Vascular Remodeling in Response to Altered Blood Flow Is Mediated by Fibroblast Growth Factor-2

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Abstract—Vascular structures adapt to changes in blood flow by adjusting their diameter accordingly. The factors mediating this process are only beginning to be identified. We have recently established a mouse model of arterial remodeling in which flow in the common carotid artery is interrupted by ligation of the vessel near the carotid bifurcation, resulting in a dramatic reduction in vessel diameter as a consequence of inward remodeling and intimal lesion formation. In the present study, we used this model to determine the role of fibroblast growth factor-2 (FGF-2) in the remodeling response by maintaining neutralizing serum levels of a mouse monoclonal antibody against FGF-2 for 4 weeks. Morphometric analysis revealed that intimal lesion formation was not affected by the antibody. However, lumen narrowing was significantly inhibited, resulting in a greater than 3-fold increase in lumen area in anti–FGF-2–treated animals compared with controls. Treatment with anti–FGF-2 antibody significantly inhibited the reduction in vessel diameter (inward remodeling) and shortening of the internal elastic lamina in the ligated vessel. In addition, anti–FGF-2 treatment also caused outward remodeling of the contralateral carotid artery. These findings identify FGF-2 as an important factor in vascular remodeling, and its effects are likely to be mediated by increasing vascular tone. The results are consistent with the recent observation of reduced vascular tone in the FGF-2–deficient mouse. (Circ Res. 1999;84:323-328.)

Key Words: basic fibroblast growth factor ■ fibroblast growth factor-2 ■ intima ■ smooth muscle

Vascular remodeling is a well-described response of blood vessels to both physiological and pathological stimuli, leading to either vessel enlargement (positive or outward remodeling) or reduction (negative or inward remodeling). Examples of increased flow and/or lumen enlargement have been described in fetal development and in clinical situations after graft placement or angioplasty. All studies found that neointimal proliferation or intimal area after angioplasty showed little correlation with restenosis (measured by lumen area) because of permanent changes in vascular geometry. In humans, intravascular ultrasound has failed to show a correlation between lumen area (percentage of stenosis) and neointima area. vascular remodeling, as measured by a decrease in vessel area, was shown to account for the majority of the restenosis process.

Two important mechanisms by which hemodynamic forces may participate in remodeling are by regulation of endothelial cell function and matrix composition and organization within the vessel. Fluid flow is a prominent mediator of endothelial cell structure and function. Endothelial cell responses stimulated by flow participate in vessel development, remodeling, tone, and atherosclerosis. Flow alters endothelial cell properties such as orientation in the vessel wall, distribution of cytoskeletal elements, and expression of genes, including platelet-derived growth factor (PDGF) A and B chains, as well as release of endothelial-derived relaxing factor.

Studies by Guyton and Hartley and Langille and O’Donnell established ligation of rat and rabbit carotid arteries, respectively, as useful models of flow-dependent remodeling. In these models, flow was decreased in one carotid artery and over 4 to 12 weeks, the vessel diameter decreased in the flow-restricted vessel and increased in the contralateral flow-augmented vessel. In addition, Langille assessed the role of the endothelium by either gently denuding endothelium or gently disrupting endothelial membranes. Both procedures totally prevented alterations in vessel diameter. Thus, it appears that the endothelium is a critical mediator of the flow-dependent remodeling response.

