Placental growth factor increases regional myocardial blood flow and contractile function in chronic myocardial ischemia

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1Division of Clinical Cardiology and Department of Cardiovascular Sciences, Gasthuisberg University Hospitals, Leuven, Belgium; 2Vesalius Research Center, Flemish Institute of Biotechnology, University of Leuven, Leuven, Belgium; 3Department of Radiology, Gasthuisberg University Hospitals, Leuven, Belgium; and 4Department of Pathology, Gasthuisberg University Hospitals, Leuven, Belgium

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Liu X, Claus P, Wu M, Reyns G, Verhamme P, Pokreisz P, Vandenwijngaert S, Dubois C, Vanhaecke J, Verbeken E, Bogaert J, Janssens S. Placental growth factor increases regional myocardial blood flow and contractile function in chronic myocardial ischemia. Am J Physiol Heart Circ Physiol 304: H885–H894, 2013. First published January 11, 2013; doi:10.1152/ajpheart.00587.2012.—Placental growth factor (PIGF) has a distinct biological phenotype with a predominant proangiogenic role in disease without affecting quiescent vessels in healthy organs. We tested whether systemic administration of recombinant human (rh)PIGF improves regional myocardial blood flow (MBF) and systolic function recovery in a porcine chronic myocardial ischemia model. We implanted a flow-limiting stent in the proximal left anterior descending coronary artery and measured systemic hemodynamics, regional myocardial function using MRI, and blood flow using colored microspheres 4 wk later. Animals were then randomized in a blinded way to receive an infusion of rhPIGF (15 μg·kg−1·day−1, n = 9) or PBS (control; n = 10) for 2 wk. At 8 wk, myocardial perfusion and function were assessed. Infusion of rhPIGF transiently increased PIGF serum levels >30-fold (1,153 ± 180 vs. 33 ± 18 pg/ml at baseline, P < 0.001) without affecting systemic hemodynamics. From 4 to 8 wk, rhPIGF increased regional MBF from 0.46 ± 0.11 to 0.85 ± 0.16 ml·min−1·g−1, with a concomitant increase in systolic wall thickening from 11 ± 3% to 26 ± 5% in the ischemic area. In control animals, no significant changes from 4 to 8 wk were observed (MBF: 0.45 ± 0.07 to 0.49 ± 0.08 ml·min−1·g−1 and systolic wall thickening: 14 ± 4% to 18 ± 1%). rhPIGF-induced functional improvement was accompanied by increased myocardial neovascularization, enhanced glycogen utilization, and reduced oxidative stress and cardiomyocyte apoptosis in the ischemic zone. In conclusion, systemic rhPIGF infusion significantly enhances regional blood flow and contractile function of the chronic ischemic myocardium without adverse effects. PIGF protein infusion may represent an attractive therapeutic strategy to increase myocardial perfusion and energetics in chronic ischemic cardiomyopathy.

chronic myocardial ischemia; growth factors; angiogenesis

CORONARY ARTERY DISEASE is the leading cause of death and disability in the developed world (36). The prevalence and severity of chronic myocardial ischemia in patients with coronary artery disease emphasize the limitations of current revascularization strategies. In most patients with advanced atherosclerosis (3, 37), the native angiogenic response fails to create an effective biological bypass, highlighting the need for improved therapies for refractory ischemia and ischemic myocardial dysfunction (4, 5). Vascular growth in ischemic tissue is a homeostatic response to maintain O2 tension and nutrient supply and is tightly regulated by angiogenic growth factors. Preclinical studies (15, 21, 29) have shown that administration of single angiogenic growth factors, including FGF2 or VEGF165, transiently improves myocardial flow and function in porcine and canine models. However, clinical experience with these agents has been less successful, in part attributable to suboptimal delivery or dose-dependent side effects (16, 19, 22, 23, 33).

