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Growth Factors as a Potential New Treatment for Ischemic Heart Disease

CHRISTOPHE BAUTERS, M.D.

Service de Cardiologie B et Hémodynamique, Hôpital Cardiologique, Université de Lille, Lille, France

Summary: Growth factors such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) exert important effects on endothelial cells in vitro and in vivo. This article reviews the effect of these two growth factors on endothelial dysfunction in various animal models of vascular disease: (1) collateral circulation supplying an ischemic territory, (2) balloon injury, and (3) diet-induced experimental atherosclerosis. Endothelial dysfunction may limit the beneficial effects of collateral vessels on tissue perfusion. Administration of VEGF or basic FGF (bFGF) augments collateral development in different models of hindlimb ischemia by enhancing neovascularity and by facilitating the recovery of endothelial function in the collateral circulation. Similarly, studies performed after balloon angioplasty have demonstrated abnormal responses of previously dilated sites to endothelium-dependent agonists. Administration of VEGF or bFGF increases endothelial regrowth and normalizes endothelium-dependent responses after experimental angioplasty. Finally, endothelium-dependent relaxation is impaired in diet-induced experimental atherosclerosis. It was recently demonstrated that hypercholesterolemic rabbits treated with bFGF had significantly better endothelium-dependent responses than those not treated with bFGF. These results show that in vivo administration of the endothelial cell growth factors VEGF and bFGF leads to significant improvement in endothelium-dependent responses and supports the concept of using these growth factors as a new therapeutic strategy for patients with vascular diseases.

Key words: animal models, atherosclerosis, basic fibroblast growth factor, collateral circulation, endothelial dysfunction, vascular endothelial growth factor

Address for reprints:

Christophe Bauters, M.D.
Service de Cardiologie B
Hôpital Cardiologique
Bvd. du Professeur J. Leclercq
59037 Lille Cedex, France

Introduction

Endothelial dysfunction has been implicated in the pathogenesis of many diseases affecting the cardiovascular system. Experimental and clinical studies have shown that endothelial dysfunction may play a key role in diverse conditions such as abnormal arterial vasomotion, thrombosis, and neointimal proliferation.[1,2] Endothelial dysfunction is a characteristic feature of atherosclerotic vessels,[3,4] arteries subjected to mechanical injury,[5,6] and collateral vessels that develop in response to severe ischemia.[7,8]

Endothelial cell growth factors such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) are important growth factors for endothelial cells in vitro. Whereas VEGF is specific for endothelial cells, FGFs also potently stimulate growth of other cell types, such as smooth muscle cells.

Recent studies have demonstrated the feasibility of using endothelial cell growth factors in vivo. Basic FGF (bFGF) and VEGF increase the development of collateral vessels in ischemic models[9–11] and enhance the extent of endothelial regrowth after arterial injury (Table I).[12,13] The marked anatomic improvement associated with administration of endothelial cell growth factors has led to speculation regarding the possible effects of these factors on endothelial dysfunction.

This article briefly addresses the effects of FGF and VEGF on endothelial dysfunction in three animal models of vascular disease: (1) the collateral circulation supplying an ischemic territory, (2) regenerated endothelium after arterial injury, and (3) the atherosclerotic rabbit model. In these experimental models, in vivo administration of these endothelium-dependent growth factors leads to significant improvement in endothelial dysfunction (Table II). The mechanisms underlying this beneficial effect remain speculative but may include growth factor-induced production of nitric oxide.

Endothelial Dysfunction in the Collateral Circulation

When a major artery becomes obstructed, blood flow to the ischemic tissue often depends on collateral vessels. When spontaneous development of collateral vessels is insufficient for allowing normal perfusion of the tissue at risk, residual ischemia occurs. A growing body of evidence indicates that ab-

TABLE I Use of endothelial cell growth factors in cardiovascular disease

1. Increase the development of collateral circulation
2. Increase reendothelialization after arterial injury

normal vascular reactivity may limit the beneficial effects of collateral vessels on tissue perfusion. Previous studies have demonstrated that this abnormal reactivity occurs, at least in part, as the consequence of dysfunctional endothelium.[7, 8, 14]

The basis for the impaired endothelial regulation of collateral vessels is not known. Studies of coronary collaterals have suggested two possible explanations.[15] The first involves the possibility that the collateral circulation fails to develop at a sufficient rate to prevent ischemic damage to endothelial cells of the recipient, downstream, reconstituted microvasculature. The second suggests that receptor-mediated production or release of endothelium-derived relaxing factor (EDRF)/nitric oxide (NO) may be regulated by perfusion pressure within the recipient vasculature; compromised perfusion pressure may further compromise abnormal endothelium-dependent flow.