We have recently established and characterized a mouse model of arterial remodeling. In this model, flow in the common carotid artery is interrupted by ligation of the vessel near the carotid bifurcation, resulting in a dramatic reduction in vessel diameter and formation of an intimal lesion. Neointima formation and the influx of inflammatory cells in this model are markedly reduced in P-selectin–deficient mice, while the reduction in vessel diameter is not affected by the lack of P-selectin. Additional specific factors that mediate
the remodeling response are beginning to emerge. Several studies have implicated nitric oxide (NO) as an inhibitor of remodeling events. Using a mouse model, Rudic et al recently reported that endothelial-derived NO is involved in this process. The authors reported that ligation of the external carotid artery in endothelial nitric oxide synthase (eNOS)–deficient mice caused thickening of the wall of the ipsilateral common carotid artery accompanied by a hyperplastic response of the vessel. This response was not seen in wild-type control mice. Our studies demonstrated that alterations in blood flow also lead to changes in gene expression of growth factors that are known to modulate proliferation and migration of smooth muscle cells (SMCs). Using in vivo techniques, we demonstrated that a 90% reduction in flow in the rat carotid artery caused a dramatic increase in PDGF-A and PDGF-B mRNA expression in the endothelium of the carotid artery with reduced flow. No change in expression of these genes was seen in the endothelium of the contralateral carotid artery with reduced flow. No change in expression of these genes was seen in the endothelium of the contralateral carotid artery that is expected to experience a compensatory increase in flow. Fibroblast growth factor-2 (FGF-2), which is known to mediate diverse biological effects including angiogenesis, bone formation, mitogenesis, migration, wound healing, neuronal survival, tumor growth, as well as vascular lesion formation in injured arteries, has very recently been identified as a crucial factor in the regulation of vascular tone. Zhou et al demonstrated that FGF-2–deficient mice display low blood pressure and decreased vascular contractility. Interestingly, like wild-type mice, these FGF-2–deficient mice exhibited a hyperplastic response of the vessel. This response was not seen in wild-type mice. To examine the role of FGF-2 in vascular remodeling, the present study used the carotid artery ligation model in mice in which circulating levels of a neutralizing mouse monoclonal antibody against FGF-2 or a control antibody were maintained for 4 weeks. Morphometric analysis revealed that treatment with the FGF-2 antibody significantly inhibited lumen narrowing and negative remodeling while neointima formation was not affected by the antibody. These findings demonstrate that FGF-2 plays an important role in vascular remodeling.

Materials and Methods

Animals

All animal studies were approved by the Institutional Animal Care and Use Committee. Twenty female FVB mice (3 to 4 months old, Jackson Laboratories, Bar Harbor, Maine) weighing 25 to 30 g were used in all experiments. The animals were anesthetized by intraperitoneal injection with a solution of xylazine (5 mg/kg, AnaSed, Lloyd Laboratories, Shenandoah, Iowa) and ketamine (80 mg/kg body weight, Ketaset, Aveco Co, Inc, Fort Dodge, Iowa). The left common carotid artery was dissected and ligated near the carotid bifurcation. A group of animals was injected with 0.5 mg of a neutralizing mouse monoclonal antibody against FGF-2 (clone 254F1) that does not cross-react with FGF-1, and additional injections (0.5 mg each) were given 7, 14, and 21 days later. Control animals received equivalent injections of nonimmune mouse IgG at the same time points. All animals were killed 28 days after ligation of the carotid artery. For monitoring antibody levels, serum samples were obtained by retro-orbital bleeds before surgery and once per week for the following 4 weeks. All animals were fixed for 5 minutes by perfusion with 4% paraformaldehyde in 0.1 mol/L sodium phosphate buffer (pH 7.3) as previously described. The left and right common carotid arteries were embedded in paraffin, and serial sections (5 µm thick) were cut for analysis by morphometry. Ten or more sections spanning the entire length of the vessel within 1 mm of the ligation were analyzed for morphometry. The mean for each animal was calculated.

Blood Pressure and Flow Measurements

Blood pressure was measured in 8-week-old FVB mice anesthetized with ketamine/xylazine with a catheter placed in the femoral artery. Systolic, diastolic, and mean blood pressure were recorded in mice 24 hours after intraperitoneal injection with 0.5 mg of monoclonal antibody 254F1 and in control mice (n=5 mice per group). Flow in the mouse carotid artery was measured near the bifurcation with a 0.5-mm perivascular ultrasonic flow probe connected to a Transonic blood flow meter.

Morphometry

Morphometric analysis was carried out on the ligated common carotid artery and on the contralateral common carotid artery 4 weeks after ligation. Digitized images of these vessels were analyzed using image analysis software for Apple Macintosh computers (NIH Image 1.60). The circumference (length) of the lumen, internal elastic lamina (IEL), and external elastic lamina (IEL) were determined by tracing along the luminal surface, IEL, and EEL, respectively. Assuming that the structure was circular, we used these measurements to calculate the lumen area. The medial area was calculated by subtracting the area defined by the IEL from the area defined by the EEL, and intimal area was determined by subtracting lumen area from the area defined by the IEL.

