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The Effect of Intracoronary Fibroblast Growth Factor-2 on Restenosis after Primary Angioplasty or Stent Placement in a Pig Model of Atherosclerosis

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Summary

Background: Therapeutic angiogenesis, if combined with primary percutaneous transluminal coronary angioplasty or stent placement, could improve the outcome of patients suffering from multifocal coronary disease.

Hypothesis: Because of the concern that angiogenic growth factors might promote restenosis, we studied the effect of a single intracoronary administration of recombinant fibroblast growth factor (rFGF)-2 on restenosis after balloon angioplasty and stent placement in a pig model of coronary atherosclerosis.

Methods: In 24 Yucatan minipigs, coronary lesions were induced by arterial injury and 3 months of atherogenic diet. After 3 months, repeat catheterization was performed with balloon dilation or stent placement at the injured sites, with a follow-up of 6 weeks. Results were monitored using quantitative angiography, intravascular ultrasound (IVUS), and histomorphometry.

Results: Intracoronary rFGF-2 2 μg/kg did not affect neointima formation or remodeling in this model. A small but significant aggravation of late lumen loss was observed in the reference segments of the rFGF-2-treated group. Angiographic and echographic late lumen loss, intimal hyperplasia, and arterial remodeling, as well as histologic neointima were all similar in the rFGF-2- and the vehicle-treated group. Confirming earlier studies from our group and those of others, stented arteries compared with balloon-dilated arteries had increased angiographic late lumen loss, a trend toward increased intimal hyperplasia and decreased remodeling.

Conclusion: We conclude that rFGF-2 does not aggravate restenosis after balloon dilation or stenting in this pig model of coronary atherosclerosis. Future combinations of angioplasty and therapeutic angiogenesis in a single session should be pursued as a feasible and safe strategy.

Key words: angiogenesis, pig, balloon angioplasty, stent, restenosis, growth factors, coronary disease

Introduction

Therapeutic angiogenesis has gained considerable attention as an alternative strategy for revascularizing ischemic myocardium that is not amenable to catheter-based revascularization or coronary artery bypass grafting (CABG).[1–4] Recently, therapeutic angiogenesis with vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (FGF-2) has entered clinical trials as an adjunctive therapy to CABG.[5–7] Likewise, patients who are treated with stent placement or percutaneous transluminal coronary angioplasty (PTCA), might benefit from adjunct angiogenic growth factor treatment to target ischemic areas not covered by the intervention. However, since FGF-2 is a nonselective mitogen, stimulates proliferation of smooth muscle cells,[8] and was associated with intimal hyperplasia,[9] FGF-2 might enhance restenosis. Studies in nonatherosclerotic rat, rabbit, or canine animal models on the effects of recombinant fibroblast growth factor (rFGF-2) on intimal hyperplasia after balloon angioplasty are contradictory[10–12] and might have limited relevance to the response of a coronary artery with an advanced atherosclerotic lesion to stents. Therefore, we decided to study the healing response of coronary arteries after PTCA and stent placement with adjunctive treatment of rFGF-2 in an established pig model of atherosclerosis.[13–15]

Methods

Yucatan Model of Atherosclerosis

All animals underwent three procedures: initial injury, an intervention and treatment session, and the final study.

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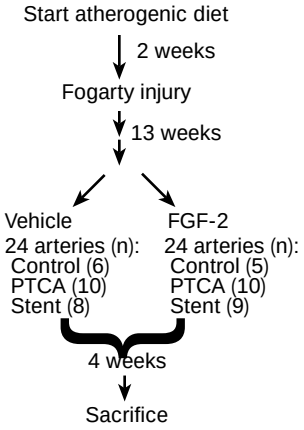

FIG. 1 Layout of the study. PTCA = percutaneous transluminal coronary angioplasty, FGF-2 = fibroblast growth factor-2.

