Cardiac-Specific Overexpression of Fibroblast Growth Factor-2 Protects Against Myocardial Dysfunction and Infarction in a Murine Model of Low-Flow Ischemia

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**Background**—Preconditioning the heart before an ischemic insult has been shown to protect against contractile dysfunction, arrhythmias, and infarction. Pharmacological studies have suggested that fibroblast growth factor-2 (FGF2) is involved in cardioprotective induction. However, because of the number of FGFs expressed in the heart and the promiscuity of FGF ligand-receptor interactions, the specific role of FGF2 during ischemia-reperfusion injury remains unclear.

**Methods and Results**—FGF2-deficient (Fgf2 knockout) mice and mice with a cardiac-specific overexpression of all 4 isoforms of human FGF2 (FGF2 transgenic [Tg]) were compared with wild-type mice to test whether endogenous FGF2 elicits cardioprotection. An ex vivo work-performing heart model of ischemia was developed in which murine hearts were subjected to 60 minutes of low-flow ischemia and 120 minutes of reperfusion. Preischemic contractile function was similar among the 3 groups. After ischemia-reperfusion, contractile function of Fgf2 knockout hearts recovered to 27% of its baseline value compared with a 63% recovery in wild-type hearts (P<0.05). In FGF2 Tg hearts, an 88% recovery of postischemic function occurred (P<0.05). Myocardial infarct size was also reduced in FGF2 Tg hearts compared with wild-type hearts (13% versus 30%, P<0.05). There was a 2-fold increase in FGF2 release from Tg hearts compared with wild-type hearts (P<0.05). No significant alterations in coronary flow or capillary density were detected in any of the groups, implying that the protective effect of FGF2 is not mediated by coronary perfusion changes.

**Conclusions**—These results provide evidence that endogenous FGF2 plays a significant role in the cardioprotective effect against ischemia-reperfusion injury. (Circulation. 2003;108:3140-3148.)

**Key Words:** ischemia ▪ myocardial infarction ▪ myocardial stunning ▪ growth substances

Cardiovascular disease (CVD) is the primary cause of death in the industrialized world. Approximately 73 million Americans have some form of CVD, with coronary (ischemic) heart disease comprising 18% of all CVD and 54% of all CVD deaths.1 Experimental and clinical studies have shown that numerous interventions, including brief periods of ischemia or hypoxia and certain endogenous mediators or pharmacological agents, are capable of protecting the heart against ischemia-induced myocardial contractile dysfunction, arrhythmias, and infarction.2,3 This cardioprotective phenomenon may have great clinical relevance and potential therapeutic applications in patients with coronary artery disease. An ideal therapeutic approach for enhancing the viability of ischemic myocardium and improving cardiac function is to increase this short-term protection and at the same time stimulate vessel growth to the ischemic region.

Fibroblast growth factor-2 (FGF2) has been implicated in multiple protective pathways that increase cell survival and promote neovascularization.4 Phase I clinical trials using FGF2 treatment in patients with severe ischemic heart disease have been implemented recently to test the efficacy, safety, and dosage of this growth factor as a medical therapy for coronary artery disease.5–7 There is significant pharmacological evidence that implicates FGF2 as a cardioprotective agent independent of stimulating neovascularization.4 These pharmacological data, however, are inherently limited by the large array of FGF isoforms and receptors8 (ie, 23 FGFs have been identified, with 10 localized to the heart9,10) and by the possibility that the inhibitors/antibodies used experimentally may act via nonspecific mechanisms. Therefore, the specific role of FGF2 in maintaining cardiac function or tissue integrity during prolonged ischemic insults remains unclear. Manipulation of the Fgf2 gene in the whole animal is essential for resolution of these issues with respect to myocardial ischemia and cardioprotection.

