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Articles

Synergistic Effect of Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor on Angiogenesis In Vivo

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Background Recent studies have suggested that vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) may have synergistic effects on the induction of angiogenesis in vitro. Therefore, we investigated the hypothesis that the simultaneous administration of VEGF and bFGF, each having been previously shown to independently enhance collateral development in an animal model of hind limb ischemia, could have a synergistic effect in vivo.

Methods and Results Ten days after surgical induction of unilateral hind limb ischemia, New Zealand White rabbits were randomized to receive either VEGF 500 μg alone (n=6), bFGF 10 μg alone (n=7), VEGF 500 μg, immediately followed by 10 μg bFGF (n=7), or vehicle only (control animals, n=8) in each case administered

intra-arterially via a catheter in the internal iliac artery of the ischemic limb. BP ratio (BPR, ischemic/healthy limb) at day 10 for the VEGF+bFGF group was 0.82±0.01, much superior ($P<.0005$) to that of either the VEGF group (0.52±0.02) or the bFGF group (0.57±0.02). This outcome persisted at day 30: BPR in the VEGF+bFGF group (0.91±0.02) exceeded that of the control group (0.49±0.05, $P<.0001$), the VEGF group (0.65±0.03, $P<.0005$), or the bFGF group (0.66±0.03, $P<.0005$). Serial angiography demonstrated a progressive increase in luminal diameter of the stem collateral artery and the number of opacified collaterals in the thigh of the ischemic limbs in all groups. Stem artery diameter with VEGF+bFGF (1.34±0.07 mm) on day 30 was significantly ($P<.05$) greater than with either VEGF (1.09±0.09) or bFGF (1.18±0.06) alone. Capillary density was significantly greater ($P<.05$) in VEGF+bFGF animals (275±20 mm^2) compared with VEGF (201±8) or bFGF (209±15).

Conclusions Combined administration of VEGF and bFGF stimulates significantly greater and more rapid augmentation of collateral circulation, resulting in superior hemodynamic improvement compared with either VEGF or bFGF alone. This synergism of two angiogenic mitogens with different target cell specificities may have important implications for the treatment of severe arterial insufficiency in patients whose disease is not amenable to direct revascularization.

Key Words: growth substances • ischemia

Introduction

A significant proportion of patients who suffer from severe lower extremity vascular insufficiency have disease so extensive that direct revascularization techniques cannot be undertaken successfully. In such patients, therapeutic strategies designed to augment native collateral vessel blood flow represent a novel means of achieving distal limb perfusion. Recent studies have established the feasibility of using recombinant angiogenic growth factors to enhance angiogenesis and collateral artery development in animal models of hind limb ischemia. Endothelial cell growth factor,[1] basic fibroblast growth factor (bFGF),[2] and vascular endothelial growth factor (VEGF)[3,4] have all been shown to enhance angiogenesis in the rabbit ischemic hind limb.

While VEGF and bFGF have certain features in common, such as their affinity for heparin, they are distinguished by the fact that VEGF is a secreted mitogen whose

receptors are found exclusively on endothelial cells; in contrast, bFGF is not secreted and stimulates the growth of smooth muscle cells and fibroblasts as well as endothelial cells. Two in vitro studies[5,6] have recently demonstrated that combined administration of VEGF and bFGF to endothelial cell cultures in three-dimensional collagen gels results in much greater and more rapid capillary tubule formation than the additive effects of either mitogen alone. The purpose of the present study was to determine whether such synergism could be demonstrated in vivo in an animal model of hind limb ischemia in which both VEGF and bFGF had previously been shown to stimulate collateral growth and to enhance limb perfusion.