The animals were preanesthetized with ketamine 10 mg/kg, intubated, and ventilated with room air and isoflurane 2–3%. Peri- and postoperative treatment consisted of bretylium tosylate 4 mg/kg intravenously (IV), IV heparin to maintain an activated clotting time > 300 s, a single intramuscular dose of 0.02 mg/kg buprenorphine, and cefazolin 0.5 gm IV. Antiplatelet therapy started 1 day before intervention with acetylsalicylic acid (325 mg, 2 days) and ticlopidine hydrochloride (250 mg, b.i.d., 14 days). A #8 Fr sheath (Cordis, Miami, Fla., USA) was advanced through a femoral cutdown, which was repaired for second use during the intervention. All animals received humane care in compliance with the Institutional Animal Care and Use Committee at Beth Israel Deaconess Medical Center and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Male Yucatan minipigs (10–15 kg) received an atherogenic diet (Purina minipig starter diet with 1.5% cholesterol and 6% peanut oil) that was continued until final study. Two weeks later, coronary angiograms were made and the left coronary circumflex (LCx) and the right coronary artery (RCA) were injured with a standard angioplasty balloon (Guidant, Santa Clara, Calif., USA), at 8 atm with a dilation ratio of 1.2.

Thirteen weeks later, animals received intervention and treatment randomized for artery and animal, respectively, prior to baseline studies. Baseline selective quantitative coronary angiography (QCA) and intravascular ultrasound (IVUS) of the LCx and RCA were made. Stent placement or PTCA was performed at the site of maximum angiographic stenosis or maximal plaque load on IVUS. After intervention, QCA and IVUS were repeated.

At the final study 6 weeks after intervention, QCA and IVUS of the LCx and RCA were repeated, the animals euthanized, and the hearts excised.

Study Design and Drug Administration (Fig. 1)

The RCA and LCx were randomly assigned to PTCA or stenting, and in each group four arteries were assigned to "no intervention." Following intervention, 12 animals were infused (10 min per artery, #2.5 Fr coronary infusion catheter, ACS, Temecula, Calif., USA) with bovine recombinant fibroblast growth factor (rFGF-2, Chiron Corporation, Emeryville, Calif., USA) at a total dose of 2 µg/kg, equally distributed over the two treated arteries. Twelve animals received vehicle. The rFGF-2 vehicle consisted of 10 mM sodium citrate, 10 mM thioglycerol, 135 mM sodium chloride, and 100 mM EDTA, pH 5.0.

Balloon Angioplasty and Stent Placement

A #8 Fr guiding catheter (Cordis) was placed in the left or right coronary ostium. For PTCA, a standard angioplasty balloon, 3–4.5 mm wide, 20 mm long, (Guidant) was introduced over a 0.014" guidewire to the selected site of angioplasty. Three 1 min inflations were performed at a nominal pressure to a dilation ratio of 1.2 according to the manufacturers table and artery diameter on IVUS.

Palmaz-Schatz biliary stents (5–9 mm wide, 15 mm, Johnson and Johnson, Warren, N.J., USA) were crimped on a angioplasty balloon. After placement over a 0.014" guidewire, the stent was deployed by a 10 s inflation at 4 atm. If necessary for a dilation ratio of 1.2 or optimal apposition of the stent, postdilatation was performed.

Coronary Angiography

Coronary angiograms were made at initial injury, before and after intervention, and at follow-up (FU). Before angiography, 2 ml of a 125 µg/ml nitroglycerin and 2.5 mg/ml papaverine cocktail (NP cocktail) was injected intracoronary. Side branches were used to localize the site of injury and intervention during follow-up angiography and intervention. Cine QCA (Model LU, GE Medical, Milwaukee, Wis., USA) was performed in standard right and left anterior oblique projections (contrast: Renografin, Squibb, Princeton, N.J., USA) using a #7-FR JR4 diagnostic angiography catheter (Cordis). The cine films were analyzed off-line, using 4× magnified end-diastolic frames and digital calipers. The tip of the guiding catheter was used for calibration.

The site with minimum lumen diameter (MLD) prior to the intervention (PRE) was used for calculation of acute gain, late lumen loss, angiographic stenosis, and dilation ratio. The reference lumen diameter (RLD) was calculated as the average of proximal and distal reference. Angiographic acute gain was defined as post minus preintervention MLD ($MLD_{POST} - MLD_{PRE}$), and late lumen loss was defined as $MLD_{POST} - MLD_{FU}$ or $MLD_{PRE} - MLD_{FU}$ for "no intervention" arteries. Angiographic stenosis was calculated as (1-MLD/RLD) × 100. Dilation ratio = balloon diameter on fluoroscopy/RLD.