Our laboratory has previously generated mice that carry either a targeted disruption (Fgf2 knockout [KO])11 or a
cardiac-specific overexpression (FGF2 transgenic [Tg]) of the Fgf2 gene to study the role of FGF2 in cardiovascular function and disease. The Fgf2 KO mice are viable and fertile. However, cardiovascular features of Fgf2 KO mice include thrombocytosis, decreased mean arterial blood pressure with no significant loss of cardiac function, and decreased portal vein vascular smooth muscle tone. Recently, in Fgf2 KO mice subjected to chronic pressure overload via aortic coarctation, we reported a marked reduction of cardiac hypertrophy, suggesting a role for FGF2 in the cardiac growth response to hemodynamic load.12 Sullivan et al13 have recently shown that targeted disruption of the Fgf2 gene does not affect vascular growth in a mouse model of hindlimb ischemia. The cardiac-specific FGF2 Tg mice, where FGF2 is overexpressed 25- to 35-fold, have normal postnatal heart development, no spontaneous cardiac hypertrophy, and no alterations in cardiac vascular density compared with wild-type (Wt) mice.

With the availability of these genetically modified mice and the pharmacological/in vitro evidence for FGF2 in eliciting a cardioprotective effect, we tested the hypothesis that this endogenous growth factor has an integral role in protecting the heart against ischemia-reperfusion injury. The results demonstrate that FGF2 produces a cardioprotective action against end points of ischemia-reperfusion injury (ie, cardiac dysfunction and myocardial infarction), independent of its angiogenic/vascular functions.

Methods

Mice were housed in a pathogen-free facility and handled in accordance with standard use protocols, animal welfare regulations, and the NIH Guide for the Care and Use of Laboratory Animals. All protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee. Wt mice, Fgf2 KO mice in which all isoforms of FGF2 are absent, and 2 lines of mice with a cardiac-specific overexpression of all 4 isoforms of human FGF2 (FGF2 Tg myosin heavy chain [MHC] 20 and FGF2 Tg MHC25) were randomly assigned to the present study (for generation of mice, see the online supplement available at http://www.circulationaha.org). Twelve Wt mice (Black Swiss/129) and 11 Fgf2 KO mice and 18 Wt mice (FVB/N), 13 FGF2 Tg mice (MHC20), and 10 FGF2 Tg mice (MHC25) completed the ischemia-reperfusion studies. Exclusion from the study was based on signs of aortic or pulmonary leaks (ie, low aortic flow and high venous PO2) in the work-performing heart preparation.

Isolated Work-Performing Heart Preparation

Age (10 to 12 weeks)- and sex-matched Wt, Fgf2 KO, and FGF2 Tg mice were anesthetized with sodium pentobarbital (80 mg/kg IP). The isolated work-performing heart methodology was used as described previously14 (see the online supplement).

Model of Low-Flow Ischemia

After a 30-minute equilibration period at a basal cardiac workload of 250 mL/min · mm Hg, the venous return was quickly (<30 seconds) reduced by 1-mL increments to a flow of 1 mL/min for 30 or 60 minutes to elicit a low-flow, global ischemic insult. To maintain the work demand on the heart during ischemia, the heart was paced 10 to 15 beats per minute above its intrinsic heart rate. Coronary blood flow was reduced ~90% of its original rate (from 2.3 to 0.2 mL/min) during this global ischemia, mimicking a severe coronary artery stenosis. After 30 or 60 minutes of low-flow ischemia, venous return was quickly increased by 1-mL increments to a flow of 5 mL/min, and reperfusion occurred for 30 or 120 minutes. Functional data, perfusate gases, and coronary effluent were obtained at designated time points of baseline, low-flow ischemia, and reperfusion (Figure 1). Coronary flow changes, myocardial oxygen consumption, percent recovery of function, and left ventricular developed pressure as well as indices of diastolic and systolic function (eg, end-diastolic pressure, half relaxation time, and time to peak pressure) were assessed at baseline, ischemia, and reperfusion.

Measurement of Infarct Size

Infarct size was determined by the histochemical stain, 2,3,5-triphenyltetrazolium chloride (TTC), which delineates viable versus necrotic tissue.15 After the 60-minute ischemia/120-minute reperfusion injury study, Wt, Fgf2 KO, and FGF2 Tg hearts were perfused with warmed, 1% TTC stain (pH 7.4) via the aortic cannula. The hearts were frozen, sliced transversely, digitally photographed, and weighed. The area at risk and infarct zone were determined by computer morphometry (NIH imaging software, 1.61 version). Infarct size was depicted as a percent of the area at risk.

Histology

Formalin (4%)-fixed hearts from sham-treated and 60-minute ischemia/120-minute reperfusion–treated Wt, Fgf2 KO, and FGF2 Tg mice were embedded in paraffin and sectioned serially (5 μm). H&E staining was used for histologic and pathological examination of hearts.