Administration of VEGF or bFGF augments collateral development in various models of hindlimb or myocardial ischemia.[9–11] Because persistent impairment in endothelium-dependent relaxation would constitute an important limitation of this promising therapeutic approach, we investigated the effects of VEGF therapy on endothelium-dependent blood flow in a rabbit model of hindlimb ischemia.[16] Ischemia was induced by ligation of the external iliac artery and excision of the femoral artery in one limb of New Zealand White rabbits (Day 0). Flow velocity was measured using a Doppler guidewire at rest and following injection of serotonin and of acetylcholine. In control animals studied at Days 10 and 40, serotonin induced a decrease in hindlimb blood flow (67 ± 6% from baseline and 29 ± 2% from baseline, respectively); by contrast, in animals treated with a bolus dose of VEGF into the internal iliac artery at Day 10 and studied at Day 40, serotonin induced an increase in flow (119 ± 8% from baseline; $p < 0.05$ vs. controls). Acetylcholine induced a moderate increase in flow in control animals (152 ± 15% at Day 10, 177 ± 14% at Day 40) but a major increase in flow in animals treated with VEGF (254 ± 25%; $p < 0.05$ vs. controls) (Fig. 1).[16] These data suggest that VEGF not only augments neovascularity in this animal model but also facilitates the recovery of endothelial function of the collateral circulation.

At least two mechanisms could explain an improvement in endothelial function of the collateral-dependent limb after VEGF therapy. The first possibility relates to the characteristics of flow and perfusion pressure in arterioles distal to collaterals. The improved perfusion pressure associated with VEGF therapy may have reversed endothelial dysfunction. A second and intriguing possibility relates to a direct improvement of endothelial function by VEGF. Beyond its mitogenic effects, VEGF may also modulate qualitative aspects of endothelial cell function.[17] It is tempting to speculate that VEGF may affect the phenotype of endothelial cells in the collateral-dependent limb and thereby restore normal endothelial function. Similarly, in the case of bFGF, recent in vitro studies have demonstrated that long-term administration of this endothelial cell mitogen preserves endothelial function in the coronary microcirculation perfused via collateral vessels.[18]

Endothelial Dysfunction after Arterial Injury

Percutaneous transluminal coronary angioplasty is a technique widely used in patients with atherosclerotic coronary artery disease. Restenosis occurring within the first 6 months remains the major problem limiting the long-term efficacy of the procedure. Two important mechanisms have been implicated in the pathogenesis of restenosis: neointimal hyperplasia and vessel remodeling. Neointimal hyperplasia, which results primarily from a growth response of the smooth muscle cells, is maximal at 1 to 4 weeks after the initial injury. Neointimal formation involves different steps: activation, proliferation, and migration of smooth muscle cells, and the production of extracellular matrix.[19] Arterial remodeling (i.e., changes in vessel size) also plays a major role in restenosis. The changes in vessel size may be bidirectional: some lesions show an increase (enlargement) whereas others show a decrease (constriction) in vessel size.[20]

TABLE II Summary of the effects of endothelial cell growth factors in experimental models of vascular diseases

Model	Endothelial regrowth	Endothelial function	Neointima	References
VEGF				
Arterial injury	↑	↑	↓	Asahara *et al.* (13, 28)
Atherosclerosis		↑	↓	Asahara *et al.* (34)
Collaterals		↑		Bauters *et al.* (16)
bFGF				
Arterial injury	↑	↑	↔	Meurice *et al.* (24)
Atherosclerosis		↑	↔	Meurice *et al.* (33)
Collaterals		↑		Sellke *et al.* (18)

Abbreviations: bFGF = basic fibroblast growth factor, VEGF = vascular endothelial growth factor, ↑ = increase, ↓ = decrease, ↔ = no change.

