Proof of concept that gene therapy can improve collateral function was demonstrated by Giordano et al. [41]. They found in a porcine model of myocardial ischemia (ameroid occlusion of the circumflex coronary artery) that a single dose i.e. administration of an adenoviral vector carrying the FGF5 transgene into the non-occluded right coronary increased myocardial flow and function. Surprisingly, they found that about 95% first pass myocardial uptake was achieved with i.e. administration. Hammond et al. have since demonstrated that FGF4 produces similar effects in restoring myocardial flow and function. Other investigators have also performed studies employing the rabbit hind limb model of ischemia and have reported that injection into the femoral artery of the VEGF<sub>165</sub> transgene carried in a plasmid vector improves collateral flow [42].

2.3. Direct intramyocardial injection

No matter how efficient first pass uptake is, a considerable proportion of an angiogenesis factor injected into an artery supplying the target tissue will enter the systemic circulation and thereby expose non-target tissues to its biologic effects [43]. While there is no definitive evidence yet that such systemic spillover will produce serious side effects, there is always that possibility (see below). It would therefore appear that if direct intramuscular injection of the angiogenesis factor, either by the transepicardial or transendocardial route, does result in enhanced collateral flow, such an approach might be preferable.

A protein, injected once intramuscularly, would be unlikely to persist in the tissue long enough to exert an important biologic effect [43]. Although multiple injections of protein might well improve collateral flow [32], such a strategy has practical limitations. Therefore, once it was demonstrated that an adenoviral vector carrying a reporter transgene efficiently expresses its gene product after intramyocardial injection [44], this approach to gene delivery was explored as an approach for gene therapy.

Proof of concept that intramyocardial injection could enhance collateral flow and improve impaired myocardial function was demonstrated in a porcine model of myocardial ischemia. This was achieved by the transepicardial injection of an adenoviral vector carrying the VEGF<sub>165</sub> transgene performed following thoracotomy [45]. The feasibility of catheter-based transendocardial delivery of angiogenesis genes has recently been shown [46,47], demonstrating that the direct injection of angiogenesis factors into the myocardium can be accomplished without the need of thoracotomy.

2.4. Clinical trials

At the time of this writing the results of several clinical trials have been reported. These are summarized in Table 1 [48–58], which shows the results of the coronary artery disease (CAD) studies, and Table 2 [59–62], which depicts the results of peripheral vascular disease studies. Several are just anecdotal reports, and although efficacy claims were made or implied, such studies provide no information regarding either safety or efficacy. Most of the other
Table 1
Coronary artery disease*

<table>
<thead>
<tr>
<th>Author</th>
<th>Reference</th>
<th>n</th>
<th>Angiogenic factor</th>
<th>Route of administration</th>
<th>Randomized</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schumacher et al.</td>
<td>[48]</td>
<td>20</td>
<td>aFGF protein</td>
<td>I.m.; thoracotomy</td>
<td>Yes</td>
<td>Improved</td>
</tr>
<tr>
<td>Seilke et al.</td>
<td>[49]</td>
<td>8</td>
<td>bFBF-protein</td>
<td>Hep-al pellets; thoracotomy</td>
<td>No</td>
<td>Improved</td>
</tr>
<tr>
<td>Laham et al.</td>
<td>[50]</td>
<td>24</td>
<td>bFBF-protein</td>
<td>Hap-al pellets; thoracotomy</td>
<td>Yes</td>
<td>Improved</td>
</tr>
<tr>
<td>Unger et al.</td>
<td>[51]</td>
<td>25</td>
<td>bFGF protein</td>
<td>Intracoronary</td>
<td>Yes</td>
<td>Safe</td>
</tr>
<tr>
<td>Laham et al.</td>
<td>[52]</td>
<td>52</td>
<td>bFGF protein</td>
<td>Intracoronary</td>
<td>No</td>
<td>Improved</td>
</tr>
<tr>
<td>Simmons et al.</td>
<td>[53]</td>
<td>337</td>
<td>bFGF protein</td>
<td>Intracoronary</td>
<td>Yes</td>
<td>No effect</td>
</tr>
<tr>
<td>Rosengart et al.</td>
<td>[54]</td>
<td>21</td>
<td>VEGF&lt;sub&gt;121&lt;/sub&gt; gene-Adeno</td>
<td>I.m.; thoracotomy</td>
<td>No</td>
<td>Improved</td>
</tr>
<tr>
<td>Losordo et al.</td>
<td>[55]</td>
<td>5</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt; gene-plasmid</td>
<td>I.m.; thoracotomy</td>
<td>No</td>
<td>Improved</td>
</tr>
<tr>
<td>Gibson et al.</td>
<td>[56]</td>
<td>28</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt; protein</td>
<td>Intravenous</td>
<td>No</td>
<td>Improved</td>
</tr>
<tr>
<td>Henry et al.</td>
<td>[57]</td>
<td>15</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt; protein</td>
<td>Intracoronary</td>
<td>No</td>
<td>Improved</td>
</tr>
<tr>
<td>Henry et al.</td>
<td>[58]</td>
<td>178</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt; protein</td>
<td>I.c. plus i.v.</td>
<td>Yes</td>
<td>No effect</td>
</tr>
</tbody>
</table>