Coronary Intravascular Ultrasound

For IVUS, we used a #2.9 F Microrail 30 MHz coronary imaging catheter (CVIS Inc., Sunnyvale, Calif., USA). The NP cocktail was injected into each artery. The catheter was ad-

vanced over a 0.014" guidewire, with the tip distal from the lesion/intervention site and manually withdrawn while imaging. At MLD, two peripheral sites of the lesion, and at the distal and proximal reference, the catheter was held for still images. Off-line video-tape analysis and quantification of a proximal and a distal reference and three evenly distributed cross sections at the level of intervention were performed using software available in the CVIS console. Lumen area (LA), media-bounded area (MBA), and stent area (SA) were manually traced. Precise sites of measurements were localized by fluoroscopy of the IVUS catheter tip with reference to side branches. Prior to stent placement or PTCA, LA and MBA were used to calculate area stenosis ($100 \times LA_{MLD}/MBA_{MLD}$), LA stenosis ($100 \times \{1\text{-}LA_{MLD}/LA_{REF}\}$), and plaque area (PA = MBA − LA). The immediate gain of IVUS was defined as $LA_{POST} - LA_{PRE}$ and late loss as $LA_{POST} - LA_{FU}$ (mm^2) or $LA_{PRE} - LA_{FU}$ for "no intervention" arteries. Intima gain was calculated from MBA or SA and LA at follow-up and at intervention, assuming constant plaque load and within-stent growth of neointima: for PTCA, intima gain = $(MBA - LA)_{FU} - PA$ and for stent, intima area gain = (SA − LA).

Tissue Analysis

After excision, hearts were pressure perfused with paraformaldehyde 4% for 2 h. Arterial segments were dissected and stored overnight in 20% sucrose at 4ºC. Five mm tissue blocks were embedded in OCT, frozen, and stored at −80ºC. Serial sections (5 µm) were made with hematoxylin and eosin (H&E) and elastin van Gieson stains. Stent struts were microscopically removed. Photomicrographs were digitally acquired (Spot camera, Diagnostic Instruments, Sterling Heights, Mich., USA) and stored. Morphometric measurements (Bioscan Optimas 6.0, Edwards, Wash., USA) were performed by manually tracing perimeters of lumen, intima, preexistent plaque (in stents), media, and adventitia.

Data Analysis and Statistical Analysis

All measurements and data analyses were performed by observers blinded to treatment. Data in text, tables, and charts are means(standard error of the mean. "No intervention" arteries were too few to incorporate into the statistical analysis. Analysis of variance (ANOVA) was used to study the influence of treatment and intervention. Bonferroni correction on post hoc tests was used to evaluate differences between groups. Since late loss (angiographic or echographic) usually correlates with immediate gain,[16] we tested regression models with immediate gain, intervention, and treatment as independent and late loss as dependent variables.

Results

Hemodynamics

Intracoronary rFGF-2 infusion at 2 µg/kg did not affect heart rate or blood pressure. Mean arterial blood pressure (MAP) was 81 ± 5 mmHg before and 82 ± 5 mmHg 20 min after start of FGF-2 infusion. In the vehicle group, MAP was 73 ± 4 mmHg before and during the infusion. Heart rate was also constant during infusion.

Angiography

In five animals (vehicle/stent: two, vehicle/control: two, FGF-2/control: one), the angiographic data were not available. The size of the arteries (RLD before intervention, Table I) was