Placental growth factor (PIGF) has a distinct biological profile with a predominant role in pathological angiogenesis without affecting quiescent vessels in healthy organs (6, 25). Genetic studies (6, 8) in mice have shown that PIGF, a member of the VEGF family, binds to VEGF receptor (VEGFR)-1 (Flt-1) but not to VEGFR-2 (Flk1) and can accelerate murine wound healing without increasing vascular permeability, a potentially attractive therapeutic strategy for chronic ischemia-induced myocardial dysfunction. The aim of this study was to evaluate the therapeutic potential of recombinant human (rh)PIGF in a porcine model of chronic ischemic cardiomyopathy, representative of human disease. We evaluated whether sustained systemic administration of rhPIGF (15 μg·kg−1·day−1) improves myocardial neovascularization and enhances cardiac perfusion and regional function during ischemic stress.

MATERIALS AND METHODS

Experiments in pigs were performed in accordance with Belgium National Institute of Health guidelines for the care and use of laboratory animals, and the protocol of this double-blind randomized controlled study was approved by the local Ethics Committee on Animal Research (Ethische Commissie Dierproeven) of the University of Leuven (Leuven, Belgium).

Animal preparation and catheterization. Domestic cross-bred pigs of either sex (Sus scrofa, weight: 22–25 kg) were premedicated with aspirin (300 mg/day, Dispril, Reckitt Benckiser, Brussels, Belgium) and clopidogrel (300 mg/day, Plavix, Sanofi, Paris, France) 1 day before and on the day of the procedure. Pigs were sedated using azaperone (3 mg/kg im, Stresnil, Janssen Pharmaceuticals, Beerse, Belgium) and anesthetized using ketamine (1 mg/kg iv, Eurovet, Heusden-Zolder, Belgium) and then intubated and mechanically ventilated using 50% O2. Continuous electrocardiographic monitoring of heart rate, rhythm, and S-T segment changes was performed. Coronary angiograms were performed after an intra-
coronary administration of nitroglycerin (200 µg) at baseline and 4- and 8-wk followups.

At baseline, after an intravenous bolus of heparin (10,000 IU, Heparine Leo, Leo Pharma, Wilrijk, Belgium) and acetylsalicylic acid (450 mg), a tight proximal left anterior descending coronary artery (LAD) stenosis was induced by implantation of a flow-limiting stent. In the central part of this homemade stent, flow limitation was obtained by wiring a 0.017-mm stainless steel wire for 15 convolutions, which effectively prevented expansion of the central portion of the stent upon balloon inflation (Fig. 1). At 8 wk, pigs were euthanized using an overdose of propofol and saturated KCl. The left ventricle (LV) distal to the stent was sectioned into five slices perpendicular to the myocardial long axis from the base to apex for infarct staining, microsphere analysis of myocardial blood flow, quantitative PCR, and histology.

Assessment of hemodynamics, perfusion, and function. During catheterization at 4 and 8 wk, a 5-Fr pressure transducer catheter (Millar Instruments) was inserted into the LV for hemodynamic measurements. After hemodynamic data were obtained, a 6-Fr pigtail catheter was inserted in the LV, and 2 million colored microspheres (15-µm diameter, Triton Technologies) were injected to measure regional myocardial blood flow (20). Absolute blood flow was quantified by comparing microsphere concentrations in different myocardial regions with those measured in a reference blood sample drawn from the carotid artery using an automated pulback. The withdrawal pump was set at a speed of 7 ml/min, started 10 s before microsphere injection, and maintained for 3 min. Two reference tissue samples were obtained from the right and left kidneys to test homogeneity of the microsphere distribution. Myocardial samples from the ischemic and remote areas (posterior wall) and reference blood and kidney samples were analyzed using a luminescence spectrophotometer (Agilent 8453E UV-visible spectroscopy system) (20).

Cardiac MRI was performed on a 3-T system (TRIO-Tim, Siemens) at 4 and 8 wk using electrocardiographic triggering and cardiac-dedicated surface coils. Global and regional function were assessed with breathhold cine MRI in the vertical and horizontal long and short axes, covering the complete LV by 6-mm-thick slices. Myocardial viability was evaluated with late contrast-enhanced (LE) MRI.

