Physiologically Assessed Coronary Collateral Flow and Intracoronary Growth Factor Concentrations in Patients With 1- to 3-Vessel Coronary Artery Disease

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**Background**—The purpose of this study was to test the hypothesis that there is a relation between collateral flow and intracoronary concentrations of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) and that the combined concentrations of both growth factors and the extent of coronary artery disease (CAD) play a role as covariables in such an association.

**Methods and Results**—In 76 patients undergoing balloon angioplasty, a collateral flow index (CFI, no units) was determined with sensor-tipped guidewires. Simultaneously, serum concentrations of bFGF and VEGF, obtained at the aortic root from the ostium of the collateralized coronary artery (n=76) and from the distal position of the occluded coronary artery (n=34), were determined. There was a direct correlation between CFI and distal VEGF (r=0.33, P=0.05) but not bFGF concentrations. Focusing on the proximal sampling site, there was a direct correlation between CFI and both bFGF (r=0.29, P=0.01) and VEGF concentrations (r=0.44, P<0.0001). The sum of the concentrations of both growth factors was directly associated with CFI irrespective of the proximal (r=0.51, P<0.0001) or distal sampling site (r=0.34, P=0.048). There was a trend toward higher proximal VEGF concentrations in patients with higher numbers of coronary stenotic lesions (r=0.25, P=0.03).

**Conclusions**—In patients with CAD, there is an association between a directly measured index of collateral flow and intracoronary concentrations of bFGF and VEGF. This direct relation is dependent on the site of blood sampling within the coronary artery tree. The association is closest when the combined bFGF and VEGF concentrations are taken into account. In the case of VEGF, it is influenced by the degree of coronary atherosclerosis. (Circulation. 1999;100:1945-1950.)

Key Words: coronary disease ▪ collateral circulation ▪ growth substances

During the past 2 decades, evidence has accumulated favoring a clinically relevant role of the collateral circulation in patients with coronary artery disease (CAD). Accordingly, study of the human collateral growth adaptation appears to be important, because insight into these evolving mechanisms is essential for establishing new therapeutic modalities promoting collateral development. On the basis of experimental data, the sprouting of coronary collateral capillaries is considered to be induced by a chemical signal from the ischemic myocardium, leading to DNA synthesis and to mitosis of vascular cells. Angiogenic growth factors have been isolated from human cardiac tissue, and they were found in elevated concentrations among patients with acute myocardial infarction and unstable angina pectoris. Patients undergoing coronary artery bypass surgery for unstable angina pectoris have recently been shown to have elevated pericardial fluid concentrations of basic fibroblast growth factor (bFGF) compared with patients undergoing cardiac surgery for reasons other than CAD. Recently, the first angiographic indication has even been provided that treatment with bFGF of patients with CAD might be effective.

In patients suffering from stable, effort-induced angina pectoris, repeated myocardial ischemic stimuli for angiogenic growth factor production can be expected to continuously sustain collateral growth and remodeling. Direct and quantitative physiological measurements of the collateral circulation in patients with stable CAD and simultaneously determined growth factor concentrations have not been performed. However, they are indispensable to elucidate which angiogenic factors effective, alone or in combination, in promoting collateral growth and which are least strongly associated with atherogenesis or the extent of CAD. Therefore, we tested the hypotheses that there is a relation between quantitative measures of collateral flow and bFGF and vascular endothelial growth factor (VEGF) concentrations and that the combined concentrations of both growth factors

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and the extent of CAD play a role as covariables in such an association.

Methods

Patients

Seventy-six patients (63±10 years old; 66 men, 10 women) with 1- to 3-vessel CAD were included in the study. All underwent percutaneous transluminal coronary angioplasty (PTCA) of 1 stenotic lesion because of symptoms related to stable CAD. Patients were prospectively selected on the basis of the following criteria: (1) any angiographic degree of coronary collaterals, (2) no previous infarction in the myocardial area undergoing PTCA, (3) no baseline ECG ST-segment abnormalities, (4) no clinical or laboratory signs of acute or chronic inflammatory illness, and (5) absence of overt neoplastic disease. The present investigation was approved by the institutional ethics committee, and the patients gave informed consent to participate in the study.