TABLE I Angiographic parameters

Intervention	No intervention		PTCA		Stent	
Treatment	Vehicle	rFGF-2	Vehicle	rFGF-2	Vehicle	rFGF-2
N	4	4	9	10	6	9
Pre RLD (mm)	2.80 ± 0.15	2.91 ± 0.08	2.75 ± 0.11	2.62 ± 0.14	2.67 ± 0.07	2.44 ± 0.15
Pre-MLD (mm)	2.40 ± 0.17	2.78 ± 0.17	2.57 ± 0.11	2.49 ± 0.13	2.58 ± 0.10	2.25 ± 0.09
Pre-diam stenosis (%)	9.6 ± 3.6	4.6 ± 5.7	5.7 ± 1.9	4.2 ± 3.1	3.1 ± 1.4	7.6 ± 3.5
Dilation ratio	NA	NA	1.16 ± 0.06	1.22 ± 0.13	1.23 ± 0.01	1.19 ± 0.06
Post-RLD (mm)	NA	NA	2.54 ± 0.13	2.65 ± 0.12	2.28 ± 0.24	2.48 ± 0.27
Post-MLD (mm)	NA	NA	2.81 ± 0.06	2.59 ± 0.11	2.78 ± 0.11	2.52 ± 0.20
Post-diam stenosis (%)	NA	NA	−10.1 ± 0.7	2.3 ± 0.5	−4.1 ± 0.6	−2.1 ± 1.1
FU-RLD (mm)	2.79 ± 0.39	2.99 ± 0.23	2.77 ± 0.07	2.39 ± 0.10	2.25 ± 0.21	2.27 ± 0.25
FU-MLD (mm)	2.61 ± 0.36	2.68 ± 0.23	2.57 ± 0.12	2.26 ± 0.13	1.76 ± 0.20	1.54 ± 0.24
FU-diam stenosis (%)	9.4 ± 4.7	10.4 ± 6.0	8.2 ± 2.9	5.7 ± 3.7	20.2 ± 7.4	33.0 ± 15.2
Ref late loss (mm)	NA	NA	−0.22 ± 0.15	0.26 ± 0.08 [a]	0.03 ± 0.2	0.21 ± 0.11 [a]
Immediate gain (mm)	NA	NA	0.25 ± 0.08	0.08 ± 0.11	0.19 ± 0.05	0.26 ± 0.09
Late loss (mm)	0.09 ± 0.09	−0.04 ± 0.04	0.25 ± 0.14	0.33 ± 0. 10	1.02 ± 0.11 [b]	0.98 ± 0.11 [b]

[a] $p = 0.033$ for treatment effect (ANOVA).
[b] $p < 0.001$ (ANOVA).
For all unmarked data, $p > 0.2$.
Abbreviations: PTCA = percutaneous transluminal coronary angioplasty, NA = not applicable, RLD = reference lumen diameter, MLD = minimum lumen diameter, FU = follow up, rFGF-2 = recombinant fibroblast growth factor-2, N = number of arteries.

not different between the treatment groups (ANOVA, p = 0.364). Stenoses were also comparable (p = 0.672). A small angiographic late loss in the reference segments of treated arteries due to transient vasospasm was maximally 0.03 mm in the vehicle group and 0.21 to 0.26 mm in the FGF-2 group (Table I, ANOVA, p = 0.033 for treatment effect, not significant for intervention effect).

Dilation ratio and immediate gain determine the extent of arterial wall injury and thus the resultant late loss. Dilation ratios were very similar in the treatment and intervention groups (Table I, p = 0.847 and p = 0.789, respectively). Immediate gain was also not different for either intervention (p = 0.593) or treatment (p = 0.521). Angiographic late loss was higher in the stent than in the PTCA groups (Table I, Fig. 2, p < 0.001) but similar in the vehicle or FGF-2 groups (p = 0.869). In a regression model with immediate gain, intervention and treatment effects on late loss, immediate gain, and intervention were significant determinants of late loss (beta coefficients 0.55, p = 0.05 and 0.67, p < 0.001, respectively) and the treatment effect was not (beta: 0.07, p = 0.63). Power calculation revealed that the study had a 80% power to detect a difference in late loss of 0.4 mm between treatment groups.

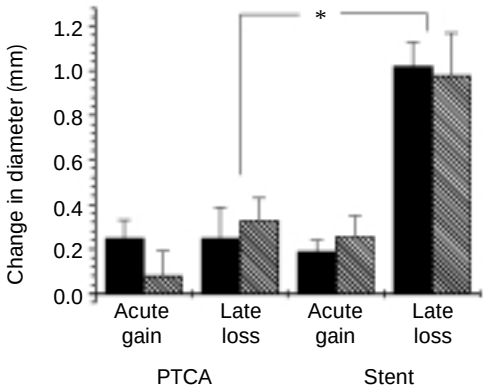

FIG. 2 Angiographic results of PTCA and stented arteries from the vehicle group (black bars) and the rFGF-2 group (hatched bars). * p < 0.001. Abbreviation as in Fig. 1.