A complete description of the MRI sequences has been previously reported (41). All MRI results were analyzed using dedicated software by two investigators (X. Liu and P. Claus) unaware of treatment allocation. For the calculation of myocardial infarct size, endocardial and epicardial borders and LE regions were contoured. Myocardial infarct size was calculated as total LE volume normalized to LV myocardial volume. For the assessment of regional and global LV function, endocardial and epicardial borders were traced in end-diastolic and end-systolic short-axis slices. We calculated LV end-diastolic volume, LV end-systolic volume, stroke volume, and ejection fraction as an index for global function. All volumes were reported indexed for body surface area. Myocardial wall thickening was measured as an index of regional function in remote and ischemic cores after the American Heart Association segmentation model (exclusion of the two most basal and apical slices to avoid measure-

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**Table 1. Primers used for quantitative PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>pVEGF receptor-1</td>
<td>Forward: 5'-CCTCCCAAGAACAGACATG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CCACAACACAGTATCCACT-3'</td>
</tr>
<tr>
<td>pFGF2</td>
<td>Forward: 5'-ACACGAAACGATCCACT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CCTCCCAAGGGATCCTAG-3'</td>
</tr>
<tr>
<td>pVEGF</td>
<td>Forward: 5'-ACACGAAACGATCCACT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CCTCCCAAGGGATCCTAG-3'</td>
</tr>
<tr>
<td>pHIF-1a</td>
<td>Forward: 5'-ACACGAAACGATCCACT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CCTCCCAAGGGATCCTAG-3'</td>
</tr>
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p, porcine; HIF, hypoxia-inducible factor; HGF, hepatocyte growth factor.
ments errors because of the presence of the LV outflow tract and partial volume effects) (26). At 4 wk, the segment in the LAD perfusion territory with the most reduced function was considered as representative for the ischemic region and followed over time.

**Administration of PlGF.** After the confirmation of LV dysfunction using cine MRI, animals were randomized to a 2-wk chronic intravenous infusion of rhPlGF or PBS (control; Fig. 1) via osmotic minipumps. rhPlGF protein was produced at Eurogentec under the European Sixth Framework Program Vasoplus. Circulating hPlGF levels were quantified using a standard PlGF immunoassay. Plates were coated overnight (4°C) with a monoclonal antibody specifically recognizing hPlGF, blocked for 1 h at room temperature with 1% BSA, washed, and incubated with a secondary biotinylated polyclonal goat anti-hPlGF antibody. Bound hPlGF was detected after an incubation with a streptavidin-horseradish peroxidase substrate. Standard dilutions of hPlGF-1 served as positive control samples.

**Chemical, histopathological, and molecular analyses.** Cardiac necrosis markers and liver function tests were analyzed at stent implantation and at 4 and 8 wk followups. Cardiomyocyte necrosis, hypertrophy, metabolism, neovascularization, and capillary density were evaluated on 5-μm sections from paraffin-embedded biopsy specimens of the ischemic and remote zones. Periodic acid-Schiff (PAS) staining was used to assess glycogen content. Standard endothelial cell (CD31) and smooth muscle cell [smooth muscle actin (SM α-actin)] markers were used to label capillaries and small muscularized vessels. The degree of neovascularization (CD31) and fibrosis (Sirius red) was semiquantitatively evaluated by an experienced pathologist (E. Verbeken) blinded to treatment allocation using a scoring system with a scale of 0–3 (where 0 = absent, 0.5 = minimal, 1 = mild, 2 = moderate, and 3 = severe) (37). Vascular density and glycogen content were assessed by counting SM α-actin-positive vessels and PAS-positive areas in 10 randomly selected high-power fields (HPFs) in the ischemic and remote myocardium. Cross-sectional area and perimeter and diameter of cardiac myocytes and their nuclei from the ischemic and remote zones were measured on 10 randomly selected HPFs.

Cardiomyocyte apoptosis was evaluated using cleaved caspase-3 (Cell Signaling) and TUNEL. The apoptotic rate was expressed as the percentage of caspase-3 and TUNEL-positive nuclei over the total number of nuclei, which was calculated from 10 randomly selected HPFs in the respective zones. Oxidative stress was evaluated using 8-hydroxy-2'-deoxyguanosine (8-OHdG) immunohistochemistry and immunoblot analysis for nitrotyrosine (Sanbio). Oxidative stress was expressed as 8-OHdG-positive nuclei over the total nuclei from 10 randomly selected HPFs.