Study Outline

The study population was divided into 2 groups according to the intracoronary (IC) Doppler- or pressure-derived collateral flow index (CFI): group CFI≥0.3 and group CFI<0.3. In all 76 patients, CFI and bFGF as well as VEGF concentrations were determined simultaneously. Growth factor concentrations were determined at the coronary ostium (proximal sampling site) in all cases and (because of technical sampling difficulties) distal to the occluded stenosis in 34 cases.

Cardiac Catheterization and Coronary Angiography

Patients underwent left heart catheterization for diagnostic purposes. Aortic pressure was measured with the PTCA guiding catheter. Biplane left ventriculography was performed, followed by coronary angiography. Coronary artery stenoses were estimated quantitatively as percent diameter reduction. Angiographic collateral degrees (0 to 3) were determined before PTCA: 0, no filling by contrast of the distal vessel via collaterals; 1, small side branches filled; 2, major side branches of the main vessel filled; and 3, main epicardial vessel filled by collaterals.

Coronary Collateral Assessment

In all study patients, coronary collateral flow relative to normal antegrade flow through the nonoccluded coronary artery (CFI) was determined by use of either IC velocity or pressure measurements, whereby both values are interchangeable.

Doppler-Derived CFI

The velocity-derived CFI was determined with a 0.014-in Doppler guidewire (Flowire, Endosonics) located distal to the stenosis to be dilated (Figure 1). CFI was calculated as the ratio of flow velocity time integral distal to the occluded stenosis (V_\text{occl}, \text{cm}) divided by that obtained at the identical location after PTCA (ie, not occluded, V_\text{nonoccl}, \text{cm}): V_\text{occl}/V_\text{nonoccl} (Figure 1). In patients revealing temporally shifted bidirectional velocity signals, antegrade and retrograde Vi were added to obtain V_{\text{total}}. The validation of this device to measure relative collateral flow has been described elsewhere.

Pressure-Derived CFI

A 0.014-in fiberoptic pressure monitoring wire (Pressureguide, Radi Medical) was set at zero, calibrated, advanced through the guiding catheter, and positioned distal to the stenosis to be dilated. The IC pressure-derived CFI was determined by simultaneous measurement of mean aortic pressure (P_\text{a}, mm Hg, via the angioplasty guiding catheter) and the distal coronary artery pressure during balloon occlusion (P_\text{occl}, mm Hg, Figure 1). Central venous pressure (CVP) was estimated to be 5 mm Hg. CFI was calculated as (P_\text{aold}−\text{CVP})/(P_\text{aold}−\text{CVP}).

Determination of Growth Factor Concentrations

The blood samples obtained distal to the occluded, collateralized coronary artery and from the ostium of the vessel (Figure 1) were collected in sterile tubes (anticoagulant: EDTA), placed on ice, treated by centrifugation at 3000g for 10 minutes at 4°C, and frozen at −40°C. Concentrations of bFGF were measured by an ELISA with a murine monoclonal antibody specific for bFGF (Quantikine, R&D Systems). This assay was performed by use of the quantitative sandwich enzyme immunoassay technique. The aforementioned antibody had been coated onto the microtiter plate. Standards and samples were pipetted into the wells, and any bFGF present was bound by the immobilized antibody. The wells were covered with the adhesive strip provided and incubated for 2 hours at room temperature. After any unbound proteins had been washed away with a 25-fold concentrated solution of buffered surfactant, an enzyme-linked polyclonal antibody specific for bFGF was added to the wells to sandwich the bFGF immobilized during the first incubation. The wells were incubated again for 2 hours at room temperature. After
removal of unbound antibody-enzyme reagent, a substrate solution of hydrogen peroxide and tetramethylbenzidine was added to the wells and color-developed in proportion to the amount of bFGF bound in the initial step. The color development was stopped by addition of 2N sulfuric acid, and the intensity of the color was measured with a spectrophotometer at 450 nm. A curve had been prepared, plotting the optical density versus the concentration of recombinant human bFGF in the 7 standard wells. By comparing the optical density of the samples with this standard, the concentration of bFGF in the unknown samples could be determined. VEGF concentrations were measured with a method similar to that for bFGF, ie, with a monoclonal antibody specific for VEGF and an enzyme-linked polyclonal antibody against VEGF for the quantitative sandwich immunoassay technique (Quantikine, R&D Systems). The measurement of concentrations of bFGF and VEGF was repeated on a different day with another ELISA kit. The difference in values between the 2 measurements was within ±10%.

