

Efficacy of Intracoronary Versus Intravenous FGF-2 in a Pig Model of Chronic Myocardial Ischemia

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Background. Therapeutic angiogenesis in ischemic myocardium has been shown to be a feasible and effective strategy to improve regional blood flow and myocardial function. However, the optimal mode of growth factor administration still needs to be established.

Methods. Using a pig model of chronic myocardial ischemia, we evaluated the efficacy of intravenous and intracoronary infusion of FGF-2 at 2 and 6 $\mu\text{g}/\text{kg}$ compared with a vehicle control. Improvement in myocardial perfusion and function was assessed by angiography, colored microspheres, and function and perfusion magnetic resonance imaging.

Results. Intracoronary 6- $\mu\text{g}/\text{kg}$ FGF-2 increased angiographic collaterals ($p = 0.046$) and increased regional

blood flow to the ischemic area from 0.36 ± 0.07 to 0.59 ± 0.08 mL/min/g at stress (vs control, $p = 0.032$). Also, after 6 $\mu\text{g}/\text{kg}$ intracoronary FGF-2, ejection fraction, regional wall motion, and thickening improved significantly by $9.9\% \pm 1.9\%$, $126\% \pm 39\%$, and $13.8\% \pm 3.6\%$, respectively. Intravenous FGF-2 and intracoronary 2 $\mu\text{g}/\text{kg}$ FGF-2 were ineffective.

Conclusions. A single 6- $\mu\text{g}/\text{kg}$ intracoronary treatment with FGF-2 resulted in significant improvement in collateralization and regional and global function of chronically ischemic myocardium. Single intravenous infusion of FGF-2 was not effective in this model.

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Various growth factors, such as basic fibroblast growth factor (bFGF or FGF-2) and vascular endothelial growth factor (VEGF), are being studied as a safe and potentially effective therapy to reduce myocardial ischemia either in conjunction with bypass surgery or percutaneous catheter-based recanalization, or as a monotherapy in "no option" patients [1-3]. All the current strategies involve invasive approaches in high-risk patients to deliver the growth factors locally. At this stage, however, the optimal dose regimen and delivery technique for these angiogenic agents are not clear and little data on the efficacy of intravenous delivery exist.

In preclinical studies, in pig or canine models of chronic myocardial ischemia, others and we have shown that continuous [4] and single intrapericardial [5] or repeated intracoronary [6] administration improved regional blood flow in the ischemic territory. Improvement in cardiac function was observed only after periadventitial delivery [4] and intrapericardial delivery [5]. With the exception of periadventitial and pericardial delivery, most of the delivery strategies are limited to perioperative procedures. To expand the patient population that might benefit from therapeutic angiogenesis by FGF-2, a better applicable and effective mode of delivery needs to be defined. The general appreciation of the binding of FGF-2 to extracellular matrix proteins, especially in the presence of an ischemia or inflammation, thus creating a local reservoir of active growth factor, prompted us to study the effect of a single injection of FGF-2.

In this study, we studied the efficacy of single intravenous and single intracoronary FGF-2 infusion with respect to myocardial perfusion and function in a pig model of chronic myocardial ischemia.

Material and Methods

Male Yorkshire pigs ($n = 57$; Parsons, Hadley, MA) weighing 15 to 30 kg were used for this study. The chronic ischemia model consisted of three phases as previously described [4, 7]. In brief, for ameroid surgery and catheterization at 3 and 6 weeks, the animals were anesthetized with Ketamine 20 mg/kg IM and pentothal 10 mg/kg IV, intubated, mechanically ventilated, and further anesthetized with 1.5% to 2.5% isoflurane in room air. Postoperatively, all animals received antibiotics and analgesics for 48 hours. Animal care was performed according to the National Institutes of Health's *Guidelines for the Care and Use of Laboratory Animals*, and the protocol was approved by the Institutional Animal Care Committee.