Intravascular Ultrasound

Forty-three arterial segments had complete serial scans (Fig. 3). Arteries in the vehicle/PTCA group were slightly bigger than in other groups (Table II, ANOVA: p = 0.046), but area stenosis and lumen stenosis were similar.

Immediate gain was slightly but not significantly higher after stenting than after PTCA, and similar for the vehicle and FGF-2 groups (Table II, Fig. 4, ANOVA, p = 0.108 for intervention and p = 0.956 for treatment effect). No treatment effect was seen on late loss (p = 0.585), intima gain (p = 0.717), or MBA loss (p = 0.765). Regression of echographic immediate gain and treatment on echographic late loss again showed a significant relation with immediate gain (beta: 0.87, p < 0.001), but not with treatment (beta: 0.34, p = 0.579). Stent placement resulted in significantly less MBA loss (Fig. 4, p = 0.05) than PTCA, but showed a trend toward increased intima gain (p = 0.108). Consequently, late loss was similar in the stented and PTCA-treated arteries (p = 0.744). Late loss in the reference segments was similar in the vehicle and FGF-treated animals and was attributed for 60–75% and 100% to neointima formation in PTCA and stent-treated arteries, respectively.

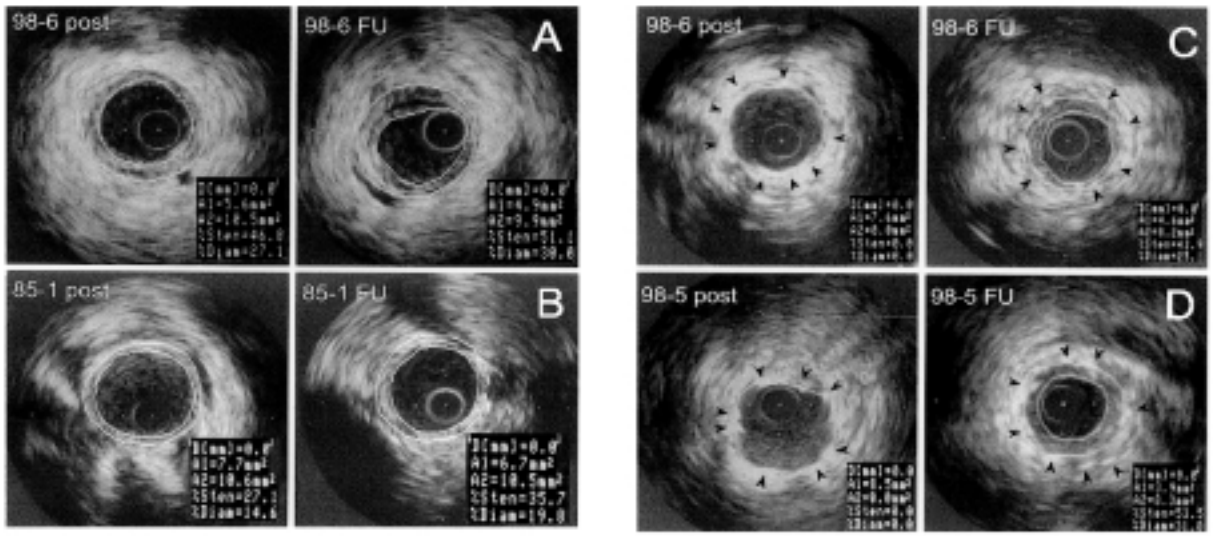

FIG. 3 Intravascular ultrasound (IVUS) cross sections from coronary arteries treated by PTCA (A, B) or stent placement (C, D), and with vehicle- (A, C) or rFGF-2- (B, D) treated coronary artery immediately after (left panels, post) intervention and at follow-up (right panels, follow-up [FU]) 6 weeks later. Lumen and media-bounded areas were manually traced and their measures displayed (A1 and A2, respectively) in the insets. Abbreviation as in Fig. 1.