Myocardial protein extracts were separated by SDS-PAGE using standard techniques. The following antibodies were used: mouse anti-actin monoclonal antibody (Millipore), Akt, phosphorylated (p)Akt, glycogen synthase kinase (GSK)-3β, pGSK-3β, and caspase-3. In addition, transcript levels of genes encoding Flt-1 (VEGFR-1), FGFR2, VEGF,

![Diagram](http://ajpheart.physiology.org/)
IGF-I, PDGF-B, VEGFR-2, VEGF-A, hypoxia-inducible factor-1a, and hepatocyte growth factor were analyzed using quantitative PCR (Table 1).

### Statistical analysis
Statistical analysis was performed using SAS statistical software (SAS version 9.2, SAS Institute). Data are expressed as means ± SE. ANOVA and a Bonferroni’s posthoc test were used to analyze differences between groups. Repeated-measures ANOVA was used to test serial data. When data did not follow a normal distribution, Kruskal-Wallis nonparametric statistics were reported, and differences between groups were identified using Mann-Whitney or Wilcoxon tests.

### RESULTS

**Pharmacokinetic and safety experiments after intravenous PlGF infusion.** Serum PlGF levels were measured in two healthy pigs on day 0 and on days 5, 10, and 14 after minipump implantation. Circulating PlGF levels were significantly elevated on day 5 (1,106 pg/ml) compared with baseline (11 pg/ml) and remained elevated on days 10 and 14 (654 and 665 pg/ml, respectively).

During chronic ischemia, serum PlGF levels at 4 wk (before minipump implantation) and 8 wk were comparable between control and PlGF treatment (45 ± 7 vs. 84 ± 49 and 52 ± 18 vs. 46 ± 18 pg/ml, respectively). However, PlGF-treated pigs had ∼30-fold higher circulating PlGF levels than control pigs 4–6 days after minipump implantation (33 ± 18 vs. 1,153 ± 180 pg/ml, *P < 0.001*). Cardiac necrosis markers and liver function tests remained unchanged in both groups.

**Study followup.** Thirty pigs were enrolled in the study followup (4 sham-operated pigs and 26 stented pigs; Fig. 2). Of the 26 stented pigs, 3 pigs died of acute ischemic complications, 1 pig died during baseline MRI, and 3 pigs were excluded after 4 wk because of an absence of coronary stenosis (*n* = 1) or total vessel occlusion (*n* = 2). In 19 pigs blindly randomized to PlGF or PBS treatment, no adverse events were observed during or after treatment.

**PlGF improves regional myocardial blood flow and systolic function.** Compared with sham-operated animals, heart rate was elevated in all stented animals at 4 and 8 wk, whereas mean arterial pressure did not differ (Table 2).

At 4 wk, myocardial blood flow was reduced by half in the ischemic territory in both groups and increased thereafter significantly after PlGF infusion, but not in the control group (Table 3). Reference blood flow to the kidneys was similar in all groups at 4 and 8 wk.

Stent implantation induced very small infarction in five of nine scanned animals in the control group (median: 6% vs. 5–6% and in five of eight scanned animals in the PlGF-treated group (median: 7%, range: 2–11%). The size of myocardial necrosis was similar in both groups and remained unchanged at 8 wk (control group: median 6% and range 5–6% vs. PlGF-treated group: median 5% and range 2–11%).

**Stent implantation.** Stent implantation reduced systolic wall thickening (SWT) in the ischemic territory to the same extent in both groups at 4 wk. Subsequent PlGF transfer significantly and selectively increased SWT in the ischemic areas at 8 wk, whereas PBS treatment had no effect (Fig. 3). In sham-operated animals, there were no significant changes in SWT over time in the LAD perfusion territory (55 ± 1% at 4 wk vs. 53 ± 1% at 8 wk) or in segments corresponding to remote areas in stented pigs (57 ± 1% at 4 wk vs. 54 ± 2% at 8 wk). The reduction in SWT in stented pigs at 4 wk was associated with an increased LV end-systolic volume index and reduced stroke volume index. At 8 wk, there were no significant differences in global LV function (Table 4).

