Treatment With Recombinant Placental Growth Factor (PlGF) Enhances Both Angiogenesis and Arteriogenesis and Improves Survival After Myocardial Infarction

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Background: Placental growth factor (PlGF), a homolog of vascular endothelial growth factor, is reported to stimulate angiogenesis and arteriogenesis in pathological conditions. It was recently demonstrated that PlGF is rapidly produced in myocardial tissue during acute myocardial infarction (MI). However, the effects of exogenous PlGF administration on the healing process after MI are not fully understood. The purpose of the present study was to examine whether PlGF treatment has therapeutic potential in MI.

Methods and Results: Recombinant human PlGF (rhPlGF; 10 μg) was administered continuously for 3 days in a mouse model of acute MI. rhPlGF treatment significantly improved survival rate after MI and preserved cardiac function relative to control mice. The numbers of CD31-positive cells and α-smooth muscle actin-positive vessels in the infarct area were significantly increased in the rhPlGF group. Endothelial progenitor cells (Flk-1+Sca-1+ cells) were mobilized by rhPlGF into the peripheral circulation. Furthermore, rhPlGF promoted the recruitment of GFP-labeled bone marrow cells to the infarct area, but only a few of those migrating cells differentiated into endothelial cells.

Conclusions: Exogenous PlGF plays an important role in healing processes by improving cardiac function and stimulating angiogenesis following MI. It can be considered as a new therapeutic molecule. (Circ J 2009; 73: 1674–1682)

Key Words: Myocardial infarction; Placental growth factor; Soluble Flt-1
Methods

Preparation of Recombinant Human PlGF (rhPlGF)
A DNA sequence encoding the mature human PlGF protein (amino acid residues 21–149 of the 149-amino acid form of PlGF) was amplified by polymerase chain reaction (PCR) with human placental cDNA (TaKaRa, Ohtsu, Japan) as a template. *Escherichia coli* BL21 was transformed with the expression vector, and the thioredoxin-PlGF fusion protein was expressed and purified essentially as previously described. Proteins were concentrated and suspended in a solution containing 20 mmol/L PB (pH 7.4), 0.5 mol/L NaCl, 1 mol/L dithiothreitol, and 8 mol/L urea, and purified by HisTrap FF (GE Healthcare Bio-Sciences, Piscataway, NJ, USA) column chromatography. Following dialysis against graded concentrations of urea containing 50 mmol/L Tris-HCl (pH 8.0), the fusion protein was resuspended in 50 mmol/L Tris-HCl (pH 8.0). After digestion by Max Enterokinase (Invitrogen, Carlsbad, CA, USA), proteins were suspended again in a solution containing 1 mol/L dithiothreitol and 8 mol/L urea and applied onto a HisTrap FF column in order to remove the tag protein. The purified PlGF was dialyzed against graded concentrations of urea containing 20 mmol/L Tris-HCl (pH 8.0). Finally, the protein solution was fractionated by gel filtration on a Superdex 75 10/300GL column (GE Healthcare Bio-Sciences).

Preparation of Recombinant Human Soluble Flt-1 (rhsFlt-1)
A DNA fragment encoding amino acids 1–338 of human Flt1 (sFlt1 (D1-3) containing 3 Ig-like domains at the N-terminus) was amplified by PCR reaction with specific primers (forward primer: 5’-CATCCATGGATCCTGAAC-TGAGTTTTAAAG-3’, reverse primer: 5’-CATGGATCC-TCAATGTTTCACAGTGATGAATGC-3’) and human placental cDNA (Clontech) as a template. The expression vector was used for transformation of BL21 star (DE3) competent cells (Invitrogen). For bacterial expression, the transformant expressing sFlt1 (D1-3) was cultured in LB medium containing 50 μg/ml of Kanamycin at 37°C. Four hours after IPTG was added to the final concentration of 1 mmol/L, the cells were collected by centrifuge. The cells were lysed by incubation with 2 mg/ml L-lysozyme and sonication. Inclusion bodies were collected by centrifuging for 20 min at 10,000 rpm, and were suspended and solubilized in 20 mmol/L PB containing 0.5 mol/L NaCl, 6 mol/L urea, 1 mmol/L DTT, and 20 mmol/L imidazole followed by His-Trap FF (GE Healthcare Bio-Sciences) column purification. Elute was dialyzed against a graded concentration of urea in 20 mmol/L Tris (pH 8.0) containing 0.5 mol/L NaCl. Following the final dialysis against 20 mmol/L Tris (pH 8.0) containing 0.5 mol/L NaCl, purified sFlt-1 was harvested.