**Study Protocol**

After diagnostic coronary angiography, an interval of 10 minutes was allowed for dissipation of the vasomotor effect of the nonionic contrast medium (Ioversol 300). IC or oral nitroglycerin was given. A multifunctional, occlusive probing catheter and the Doppler or pressure guidewire were positioned at and distal to the stenosis to be dilated, respectively. From the angioplasty guiding catheter (ostial or proximal sampling site in the aortic root, ie, arterial blood) and from the multifunctional probing catheter (distal sampling site), 4 mL of blood was collected into a tube. Simultaneously, $V_{\text{occl}}$ or $P_{\text{occl}}$ was determined to confirm coronary occlusion by the probing catheter, with the subsequent respective values during balloon occlusion used for comparison. During the entire protocol, an IC ECG obtained from the guidewire and a 3-lead surface ECG were recorded. After removal of the multifunctional probing catheter, the Doppler or pressure guidewire was used to transport the PTCA balloon. PTCA was performed. Measurements of $V_{\text{occl}}$ or $P_{\text{occl}}$ and simultaneous $P_{\text{a}}$ as well as ECG recordings were performed (Figure 1). After completion of PTCA and after cessation of reactive hyperemia, $V_{\text{occl}}$ was measured distal to the dilated stenosis in the cases in which Doppler-derived CFI was determined.

**Statistical Analysis**

Between-group comparisons of continuous demographic, angiographic, hemodynamic, growth factor, and collateral flow index data were performed by an unpaired Student’s t test. A $x^2$ test was used for comparison of categorical variables between the 2 study groups. Linear regression analysis was used for assessing the relation between collateral flow index values and distal or proximal growth factor concentrations. Mean values±SD are given. Statistical significance was defined at a value of $P<0.05$.

**Results**

**Patient Characteristics and Clinical Data**

There were no statistically significant differences between the 2 study groups regarding age of the patients, sex, frequency of cardiovascular risk factors, and the use of vasoactive and lipid-lowering substances as well as heparin immediately before PTCA. The frequency of peripheral artery disease was similar between the 2 groups.

**Angiographic and Coronary Collateral Data**

The Canadian Cardiac Society class of angina pectoris, the occurrence of myocardial infarction in non-PTCA territory, and the number of vessels affected by CAD did not differ between the study groups. There were no statistical differences between the study groups regarding the frequency of the vessels or the site of the stenosis treated by PTCA. LV ejection fraction was similar in the study groups. The duration of angina pectoris was higher in patients with CFI $\geq 0.3$ than in those with CFI $<0.3$: 14±21 weeks versus 8±12 weeks, respectively ($P=0.002$). Total coronary occlusions in the vessel to be dilated were more frequent in patients with CFI $\geq 0.3$ (7 of 10) than in those with CFI $<0.3$ (10 of 66; $P=0.0001$). Percent diameter stenosis before PTCA was 92±9% in patients with CFI $\geq 0.3$ and 83±10% in those with CFI $<0.3$ ($P=0.01$).

Qualitative and quantitative variables for the assessment of the collateral circulation were significantly different between the groups (Table).