Vascular Bed Staining

Vascular density levels were determined on nonischemic Wt, Fgf2 KO, and FGF2 Tg hearts (4 hearts per group, 10 to 12 weeks of age) using antibodies against α-smooth muscle actin and von Willebrand’s factor (see the online supplement available at http://www.circulationaha.org).
Determination of FGF2 Release
Quantitative determination of FGF2 release in coronary effluent collected at baseline, ischemia, and reperfusion (see the online supplement) was assessed by ELISA (R&D Systems). FGF2 concentration (pg/mL) in perfusates was normalized for coronary flow rate and heart weight (pg/min per g heart weight).

Western Blots to Detect Level of FGF2
Protein levels of FGF2 were determined in nonischemic Wt, Fgf2 KO, and Fgf2 Tg hearts (10 to 12 weeks of age) via Western blot analysis (see the online supplement).

Statistical Analysis
All values are expressed as mean±SEM. Multiple groups were compared using 1-way ANOVA followed by a Student t test. Differences at various time points were compared using 2-way ANOVA for time and treatment with repeated measures with the Fisher least-significant-difference post hoc test. Regression analysis was performed to compare coronary flow with +dP/dt or infarct size. Statistical differences were considered significant when P<0.05 (see the online supplement).

Results
Cardiac Phenotype and FGF2 Protein Expression in Fgf2 Knockout and FGF2 Transgenic Mice
Ratios of heart weight to body weight (mg/g) were similar for all 3 genotypes (Wt, 5.0±0.1 versus Fgf2 KO, 5.3±0.2; Wt, 5.2±0.1 versus FGF2 Tg MHC20, 5.3±0.1 versus FGF2 Tg MHC25, 5.4±0.2), indicating no spontaneous hypertrophy or inhibition of cardiac growth during development. Fgf2 KO hearts have normal cardiac function,11 and overexpression of the human FGF2 gene in the heart does not affect cardiac-specific gene expression (ie, α-myosin heavy chain, β-myosin heavy chain, L-type Ca2+ channels, and ryanodine receptor) (data not shown).

Multiple isoforms of FGF2 have been identified, representing alternative translation products from a single gene.16 Western blots performed on cardiac tissue from FGF2 Tg mouse lines MHC20 and MHC25 showed that the human FGF2 protein isoforms (18 kD and 22 to 34 kD) are expressed 5- to 25-fold, respectively, above Wt mouse FGF2 protein levels (Figure 2B). No FGF2 protein was detected in Fgf2 KO mice (Figure 2A).

Effects of Fgf2 Ablation or Cardiac-Specific FGF2 Overexpression on Recovery of Postischemic Cardiac Function
Wt, Fgf2 KO, and Fgf2 Tg hearts were subjected to either 30 or 60 minutes of low-flow ischemia and 30 or 120 minutes of reperfusion, respectively (Figure 1). Low-flow ischemia models simulate the ischemia-reperfusion injury profiles observed clinically resulting from coronary artery disease and cardiac surgical interventions.17