a Abbreviations: epi, epicardial; im, intramuscular; hap-al, heparin alginate; ic, intracoronary; iv, intravenous; adeno, adenovirus.

Table 2
Peripheral vascular disease

<table>
<thead>
<tr>
<th>Author</th>
<th>Reference</th>
<th>No. of patients</th>
<th>Angiogenic factor</th>
<th>Route of administration</th>
<th>Randomized</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isner et al.</td>
<td>[59]</td>
<td>1</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt; gene-plasmid</td>
<td>Via hydrogel balloon</td>
<td>No</td>
<td>Improved</td>
</tr>
<tr>
<td>Baumgartner et al.</td>
<td>[60]</td>
<td>9</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt; gene-plasmid</td>
<td>Intramuscular</td>
<td>No</td>
<td>Improved</td>
</tr>
<tr>
<td>Isner et al.</td>
<td>[61]</td>
<td>6</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt; gene-plasmid</td>
<td>Intramuscular</td>
<td>No</td>
<td>Improved</td>
</tr>
<tr>
<td>Lazarous et al.</td>
<td>[62]</td>
<td>19</td>
<td>bFGF protein</td>
<td>Intra-arterial</td>
<td>Yes</td>
<td>Safe</td>
</tr>
</tbody>
</table>

studies are Phase I trials — and although some of the authors suggest that treatment benefit was evident, the design of the studies permits no such conclusions to be drawn. The studies accomplished what Phase I studies are designed to accomplish — they demonstrated safety and feasibility. The design of only two of the studies rose to a Phase II status, as they had most of the elements that would permit at least tentative efficacy conclusions to be drawn — the studies of Henry et al. [58], and that of Simons et al. [53]. These were randomized, double-blinded, and reasonably powered to demonstrate efficacy, at least if a large treatment effect occurred. Interestingly, each of these studies demonstrated no treatment effect on the primary end-point (treadmill exercise performance). Importantly, these studies showed that exercise performance improved, but it improved in the untreated patients as much as it did in the treated patients.

It should be pointed out that these negative Phase II study results followed the prior presentation at national meetings of each of these studies in their non-randomized Phase I iterations; each Phase I study was reported as showing very encouraging positive results [52,56,57]. Indeed, to show how misleading non-randomized studies can be, the exercise–dobutamine/dipyridamole myocardial perfusion (SPECT) studies of a subset of the VEGF treated Phase I study patients (14 patients) were separately analyzed [63]. Studies were conducted before as well as 30 and 60 days after VEGF administration. Multiple endpoints were analyzed with only two of the analyses showing a statistically significant ($P<0.05$) improvement. The authors concluded that the Phase I data support the concept that VEGF improves myocardial perfusion at rest.