Serum Levels of FGF-2 Antibody

Levels of active FGF-2 antibody in mouse sera were determined by measuring the ability of the sera to inhibit FGF-2 function in a binding assay. Binding of FGF-2 to its high-affinity binding receptor was quantified using a previously published procedure. Briefly, a recombinant fusion protein composed of the 2-loop extracellular portion of FGF receptor-1 (FGFR1) and the heavy chain of mouse IgG2a was captured onto microtiter wells (Immulon 4) coated with IgG2a-specific goat anti-mouse antibody (Southern Biotechnology Inc, Birmingham, Ala). [125I]-FGF-2 (3 ng/mL; Biomedical Technologies Inc, Stoughton, Mass) in binding buffer (PBS, 1 mg/mL BSA) was added to the wells and incubated for 90 minutes at 22°C. For inhibition studies, the [125I]-FGF-2 was first incubated with serum samples for 30 minutes at 37°C before addition to the receptor. Nonspecific binding was determined as the amount bound in the presence of 1000-fold excess of unlabeled FGF-2. Incubations were terminated by washing the wells 3 times with ice-cold PBS. Bound FGF-2 was measured in a gamma counter.

Statistical Analysis

Student t test was used to compare the means between animals injected with the anti–FGF-2 antibody and the nonimmune IgG (intimal area, lumen area, left carotid medial area, length of IEL, and left carotid EEL). Means were considered significantly different if P≤0.05. Mann-Whitney U test was used for groups of data that did not have a normal distribution (right carotid medial area and right carotid EEL).

Results

We have previously described a mouse model of vascular remodeling in which the left common carotid artery is permanently ligated near the carotid bifurcation. This procedure completely interrupts net flow in this vessel, causing the flow to decrease from 0.58±0.05 mL/min (mean±SEM) in normal vessels to zero in ligated vessels. In the FVB mouse strain, this leads to a decrease in lumen size as a result of neointima formation and inward remodeling of the vessel. In the present study, we determined the role of FGF-2 in the remodeling events by administering a neutralizing murine
medial hypertrophy, extensive neointimal lesion formation, and shrinking of the IEL (arrowheads). Treatment with anti–FGF-2 caused dilation of the unmanipulated carotid artery (C) and inhibited lumen narrowing and shrinking of the IEL (arrowheads) in the ligated vessel (D). Blood but no clot formation is found in the lumen. Hematoxylin/eosin stain, original magnification ×200. Figure 2. Representative photomicrographs of cross-sectioned mouse carotid arteries from animals treated with nonimmune IgG (A and B) and anti–FGF-2 antibody (C and D) for 4 weeks. Unmanipulated right carotid artery (A) shows a thin media, whereas remodeling in the ligated carotid artery (B) reveals medial hypertrophy, extensive neointimal lesion formation, and shrinking of the IEL (arrowheads). Treatment with anti–FGF-2 caused dilation of the unmanipulated carotid artery (C) and inhibited lumen narrowing and shrinking of the IEL (arrowheads) in the ligated vessel (D). Blood but no clot formation is found in the lumen. Hematoxylin/eosin stain, original magnification ×200.

Values are mean±SEM; n indicates number of animals per group. Note that the medial area increased ~2-fold upon ligation of the vessel. Values comparing control antibody and anti–FGF-2 antibody in all categories were not significantly different.
strains of mice; however, there were no significant differences between control and antibody-injected groups in systolic blood pressure (75 ± 2.7 mm Hg and 70 ± 3.5 mm Hg, respectively), diastolic blood pressure (43 ± 2.3 mm Hg and 43 ± 2.5 mm Hg, respectively), and mean blood pressure (56.8 ± 2.6 mm Hg and 53.2 ± 2.8 mm Hg, respectively).