Animals
C57BL/6 mice were purchased from SLC (Shizuoka, Japan). Transgenic mice (C57BL/6 background) that ubiquitously express enhanced GFP (GFP mice) were a generous gift from Dr Masaru Okabe (Osaka University, Suita, Japan). All experimental procedures were performed in accordance with protocols approved by the Ethics Review Committee for Animal Experimentation of Nara Medical University.

Preparation of Mouse Model of MI
C57BL/6 mice (12 weeks old) were anesthetized with diethyl ether inhalation and an osmotic minipump (Durect Corp, Cupertino, CA, USA) filled with 10 μg of rhPlGF or 40 μg of rhsFlt-1 was implanted subcutaneously. A polyethylene tube connected to the osmotic minipump was extended to the peritoneal cavity and stitched to the oblique abdominal muscle using a 7-0 silk suture. The next day, coronary ligation was performed in the same mouse, as previously reported. rhPlGF was infused for 3 days and rhsFlt-1 was infused for 7 days. In total, 95 mice were used for assessing survival rate and 74 mice for histological, physiological and biochemical analyses.

Bone Marrow Transplantation (BMT)
BMT was performed as described previously. Six weeks after BMT, the mice were treated with rhPlGF (10 μg) and coronary ligation was performed. Five days after AMI, peripheral blood was collected for fluorescence-activated cell sorting analysis, and hearts were harvested for immunohistochemical examination.

Enzyme-Linked Immunosorbent Assay (ELISA)
Levels of human and murine PlGF were measured by ELISA (R&D System, Germany). The assay for human PlGF recognizes recombinant and natural human PlGF, but not murine PlGF. Murine PlGF also reacts only with natural murine PlGF. Both human and murine PlGF assays recognize free PlGF, but not PlGF bound to sFlt-1.

Binding Assay of rhPlGF With rhsFlt-1 In Vitro
To confirm whether rhsFlt-1 binds to PlGF, we performed an in vitro binding assay. rhsFlt-1, which contains a histidine-tag, was diluted with a solution consisting of 20 mmol/L imidazole, 0.5 mol/L NaCl, 20 mmol/L Tris, and 0.1 mg/ml bovine serum albumin (BSA), at 5 concentrations: 1 ng/20 μl, 10 ng/20 μl, 100 ng/20 μl, 1,000 ng/20 μl, and 10,000 ng/20 μl, respectively. The rhsFlt-1 solution was mixed with Ni-agarose gel (Qiagen, Valencia, CA, USA), which can bind sFlt-1 that is histidine-tagged. After centrifugation, the supernatant was removed and rhPlGF solution (10 ng/100 μl, 100 mmol/L NaCl, 50 mmol/L Tris, 20 mmol/L imidazole, 0.1 mg/ml BSA) was added to the precipitation including the Ni-agarose-rhsFlt-1 complex. The solution was incubated overnight at 4°C. After centrifugation in order to remove the Ni-agarose-rhsFlt-1 complex bound to rhPlGF, uncombined rhPlGF in the supernatant was measured by ELISA.

Reverse Transcriptase-PCR
The apex of the infarcted heart was homogenized and RNA was extracted using Trizol reagent (Life Technologies, Grand Island, NY, USA). Reverse transcription was performed and PCR was then conducted using ABI Prism7700 (Applied Biosystems, Carlsbad, CA, USA). The level of brain natriuretic peptide (BNP) in infarcted hearts was measured by Taqman Gene Expression Assays (Applied Biosystems).

Evaluation of Infarct Size
In order to evaluate infarct size, TTC (2,3,5-triphenyltetrazolium chloride; Sigma Chemical Co, St Louis, MO, USA) staining was performed. At 7 or 28 days after coronary ligation, the hearts were excised, washed with phosphate-buffered saline (PBS), and the ventricles were cut into transverse slices. The slices were placed on a culture plate, stained for 15 min at room temperature with 1.0 ml of 1.5% TTC solution to determine the infarct area, and photo-
Infarct area and infarct fraction were evaluated. Infarct fraction was calculated as: infarct area/(infarct area + non-infarct area). Fibrin deposition within the infarct area was evaluated by Masson-trichrome staining: areas of fibrin deposition and area of viable tissue were directly measured, and the ratio of fibrin deposition area to viable tissue area (fibrin deposit area/viable tissue area) was calculated. Sequential sections were used to analyze the TTC and Masson-trichrome staining.