**PlGF induces neovascularization in the ischemic myocardium.** Clusters of SM α-actin-positive vessels were observed in the ischemic myocardium (Fig. 4, A and B) but not in the remote myocardium. Quantitative analysis showed a significantly higher number and larger area of SM α-actin positive vessels on randomly selected HPFs in the PlGF-treated group (Fig. 4, A–D). Capillary density score, measured from the number of lectin-positive clusters, was greater in ischemic areas from the...
Table 4. MRI analysis of global LV function

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>PIGF-Treated Group</th>
<th>Sham Group</th>
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<tbody>
<tr>
<td></td>
<td>4 wk</td>
<td>8 wk</td>
<td>4 wk</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>45 ± 2</td>
<td>65 ± 2*</td>
<td>45 ± 1</td>
</tr>
<tr>
<td>LV mass index, g/m²</td>
<td>77 ± 4</td>
<td>81 ± 3</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>End-diastolic volume index, ml/m²</td>
<td>82 ± 9</td>
<td>76 ± 6</td>
<td>81 ± 5</td>
</tr>
<tr>
<td>End-systolic volume index, ml/m²</td>
<td>52 ± 7†</td>
<td>45 ± 4†</td>
<td>51 ± 4†</td>
</tr>
<tr>
<td>Stroke volume index, ml/m²</td>
<td>30 ± 2†</td>
<td>31 ± 2†</td>
<td>30 ± 2†</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>38 ± 2†</td>
<td>41 ± 2†</td>
<td>37 ± 3†</td>
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</table>

Values are means ± SE; n = 9 animals in the control group, 8 animals in the PIGF-treated group, and 4 animals in the sham group. Volumetric parameters were indexed to body surface area (BSA) using Meeh’s formula as follows: BSA (in m²) = [body weight (in kg)]²/³ × 10⁻², where k = 9.0 (see Table 7.1 in Ref. 31). *P < 0.05 vs. 4 wk; †P < 0.05 vs. the sham group.

PlGF-treated group compared with the control group (0.64 ± 0.3 vs. 0.15 ± 0.1, P < 0.05; Fig. 4, E–G).

Cardiomyocyte and nuclear perimeters were larger in the control group than in the PIGF-treated group (91 ± 1 vs. 79 ± 0.7 µm and 19.7 ± 0.2 vs. 18.4 ± 0.1 µm, respectively, P < 0.05 for both; Fig. 4, H–L), with a marked rightward shift in distribution (Fig. 4, K and L). There were no differences in cardiomyocyte or nuclear sizes, cross-sectional areas, or diameters between PIGF and sham-operated group. We also measured transcript levels of several angiogenic growth factors and their receptors in the ischemic and remote myocardium, several of which showed increased expression levels in both the ischemic and remote zones of PIGF-treated animals (Fig. 5).

PIGF preserves nutrient myocardial perfusion and is associated with reduced cardiomyocyte cell apoptosis. To investigate the effect of PIGF on myocardial nutrient blood flow, we compared glycogen deposition in ischemic and remote areas. The glycogen area (%HPF) in the ischemic zone of control was greater than the glycogen area in the PIGF-treated group (2.1 ± 0.74 vs. 0.81 ± 0.22, P < 0.05; Fig. 6, A–C). No differences in fibrosis were observed.

Immunohistochemical staining for 8-OHdG, an index of ischemia-induced oxidative DNA damage, showed a significantly greater percentage of 8-OHdG-positive nuclei in the ischemic area of the control group than in the PIGF-treated group (2.1 ± 0.74 vs. 0.81 ± 0.22, P < 0.05; Fig. 6, D–F). To investigate possible effects of PIGF on the inflammatory/immune response, immunohistochemical

![Fig. 4. PIGF treatment induces neovascularization in the ischemic myocardium. Representative sections of smooth muscle α-actin-stained [SMa; control group (A) and PIGF-treated group (B)] and lectin-stained [control group (E) and PIGF-treated group (F)] myocardial tissue in the ischemic area are shown. The number and area of SMa-positive vessels (C and D) and number of lectin-positive vessels (G) were significantly higher in the ischemic area of PIGF-treated animals than in control animals. On Sirius red-stained sections [control group (H), PIGF-treated group (I), and sham group (J), cardiomyocyte size and nuclei perimeters were larger in the ischemic area of the control group than in the PIGF-treated group, with a clear concomitant rightward shift in the distribution of cardiomyocyte perimeter (K) and nuclear perimeter (L). The dashed red lines indicate mean perimeters in the sham group. HPF, high-powered field. *P < 0.05 vs. the control group; †P < 0.05 vs. the remote myocardium.](http://ajpheart.physiology.org/)

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staining for CD45 (Fig. 6, G–I) and MAC3 (Fig. 6, J–L) was performed in tissue from the ischemic region. Semiquantitative analysis showed no increases in CD45- or MAC3-positive nuclei in PIGF-treated animals.