**Coronary Collateral Flow Data and Growth Factor Concentrations**

bFGF and VEGF concentrations as well as the sum of both concentrations irrespective of the sampling site were higher in the group with CFI $\geq 0.3$ than in the group with CFI $<0.3$ (Table). This difference reached statistical significance only for proximally obtained VEGF and the pooled values of proximally or distally obtained bFGF+VEGF. CFI values were not related to bFGF concentrations from samples taken distal to the occluded stenosis, and there was a trend toward a correlation between CFI and distal VEGF concentrations (Figure 2). Focusing on the proximal sampling site at the ostium of the collateralized vascular bed, there was a direct correlation between CFI and both bFGF and VEGF concentrations (Figure 2). The pooled concentrations of both growth factors were directly associated with CFI irrespective of the sampling site (Figure 3). The correlations for proximal and distal values were as follows: bFGF$_{\text{proximal}}$ = 16.3 + 0.63 bFGF$_{\text{distal}}$; $r=0.30$, $P=0.09$; VEGF$_{\text{proximal}}$ = 5.0 + 0.49 VEGF$_{\text{distal}}$; $r=0.46$, $P=0.009$.

There was a trend toward higher proximal VEGF concentrations in patients with more extended CAD (number of vessels diseased, #VD, ie, number of vessels with percent stenoses of $>50\%$ diameter): VEGF = 8.3 + 9.1 #VD, $r=0.25$, $P=0.09$; bFGF = 34.6 + 14.5 #VD, $r=0.30$, $P=0.03$.
Discussion

This study in patients with CAD documents, for the first time, an association between a directly measured index of collateral flow and IC concentrations of bFGF and VEGF. This relation is less close for bFGF than VEGF, it is dependent on the site of blood sampling within the coronary artery tree, the association is closest when the combined bFGF and VEGF concentrations are taken into account, and in the case of VEGF, it is influenced by the degree of coronary atherosclerosis.

Collateral Angiogenesis and Growth Factors

It has been experimentally documented that vascular growth in adult organisms advances via sprouting of capillaries (angiogenesis) and via enlargement of preexisting arteriolar connections into collateral arteries.2,15 Investigations in animal models have disclosed mechanisms leading to angiogenesis with bFGF and VEGF as the major components.3,16 Therapeutic angiogenesis in the situation of peripheral or coronary artery disease has been attempted experimentally by direct growth factor peptide injection and infusion or transfer of growth factor genes.17–19 In most of those studies, endpoint variables such as ventricular ejection fraction, myocardial infarct size, capillary density, collateral resistance, or collateral backflow have been used to assess the effect of the growth factor treatment. In the clinical setting of coronary artery disease, no study has used quantitative measurements of collateral flow to assess its relation to bFGF and/or VEGF.

Figure 2. Correlations between CFI values (horizontal axes) and growth factor concentrations from blood samples obtained distal to (vertical axes, top) and at ostium (vertical axes, bottom) of collateralized, occluded coronary artery. Left, bFGF concentrations are plotted against CFI. Right, VEGF concentrations are plotted against CFI.

Figure 3. Correlations between CFI values (horizontal axes) and summed concentrations of bFGF and VEGF from blood samples obtained at ostium of collateralized coronary artery (vertical axis, left) and distal to occluded coronary artery (vertical axis, right).

$P=0.03$. This trend was absent in the case of distal growth factor concentrations and proximal bFGF concentrations.

Growth factor concentrations were different between proximal and distal sampling sites, showing, on average, higher distal than proximal values in the case of bFGF and lower distal values for VEGF. It can only be speculated that the higher distal concentrations of bFGF were related to the catheter manipulations at the site of the stenosis, and the higher proximal concentrations of VEGF may reflect the degree of systemic atherosclerosis, for which it is a better indicator than bFGF.