After 60 minutes of low-flow ischemia, Fgf2 KO hearts recovered to 27±3% of their baseline contractile function compared with postischemic Wt hearts (63±8%, P<0.05, Figure 3A, Table 1). In contrast, FGF2 Tg hearts (MHC20 and MHC25) had a significant increase (79±6% and 88±2%, respectively) in the percent recovery of postischemic function compared with Wt hearts (57±9%, P<0.05, Figure 3B, Table 1). Other preischemic and postischemic functional parameters for Wt, Fgf2 KO, and Fgf2 Tg hearts are shown in Table 2. With the exception of a slight but significant elevation in left ventricular pressure in Fgf2 KO hearts, there were no differences in preischemic functional parameters and myocardial oxygen consumption between Wt and Fgf2 KO hearts. After ischemia-reperfusion injury, there was significant systolic and diastolic dysfunction as measured by left ventricular pressure, +dP/dt, time to peak left ventricular pressure, −dP/dt, and half relaxation time and a significant decrease in myocardial oxygen consumption in Fgf2 KO hearts compared with Wt hearts (P<0.05, Table 2). With the exception of elevated myocardial oxygen consumption in FGF2 Tg (MHC 20), there was no difference in preischemic functional parameters between Wt and FGF2 Tg hearts. Both systolic and diastolic functional parameters were significantly improved in FGF2 Tg hearts compared with Wt hearts after ischemia-reperfusion injury (P<0.05, Table 2). A similar degree of postischemic functional recovery occurred as that of cohorts assigned to the 60 minutes of ischemia and 120 minutes of reperfusion (irreversible injury) in Fgf2 KO mice (23±4%) and the 2 FGF2 Tg mouse lines (90±3% and 96±2%) subjected to 30 minutes of ischemia and 30 minutes of reperfusion (reversible injury; Table 1).
No significant alterations in vessel density or defects in vasculogenesis or angiogenesis were detected in any of the groups. The number of smooth muscle–containing blood vessels per square millimeter was similar in all 3 genotypes (Wt, 11±1 versus Fgf2 KO, 11±1; Wt, 11±1 versus FGF2 Tg MHC20, 10±1 versus FGF2 Tg MHC25, 11±1). Also, the level of capillaries per square millimeter was similar in all genotypes studied (Wt, 738±241 versus Fgf2 KO, 363±65; Wt, 760±264 versus FGF2 Tg MHC20, 315±18 versus FGF2 Tg MHC25, 651±214). During reperfusion, Fgf2 KO hearts had a significant increase in coronary flow (P<0.05, Table 1); however, this group showed no improvement in the recovery of postischemic contractile function. Cardiac-specific overexpression of FGF2 significantly improved the recovery of postischemic contractile function. n=8, Wt; n=6, Fgf2 KO. n=11, Wt; n=6, FGF2 Tg MHC20. n=5, FGF2 Tg MHC25. *P<0.05 vs Wt for the same time point.

Reduction of Myocardial Infarct Size After Ischemia-Reperfusion Injury in Hearts With an Overexpression of FGF2

Histologic examination of postischemic Fgf2 KO and Wt hearts revealed contraction bands (hypercontracted myofibers) and vacuolizations (Figures 4A through 4C), which are both indicative of cardiomyocyte damage. However, the myofibers were well-preserved in postischemic FGF2 Tg hearts (Figure 4D), suggesting that priming the heart with FGF2 maintains the integrity of the muscle during ischemia-reperfusion injury.

Myocardial infarct size was assessed after 60 minutes of ischemia and 120 minutes of reperfusion. Infarct size as a percent of the area at risk was markedly reduced in FGF2 Tg hearts (MHC20, 13±6%; MHC25, 16±4%) compared with Wt (30±3%, P<0.02, Figure 5B). Infarct size was not different between Wt and Fgf2 KO hearts (Figure 5A). Also, the degree of myocardial infarct size did not correlate with alterations in coronary flow in any of the groups (data not shown), indicating that coronary flow was not a factor in protecting against infarction in this setting or model.

Pathological assessment of murine hearts subjected to 30 minutes of ischemia and 30 minutes of reperfusion revealed
TABLE 1. Coronary Flow and Percent Recovery of Function in WT, Fgf2-Deficient (KO), and FGF2-Overexpressing (Tg) Hearts

<table>
<thead>
<tr>
<th>Coronary Flow, mL/min</th>
<th>% Recovery of Contractile Function</th>
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<tbody>
<tr>
<td>n</td>
<td>Preischemia</td>
</tr>
<tr>
<td>30 min*</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>1.9±0.5</td>
</tr>
<tr>
<td>Fgf2 KO</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>WT</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>Fgf2 Tg MHC20</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>Fgf2 Tg MHC25</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td>60 min§</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>Fgf2 KO</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td>WT</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>Fgf2 Tg MHC20</td>
<td>2.2±0.2‡</td>
</tr>
<tr>
<td>Fgf2 Tg MHC25</td>
<td>1.7±0.2</td>
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</tbody>
</table>

*After 30 minutes of low-flow ischemia and 30 minutes of reperfusion.
†P<0.05 vs preischemia.
‡P<0.05 vs postischemic Wt.
§After 60 minutes of low-flow ischemia and 120 minutes of reperfusion.
††P<0.05 vs WT.