A plastic ameroid (inner diameter, 2 to 2.5 mm; Research Instruments, Escondido, CA) was placed on the proximal left circumflex artery (LCX) or a major side branch, through a left lateral fourth intercostal thoracotomy. Three weeks (second phase, midstudy) later, right and left coronary catheterization was performed through a standard femoral cut-down after systemic anticoagulation with Heparin 100 U/kg. Intraarterial pressure and electrocardiogram were continuously recorded. Selective

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left and right angiography (General Electric, Waukesha, WI; contrast: Renografin; Squibb Diagnostics, Princeton, NJ) confirmed complete occlusion of the LCX and allowed assessment of baseline flow and the presence of collaterals in the LCX territory, according to the Rentrop scoring system from 0 to 3, as previously described [4]: 0 = none; 1 = filling of side branches of the LCX; 2 = partial filling of the LCX main artery via collateral channels; 3 = complete filling of the LCX. Angiographic analysis was blinded to treatment. For regional blood flow measurements, colored microspheres were injected into the left atrium (see below). Directly after this, function, perfusion, and collateral sensitive magnetic resonance imaging (MRI) was performed on all animals to quantify baseline regional cardiac function and perfusion before start of the treatment.

Pigs were then randomly assigned to one of the following treatments: 1) vehicle control; 2) 2 $\mu\text{g}/\text{kg}$ rFGF-2 IV; 3) 6 $\mu\text{g}/\text{kg}$ rFGF-2 IV; 4) 2 $\mu\text{g}/\text{kg}$ rFGF-2 IC; 5) 6 $\mu\text{g}/\text{kg}$ rFGF-2 IC. Five minutes before FGF-2 administrations, heparin (70 U/kg, IV) was given. Bovine recombinant FGF-2 (rFGF-2; Chiron Corporation, Emeryville, CA) was dissolved and diluted in vehicle consisting of 10 mmol/L sodium citrate, 10 mmol/L thioglycerol, 135 mmol/L sodium chloride, 100 mmol/L EDTA, pH 5.0. The intracoronary FGF-2 was equally divided and infused into the right coronary artery (RCA) and the proximal LCX using a 3F Cordis infusion catheter. Intravenous infusions were given through an ear vein. In short proximal LCX stumps, FGF-2 was delivered into the proximal part of the LAD. The vehicle control group consisted of animals that received intravenous vehicle ($n = 4$) or intracoronary vehicle ($n = 4$).

Three weeks after therapy (third phase, final study), repeat selective angiograms were made and two sets of colored microspheres were injected into the left atrium, one before (rest) and one after injection of Adenosine 1.25 mg/kg IV (stress). Function and perfusion MRI was also repeated in all animals. Finally, animals were euthanized and the hearts were excised.

Regional Blood Flow

For microspheres injection into the left atrium, a 7F JL4 catheter was retrogradely advanced across the aortic and mitral valve into the left atrium. The left atrial position of the catheter was confirmed by contrast injection and the presence of an atrial pressure waveform. At midstudy, and during the final study at rest and stress, 6×10^6 microspheres (Dye Trac; Triton Technologies, San Diego, CA) were injected according to a standard protocol [4]. Reference blood samples were drawn simultaneously. At the end of the study (final study), a mid papillary, 1-cm-thick cross section of left ventricle was taken and divided into eight radial segments. The segment in the LCX territory was further subdivided in an endocardial and epicardial piece. Tissue samples and reference blood samples were digested and the microspheres retrieved according to the manufacturers protocol. The samples were analyzed with a spectrophotometer (SU 600; Beckman, Fullerton, CA). From the optical density (OD)

measurements, the myocardial flow was calculated as blood flow: (tissue sample X; mL/min/g) = [withdrawal rate (mL/min)/weight (tissue sample X; g)] \times [OD (tissue sample X)/OD (reference blood sample)], using the Excel worksheet and macros provided by the manufacturer.