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Animal Model

We used a rabbit ischemic hind limb model that in previous studies from our laboratory and others has been shown to reproduce severe limb ischemia that persists for up to 3 months' follow-up.[7] All protocols were approved by St Elizabeth's Institutional Animal Care and Use Committee. Male New Zealand White rabbits (3.5 to 4 kg) (Pine Acre Rabbitry, Norton, Mass) were anesthetized with a mixture of ketamine (50 mg/kg) and acepromazine (0.8 mg/kg) after premedication with xylazine (2 mg/kg). A longitudinal incision was then performed, extending inferiorly from the inguinal ligament to a point just proximal to the patella. The limb in which the incision was performed—right versus left—was determined randomly at the time of surgery by the operator. Through this incision, using surgical loupes, the femoral artery was dissected free along its entire length; all branches of the femoral artery (including the inferior epigastric, deep femoral, lateral circumflex, and superficial epigastric) were also dissected free. After the popliteal and saphenous arteries were dissected distally, the external iliac artery and each of the above arteries were ligated with 4.0 silk (Ethicon). Finally, the femoral artery was completely excised from its proximal origin as a branch of the external iliac artery, to the point distally where it bifurcates to form the saphenous and popliteal arteries. The excision of the femoral artery results in retrograde propagation of thrombus to the origin of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent on collateral vessels issuing from the ipsilateral internal iliac artery.

Recombinant VEGF and bFGF

The 165-amino acid homodimeric species of recombinant human VEGF ($rhVEGF_{165}$) was purified from transfected Chinese hamster ovary cells as previously described.[8]

The purity of the material was assessed by a silver-stained SDS/PAGE gel and by the presence of a single NH_2-terminal amino acid sequence. Human recombinant bFGF purified by heparin-sepharose chromatography was obtained from Genzyme.

Intra-arterial Administration of VEGF and bFGF

Ten days postoperatively (day 0), the animals were randomized to four groups. After a baseline angiogram was performed (see below), a 3-F end-hole infusion catheter (Tracker-18, Target Therapeutics) was positioned in the internal iliac artery of the ischemic limb. A total of six rabbits received a single intra-arterial bolus dose of 500 µg VEGF in 3 mL of saline containing 0.1% albumin (Sigma Chemical Co); seven rabbits received a single intra-arterial bolus of 10 µg bFGF; seven rabbits received an intra-arterial bolus of 500 µg VEGF immediately followed by an intra-arterial bolus of 10 µg bFGF; and, finally, eight rabbits received only vehicle and were used as a control group. The doses of VEGF and bFGF were chosen on the basis of the studies cited above[2 3 4] that demonstrated augmented angiogenesis with each of these cytokines administered independently. Limitations imposed by the numbers of animals required to investigate the four treatment strategies outlined here precluded investigation of multiple doses of VEGF and bFGF in order to establish in vivo dose-response curves.

Lower Limb Calf BP Ratio

Calf BP was measured in both hind limbs on day 0 (immediately before treatment), day 10, and day 30. On each occasion, the hind limbs were shaved and cleaned, the pulse of the posterior tibial artery was identified using a Doppler probe, and the systolic BP in each limb was measured using standard techniques. The calf BP ratio (BPR) was defined for each rabbit as the ratio of systolic pressure of the ischemic limb–to–systolic pressure of the normal limb.

Angiography

Angiography was performed on day 0 (before initiation of therapy), day 10, and day 30. A 3-F infusion catheter (Tracker-18, Target Therapeutics) was introduced into the right common carotid artery through a small cutdown and advanced to the lower abdominal aorta using a 0.014-in guide wire (Hi-Torque Floppy II, Advanced Cardiovascular Systems) under fluoroscopic guidance. The tip of the catheter was advanced to the entrance of the internal iliac artery of the ischemic limb. So that all measurements of arterial lumen diameter could be compared, an intra-arterial injection of 0.25 mg of nitroglycerin (Solopak Laboratories) was given to produce maximum vasodilatation. A total of 5 mL of contrast media (Isovue-370, Squibb Diagnostics) was then injected using an automated angiographic injector (Medrad)

at a rate of 1 mL/s. Serial images of the ischemic hind limb were then recorded on 105-mm spot film at a rate of 1 film per second for 10 seconds.