One of the principal findings of our study, ie, that the pooled concentrations of bFGF and VEGF were closely associated with collateral flow irrespective of the sampling site (Figure 3), is in agreement with the only experimental investigation of this kind that found a synergistic effect of both growth factors on the blood pressure ratio between the ischemic and nonischemic limbs compared with either factor alone.9 Although the serum concentrations of bFGF and VEGF in our study were rather low, the interpretation of a synergistic effect still appears reasonable in light of the
recently published study by Baumgartner et al.\textsuperscript{23} in patients with peripheral atherosclerotic disease showing that the average serum concentration of VEGF after treatment with intramuscular injection of naked plasmid DNA pHVEGF\textsubscript{165} was 148 pg/mL. However, considering that 75\% of the collateral flow variability is related to factors other than the sum of the concentrations of bFGF and VEGF ($r^2=0.51$, Figure 3, left), alternative parameters possibly influencing both collateral flow and the growth factors examined have to be taken into account.

**Collateral Arteriogenesis and Growth Factors**

An aspect partly responsible for the variability in the bFGF+VEGF-to-CFI relation is that collateral flow as assessed in our study probably does not exactly represent the “flow” induced by the growth factors measured. A more adequate parameter for collateral flow inducible by angiogenic growth factors such as bFGF and VEGF would be myocardial perfusion via collaterals rather than epicardially measured CFI. This concept relates to the recent finding of a spatial dissociation of bFGF- and VEGF-induced capillary sprouting (angiogenesis) in an ischemic region and the growth of “conductance” collaterals (arteriogenesis).\textsuperscript{15} The measurement site of collaterals in our study corresponds more to the area of arteriogenesis rather than angiogenesis. The fact that there is a certain association between the variables determined in our study is in accordance with the currently held pathophysiological view of collateral development, whereby the ischemia-induced angiogenesis leads to a certain increase in collateral flow and to a flow-induced, nitric oxide– or even growth-factor–mediated vasodilatation in newly formed as well as preformed collaterals.\textsuperscript{2,24} Subsequently, structural vascular remodeling, probably influenced by other growth factors such as monocyte chemotactic protein-1, occurs and leads to a large epicardial collateral artery.\textsuperscript{25}

**Atherogenesis and Growth Factors**

A factor further confounding the relation between collateral flow and growth factors is the extent of coronary as well as systemic atherosclerosis. Neovascularization in atherosclerotic plaques may be meditated by the overexpression of growth factors and by local hypoxia and may contribute to the growth and rupture of plaques.\textsuperscript{26} This implies that the therapeutic administration of angiogenic peptides may have a dual effect in patients with CAD: promoting collateral formation but simultaneously exacerbating atherogenic processes. In this context, Lazarous and coworkers\textsuperscript{10} have demonstrated in dogs that bFGF enhanced coronary collateral development without increasing neointimal accumulation at sites of vascular injury, whereas VEGF did not promote collateral growth but provoked neointimal accumulation. The present data on a significant trend to more extended coronary atherosclerotic lesions in patients with higher VEGF concentrations corroborate the aforementioned experimental investigation.

**Study Limitations**

Aside from the limitations alluded to above, there are other confounders of the relation between growth factors and CFI, such as measurement errors in the assessment of collaterals. Compared with IC Doppler-derived measurements of the collateral flow index, the standard error of estimate using pressure measurements is 0.08,\textsuperscript{12} If CVP is assumed instead of directly measured for the calculation of pressure-derived collateral flow index, another source of variability is introduced, which weighs more in the lower than the upper range of collateral flow indices.

It is conceivable that angiogenic growth factors would have been more concentrated in the venous effluent at the coronary sinus than in the arterial system. Coronary sinus sampling, however, was not performed to avoid an increased risk for the patients.

The fact that the angiogenic growth factors investigated are heparin-binding peptides causes potential measurement errors in their concentration in dependence of the serum heparin concentration.\textsuperscript{2,27} The dose of heparin administered to all patients immediately before PTCA was almost identical in the study groups, which made it unlikely that growth factor concentration differences between the groups were due to varying heparin concentrations. Furthermore, determination of the growth factor concentration under different heparin concentrations in 1 of the samples did not yield varying concentrations.

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