Myocardial MRI Analysis

Arterial pulse-gated MRI was performed on anesthetized (1% to 2% isoflurane) and ventilated animals, in the body coil of a 1.5-Tesla whole-body (Siemens, Munich Germany) Vision prototype. Baseline anatomic images were obtained by a turboFLASH technique to identify coordinates for apical four-chamber, two-chamber, and short-axis views. For function studies, 24 sequential image frames were collected over 12 heartbeats during breath-hold using shared-center turboFLASH in each of the three standard views. After detection of the optimal inversion time (TI; typically 200 to 300 ms), a series of 32 diastolic images were acquired in the double-oblique four-chamber view during breath-hold, while injecting 0.05 mmol/kg gadodiamide (T1-reducing contrast agent). The series of images was viewed as a movie, to locate the zone with impaired contrast arrival. The short axis at the center of that zone (target zone) was prescribed graphically. All measurements were performed by two independent investigators blinded to treatment. Custom-designed software was used to define myocardial borders and measure wall thickness. End-systolic and end-diastolic left ventricular volumes were computed from biplane measurement (apical four-chamber and two-chamber views) as previously validated, and used to calculate left ventricular ejection fraction. Target wall motion (radial shortening) and target wall thickening were expressed as percentage of the radial length or wall thickness at the end of diastole. Both parameters were also measured at the septum, yielding normal target wall motion and target wall thickening. The area of delayed contrast arrival was defined as myocardium demonstrating distinctly slowed time (≥ 1 cardiac cycle) to half-maximal signal intensity, using a two-dimensional map of contrast intensity versus time [8].

Toxicologic Assessment of FGF-2 Administration

Before treatment and at necropsy, blood samples for hematology, coagulation, and serum chemistry were obtained from at least three fasted animals per group. Hematology parameters included hemoglobin, mean corpuscular hemoglobin concentration, hematocrit, erythrocyte count, total leukocyte count, differential, platelet count, mean corpuscular hemoglobin, and mean corpuscular volume. Serum chemistry included aspartate aminotransferase, alanine aminotransferase, gamma glutyltransferase, alkaline phosphatase, lactate dehydrogenase, total bilirubin, total cholesterol, triglycerides, blood urea nitrogen, creatinine, creatine phosphokinase, albumin, globulin, total protein, electrolytes (Na, K, and Cl), calcium, phosphorus, and glucose.

In addition, for four randomly selected animals in each treatment (not vehicle) group, tissue samples were taken from major organs and processed for histology. His-

topathological findings were graded on a scale of 1 to 4 (minimal < mild < moderate < marked), by a veterinary pathologist blinded to treatment.

Data Representation and Statistical Analysis

All data are presented as mean \pm SEM, unless indicated otherwise. Pre- and posttreatment measurements were evaluated per treatment group by a paired *t* test. In addition, analysis of variance (ANOVA) with a Bonferroni corrected post hoc analysis was done to compare differences between groups. Ordinate parameters were analyzed with a Kruskal Wallis test to evaluate differences between groups, and by the Wilcoxon test to analyze for paired evaluations within the groups. Differences with a *p* value <0.05 were considered significant.

Results

Fifty-seven animals received an ameroid constrictor and 13 animals died before initiation of treatment. Forty-four animals (control, *n* = 10; FGF 2 μ g/kg IV, *n* = 9; FGF 6 μ g/kg IV, *n* = 9; FGF 2 μ g/kg IC, *n* = 8; and FGF 6 μ g/kg IC, *n* = 8) completed the entire study.

Hemodynamic Parameters

Intravenous infusion caused a mild but significant decrease in blood pressure of 12.3 ± 3.7 mm Hg (*p* = 0.02) in the FGF 2 μ g/kg IV group and 9.6 ± 2.1 mm Hg (*p* = 0.01) in the FGF 6 μ g/kg IV group. After intracoronary infusion, the drop in blood pressure was significant only at 2 μ g/kg with 10.0 ± 2.2 mm Hg (*p* = 0.04) and not at 6 μ g/kg (6.1 ± 4.9 mm Hg, *p* = 0.25). In all groups, heart rate decreased mildly, ranging from 2 to 15 bpm, but was significant only in the FGF 2 μ g/kg IV with 9 ± 4 bpm (*p* = 0.05) and 6 μ g/kg IC group with 18 ± 6 bpm (*p* = 0.03).

Coronary Angiography

Seven follow-up angiograms, two in the control group, two in the FGF 2 μ g/kg IV, one in the FGF 6 μ g/kg IV, and two in the FGF 2 μ g/kg IC group, were not available for analysis. Collateral index had improved significantly in the 6 μ g/kg IV group and in both 2 and 6 μ g/kg IC groups (Fig 1), whereas baseline collateral index was similar (*p* = 0.119, Kruskal Wallis). For all groups pooled, collateral index resulted from left-to-left collaterals (either LAD to LCX or LCX to LCX, *n* = 37; *p* < 0.001, McNemar test) and not from right-to-left (*p* = 1.0), suggesting a localized effect of intravascular drug delivery. However, changes were not significant in any subgroup.