The present study has demonstrated that FGF2 elicits a cardioprotective effect against cardiac dysfunction and myocardial infarct size, independent of alterations in coronary perfusion or vessel density. Chronic elevation in cardiac levels of FGF2 protected the heart against reversible and irreversible injury, supporting previous pharmacological findings that exogenous administration of FGF2 attenuated contractile dysfunction or the degree of myocardial infarction. For example, isolated rodent heart studies have demonstrated that exogenous administration of FGF2 (1 to 10 μg/mL) elicited a similar percent recovery (63% to 96%) of postischemic contractile function and reduction in infarct size (10% of ventricular area at risk) to our endogenous-overexpressing FGF2 murine model. However, this is the first evidence to demonstrate that in the absence of endogenous FGF2, there is significant cardiac (systolic and diastolic) dysfunction after ischemia-reperfusion injury. Although Fg2 KO hearts had a poorer postischemic recovery of cardiac function compared with Wt hearts, myocardial infarct size

Release of FGF2 Into Coronary Effluent
Although FGF2 lacks the signal peptide sequence for its release from cells, FGF2 has been detected in the extracellular milieu. The present view is that FGF is synthesized and stored in cardiac myocytes and in nonmyocytes (ie, fibroblasts and endothelial cells) and can be actively transported out of these cells or released in response to a stressful stimulus. Therefore, FGF2 release would mediate an autocrine or paracrine effect via FGF receptor signaling to activate cardioprotective signaling pathways. The level of FGF2 release detected in the coronary effluent was significantly higher (≈2-fold) in the FGF2 Tg hearts compared with Wt hearts at baseline and during reperfusion (P<0.05, Figure 6B). No FGF2 release was detected in the coronary effluent from Fg2 KO hearts at any time point (Figure 6A). A similar pattern of FGF2 release was measured in the coronary effluent from FGF2 Tg hearts subjected to 30 minutes of ischemia and 30 minutes of reperfusion (data not shown).

Discussion
The present study has demonstrated that FGF2 elicits a cardioprotective effect against cardiac dysfunction and myocardial infarct size, independent of alterations in coronary perfusion or vessel density. Chronic elevation in cardiac levels of FGF2 protected the heart against reversible and irreversible injury, supporting previous pharmacological findings that exogenous administration of FGF2 attenuated contractile dysfunction or the degree of myocardial infarction. For example, isolated rodent heart studies have demonstrated that exogenous administration of FGF2 (1 to 10 μg/mL) elicited a similar percent recovery (63% to 96%) of postischemic contractile function and reduction in infarct size (10% of ventricular area at risk) to our endogenous-overexpressing FGF2 murine model. However, this is the first evidence to demonstrate that in the absence of endogenous FGF2, there is significant cardiac (systolic and diastolic) dysfunction after ischemia-reperfusion injury. Although Fg2 KO hearts had a poorer postischemic recovery of cardiac function compared with Wt hearts, myocardial infarct size

TABLE 2. Preischemic and Postischemic Functional Parameters for WT, Fgf2 KO, and FGF2 Tg Hearts