TABLE II Intravascular ultrasound parameters

Intervention	No intervention		PTCA		Stent	
Treatment	Vehicle	rFGF-2	Vehicle	rFGF-2	Vehicle	rFGF-2
N	4	4	9	10	7	9
Pre ref area (mm^2)	5.93 ± 1.20	6.45 ± 0.70	8.83 ± 0.59 [a]	6.98 ± 0.60	6.94 ± 0.23	6.70 ± 0.58
Pre MLA (mm^2)	5.43 ± 1.19	6.39 ± 0.86	8.06 ± 0.53	6.92 ± 0.74	5.97 ± 0.46	5.87 ± 0.43
Pre LA-stenosis (%)	8.6 ± 6.5	0.4 ± 7.0	8.0 ± 3.7	1.4 ± 4.9	13.9 ± 6.0	11.2 ± 4.5
Pre area stenosis (%)	14.2 ± 5.9	15.7 ± 6.3	13.2 ± 4.3	9.3 ± 4.1	22.8 ± 7.5	15.7 ± 4.5
Post ref area (mm^2)	NA	NA	8.42 ± 0.93	7.09 ± 0.58	7.45 ± 1.09	7.94 ± 0.90
Post MLA (mm^2)	NA	NA	8.88 ± 0.78	7.25 ± 0.77	7.18 ± 0.51	7.64 ± 0.88
Post LA stenosis (%)	NA	NA	−11.4 ± 10.1	−3.5 ± 7.1	1.5 ± 10.8	1.6 ± 8.2
FU ref area (mm^2)	6.88 ± 1.24	4.89 ± 0.27	7.47 ± 0.51	5.40 ± 0.56	6.26 ± 0.77	6.84 ± 0.50
FU MLA (mm^2)	6.47 ± 1.04	5.30 ± 0.40	6.01 ± 0.53	4.64 ± 0.37	4.75 ± 0.79	4.07 ± 0.73
FU LA stenosis (%)	3.9 ± 4.4	−8.2 ± 5.4	19.0 ± 4.7	7.0 ± 9.2	20.0 ± 24.0	42.3 ± 7.3
Ref late loss (mm^2)			0.93 ± 0.52	1.69 ± 0.55	1.19 ± 0.83	1.10 ± 0.71
Ref MBA loss (mm^2)			0.70 ± 0.35	1.04 ± 0.45	1.03 ± 0.28	1.28 ± 0.28
Immediate gain (mm^2)	NA	NA	0.82 ± 0.44	0.34 ± 0.38	1.22 ± 0.46	1.77 ± 0.76
Late loss (mm^2)	NA	NA	2.87 ± 0.85	2.61 ± 0.74	2.43 ± 0.76	3.57 ± 0.70
Intima area gain (mm^2)	NA	NA	1.47 ± 0.55	0.98 ± 0.42	2.19 ± 0.77	3.19 ± 0.73
MBA loss (mm^2)	NA	NA	1.41 ± 0.38	1.63 ± 0.89	0.24 ± 0.25 [b]	0.38 ± 0.21 [b]

[a] $p = 0.046$ vehicle versus rFGF-2.
[b] $p = 0.02$ stent versus PTCA.
For all unmarked data, $p > 0.2$.
Abbreviations: Ref = reference, MLA = minimum lumen area, LA = lumen area, FU = follow up, MBA = media bounded area, N = number of arteries, NA = not applicable.

Histology

Histomorphometric analysis of harvested arteries confirmed IVUS findings (Table III, Fig. 5). Treatment or intervention had no effect on morphometric parameters; however, in the rFGF-2-treated group, media area showed a trend toward reduction ($p = 0.06$, ANOVA). Ultrasound and histomorphometric parameters at follow-up, such as lumen area (Pearson, $r = 0.636$, $p < 0.001$) and intima area ($r = 0.580$, $p < 0.001$), correlated fairly well.