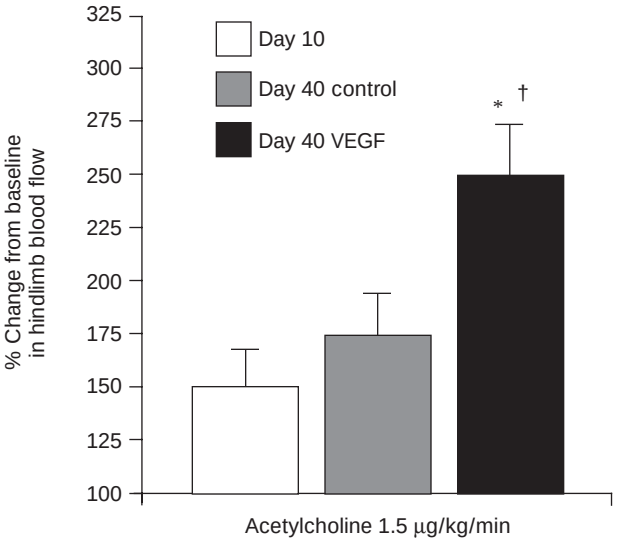

FIG. 1 Change in hindlimb blood flow in response to acetylcholine in rabbits 10 and 40 days after surgery. Note the significant difference between percent changes in hindlimb blood flow in VEGF-treated rabbits compared with control rabbits. * $p < 0.01$ versus Day 10; † $p < 0.05$ versus Day 40 control. VEGF = vascular endothelial growth factor. Reprinted from Ref. No. 16 with permission.

In the hours following experimental angioplasty, endothelial cells rapidly enter the replication cycle to restore endothelial continuity. Endothelial regeneration starts from the leading edge of the denuded area and from the ostia of collateral and/or branch arteries.[21] Even if complete reendothelialization occurs, the functional properties of the regenerated endothelium are abnormal. After vascular injury, endothelium-dependent relaxation to vasodilator agonists is depressed in arteries with regenerated endothelium while the ability of the underlying smooth muscle cells to relax or contract does not change.[5, 6] Similarly, studies performed after coronary angioplasty in men have demonstrated abnormal responses of previously dilated sites to endothelium-dependent agonists such as serotonin and acetylcholine.[22, 23]

Fibroblast growth factor and VEGF have both been used in vivo in attempts to increase endothelial regrowth after experimental arterial injury.

Fibroblast Growth Factor

Lindner *et al.*[12] first demonstrated that the in vivo administration of bFGF was associated with a significant increase in endothelial cell coverage on denuded arteries. This study provided clear evidence for the mitogenic effect of bFGF on endothelial cell replication in vivo, and further demonstrated that total endothelial cell regrowth could be achieved within 10 weeks in a rat carotid model of balloon denudation by systemic administration of bFGF. It is interesting that recombinant bFGF may achieve significant reendothelialization of denuded arteries when given at much lower doses than those used by Lindner *et al*. In a rabbit model of balloon denudation of the iliac artery, our group (Meurice *et al.*[24]) observed a significant ($p < 0.005$) increase in endothelial regrowth after administration of 2.5 µg of bFGF twice weekly for 2 weeks. Finally, the beneficial effect of FGF on endothelial regrowth is not limited to bFGF; administration of low doses of acidic FGF also promotes repair of damaged endothelium in vivo.[25]

Although these studies established the beneficial effect of FGF on endothelial cell growth in vivo, it was of critical importance to assess the function of the neoendothelium that regenerated in response to growth factor administration. As mentioned above, Meurice *et al.*[24] reported the effect of long-term (4 weeks) administration of bFGF on physiologic responses to endothelium-dependent agonists after vascular injury in a rabbit model. As stated, administration of relatively low doses of bFGF was associated with a significant increase in reendothelialization. Four weeks after denudation, endothelium-independent responses did not differ significantly between the bFGF and the control groups. In contrast, the maximal endothelium-dependent relaxation induced by acetylcholine in the bFGF-treated animals was significantly ($p < 0.05$) greater than that in the control group (Fig. 2).[24]

The mechanisms by which bFGF restored the relaxant response to acetylcholine are not completely understood, but the normalized endothelium-dependent responses observed after bFGF treatment probably do not relate solely to endothelial regrowth. Previous studies[6] that demonstrated persistent abnormal endothelium-dependent responses in rabbit iliac arteries despite complete reendothelialization suggest that bFGF, in addition to its effect on endothelial cell growth, might also modulate some qualitative aspects of endothelial cells and restore normal physiologic responses to endothelium-dependent agonists.