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>MAP</th>
<th>LVP</th>
<th>LVEDP</th>
<th>+dP/dt</th>
<th>−dP/dt</th>
<th>TPP</th>
<th>RT 1/2</th>
<th>LAP</th>
<th>MVO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preischemia</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>WT</td>
<td>339±4</td>
<td>50.2±0.1</td>
<td>90.2±1.2</td>
<td>10.0±1.1</td>
<td>3710±161</td>
<td>−2823±89</td>
<td>0.5±0.0</td>
<td>0.8±0.0</td>
<td>6.1±0.3</td>
<td>128±11</td>
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<td>Fgf2 KO</td>
<td>349±8</td>
<td>50.3±0.1</td>
<td>94.6±2.0*</td>
<td>8.4±0.6</td>
<td>3994±183</td>
<td>−3093±140</td>
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<td>0.7±0.0</td>
<td>5.9±0.3</td>
<td>122±16</td>
</tr>
<tr>
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<td>363±11</td>
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<td>96.5±1.4</td>
<td>4.9±0.3</td>
<td>3923±68</td>
<td>−3386±96</td>
<td>0.4±0.0</td>
<td>0.6±0.0</td>
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<td>Fgf2 Tg MHC20</td>
<td>358±13</td>
<td>50.1±0.1</td>
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<td>6.6±1.3</td>
<td>4025±130</td>
<td>−3290±159</td>
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<td>6.6±0.6</td>
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<td>Fgf2 Tg MHC25</td>
<td>378±11</td>
<td>50.1±0.1</td>
<td>99.0±1.6</td>
<td>7.1±2.5</td>
<td>4225±194</td>
<td>−3507±147</td>
<td>0.4±0.0</td>
<td>0.5±0.1</td>
<td>7.0±0.9</td>
<td>118±13†</td>
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<tr>
<td>120-minute reperfusion</td>
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<td></td>
<td></td>
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<tr>
<td>WT</td>
<td>342±3</td>
<td>35.7±4.6</td>
<td>63.3±8.4</td>
<td>20.9±2.8</td>
<td>2361±333</td>
<td>−1575±183</td>
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<td>Fgf2 KO</td>
<td>349±9</td>
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<td>1085±179*</td>
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<tr>
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<td>2219±358</td>
<td>−1685±231</td>
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<td>Fgf2 Tg MHC20</td>
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<td>3185±333*</td>
<td>−2152±269</td>
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<td>21.3±5.3</td>
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<tr>
<td>Fgf2 Tg MHC25</td>
<td>378±10</td>
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<td>88.9±3.1*</td>
<td>10.0±2.0*</td>
<td>3742±215*</td>
<td>−2488±113*</td>
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<td>0.7±0.1*</td>
<td>13.2±2.9</td>
<td>154±39</td>
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HR indicates heart rate (bpm); MAP, mean aortic pressure (mm Hg); LVP, peak systolic left ventricular pressure (mm Hg); LVEDP, left ventricular end-diastolic pressure (mm Hg); +dP/dt, rate of contractility (mm Hg per second); −dP/dt, rate of relaxation (mm Hg per second); TPP, time-to-peak left ventricular pressure normalized to contractility (ms/mm Hg); RT 1/2, half relaxation time normalized to relaxation (ms/mm Hg); LAP, left atrial pressure (mm Hg); and MVO2, myocardial oxygen consumption (µL/g heart wet weight). All values are mean±SEM.

*P<0.05 vs WT cohort.
†P<0.05 vs FGF2 Tg.
was similar in both groups, a finding consistent with other cardioprotective studies, demonstrating that the level of myocardial infarction is not always a predictor of postischemic improvement in left ventricular function produced by preconditioning.28,29 Conversely, overexpressing the human FGF2 gene specifically in the heart resulted in both an enhanced recovery of postischemic contractile function and a reduction in myocardial infarct size.

It has been suggested that induction of low-flow global and regional ischemia is better controlled in isolated perfused hearts (both Langendorff and work-performing preparations) of small mammalian animal models.30 Previous ex vivo murine studies of myocardial ischemia have been performed on the Langendorff isolated heart model; however, the work-performing heart preparation is a load-dependent (preload and afterload) model and has the advantage of measuring work-related indices of whole heart function during normal and ischemic episodes. In the working mode, the ischemic heart is a loaded system, which results in a supply-demand imbalance of oxygen and an accumulation of cytotoxic metabolites, whereas the Langendorff-isolated heart preparation is an unloaded, quiescent system with no significant oxygen imbalance during ischemia. Consequently, the isolated work-performing heart preparation is the model of choice for characterizing postischemic myocardial stunning.31

Figure 4. Representative Masson’s trichrome images of sham-treated Wt (A and C), Fgf2 KO (B), and FGF2 Tg (D) hearts and Wt (E and G), Fgf2 KO (F), and FGF2 Tg (H) hearts after 60 minutes of ischemia and 120 minutes of reperfusion. Contraction bands (†), vacuoles (▲), and wavy myofibrils (●) are all signs of cellular damage. Images at ×300.
FGF2 is widely detected during fetal development and in adults in many tissues including heart, and the actions of FGF2 are thought to mediate critical roles in early embryogenesis, organogenesis, maintenance of adult function, and neovascularization in response to disease and tissue injury. In the heart, FGF2 has been localized to extracellular, cytoplasmic, and nuclear environments in developmental and adult cardiac myocytes and nonmyocytes. Increased levels of FGF2 have been detected in ischemic cardiac tissue and in pericardial fluid, serum, or urine from patients with myocardial ischemia. Those increases of FGF2 are similar to the increase measured in the coronary effluent of Tg hearts before and during ischemia-reperfusion injury (≈2-fold increase, see Figure 6). Unexpectedly, the level of FGF2 release from Wt or Tg hearts was not increased additionally during reperfusion compared with the baseline measurements.