Statistical Analysis
All results are expressed as the means±SD. Differences between groups were evaluated for statistical significance using Kaplan-Meier analysis, Student’s t-test and 1-factor ANOVA. Values of P<0.05 were considered significant.

Results
Recombinant hPIGF Administration and Survival ofAMI Mice
The plasma rhPlGF level on the 3rd day of administration by
Figure 3. Echocardiography performed on the phosphate-buffered saline (PBS) (A) and recombinant human placental growth factor (rhPlGF) (B) groups 7 days after myocardial infarction. Left ventricular diastolic dimension and ejection fraction were analyzed (C,D). Reverse transcriptase-polymerase chain reaction was performed to compare the brain natriuretic peptide level in infarcted tissue of the PBS and rhPlGF groups (E). GAPDH was used as a control. Values are means±SD; **P<0.01 (comparison between PBS and PlGF groups).

Figure 4. Regenerating vessels in infarcted myocardium analyzed by immunohistochemistry 7 days after myocardial infarction. The number of CD31-positive vessels was increased by recombinant human placental growth factor (rhPlGF) administration (×200) (A–C). The number of αSMA-positive mature blood vessels was also increased after rhPlGF treatment (×100) (D–F). Values are means±SD; **P<0.01 vs phosphate-buffered saline (PBS). IZ, infarct zone; NIZ, non-infarct zone.
osmotic minipump was 1.252±460 pg/ml, which is approximately 4-fold higher than the endogenous mouse PIGF plasma level 3 days after an AMI (307±68 pg/ml) (data not shown). During the administration of rhPlGF, blood pressure did not differ significantly between the PlGF and PBS groups (data not shown). At 28 days after development of AMI, 6 of 20 mice in the PlGF group and 12 of 17 mice in the PBS group had died. The mortality rate was thus improved by 52% by rhPlGF treatment. Kaplan-Meier analysis showed that administration of rhPlGF for 3 days improved survival rate significantly (P<0.05) compared with vehicle (Figure 1).

**PIGF Improves Cardiac Function and Suppresses Remodeling**

We next evaluated the effects of rhPIGF on infarct size and cardiac function after induction of MI. TTC staining of the heart revealed that 7 days after MI, both infarct area and infarct fraction were significantly smaller in the rhPlGF group than in the PBS group (data not shown). At 28 days after development of AMI, 6 of 20 mice in the PIGF group and 12 of 17 mice in the PBS group had died. The mortality rate was thus improved by 52% by rhPlGF treatment. Kaplan-Meier analysis showed that administration of rhPlGF for 3 days improved survival rate significantly (P<0.05) compared with vehicle (Figure 1).

The ratio of fibrin deposit area to viable tissue area (fibrin deposit area/viable tissue area) in the infarct area was significantly lower in the rhPlGF group than in PBS group (1.5±1.04, 4.63±1.85, P<0.05) (Figure 2I). Echocardiographic findings at 7 days after coronary ligation showed that LVEDd was smaller in the rhPlGF group than in the PBS group (4.50±0.46 mm vs 5.14±0.38 mm, P<0.01) and LVEF was significantly higher (40.6±9.17% vs 25.7±7.23%, P<0.01) (Figures 3A–D). The level of BNP mRNA in the infarcted myocardium was significantly lower in the rhPlGF-treated group than in the PBS group (BNP mRNA/GAPDH: 0.46±0.42 vs 1.14±0.44, P<0.01) (Figure 3E).