Coronary Blood Flow

Baseline regional blood flow in the ischemic (LCX) and normal (LAD) territories was measured at rest and post-treatment (final study) at rest and stress (adenosine). Absolute ischemic flow (mL/min/g tissue) and the LCX/LAD flow ratio were determined. LAD flow at baseline, rest, and stress at the final study were similar in the five groups (ANOVA, *p* = 0.363, *p* = 0.418, and *p* = 0.331, respectively). Rest LAD flow did not change significantly over time (ANOVA, *p* = 0.266). In addition, LCX coro-

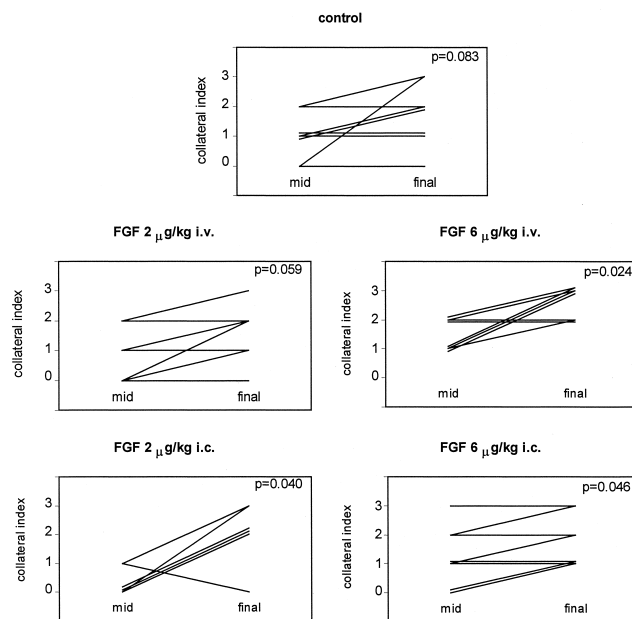


Fig 1. Line plots of collateral index scores at treatment (mid) and 3 weeks after treatment (final). Each line represents one case; to represent each experiment, the lines that overlapped completely were translated 0.1 on the y scale. The paired data were analyzed with a Wilcoxon test and the baseline collateral indices for all groups were analyzed using Kruskal Wallis.

nary blood flow at baseline (before FGF2 infusion) was similar in all five groups (ANOVA, *p* = 0.361). At the final study at rest, absolute LCX flow and the LCX/LAD ratio did not change significantly (Fig 2A). However, LCX flow at stress was significantly higher in the FGF 6 μ g/kg IC group than in controls (ANOVA, *p* = 0.039; Fig 2B).

MRI: Left Ventricular Function

Infarct size visualized as myocardium without MRI contrast uptake was measured to avoid confounding of regional function and perfusion measurements. Infarct size was similar among the five groups at either baseline (3 weeks) or final study (ANOVA, *p* = 0.594 and *p* = 0.303, respectively). Infarct size, $3.0\% \pm 4.9\%$ left ventricular area (mean \pm SD), was within the range reported for this model [7].

Left ventricular ejection fraction (EF) at baseline was similar for all treatment groups (ANOVA, *p* = 0.120). Using each animal as its own control, EF improved significantly in controls (*p* = 0.018), in the FGF 2 μ g/kg IV (*p* = 0.046), the FGF 6 μ g/kg IV (*p* = 0.001), and the FGF 6 μ g/kg IC groups (*p* = 0.001). The improvement in EF after treatment was significantly higher in the FGF 6 μ g/kg IC (*p* < 0.01) group compared with controls (Fig 3). The improvement in indexed target wall motion (target wall motion/normal wall motion) was significant only in the FGF 6 μ g/kg IV (*p* = 0.019) and the FGF 6 μ g/kg IC groups (*p* = 0.004), whereas indexed target wall thickening improved in the FGF 6 μ g/kg IC group (ANOVA, *p* = 0.007 compared with improved target wall thickening in controls, *p* = 0.001) (Table 1).