Why were the results of these latter two trials negative — one [53] testing the effects of single bolus i.c. injection of bFGF protein and the other [58] testing the effects of single bolus i.e. injection of VEGF<sub>165</sub> protein (17 or 50 ng/kg/min) followed by 4 h i.v. infusions of VEGF<sub>165</sub> (17 or 50 ng/kg/min) delivered during each of 3 days (days 3, 6 and 9)? Although, as discussed above, there are conflicting results reported in the literature as to the efficacy of these agents in regard to their mode of delivery, at least some animal studies predicted that single dose administration of VEGF or bFGF proteins would not improve collateral flow in patients, and that i.v. administration of VEGF<sub>165</sub> or bFGF would also be ineffective. Alternatively it is possible that the negative results were contributed to by too small a sample size, by insensitive tests measuring end-point change, or by other as yet unrecognized factors. Finally, the possibility always must be considered that given the inherent complexity of atherosclerotic disease in humans, enhancing collateral function in patients is a more difficult feat to accomplish than it is in experimental animals, necessitating the development of more sophisticated strategies.
3. Concept of multifactor administration

The molecular mechanisms responsible for angiogenesis are extraordinarily complex: multiple genes must coordinately express their products in appropriate amounts and in an appropriate time-dependent manner. For example, recent studies in knockout mice have demonstrated that VEGF and Ang-1 have unique and specific interactions in the induction and maintenance of new blood vessel formation during embryogenesis [64–69]. Studies in mice carrying homozygous disruption in the receptors of these ligands have demonstrated that VEGF, after binding to its receptors, induces endothelial cell (EC) proliferation, cell-cell interaction, and tube formation. The resulting vessels are immature, thin-walled, sinusoidal structures that leak, lack branching and complexity, and are devoid of a supporting network of periadventitial cells. The next sequential step in vessel development derives from the expression of Ang-1. After binding to its receptor Tie-2, Ang-1 induces vessel budding and branching, and recruits periendothelial support cells, including SMCs, an action that helps maintain the integrity and stabilization of the newly formed blood vessels. Thus, VEGF and Ang-1 are expressed sequentially during embryogenesis, and each of these ligands controls specific, complementary functions that ultimately contribute to the development of mature, functional blood vessels.

These observations raise an important question: is the administration of a single angiogenesis agent capable of inducing the development of mature collaterals in adults? The answer to this question is not entirely clear. Thus, when an adenoviral vector expressing murine VEGF was injected into different normal tissues of adult nude mice [70], the response was characterized by similar VEGF-induced effects found in the Ang-1 knockout mice studies [64–69] — angiogenesis occurred leading to the formation of enlarged, thin-walled vessels that lacked supporting pericytes and that were hyperpermeable. These immature vessels evolved over time, and the type of vessels formed appeared to be tissue specific. In some of the tissues these vessels evolved into clusters of mature-appearing muscular arteries and veins — however, in other tissues they formed disorganized vessel tangles (glomeruloid bodies), similar to structures found in vascular malformations, malignant tumors, and in benign vascular tumors. While it was not ascertained what the morphological characteristics of vessels would be if VEGF were injected into ischemic tissues, these results raise important questions as to whether VEGF, administered as a single agent, can lead to the development of mature functional collateral vessels.

The identification of many genes that are involved in angiogenesis makes it not unreasonable to conjecture that many other as yet unknown angiogenesis signaling cascades will be found, and that many of these will not merely represent redundant pathways, but will be essential to collateral development. If it is ultimately found that current strategies of enhancing collateral development, which involve administration of single angiogenesis proteins or genes, do not cause an optimal angiogenesis response; (1) will the administration of multiple factors be required to optimize an angiogenesis effect and (2) if so, what ‘multiple factor’ strategies can be employed and in what sequence should they be administered?

3.1. Hypoxia-responsive transcription factor-induced increase in multiple angiogenesis factors

There are several examples of possible strategies that are currently being tested experimentally. One interesting approach is to administer a gene encoding a factor that is not in itself angiogenic, but stimulates the expression of multiple genes that are. Thus, hypoxia inducible factors (HIF-1β, HIF-1α, and HIF-2α) are transcription factors that, as their name implies, are activated by low oxygen tension [71–75]. Once activated they bind to the promoters of multiple genes and thereby stimulate gene expression. One of the functions of the genes they stimulate, including VEGF, VEGFR1, VEGFR2, Ang-2, Tie-1, and nitric oxide synthase, can be thought of as being involved in the homeostatic responses to hypoxia.