The internal luminal diameter of the main collateral (stem) artery issuing from the internal iliac artery (Fig 1) was measured using a previously validated automated edge-detection system.[9] The film selected for analysis was scanned with a high-resolution video camera; the signal produced by the video camera was digitized and displayed on a video monitor. Center lines were traced manually for a 10-mm-long segment beginning immediately after the origin of the vessel from the internal iliac artery. The contours were subsequently detected automatically on the basis of the weighted sum of first and second derivative functions applied to the digitized brightness information. The vascular diameter was then measured 5 mm distal to the origin of the vessel from the internal iliac artery.

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Figure 1. Diagrammatic representation of animal model (left) with accompanying angiogram (right). Right, External iliac artery (EIA) typically develops thrombotic occlusion after excision of ipsilateral femoral artery. Excised femoral artery and thrombosed external iliac shaded in diagram and missing on arteriogram. Large arrow indicates multiple collateral sprouts that reconstitute distal circulation of the ischemic limb.

The analysis of collateral development was performed on the 4-second angiogram. To assess the number of collateral vessels, we used a grid overlay composed of 2.5-mm–diameter circles arranged in rows spaced 5 mm apart. This acetate overlay was placed over the angiogram recorded at the level of the medial thigh. The number of contrast-opacified arteries crossing over circles and the total number of circles encompassing the medial thigh area were counted by a single observer blinded to the treatment regimen. An angiographic score was calculated for each film as the ratio of the number of arteries crossing through the 2.5-mm–diameter circles divided by the total number of circles in the medial thigh.

Capillary Density and Capillary/Muscle Fiber Ratio

The angiogenic effect of VEGF and bFGF at the microvascular level was examined by measuring the number of capillaries in light microscopic sections taken from the ischemic hind limbs. Tissue specimens were obtained as transverse sections from the adductor muscle and the semimembranous muscle of both limbs of each animal at

the time of death (day 30). These two muscles were chosen for light microscopic analysis because (1) they are the two major muscles of the medial thigh and (2) each was originally perfused by the deep femoral artery, ligated at the time that the common/superficial femoral artery was excised. Muscle samples were embedded in OCT compound (Miles) and snap-frozen in liquid nitrogen. Multiple frozen sections (5 μm in thickness) were then cut from each specimen on a cryostat (Miles), so that the muscle fibers were oriented in a transverse fashion, and two sections were then placed on glass slides. Tissue sections were stained for alkaline phosphatase using an indoxyl-tetrazolium method to detect capillary endothelial cells as previously described[10] and were then counterstained with eosin. Capillaries were counted by a single observer blinded to the treatment regimen under a x20 objective to determine the capillary density (mean number of capillaries per square millimeter). A total of 20 different fields from the two muscles were randomly selected, and the number of capillaries counted. To ensure that analysis of capillary density was not overestimated due to muscle atrophy, or underestimated due to interstitial edema, capillaries identified at necropsy were also evaluated as a function of muscle fibers in the histological section. The counting scheme used to compute the capillary/muscle fiber ratio was otherwise identical to that used to compute capillary density.

Histological Analysis of the Internal Iliac Artery

To determine whether bolus administration of VEGF and/or bFGF could result in the induction of local neointimal thickening, a 20-mm-long segment of the internal iliac artery immediately distal to the site of catheter infusion was retrieved from three animals selected at random in each group at the time of death (day 30). The vessels were perfusion-fixed with methanol at a pressure of 100 mm Hg and embedded in paraffin. Sections were stained with hematoxylin-eosin and elastic trichrome.

Neointimal thickening was assessed in terms of intima area-to-media area ratio (I/M) using transverse sections of hematoxylin-eosin- or elastic trichrome-stained arteries. Histological sections were projected onto a digitizing board (Summagraphics Corp), and values for intima and media areas were calculated by a technician blinded to treatment regimen, using a computerized sketching program (MACMEASURE, version 1.9, NIMH).

Statistical Analysis

All results are expressed as mean±SEM. Statistical comparisons were performed with the use of ANOVA. When a significant difference was detected, multiple-comparison analysis was performed using the Student-Newman-Keuls test. Evidence

of interaction within the model was tested for using two-factor ANOVA. A value of $P<.05$ was considered to denote statistical significance.