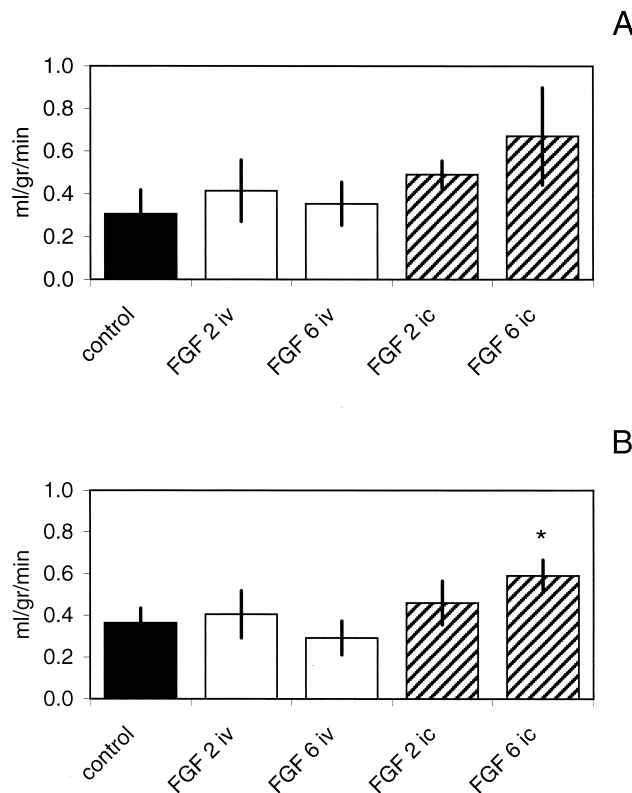


Fig 2. Regional blood flow changes in the ischemic area between midstudy and final study at rest (A) and regional blood flow at stress during the final study (B). Regional blood flow in the ischemic area under stress conditions (Adenosine 1.25 mg/kg IV) 3 weeks after treatment was higher in the FGF 6 μ g/kg IC group (* $p = 0.039$).

MRI: Perfusion

At baseline, no differences in areas of delayed arrival (ANOVA, $p = 0.140$) or collateral extent ($p = 0.103$) were found between the groups. The size of the zone of

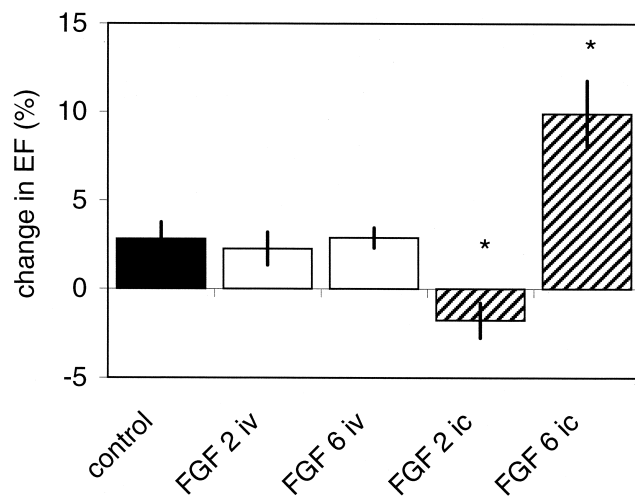


Fig 3. Change in EF from midstudy to final study. Ejection fraction was assessed by MRI. Baseline ejection fractions were similar in all groups and improved significantly by FGF 6 μ g/kg IC and deteriorated significantly in the FGF 2 μ g/kg IC group (* $p = 0.01$).

A delayed arrival decreased in the FGF 6 μ g/kg IC ($p < 0.001$; Fig 4), which was significantly different from the change in controls (ANOVA, $p < 0.001$).

Toxicologic Assessment (Data Not Shown)

There were no macroscopic or microscopic lesions related to intravenous or intracoronary administration of FGF-2. Furthermore, no changes in hematological or biochemical parameters were observed in any of the treatment groups.

Comment

Basic fibroblast growth factor (FGF-2) is a potent angiogenic growth factor that is currently under study for treatment of myocardial ischemia. FGF-2 stimulates endothelial cell proliferation, migration, and vascular tube formation in three-dimensional cultures in collagen [9] as well as angiogenesis in an in vivo Matrigel pellet assay [10]. The angiogenic efficacy of FGF-2 was confirmed in numerous animal models [11, 12].