**Discussion**

The present study sought to determine the role of FGF-2 in vascular remodeling and intimal lesion formation. We chose to use our previously established model of carotid artery ligation in FVB mice, which is characterized by extensive neointima formation and inward remodeling of the vessel occurring over a period of 4 weeks. Thrombus formation occurs only in the most distal segment of the vessel within 1 to 2 mm of the ligation. This portion of the vessel was excluded from the analysis. Using a mouse monoclonal antibody in the mouse species allowed us to maintain circulating antibody levels for the entire experimental period. The possibility of an immune response that causes antibody to be cleared from circulation was thus markedly reduced. Antibodies from different species are often rapidly eliminated starting in the second week of treatment. In the present study, several interesting observations were made. Consistent with our earlier findings in the rat balloon injury model, the intimal lesion formation was not inhibited by the neutralizing anti–FGF-2 antibody, despite the inhibition of early SMC proliferation. Similarly, with use of a rabbit balloon injury model, Parish et al. confirmed that the late-stage neointimal lesion is not inhibited by anti–FGF-2, even though earlier time points showed a reduction in lesion size. In the present study, we did not examine the effects of FGF-2 antibody on early SMC proliferation, and because lesion formation in this model occurs relatively slowly compared with balloon injury models, it will be difficult to detect potential effects on SMC

**Figure 3.** Morphometric analysis of lumen areas in the right (unmanipulated) and left (ligated) mouse carotid artery 4 weeks after ligation. Treatment with anti–FGF-2 antibody markedly inhibited lumen narrowing in the ligated arteries (A), but the lumen was also larger in the contralateral carotid artery (B). Lumen area ratios (C) were significantly increased after anti–FGF-2 treatment. Data represent mean ± SEM; n indicates number of animals per group.

**Figure 4.** Morphometric analysis of IEL and EEL in the right (unmanipulated) and left (ligated) mouse carotid artery 4 weeks after ligation. Treatment with anti–FGF-2 antibody inhibited shortening of the IEL in the ligated vessels (A), and the IEL of the contralateral vessel (B) was also significantly longer in the anti–FGF-2 group. Remodeling index showed significant inhibition of negative remodeling (C) with anti–FGF-2 antibody treatment. No differences were seen among EEL lengths (D and E). Data represent mean ± SEM; n indicates number of animals per group.
proliferation. The cell density of the neointima as determined by the number of nuclei per unit area was similar between groups, suggesting that cell proliferation may not have been affected by the anti–FGF-2 antibody in this model.

Highly relevant are the recent observations made in the FGF–2–deficient mouse.40 These mice formed neointimal lesions in response to vascular injury and vascular tone, as well as blood pressure being reduced.40 Administration of the FGF-2 antibody in the present study had dramatic inhibitory effects on inward remodeling, ie, shortening of the IEL and loss of lumen area. These findings are consistent with the concept of FGF-2 as a mediator of vascular tone, although the exact mechanisms by which FGF-2 affects vascular tone still have to be identified.40 The contractile apparatus or the coordination of humoral vasoreactive signals are potential targets for FGF-2.40 Reduced inward remodeling in the anti–FGF-2–treated mice was not the result of decreased blood pressure that has been described in the FGF–2–deficient mice, given that there was no significant difference in blood pressure between groups in our study. At this time, it is unclear what the source of the FGF-2 might be that regulates vascular tone. Because the ligation model is not a denuding model, it is possible that FGF-2 produced locally in the endothelium or SMCs45 might be responsible for these effects in addition to potential levels of circulating FGF-2 found in plasma.

Interesting observations were also made regarding the effect of the anti–FGF-2 antibody on the unmanipulated right carotid arteries. As pointed out earlier, the average body weight of the animals used in the anti–FGF-2 group and the control group was not different, and all animals were perfusion-fixed under physiological pressure. Both the lumen area and the length of the IEL were significantly increased in plasma.

Reduced inward remodeling in the anti–FGF-2–treated mice was not the result of decreased blood pressure that has been described in the FGF–2–deficient mice, given that there was no significant difference in blood pressure between groups in our study. At this time, it is unclear what the source of the FGF-2 might be that regulates vascular tone. Because the ligation model is not a denuding model, it is possible that FGF-2 produced locally in the endothelium or SMCs45 might be responsible for these effects in addition to potential levels of circulating FGF-2 found in plasma.

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References


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