Results

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Calf BPR

As shown in Fig 2, calf BPR was similar in all groups at day 0. By day 10, the BPR had improved in all groups. Although VEGF alone and bFGF alone each resulted in a BPR significantly greater ($P<.05$) than that of the control group, the effect of VEGF+bFGF was significantly greater than the results seen with either mitogen alone. The BPR for the VEGF+bFGF group, 0.82±0.01, was much superior ($P<.0005$) to that of either the VEGF group (0.52±0.02) or bFGF group (0.57±0.02). Similarly, at day 30, the superiority of the BPR in the VEGF+bFGF group, 0.91±0.02, persisted in comparison with that of either the control group (0.49±0.05, $P<.0001$), the VEGF group (0.65±0.03, $P<.0005$), or the bFGF group (0.66±0.03, $P<.0005$). Furthermore, when two-factor ANOVA was performed, the BPR on day 10 was significantly greater ($P<.0004$) in the VEGF+bFGF group compared with either mitogen alone, implying that there may be interaction between the VEGF and bFGF when used together.

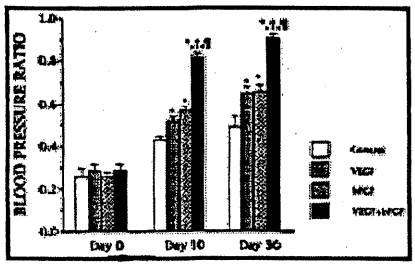

Figure 2. Bar graph shows effect of VEGF, bFGF, and VEGF+bFGF on blood pressure ratio (systolic pressure of the ischemic limb–o-systolic pressure of the normal limb) at days 0, 10, and 30. *$P<.05$ vs control group; †$P<.05$ vs VEGF group; ¶$P<.05$ vs bFGF group.

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Quantitative Angiography

Serial angiographic examinations documented a progressive increase in the luminal diameter of the principal stem collateral artery issuing from the internal iliac artery. No differences among the three experimental groups were detected at days 0 and 10. By day 30, however, the mean diameter of the stem artery in the VEGF+bFGF group (1.34±0.07 mm) exceeded that of the VEGF group (1.09±0.09 mm) as well as the group receiving bFGF (1.18±0.06 mm) (Fig 3).

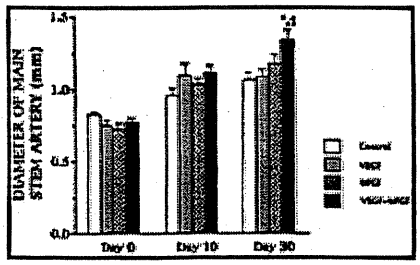

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Figure 3. Bar graph shows effect of VEGF, bFGF, and VEGF+bFGF on diameter of the main collateral (stem) artery at days 0, 10, and 30. **P*<.05 vs control group; †*P*<.05 vs VEGF group.

Serial assessment of the number of angiographically visible collateral vessels (angiographic score) revealed a progressive increase throughout the follow-up period in all three experimental groups. By day 30, the angiographic score for all three treatment groups was significantly greater than that measured for the control group (*P*<.01) (Fig 4). In the case of this particular index, however, the angiographic score of the VEGF+bFGF group was not significantly different from the scores of the VEGF and bFGF groups alone.

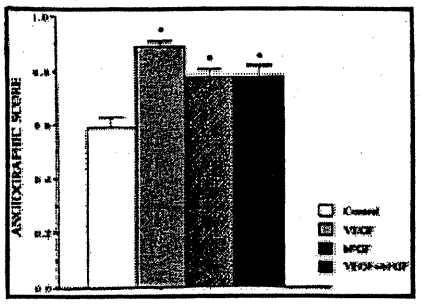

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Figure 4. Bar graph shows effect of VEGF, bFGF, and VEGF+bFGF on angiographic score at day 30. **P*<.05 vs control group.