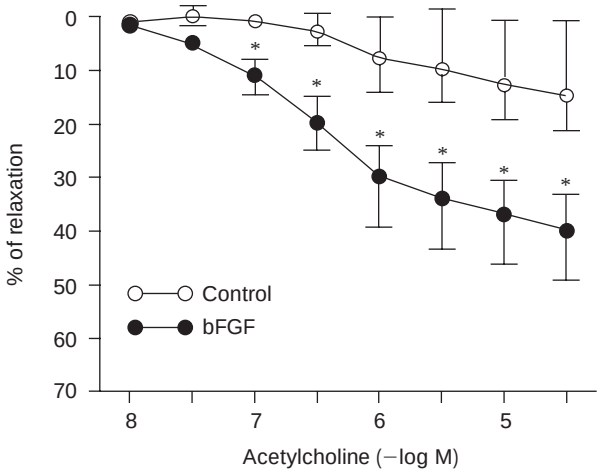

FIG. 2 Endothelium-dependent relaxation in response to acetylcholine in rabbit arteries denuded 4 weeks previously (animal model of balloon injury). The control group (top line) demonstrated almost no relaxation in response to acetylcholine, whereas the group that received bFGF showed significantly (* $p < 0.05$) increased relaxation in response to acetylcholine, indicating improvement in endothelial function. Reprinted from Ref. No. 24 with permission.

The effect of FGF administration on neointimal thickening after arterial injury is not clear. Lindner *et al.*,[26] using high doses of bFGF (12 µg/day for 2 weeks in a rat model), found an increase in neointimal thickening. By contrast, Bjornsson *et al.*,[25] using low doses of acidic FGF in the same model, observed an inhibition of neointimal thickening. Finally, in the study by Meurice *et al.*[24] discussed previously, a similar degree of neointimal thickening was observed in control and treated rabbits 4 weeks after injury. Taken together, these studies suggest that the final effect of FGF on neointimal thickening may be the consequence of a balance between stimulatory and inhibitory effects on smooth muscle cell growth. Experimental studies support the idea that certain functions of the endothelium, such as production of NO, are critical to the prevention of luminal narrowing by neointimal thickening;[27] accelerated reendothelialization may thus reduce neointimal formation. On the other hand, bFGF, as a potent growth factor for smooth muscle cells, may directly enhance neointimal formation. Variables such as the dose used, the duration of treatment, and the animal model studied may explain discrepancies among studies.

Vascular Endothelial Growth Factor

Asahara *et al.*[13] investigated the hypothesis that a single, direct application of VEGF to the intimal surface of a balloon-injured artery could accelerate reendothelialization. In this study, VEGF (100 µg) was given locally after balloon injury of the rat carotid artery. At 2 weeks and 4 weeks after injury, the extent of reendothelialization was markedly superior in the VEGF group compared with the control group (Fig. 3). It is interesting that neointimal thickening was correspondingly attenuated to a statistically significant degree ($p < 0.05$) in arteries treated with VEGF. In addition, histochemical analyses demonstrated a lower frequency of proliferating cells in the neointima of VEGF-treated animals. VEGF thus appears to be as potent as FGF in inducing endothelial regrowth; its specificity for endothelial cells may represent a potential advantage over FGF because the indirect inhibition of smooth muscle cell growth by the regenerated endothelium will not be antagonized by direct stimulation.

More recently, the effects of direct gene transfer of VEGF after angioplasty were investigated in a rabbit model.[28] In this study, New Zealand White rabbits underwent simultaneous balloon injury and gene transfer with phVEGF165, encoding the 165-amino acid isoform of VEGF. Gene expression was observed as early as 36 h post transfection and persisted through 2 weeks, before diminishing at 3 weeks. An increase in serum concentration of VEGF was observed 5 days after transfection. Planimetric analysis disclosed near-complete reendothelialization by 7 days among VEGF-transfected arteries, while the extent of reendothelialization in control arteries was < 50% complete at 7 days and remained nearly 20% incomplete at 4 weeks. A complete assessment of the consequences of reendothelialization showed that (1) treated arteries recovered near-normal endothelium-dependent responses within 1 week, whereas control arteries demonstrated persistently impaired endothelium-dependent responses at 4 weeks post injury; (2) VEGF-treated arteries had less neointimal thickening and, consequently, a greater luminal diameter on angiography than control arteries; and (3) thrombotic occlusion developed less frequently in animals transfected with phVEGF165 than in control animals.