Although the first phase I clinical trial has shown the safety of intracoronary FGF-2 over a wide range of dosages [3], the optimal dose regimen and delivery strategy for efficacy with respect to cardiac angiogenesis have not been established yet. We chose a porcine ameroid model for preclinical testing of delivery strategies because of several unique aspects. First, the ameroid occluder results in consistent and gradual occlusion of the LCX, resulting in minimal myocardial necrosis, but reduced regional myocardial function, which is detectable with various noninvasive imaging modalities. Because an effect of estrogen on cardiac angiogenesis cannot be ruled out and synchronization of these studies with the menstrual cycle is logistically impossible, we excluded females from this study. We have shown previously that FGF-2 was effective in this model when given either periaortally in a sustained-release formulation [4] or by single intrapericardial injection [5]. In a similar model in dogs, daily intracoronary injections of FGF-2 [6] also induced increased vascularity of ischemic myocardium. Although very encouraging, there are little data considering the efficacy of single intravascular administration of angiogenic growth factors.

In this study, in which we compared the efficacy of intravenous and intracoronary delivery of 2 or 6 μ g/kg FGF-2, blood supply to the myocardium, as assessed by the colored microsphere method, improved by the high-dose (6 μ g/kg) intracoronary FGF-2. Although this effect was only significant at stress, the same trend was seen for regional blood flow at rest. Both intravenous FGF-2 doses as well as the 2- μ g/kg dose were ineffective. This change in regional blood flow was confirmed by perfusion and collateral-sensitive MRI, and had functional significance because it was accompanied by an increase in EF and improvement in target wall motion and target wall thickening in the high-dose intracoronary group. The effect on EF was added to the natural tendency to grow collaterals and improve perfusion and function of ischemic myocardium.

In other studies with different end points, a single

Table 1. Indexed Target Wall Motion (ITWM) and Target Wall Thickness (ITWT) and Change From Mid to Final Study

Treatment	n	ITWM, Baseline	ITWM, Final	Change ITWM (%)	ITWT, Baseline	ITWT, Final	Change ITWT (%)
control	9	0.32 ± 0.07	0.36 ± 0.07	30.6 ± 20.8	0.54 ± 0.06	0.59 ± 0.08	1.4 ± 1.7
FGF 2iv	8	0.34 ± 0.06	0.46 ± 0.07	51.8 ± 25.6	0.43 ± 0.09	0.43 ± 0.11	3.8 ± 3.3
FGF 6iv	9	0.28 ± 0.08	0.44 ± 0.11	108.2 ± 44.4 ^a	0.23 ± 0.05	0.33 ± 0.07	4.2 ± 2.5
FGF 2ic	7	0.46 ± 0.13	0.47 ± 0.11	22.7 ± 26.5	0.46 ± 0.14	0.44 ± 0.13	-2.2 ± 2.5
FGF 6ic	8	0.36 ± 0.08	0.67 ± 0.12	126.4 ± 39.0 ^{a,b}	0.21 ± 0.07	0.31 ± 0.07	13.8 ± 3.6 ^{a,b}

Data are mean ± SD.

^a Baseline versus final. ^b Compared to change.

ITWM = indexed target wall motion; ITWT = indexed target wall thickening.

intracoronary injection of FGF-2 has been effective [13, 14]. The current study, however, presents the first evidence that a single intracoronary injection of 120 to 150 µg FGF-2 improves regional blood flow as well as regional and global cardiac function. The ineffectiveness of intravenous FGF-2 might result from less favorable pharmacokinetics. Several studies have reported a 3- to 10-fold lower recovery of radiolabeled FGF-2 from the myocardium after intravenous administration than after intracoronary injection [15, 16], which in turn has a lower recovery and shorter redistribution times than intrapericardially delivered FGF-2 [5, 17]. FGF-2 might be retained in the myocardium by a high-capacity, low-affinity sink provided by heparan sulfates in the matrix and on the surface of endothelial cells, which are upregulated by ischemia [18]. In addition, expression of FGF-R1 receptors, which are the primary transducers of FGF-2 signaling, is also upregulated by ischemia [19, 20].

In this animal study, in accordance with the phase I clinical trial, intravenous FGF-2 and 2 µg/kg intracoronary FGF-2 had no major hemodynamic, hematologic, or biochemical side effects.