Shown in Fig 5 are representative examples of angiograms recorded 4 seconds after contrast injection on day 30 in each of the three treatment groups. Note increased caliber of stem artery and its medium-size derivatives in the angiogram taken from an animal in the VEGF+bFGF group.

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Figure 5. Representative angiographic findings at day 30 after injection of VEGF (A), bFGF (B), and VEGF+bFGF (C). Note increase in angiographic luminal diameter of stem artery and its medium-size derivatives in angiogram (C) recorded from an animal in the VEGF+bFGF group.

Capillary Density and Capillary/Muscle Fiber Ratio

To further evaluate the effect of combined combined administration of VEGF and bFGF on revascularization of the ischemic limb, the medial thigh muscles of the ischemic limbs were examined histologically at day 30 as described above. As shown in Fig 6, administration of VEGF or bFGF produced mean values for capillary density (201±8 mm^2, 209±15 mm^2, respectively) and capillary/muscle fiber ratio (0.55±0.01, 0.58±0.04) that were significantly greater than those of the control group (156±10 and 0.39±0.04, respectively). The combined combined administration of VEGF and bFGF resulted in both a capillary density (275±20 mm^2) and a capillary/muscle fiber ratio (0.86±0.06) that were significantly higher ($P<.05$) than the corresponding values observed with either VEGF or bFGF alone.

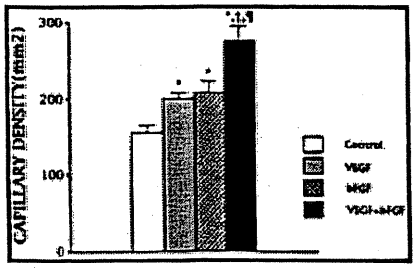

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Figure 6. Bar graph shows effect of VEGF, bFGF, and VEGF+bFGF on capillary density at day 30. *$P<.05$ vs control group; †$P<.05$ vs VEGF group; ¶$P<.05$ vs bFGF group.

Histological Analysis of the Internal Iliac Artery

Histological cross sections of the internal iliac artery were analyzed at day 30 in

three animals selected at random from each group. A minimal degree of neointimal thickening was observed in all three groups; no specific relation to treatment regimen was apparent from analysis of the I/M ratios: VEGF+bFGF=0.040±0.024; VEGF=0.029±0.015; and bFGF=0.033±0.017 (*P*=NS).

Finally, the synergism of combined administration of VEGF and bFGF was tested by comparing the percentage increase in each treatment group with the control group for BPR, diameter change in the stem artery, and capillary density. As Fig 7 illustrates, the results obtained after combined administration of VEGF+bFGF exceeded the sum of the results achieved by administration of each growth factor alone for each parameter indicated.

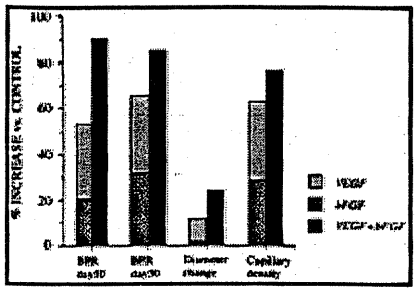

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Figure 7. Bar graph shows synergistic effect of VEGF+bFGF on blood pressure ratio (BPR) at day 10 and day 30, change in angiographic luminal diameter of stem artery, and change in capillary density, in each case presented as percent increase above ischemic, untreated control animals. For each parameter, the paired bars contrast the additive result of VEGF and bFGF administered alone (left, composite bar) vs effect of VEGF+bFGF administered together (right, black bar). In each case, the percent increase over control values achieved after combined administration of VEGF and bFGF exceeds additive effects of VEGF and bFGF alone.