Endothelial Dysfunction in Atherosclerotic Vessels

Diet-induced experimental atherosclerosis impairs endothelium-dependent relaxation in vitro and in vivo but has no effect on endothelium-independent relaxation.[3, 4] Several mechanisms may explain the abnormal endothelium-dependent responses in atherosclerotic vessels. Hypercholesterolemia induces extensive morphologic changes in the endothelial cell layer, including areas of deendothelialization that may reduce the total number of endothelial cells.[4] Alternatively, the thickened intima of atherosclerotic vessels could serve as a physical barrier to EDRF/NO. However, the fact that hypercholesterolemia can also impair endothelium-dependent relaxation in the absence of gross structural changes[29, 30] suggests that abnormal endothelium-dependent responses are more likely related either to reduced synthesis or enhanced destruction of EDRF/NO. This latter possibility is supported by studies showing that the EDRF/NO precursor L-arginine restores normal endothelial relaxation in hypercholesterolemic rabbits[31] and that excessive endothelial production of superoxide anion can inactivate NO in similar models.[32]

We recently tested the hypothesis that sustained administration of bFGF in hypercholesterolemic rabbits might restore

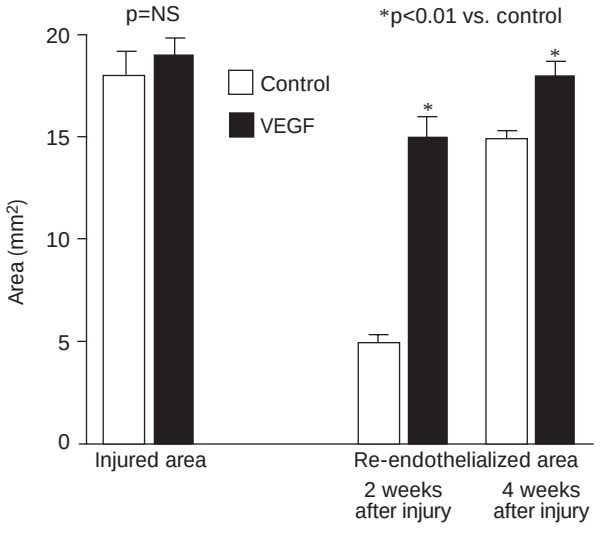

FIG. 3 Reendothelialization in rat carotid artery following balloon angioplasty. The group of rats that received VEGF showed a significantly greater degree of reendothelialization than did rats in the control group. Reprinted from Ref. No. 13 with permission.

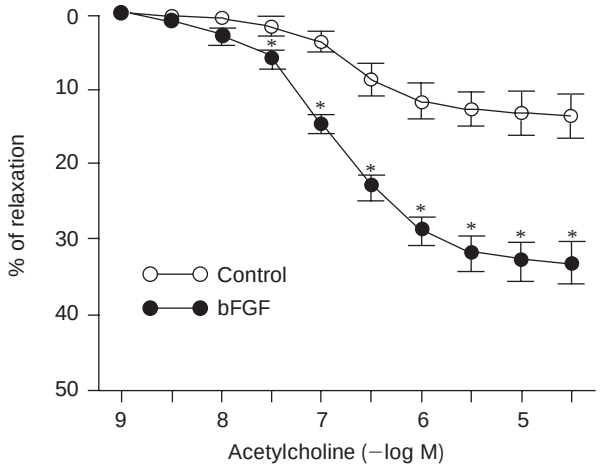

FIG. 4 Endothelium-dependent responses to acetylcholine in hypercholesterolemic rabbits treated either with placebo (control group) or twice-weekly intravenous boluses of bFGF. Hypercholesterolemic rabbits treated with bFGF had significantly (* $p < 0.05$) better endothelium-dependent relaxation than did control animals. Reprinted from Ref. No. 33 with permission.

normal physiologic responses to endothelium-dependent agonists.[33] After feeding on a 2% hypercholesterolemic diet for 6 weeks, the animals received twice-weekly intravenous boluses of either bFGF or placebo for 3 weeks and were sacrificed for assessment of in vitro vasoreactivity and for histologic analysis. Hypercholesterolemic rabbits treated with bFGF had significantly ($p < 0.05$) better endothelium-dependent responses than did untreated animals (Fig. 4). Endothelium-independent responses did not differ significantly between the two groups. A similar degree of plaque formation was observed in the two groups.

These results in the hypercholesterolemic rabbit are concordant with those of previous studies demonstrating that administration of endothelial cell growth factors may help to restore normal responses in other models of endothelial dysfunction. Although an anatomic effect on the endothelial cell cannot be excluded, our data suggest that administration of endothelial cell growth factors is also associated with functional changes at the endothelial level.

Conclusions

In vivo administration of endothelial cell growth factors leads to significant improvement in endothelium-dependent responses. This effect is observed with bFGF and VEGF in various animal models of endothelial dysfunction, such as the collateral circulation, the regenerated endothelium after arterial injury, and experimental atherosclerosis (Table II). While the precise mechanisms underlying this beneficial effect remain to be determined, the use of endothelial cell growth factors may represent a new therapeutic strategy for patients with vascular diseases.

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