Administration of genes encoding one of the HIF transcription factors (or encoding molecules that interfere with the degradation of such factors [76], by activating multiple genes involved in angiogenesis, might therefore be an excellent strategy to test the concept that it takes the administration of more than a single agent to cause an optimal angiogenesis response. Employing a multifactor approach that is based on a normal cellular response mechanism, such as overexpressing one of the HIF family of transcription factors in ischemic cells, offers a reasonable chance that necessary and sufficient angiogenesis factors will be expressed, and that such expression will occur in a sequence and concentration-appropriate manner.

3.2. Delivery of cells (or factors attracting cells) that express multiple angiogenesis factors

Another multifactor approach is to attract or deliver to ischemic tissue cells that nature has imbued with the capacity to express multiple angiogenesis factors in appropriate sequence and concentration. One type of cellular approach is being tested by investigators from the Max Planck Institute, who have demonstrated the importance of the monocyte in promoting arteriogenesis [77–79]. The monocyte, upon phenotypic differentiation to a tissue macrophage, expresses such potentially angiogenesis factors as VEGF, nitric oxide, MCP-1, and various cytokines. This group has shown in the rabbit hindlimb model of ischemia that the administration of agents that increase the targeting of monocytes to ischemic tissue (administration of MCP-1) improves collateral flow.
Our laboratory has used another multifactor cellular approach. We hypothesized that bone marrow cells have angiogenesis properties that will enhance collateral flow in ischemic tissue; in testing this concept we found that in culture these cells do in fact secrete angiogenesis factors, including VEGF, MCP-1 and bFCF, and that when the conditioned medium derived from these cells is applied to endothelial cells, the endothelial cells proliferate, migrate, and form tubes. Most importantly, we found that when autologous bone marrow is injected transendocardially into ischemic porcine myocardium, collateral flow and myocardial function improve significantly [80].

4. Target site for delivery of angiogenesis factors

Regional myocardial ischemia leads to changes in the ischemic tissue that in some manner induces collateral growth. Implicit in most angiogenesis studies has been the assumption that it is the ischemic tissue that should be targeted for delivery of therapeutic agents. In this regard, recent work [77–79] has called attention to the difference between angiogenesis (the sprouting of endothelial cells from existing vessels to form a new capillary network) and arteriogenesis (the remodeling and expansion of existing vessels). Based on these considerations a compelling case can be made that it is the process of arteriogenesis, rather than the development of capillaries in the ischemic region, that is critical to improved collateral function. If this is indeed the case, then an alternative paradigm for therapeutic targeting becomes possible, as some of the vessels involved in arteriogenesis may lie outside the ischemic zone.

This concept is illustrated in Fig. 1, which shows how ischemia occurring in a relatively distant site could lead to the enlargement of potential collaterals that do not carry flow and that do not actually reside within the ischemic tissue. (Such distant collaterals are seen for example, in patients with coarctation of the aorta, in which ischemia occurs in the legs but collaterals develop in the thorax.) Among the multiple genes activated by shear stress are cytokines, MCP-1, adhesion molecules, and NOS. The Max Planck Institute investigators postulate that MCP-1 attracts monocytes to this focus of increased shear stress, the monocytes adhere to the adhesion molecules and then enter the subendothelial space, where they differentiate into macrophages. The macrophages express multiple products, including MCP-1, cytokines, and the angiogenesis factors VEGF and FGF. In this paradigm the monocyte, which is targeted to the small collaterals being subjected to increased shear stress, because of stress-induced monocyte-attracting factors, becomes the critical cell mediating arteriogenesis, and thus collateral development (Fig. 2).