Discussion

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Recent studies have demonstrated the therapeutic potential of administering various angiogenic growth factors to augment revascularization of the ischemic limb,[1 2 3 4] as well as myocardium.[11 12 13] Endothelial cell growth factor,[1] bFGF,[2] and VEGF,[3 4] administered by repeat intramuscular injection[1 2 3] or as an intra-arterial bolus,[4] have all been shown to substantially increase collateral development in the rabbit ischemic hind limb. To the best of our knowledge, the current study is the first in vivo demonstration that the combination of two angiogenic mitogens with differing target cell specificities can produce a significantly greater and more rapid improvement in collateral circulation than

administration of either mitogen alone. These findings may have important practical implications for the treatment of patients with severe limb ischemia.

That such an approach might be feasible was suggested by several studies that provided evidence for a synergistic effect of growth factors on angiogenesis in vitro. Pepper et al[14] demonstrated that in the presence of direct-acting angiogenic cytokines such as bFGF and VEGF, TGF-β_1 potentiates the in vitro angiogenic response in a dose-dependent manner. Similar findings were reported subsequently by Gajdusek et al.[15] While recent work from our own laboratory[16] has shown that TGF-β_1 upregulates transcription of both bFGF and VEGF in vascular smooth muscle cells, the absence of smooth muscle cells from the in vitro systems employed by Pepper and Gajdusek suggests that the observed synergism did not simply represent upregulation of a direct cytokine (bFGF/VEGF) by an indirect cytokine (TGF-β_1). Pepper et al[5] then demonstrated that adding VEGF or bFGF simultaneously to microvascular endothelial cells grown on the surface of three-dimensional collagen gels induced an angiogenic response that was greater than additive, and which occured with greater rapidity than the response to either cytokine alone. Goto et al[6] also measured the combined effect of VEGF and bFGF on the proliferative and morphological changes exhibited by bovine capillary endothelial cells cultured in a gel of type I collagen; when VEGF and bFGF were added simultaneously, both the number of cells and the number of cord-like structures formed were greater than the sum of those stimulated with either growth factor alone.

Our in vivo results are in agreement with these in vitro experiments. Compared with the magnitude of spontaneous collateral formation seen in the ischemic, untreated control group, combined administration of VEGF and bFGF produced increases in BPR, stem artery diameter, and capillary density that were greater than the sum of the effects observed with either growth factor alone. In addition, statistical analysis looking for a greater than additive effect that would imply some sort of interaction between the two mitogens revealed such an effect in the day 10 posttreatment BPR . Although such an interactive mechanism of action could not be demonstrated statistically in the other parameters measured, the accelerated effect on distal limb BP seen early after administration of VEGF+bFGF is of potential clinical significance in terms of more rapid restoration of perfusion to critically ischemic limbs.

Anatomic evidence of neovascularity was investigated at two levels. Necropsy examination documented an increase in vascularity at the capillary level,

consistent with the classic definition of angiogenesis formulated by Klagsbrun and Folkman.[17] We also assessed the diameter and number of angiographically visible collateral vessels by systematic quantification of the angiographic examinations. These analyses documented that the angiographic luminal diameter of the stem artery was significantly greater in the group treated with VEGF+bFGF than VEGF or bFGF alone. While the angiographic scores of VEGF, bFGF, and VEGF+bFGF were superior to those observed in control animals, combined administration of these cytokines did not achieve the same magnitude of improvement compared with independent cytokine treatment in angiographic score demonstrated for the other indexes cited above.

The potential therapeutic benefit of stimulating formation of larger, more "mature" collaterals is suggested by the hemodynamic evidence of increased downstream perfusion pressure documented on serial measurements of the lower limb BPR. The dramatic increase in BPR in the VEGF and bFGF groups between day 0 and day 10 compared with that seen after administration of either growth factor alone is likely to be a consequence of increased blood flow to the distal limb through the enlarged collateral bed in the combined therapy group. This conclusion is supported by recent findings in our laboratory in which the combination of VEGF+bFGF has been found to have a synergistic effect on maximum flow reserve in this same animal model, compared with bFGF or VEGF alone (C. Bauters, unpublished observations, 1994). Maximum flow reserve was calculated from velocity waveforms obtained using a Doppler wire positioned in the internal iliac artery (the main source of blood flow to the ischemic limb in our model) according to the method described by Bauters et al.[18] The physiological consequences of increased capillary density within the ischemic muscles were not assessed independently in the present study. It seems plausible, however, that such increased microvascular capacity should, for a similar level of blood flow, allow better oxygen extraction by the ischemic muscles and, consequently, better muscle performance.