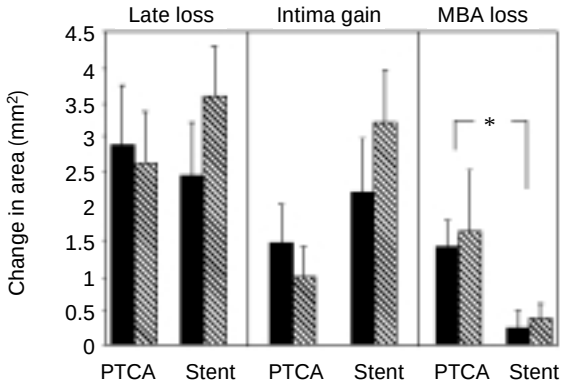

FIG. 4 Intravascular ultrasound results of immediate gain and late loss in the PTCA and stented arteries from the vehicle group (black bars) and the rFGF-2 group (hatched bars). The data correspond with those in Table V. * $p = 0.05$. MBA = media-bounded area; other abbreviations as in Fig. 3.

Discussion

Clinical Background

In numerous preclinical experiments with either $VEGF_{165}$[11, 17, 18] or with FGF-1,[19, 20] FGF-2,[21, 22] or FGF-5,[23] blood flow to ischemic myocardium could be enhanced by angiogenesis, which resulted in improved myocardial function in some studies.[17, 20, 23] Surprisingly, this functional angiogenesis was triggered and sustained by a single adminis-

TABLE III Morphometric results of recombinant fibroblast growth factor (rFGF-2) treatment and intervention

	Vehicle		rFGF-2	
Treatment	PTCA	Stent	PTCA	Stent
N	9	6	10	9
Adventitia (mm^2)	3.17 ± 0.39	1.81 ± 0.23	2.64 ± 0.49	2.09 ± 0.21
Media (mm^2)	1.61 ± 0.24	1.31 ± 0.22	1.09 ± 0.15	1.01 ± 0.15
Intima (mm^2)	1.75 ± 0.65	1.96 ± 0.51	2.01 ± 0.47	3.19 ± 0.59
Lumen (mm^2)	4.60 ± 0.36	2.72 ± 0.31	3.16 ± 0.37	3.50 ± 0.35

Abbreviations as in Table I.

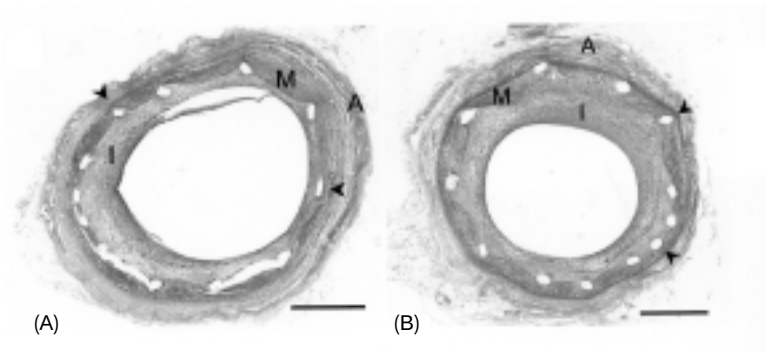

FIG. 5 Photomicrographs of stented arteries from a vehicle- (A) and rFGF-2- (B) treated animal stained with elastin van Gieson. The arteries are the same as used for Figure 2. A = adventitia, M = media, I = intima. The arrowheads indicate voids of stent struts. Bar = 750 μm.

tration of FGF-2.[24] During the last 2 years, the clinical safety of growth factor administration was demonstrated for FGF-1, FGF-2, and $VEFG_{165}$ either as adjunct to CABG with local delivery or as stand-alone systemic therapy,[5, 7, 25] allowing the implementation of larger scale phase II/phase III trials. In a phase I trial with single intracoronary FGF-2 administration, 1.5 μg/kg/artery (to a total dose of 3 μg/kg) was the lowest that improved perfusion on single-photon emission computed tomography (SPECT).[26]

In this study, we further support the feasibility and safety of such a strategy by showing that restenosis is not enhanced by a single intracoronary administration of 2 μg/kg FGF-2. This was true both for PTCA and for in-stent restenosis in an atherosclerotic environment. The higher neointima in the stented arteries treated with FGF-2 was attributed to a difference in acute gain, since the treatment effect was not significant in a regression model that corrected for the influence of acute gain.