Given these concepts, the question arises as to whether optimal efficacy for enhancing collateral function requires that the angiogenesis agent be delivered into the site of ischemia, or whether it must be delivered to the zone in which the potential collaterals reside, which could be distant from the site of ischemia. We believe that arteriogenesis probably does play the key role in collateral enhancement, and therefore that counting capillaries as a means of proving the success of an angiogenesis intervention is meaningless. However, we also believe that potential collaterals probably reside throughout the myocardium, and that angiogenesis factors, insofar as they are capable of improving collateral flow, will probably improve collateral flow whether delivered to the ischemic region, the surrounding contiguous non-ischemic region, or...
both. The enhancement of collateral flow by interventions in which angiogenesis factors are delivered locally into the ischemic region suggests the validity of this belief [49]. However, definitive assessment of this important conundrum will be necessary before reliable strategies for therapeutic angiogenesis can be definitively developed. In addition, we do not as yet know the functional overlap and the functional differences between the cytokines that induce angiogenesis vs. those that promote arteriogenesis. This is another issue that will have to be addressed before we will be able to reliably identify the optimal intervention(s) that will enhance collateral flow.

5. Yellow flags — the potential for deleterious effects

For most potent therapeutic interventions, therapeutic efficacy is rarely free of the potential for harmful effects to occur. The biological activities of most of the angiogenesis agents currently being tested clinically are very potent, and it is likely that the same activities that lead to a therapeutic effect could also cause unwanted side effects. It is therefore probable that some side effects consequent to the cellular effects of these agents will inevitably occur. If this is true, then the critical question we will have to address in large clinical trials is whether the incidence of these risks is sufficiently low so that the risks will be outweighed by the therapeutic benefits.

Among the side effects that might occur as a result of the biologic effects of these agents is the development of new blood vessels in non-targeted tissues, a complication that would be particularly devastating if it were to occur, for example, in the retina. It is possible that this particular complication may not develop unless a tissue is ‘primed’ to respond with an angiogenesis response. That is, quiescent cells have low constitutive expression of receptors for the VEGF and FGF family of agents — thus, unless the tissue is exposed to very high doses of the ligands for prolonged periods, it is possible that normal tissue is resistant to the neovascularization effects of angiogenesis factors, a result suggested by the study by Banai et al. [81]. In this regard, a patient with diabetic retinopathy does have vascular cells that are ‘primed,’ insofar as it has been demonstrated that there are increased levels of one of the receptors for VEGF [82–84].

Other VEGF-specific complications could develop as a result of the potent activity of VEGF as an inducer of vascular permeability [10–14,85,86]. Although angiogenesis and vascular permeability might be considered two separate biological activities, it is also possible that the vascular permeability properties of VEGF are essential for angiogenesis to occur.

Whatever the interrelation between these two actions, if vascular permeability increases in tissues other than the tissue targeted for angiogenesis, serious consequences could accrue. That this could occur was demonstrated in a recent study in which the effects of overexpression of VEGF (achieved by injecting an adenovirus carrying the VEGF transgene) in adult mice was investigated [87]. The mice, as expected, developed elevated circulating levels of VEGF following injection of the adenoviral vector. However, a high percentage died within days, developing increased vascular permeability and severe multiple organ edema.

Other potential complications based on biological activities are the expansion and induction of instability of atherogenic plaque, and the growth of tumors. For example, Flugelman et al. demonstrated an association between unstable angina and the intra-plaque presence of aFGF and bFGF [88]. They suggested that these agents might play a role in plaque instability. In addition, the broad range of cells on which the FGF family of agents exert mitogenic effects could result in the growth of cells resident within plaques or of malignant cells.

Although the direct mitogenic effects of VEGF are largely limited to endothelial cells, it is of note that VEGF and its receptors, VEGFR1 and VEGFR2 (flt-1 and Flk-1), are overexpressed in atherosclerotic lesions [89]. Moreover, a number of non-endothelial tumor cells have been found to possess low levels of functional VEGFR-1 and VEGFR-2 [90]. Also of possible relevance is the fact that the uterus possesses functional VEGF receptor tyrosine kinases [91], and that VEGF is mitogenic for uterine smooth muscle. These observations raise the possibility that the atherosclerotic lesion, certain tumors, and the common leiomyoma (fibroid), could at least theoretically respond to direct exogenous stimulation by VEGF.