Studies have demonstrated that administration of FGF2 to nonischemic rat hearts induced a negative inotropic action, suggesting that this growth factor may contribute to myocardial dysfunction associated with ischemia-reperfusion injury or, conversely, may contribute to the cardioprotective phenotype through suppression of energy requirements. Cardiac contractility and heart rate are 2 of the 4 major determinants of myocardial oxygen demand and can influence the degree of ischemic injury. We did not observe any significant basal heart rate or cardiac function differences, as measured by +dP/dt and −dP/dt, in Tg KO or Tg Tg hearts compared with Wt hearts (see Table 2); however, the Tg Tg hearts recovered significantly better than Wt or FGf2 KO hearts. This suggests that FGF2 overexpression may be providing a short-term or long-term defense against ischemia-reperfusion injury either by preserving energy production or upregulating potential cardioprotective pathways. There is recent evidence that increased levels of ATP and creatine phosphate were detected in FGF2-treated rat hearts after ischemia-reperfusion injury, which supports our findings that FGF2 may preserve energy by maintaining appropriate oxygen supply/demand balance, thereby reducing myocardial damage. Furthermore, FGF2 is known to signal through 2 different protein kinase pathways, protein kinase C and the mitogen-activated protein kinases. Studies are presently underway to identify those cardioprotective signaling pathways involved in FGF2-induced cardioprotection in this low-flow (ie, coronary artery stenosis) ischemia setting. For example, protein kinase C or inducible nitric oxide synthase, both signaling pathways implicated in other models of cardioprotection, have recently been demonstrated to be involved in postischemic recovery of contractile function when hearts were treated with FGF22, suggesting that these signaling pathways may also mediate the protective effect in our FGF2 murine model of ischemia-reperfusion injury.

Answers to questions concerning functional differences between endogenously expressed and exogenously applied FGF2 and how this relates to ischemia-reperfusion injury and cardioprotection remain elusive. For example, Sheikh et al demonstrated that striated muscle overexpression of the rat Fgf2 cDNA coding for the low-molecular-weight isoform resulted in a reduction in lactate dehydrogenase release, a biomarker indicating cell injury, without improvement in postischemic recovery of contractile function during global ischemia. However, administration of FGF2 before ischemia attenuated myocardial stunning in the mouse heart, suggesting a role of FGF2 in maintaining cardiac function after ischemia-reperfusion injury. We have demonstrated that the presence of all of the FGF2 isoforms protects the murine heart against both cardiac dysfunction and myocardial infarction. The differences among the mouse models suggest that expression of or treatment with a particular FGF2 isoform may dictate the cardioprotective end point affected.

FGF2 is a potent angiogenic factor; however, in our model, there were no significant differences in blood vessel (smooth muscle-containing) or capillary number between Tg Tg and Wt hearts. This observation is opposite that in the study by Sheikh et al, in which overexpression of the low-molecular-weight FGF2 isoform resulted in a significant increase in capillary density compared with Wt murine hearts, again implicating different biologic functions of the FGF2 protein isoforms. Also, our findings demonstrated that vascular growth was not affected in KO KO hearts, which was
consistent with a Fgf2 KO mouse study of vascular growth in skeletal muscle.13 These findings indicate that other angiogenic factors may mediate angiogenesis and vasculogenesis or that angiogenesis is not triggered in the myocardium until an ischemic or hypoxic event occurs, which then activates growth factor release and angiogenic signaling. Furthermore, we reported that there was no correlation between recovery of function or myocardial infarct size and coronary flow. Our findings are similar to other studies in which the cardioprotective effect of molecules such as adenosine or proteins such as ATP-sensitive potassium channels did not correlate with hemodynamic alterations.37

In summary, our results demonstrate an important role for FGF2 in the cardioprotective processes participating in post-ischemic recovery of cardiac function and in the reduction of myocardial infarct size. These cardioprotective actions of FGF2 occurred rapidly and are independent of its mitogenic/angiogenic activities. Overall, our findings may provide insight into the specific pathways in which FGF2 is involved in cardioprotection and could lead to the development of novel therapeutic strategies to protect the heart in patients susceptible to coronary artery disease.

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