To explore molecular mechanisms of PIGF-mediated cardioprotection, we measured Akt and GSK-3β protein levels and phosphorylation states in the ischemic region (Fig. 7). In the PIGF-treated group, a twofold higher ratio of pAkt to Akt

Fig. 5. PIGF treatment increases expression levels of angiogenic growth factors and receptors in myocardial tissue of hearts subjected to chronic ischemia. Expression levels of angiogenic growth factors and receptors in the ischemic and remote myocardium of sham, control, and PIGF-treated pigs are shown. p, porcine; VEGFR, VEGF receptor; HIF, hypoxia-inducible factor; HGF, hepatocyte growth factor; Ct, threshold cycle. *P < 0.05 vs. the control group; †P < 0.05 vs. the sham group.

Fig. 6. PIGF treatment is associated with reduced glycogen deposition and cellular injury in the ischemic myocardium. Glycogen content, measured on periodic acid-Schiff-stained myocardial tissue in the ischemic border [control group (A) and PIGF-treated group (B)], was significantly lower in PIGF-treated animals (C). Oxidative stress was measured on 8-hydroxy-2′-deoxyguanosine (8-OHdG)-stained sections from ischemic tissue [control group (D) and PIGF-treated group (E)]. The percentage of 8-OHdG-positive nuclei was significantly lower in PIGF-treated animals (F). Inflammation was measured using CD45 [control group (G) and PIGF-treated group (H)] and MAC3 [control group (J) and PIGF-treated group (K)]. No significant differences in inflammation response in the ischemic region were observed (I and L). *P < 0.05 vs. the control group; †P < 0.05 vs. the remote myocardium.
and pGSK-3β to GSK-3β was observed than in corresponding ischemic regions of the control group, whereas in remote regions or in healthy animals no differences in phosphorylation status were detected with PIGF treatment (Fig. 7). Finally, PIGF was associated with reduced apoptosis, as evidenced by twofold lower caspase 3 expression (Fig. 8, A and B) and a significantly lower percentage of TUNEL- and caspase-3-positive nuclei in the ischemic area compared with the control group (Fig. 8, C–H).

**DISCUSSION**

This study shows that systemic administration of rhPIGF enhances myocardial perfusion, regional LV function, and energy metabolism in the chronic ischemic myocardium. Resting blood flow to the anterior wall was reduced by half, and subsequent ischemia-induced LV dysfunction was documented with MRI in a preclinical porcine model. Chronic infusion of rhPIGF was well tolerated and enhanced myocardial blood flow and neovascularization in the ischemic territory. Importantly, enhanced perfusion after PIGF administration resulted in improved systolic function recovery in the ischemic area, which was associated with reduced cardiomyocyte apoptosis.

**Growth factor-mediated therapies for ischemic cardiomyopathy.** Therapeutic angiogenesis for ischemic cardiovascular disease is based on the concept that stimulated coronary collateral development from existing microvessels can alleviate myocardial ischemia and enhance functional recovery. Animal models of myocardial infarction (15) or ameroid constrictor-induced chronic myocardial ischemia (2, 14, 15, 21, 29, 38) have shown increased collateral blood flow after administration of members of the FGF and VEGF growth factor families (2, 14, 15, 21, 29, 38) and variable improvements in LV function (21, 32). Subsequent studies (13, 16, 19, 22, 23, 32, 33) using FGF or VEGF in patients with ischemic heart disease failed to recapitulate preclinical findings, in part because of ineffective delivery strategies, insufficient duration of biological activity after short-term intracoronary administration of recombinant proteins, or dose-related side effects. The latter were attributed to systemic exposure to potent growth factors, resulting in hypertension, glomerulotoxicity, and anemia due to bone marrow suppression (13, 16, 32).