There is also increasing evidence suggesting that growth of microvessels into plaque or tumors, through angiogenesis processes, is critical to growth of both tumor and plaque [92–97]. Thus, microvascular angiogenesis per se, an activity inherent in most angiogenesis factors, could predispose to plaque or tumor growth. In addition, the potent vascular permeability effect of VEGF could result in exposing a plaque or tumor to many cytokines and growth factors that normally are confined to the plasma, and through this indirect mechanism stimulate their growth.

It must be emphasized that there have been no conclusive reports in clinical studies demonstrating that angiogenesis agents actually induce new tumor development, increase growth of in situ tumors, or increase plaque size. However, several experimental studies have demonstrated that prolonged exposure of skeletal muscle or myocardium to high local levels of VEGF or the FGF family of peptides can cause hemangioma-like tumors and vascular malformations [81,89,98–105], and can increase neointimal development [106–109].

Experimental studies have also demonstrated that high doses of bFGF can lead to the development of thrombocytopenia and renal toxicity [51]. In addition, the immune surveillance system is not normally exposed to
large amounts of these proteins. It is therefore possible that antibodies can develop to these cytokines, and that these could either impair the efficacy of repeated administration of the agents, or even possibly lead to immunopathogenic processes. It should also be noted that one of the clinical trials in progress employs FGF2 of porcine origin [52,53]; although the high homology between the FGFs in different species makes it unlikely that recognition of non-self protein will occur, this certainly is not beyond the realm of possibility.

FGF and VEGF proteins, administered acutely, can produce hypotension through, at least in part, a nitric oxide-mediated pathway [110–112] and, in the case of FGF2, through a potassium channel-mediated mechanism [113]. The hypotensive effect has resulted in the death of pigs that had chronic myocardial ischemia and that were treated with the intracoronary injection of VEGF165 protein [38], and in a prolonged hypotensive episode of a patient entered into a Phase I study testing the safety of intracoronary administration of bFGF [51]. This complication appears to occur only when high systemic levels of bFGF and VEGF develop rapidly. Thus, it would appear to be of no or little concern if bFGF and VEGF proteins are not administered rapidly and of no concern when the factors are given as genes — which express the proteins they encode slowly.

Lastly, we also need to consider, in the case of gene therapy employing viral vectors, the potential for the vectors themselves to cause deleterious effects. The administration of large amounts of virus can lead to massive immune responses that could cause serious, even fatal, immunopathology. Such responses are unlikely given the amount of adenovirus administered in current clinical cardiovascular protocols. However, the foreign proteins presented by the virus, even when administered in relatively small amounts, will probably induce immune responses that conceivable could decrease subsequent sensitivity to the beneficial effects of the transgene delivered by the virus if repeatedly administered, or could possibly lead to immune-mediated tissue damage.

6. Conclusions

Much has been learned about angiogenesis and the effects of angiogenesis agents over the past 2 decades, but much still needs to be learned. Given the large number of positive animal studies reporting success in enhancing collateral flow, we believe that eventually angiogenesis interventions will also be successful in patients. However, atherosclerosis is a complex disease, and it is not clear whether the strategies that appear to work in experimental animals will also work in patients. There are many questions still to be answered, among which are what angiogenesis factor(s) will turn out to produce an optimal effect, will the protein or gene be a superior means of delivering the factor, does optimization of response require a multiple-factor strategy, what is the optimal delivery strategy, are there as yet unforeseen obstacles to overcome, and will there be serious side effects?

As far as the latter question is concerned, as noted, the angiogenesis agents being tested have potent biological activities and thus certainly have the potential for such effects. So too, in the case of gene therapy utilizing viral vectors, do the vectors themselves. It is thus highly unlikely that trials will be totally free of serious complications. The terms of the argument will probably best be framed by risk vs. benefit considerations: therefore, carefully controlled large clinical trials must be completed to ascertain whether the incidence of serious complications is sufficiently low such that the attendant risks of therapy are outweighed by the benefits attained. In the end, as scientists we must be vigilant that we are not seduced by hype, and that we judge the merits of this potential important and novel therapeutic approach to obstructive arterial disease in a dispassionate and objective manner.

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