The fundamental basis for the synergistic effect demonstrated in the present study remains to be elucidated. While VEGF is endothelial-cell specific,[19] bFGF is also a potent mitogen for a variety of other cell types, including smooth muscle cells.[20] Wilting et al,[21] for example, compared the effects of VEGF and bFGF on in vivo angiogenesis in the chick chorioallantoic membrane and demonstrated that VEGF induced vigorous endothelial cell proliferation, whereas bFGF elicited primarily fibrocyte proliferation with only minor endothelial cell proliferation. A direct stimulation of smooth muscle cells by bFGF might be responsible for some of the in

vivo effects observed in the current study, such as the statistically significant increase in the angiographic luminal diameter of the stem artery.

Conversely, the increase in capillary density and capillary/muscle fiber ratio in the ischemic muscles is more likely to be a consequence of a synergistic effect on endothelial cells similar to that observed in the in vitro experiments. It is important in this regard to underscore the independent receptor systems responsible for signaling the mitogenic effects of VEGF and bFGF in endothelial cells. Two tyrosine kinases, the *fms*-like tyrosine kinase (Flt-1)[22] and the kinase domain region (KDR)[23 24] proteins, have been shown to bind VEGF with high affinity. An independent dual receptor system comprising cell surface heparan sulfate proteoglycans and high-affinity tyrosine kinase receptors modulates the activity of bFGF.[25] Thus, amplification of the endothelial response to these mitogens is feasible based on their independent receptor systems alone.

Park et al[26] have recently proposed another possible basis for synergism among angiogenic growth factors. They demonstrated that the 152-amino acid isoform of placenta growth factor (PlGF) potentiates the action of low, marginally efficacious concentrations of VEGF on endothelial cell growth in vitro and in vivo potentiates Evans blue dye extravasation observed in a Miles assay. While PlGF binds with high affinity to Flt-1, it does not bind to the KDR receptor. The latter receptor appears to account for most of the bioactivity induced by VEGF. These authors thus suggested that potentiation in this case might be the result of the Flt-1 receptor behaving as a "decoy," ie, binding either ligand, but having little or no transducing activity. PlGF might therefore act to release VEGF from Flt-1 and to increase its availability to the more relevant KDR receptor. Because PlGF failed to potentitate low doses of bFGF, these findings were interpreted to be specific for VEGF. Whether an as yet unidentified high-affinity receptor for VEGF/bFGF could similarly contribute to the synergism demonstrated in the current study remains to be investigated.

We assessed the extent of neointimal thickening in the internal iliac artery at day 30 and found no evident differences among the three experimental groups. Whereas VEGF is an endothelial cell-specific mitogen, Lindner et al[20] have demonstrated that systemic administration of bFGF is a potent mitogen for smooth muscle cells in arteries denuded by a balloon catheter: Prolonged administration of bFGF (12 μg/day for 2 weeks) after balloon denudation of the rat carotid artery caused an approximate twofold increase in intimal thickening. In the current study, the use of single-bolus administration, a lower dose of bFGF (10 μg/rabbit), and no prior balloon denudation may explain the absence of neointimal thickening. In addition,

a recently described protective effect of VEGF in diminishing intimal hyperplasia by expediting endothelial "repaving" after balloon injury[27] may further offset any potentially adverse effects of bFGF. These results suggest that it might be possible to determine a dosing regimen of angiogenic growth factors that will increase vascularity while causing minimal deleterious effects in terms of local progression of atherosclerosis.

In conclusion, this study demonstrates that combined administration of VEGF and bFGF produces a greater and more rapid improvement in angiogenesis than administration of either VEGF or bFGF alone. This synergism may have important clinical implications, especially in situations such as severe limb ischemia where the time of response to treatment often determines the prognosis.

Acknowledgments

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