Here, we tested the potential of sustained systemic delivery of PIGF to stimulate perfusion and improve regional contractile function in the chronic ischemic myocardium. PIGF acts via VEGFR-1 (Flt-1) binding on endothelial cells, smooth muscle cells, macrophages, and bone marrow progenitor cells and has a unique role in vascular development under pathological conditions. In rodent models of limb ischemia, sustained infusion of PIGF for 3–7 days accelerated collateral growth in ischemic limbs without prohibitive side effects (5, 6, 28) and enhanced functional recovery (25). Similarly, after permanent coronary artery ligation in mice, a single high-dose intramuscular injection induced angiogenesis in the border zone of the infarct (35). While these permanent ligation models are less representative of human disease, our porcine coronary stenosis model recapitulates some of the structural and functional characteristics of ischemic cardiomyopathy in patients (10) and integrates MRI-based functional assessments, which are routinely used in clinical practice.

**PIGF enhances neovascularization and perfusion in the ischemic myocardium.** PIGF administration improved regional blood flow to the ischemic myocardium. We confirmed that the

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**Fig. 7.** Immunoblot analysis of Akt and glycogen synthase kinase (GSK)-3β (A) in total protein extracts from ischemic and remote tissue and in two normal animals 14 days after PIGF infusion in the pharmacokinetic analysis. In ischemic segments, densitometry revealed a significantly increased pAkt-to-Akt ratio (B) in PIGF-treated animals compared with control animals, whereas in nonischemic tissue, the phosphorylation status was comparable. In control animals, the pGSK-3β-to-GSK-3β ratio was reduced in ischemic tissue (C) compared with nonischemic regions or with the healthy myocardium. In contrast, pGSK-3β-to-GSK-3β ratios did not decrease in ischemic tissue of PIGF-treated animals. *P < 0.05 vs. the control group.
chronic ischemic burden per se did not trigger a major endogenous PlGF release at 4 wk, which could confound the observed neovascularization. Endogenous PlGF release typically and transiently accompanies acute ischemia and infarction (18), but reported levels are 30-fold less than levels measured here. High circulating PlGF levels induced characteristic morphological features of angiogenesis and arteriogenesis in the ischemic porcine myocardium (Fig. 4). Potential mechanisms include the muscularization of small neovessels via the release of smooth muscle cell mitogens from activated endothelial cells and fibroblasts (1), from infiltrating inflammatory cells (7), or via direct Flt-1 receptor binding on smooth muscle cells (24) and stimulated growth of arterial collaterals (39). In this study, we did not detect a difference in infiltrating monocytes or macrophages in the ischemic tissue 4 wk after PlGF infusion.

Interestingly, we detected increased expression levels of several growth factors and receptors in PlGF-treated animals (Fig. 5), the mechanism of which is unknown. Preclinical studies have shown that some of these growth factors, such as hepatocyte growth factor, in turn increase the expression of VEGFRs and c-Met in endothelial cells (30, 40) and cooperate with VEGF-A165 to amplify the vasculoproliferative response (12, 34). To what extent such growth factor cooperativity mediates the effects of PlGF in the ischemic myocardium remains to be determined. We also observed higher pAkt and pGSK-3β protein levels in PlGF-treated animals (Fig. 7, B and C), and several groups (17, 27) have previously reported that GSK-3β, when phosphorylated by Akt at Ser9, is strongly cardioprotective and associated with reduced apoptosis.

Study limitations. We studied juvenile pigs, precluding direct extrapolation to patients with advanced age, comorbidities, and atherosclerosis, in whom angiogenic growth factors could induce intimal hyperplasia and progression of coronary atherosclerotic lesions (9). We used a single sustained delivery of rhPlGF and cannot exclude more effective doses or duration of delivery.

Similarly, we only measured regional function 4 wk after PlGF infusion. Hence, the question whether a longer followup would further normalize SWT and translate into improved global function recovery remains to be addressed in future studies.

Conclusions. In summary, prolonged systemic administration of rhPlGF in a porcine model of chronic ischemic cardiomyopathy significantly improves regional myocardial blood flow and contractile function without major adverse effects. PlGF appeared to be well tolerated, and its neovascularization capacity and metabolic effects represent an attractive therapeutic strategy in patients with chronic ischemic cardiomyopathy and refractory myocardial ischemia.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