Clinical Implications

If a single intracoronary infusion of FGF-2 proves to be effective in patients with chronically ischemic myocardium,

this strategy will greatly increase the number of patients that might benefit from adjunctive growth factor therapy, especially in view of the minimal side effects [3]. Each patient undergoing percutaneous revascularization is a candidate for angiogenic therapy because most interventions are local and aimed at the most severe stenoses in epicardial arteries. The additional benefit of myocardial salvage during reperfusion injury by FGF-2 further emphasizes the potential value of this adjunct pharmacotherapy [21].

Conclusions

It is concluded that a single 6-µg/kg intracoronary FGF-2 delivery results in significant improvement in collateralization and regional and global function of chronically ischemic myocardium. A single intravenous infusion of FGF-2 is ineffective in the doses tested. A phase II clinical trial of patients with coronary artery disease designed to evaluate this intracoronary therapeutic strategy is currently underway.

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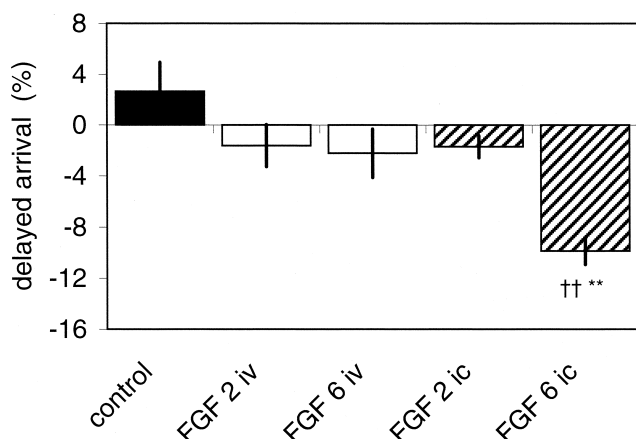


Fig 4. Perfusion-sensitive MRI. Change in delayed arrival from midstudy to final study. Delayed arrival decreased in the FGF 6 µg/kg IC group (**p = 0.001). This decrease was significantly different from control (††p = 0.001).

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INVITED COMMENTARY

A growing number of experimental studies continue to strengthen the evidence base suggesting the feasibility of administering angiogenic growth factor as a means of "biologically" revascularizing ischemic tissues. This strategy, termed therapeutic angiogenesis, has now been shown to induce the neovascularization of a wide variety of tissues. A number of studies, including this report by Sato and associates, suggest that this neovascularization response to growth factor delivery is sufficiently robust to significantly enhance myocardial function, as well as perfusion. In fact, similarly effective results with a wide variety of angiogenic mediators have thus far made it difficult to discern a relative efficacy advantage of one of these mediators as compared with any others. As noted by Sato and associates, the most significant difference in reported results of angiogenic regimens appears to be in regard to method of delivery. For the treatment of cardiac disease, localized intracoronary or intramyocardial therapy appears to be uniformly superior to systemic therapy. While intracoronary therapy yielded significant results in the present study, other reports suggest potentially advantageous pharmacokinetics with intramyocardial as opposed to intracoronary delivery. In this regard, one unanswered question is whether the potential ease of catheter-based intracoronary delivery outweighs the potentially superior efficacy of intramyocardial delivery of angiogenic growth factors (whether delivered "surgically" via an epicardial route or percutaneously via an endocardial route).

An even more daunting challenge to the promise of angiogenic therapy is presented by the apparent discrepancy between the robust responses noted in experimental angiogenic models in "normal" tissues such as in the present study, and the somewhat less profound responses reported from initial clinical trials. It is unknown whether this discrepancy represents a "false negative" due to the limitations of clinical diagnostic methodologies, or whether the challenge of inducing angiogenesis in a potentially unfavorable milieu (elderly, diabetic, hypercholesterolemic, atherosclerotic subjects) is a true barrier to clinical success. More stringent experimental studies and clinical trials, possibly involving more sophisticated angiogenic "cocktails" or sequenced growth factor administration regimens, in addition to the adaptation of refined diagnostic modalities, will likely be needed before this question can be answered. While the initial data regarding angiogenic therapies remain encouraging, much work remains ahead.

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