Animal Model of Atherosclerosis

The study was performed in an established model of coronary atherosclerosis,[13, 14] which closely mimics human pathology and the response to arterial injury[13, 15, 27, 28] in spite of mild disease. A dilation ratio of 1.2 was used to avoid overdistension, which resulted in a healing response comparable to that after PTCA or stenting in human atherosclerotic arteries.[28]

Fibroblast Growth Factor-2 and Arterial Healing

In stented arteries, neointima formation is the primary cause of restenosis. Since FGF-2 is a mitogen for endothelial cells, smooth muscle cells, and other mesenchymal cells, its effect on neointima formation is not obvious. FGF-1 and FGF-2 may reduce neointima formation by accelerating endothelial regeneration.[11, 29, 30] FGF-1 reduced intimal hyperplasia in a rat carotid artery model,[29] but no inhibitory effect of FGF-2 on intimal hyperplasia has been shown yet. Instead, in a balloon injury model in the rabbit, a single injection of FGF-2 has lead to enhancement of intimal hyperplasia.[12] Several strategies aiming at inhibition of the FGF-2/FGF-R1 pathway either through antibodies against FGF-2,[31] an FGF-2-toxin fusion protein,[32] or antisense FGF-2[10] resulted in reduction of neointima formation, also suggesting that the mitogenic effect of FGF-2 on smooth muscle cells might be dominant. However, these studies have been performed primarily in the rat carotid artery in the absence of preexistent intima and with sustained delivery of FGF-1, FGF-2, or FGF-2 inhibitors, and the present study suggests that this effect may be of little practical relevance.

Potential Side Effects of Fibroblast Growth Factor-2

A second potential side effect of FGF-2 in atherosclerotic plaque is the acceleration of plaque angiogenesis with the potential of creating a vulnerable plaque that may rupture and hemorrhage, causing an acute coronary syndrome. Indeed, hemorrhages in the vicinity of newly formed vessels of atherosclerotic plaques have frequently been identified,[33] and this may be exaggerated by FGF-2-stimulated interstitial collagenase activity.[34] We observed no plaque ruptures or hemorrhages in this model of mild disease.

Fibroblast Growth Factor-2 and Geometric Remodeling

In this study, neointima formation accounted for 51 and 38% of lumen renarrowing in the vehicle and FGF-2 PTCA group, respectively, and for virtually all of the restenosis in the stent groups. This finding is consistent with previous studies using the iliac and femoral arteries in the same model.[13, 35] The complementing portion of lumen renarrowing is attributed to remodeling, which was not influenced by FGF-2.

Limitations

This study was performed in a small number of animals. Higher numbers of observations were obtained by using both branches of the left coronary artery. Although statistical analysis accounted for more observations in one pig, the overall power of analysis was borderline (0.8 or higher). The FGF-2 was given as a single intracoronary infusion and recent clinical data (FGF Initiating Revascularization Trial [FIRST]) indicate that this mode of delivery has limited efficacy.[36] Al-

though double-blind randomized phase II trials of intracoronary FGF-2 in patients with CAD (FIRST) and intra-arterial FGF-2 in patients with peripheral vascular disease (Therapeutic Angiogenesis with FGF-2 for Intermittent Claudication [TRAFFIC]) demonstrated significant symptomatic relief and functional improvement in subgroups of FGF-2-treated patients, this effect was temporary. The final proof of efficacy of this approach, however, must await pivotal phase III trials. It remains to be shown whether longer exposures to these growth factors are more efficacious and equally safe with respect to restenosis and atherogenesis.

Clinical Implications

These results imply that intracoronary FGF-2 may be used as an adjunct to PTCA or stent placement with the intention to improve perfusion and function of ischemic areas that are not directly targeted by the intervention. In the absence of adverse effects on restenosis, a larger group of patients will benefit from such a strategy.

Conclusions

We conclude that a single intracoronary administration of rFGF-2 does not aggravate restenosis after balloon angioplasty or stent placement in a relevant model of coronary atherosclerosis. The rFGF-2 affects neither neointima formation nor remodeling to a clinically significant extent. This opens possibilities for the implementation of trials in which angioplasty is combined with rFGF-2 angiogenic therapy.

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