**Figure 5.** Co-administration of both rhPIGF and rhsFlt-1 in MI mice. Binding assay of rhPIGF with rhsFlt-1 (A). Recombinant human sFlt-1 can bind to rhPIGF in vitro. The efficacy of rhPIGF was inhibited by rhsFlt-1 in a dose-dependent manner. Human PIGF in peripheral blood was analyzed in normal mice using an ELISA kit (B). (Cont: normal mice; PIGF: rhPIGF-treated mice; PIGF + sFlt-1: co-administration of rhPIGF and sFlt-1.) rhPIGF was suppressed by rhsFlt-1 administration in vivo. Values are means±SD; *P<0.05 vs PIGF, †P<0.05 vs PIGF. Murine PIGF in peripheral blood in normal and MI mice was analyzed by ELISA kit (C). (C: normal mice; AMI: PBS-treated MI mice; PIGF: administration of rhPIGF with coronary ligation; PIGF + sFlt-1: co-administration of rhPIGF and rhsFlt-1 with coronary ligation; sFlt-1: administration of sFlt-1 with coronary ligation.) PIGF activity was suppressed by administration of sFlt-1. Values are means±SD; *P<0.05 vs Control, †P<0.05 vs PIGF. Blood pressure of rhsFlt-1- or PBS-treated mice was measured for 7 days (D). Survival of MI mice administered rhPIGF, PBS, sFlt-1, or rhPIGF and rhsFlt-1 simultaneously (E). Survival rate was improved in the rhPIGF group, but not in the other groups. Survival rate in the rhsFlt-1 group was the same as that in the PBS group. *P<0.05 vs the other groups. rhsFlt-1, recombinant human soluble Flt-1. See text and Figures 1–4 for abbreviations.
PIGF Induces Angiogenesis and Arteriogenesis
To elucidate the mechanism underlying the beneficial effects of PIGF on post-AMI pathology, we analyzed angiogenesis and arteriogenesis in the infarcted myocardium. The number of CD31-positive vascular endothelial cells in the infarct areas (including the border zone) was significantly higher in the rhPlGF group than in the PBS group (644.6 ± 90.54/mm² vs 459.0 ± 73.89/mm², P<0.01) (Figures 4A–C). Similarly, the number of α-SMA-positive vessels was increased in the same areas in the rhPlGF group relative to controls (rhPlGF: 31.6 ± 7.20/mm², PBS: 23.5 ± 7.41/mm², P<0.01) (Figures 4D–F).

Beneficial Effect of Exogenous PIGF Blocked by rhsFlt-1
In order to further evaluate the actions of exogenously administered rhPlGF, we examined whether or not its beneficial effects were blocked by the simultaneous administration of rhsFlt-1, a PIGF antagonist.

We confirmed before the experiment that rhsFlt-1 binds to PIGF in vitro and in vivo. As shown in Figure 5A, an in vitro binding assay showed that 10⁻¹² mol/L rhsFlt-1 binds to 0.17×10⁻¹² mol/L rhPlGF. When rhPlGF was administered alone, the plasma rhPlGF level was 1.252 ± 460 pg/ml. Co-administration of rhsFlt-1 decreased the plasma rhPlGF level to 753 ± 69 pg/ml, as shown in Figure 5B. The plasma level of endogenous PIGF was higher in AMI mice with rhPlGF treatment than in control mice (307 ± 68 pg/ml vs 128 ± 8.8 pg/ml). The co-administration of rhsFlt-1 also reduced the plasma level of endogenous PIGF to 121 ± 108 pg/ml (Figure 5C). Thus, rhsFlt-1 blocked both exogenous and endogenous PIGF by approximately 50%. We also proved that sFlt-1, like rhPlGF, did not affect hemodynamic function (Figure 5D).

As shown in Figure 5E, co-administration of rhsFlt-1 with rhPlGF lowered the survival rate of mice given rhPlGF alone, validating the improvement in the survival rate seen in mice treated with rhPlGF alone. The survival rate of control mice and those given only rhsFlt-1 was similar.

Soluble Flt-1 Inhibits rhPlGF Effects on Infarct Size and Angiogenesis
As described earlier, TTC staining showed that the infarct size...
area in rhPlGF-treated mice was significantly smaller than that of the PBS-treated group. However, the infarct area in the co-administered group was significantly larger than that in mice given rhPlGF alone (7.99±1.33 mm² vs 4.25±2.04 mm², P<0.01) and was similar to that in the PBS group (5.96±1.54 mm²). Interestingly, administration of rhFlt-1 alone increased the infarct area to 11.90±2.90 mm², which was significantly larger than the infarct area of the PBS group (P<0.01) (Figures 6A–E).

CD31-positive cells were counted to confirm the inhibitory effect of rhsFlt-1 on angiogenesis. In the MI mice, the number of newly formed vessels was increased in the rhPlGF group, but was decreased by co-administration of rhsFlt-1 to a quantity similar to that in the PBS group (rhPlGF: 644±90.5/mm²; rhPlGF+sFlt-1: 335±103/mm²; PBS: 407±121/mm²; sFlt-1: 287±75.7 mm²) (Figure 6F).

Mechanisms of PlGF-Induced Protective Effect on Ischemic Heart

CD31-positive cells were counted to confirm the inhibitory effect of rhsFlt-1 on angiogenesis. In the MI mice, the number of newly formed vessels was increased in the rhPlGF group, but was decreased by co-administration of rhsFlt-1 to a quantity similar to that in the PBS group (rhPlGF: 644±90.5/mm²; rhPlGF+sFlt-1: 335±103/mm²; PBS: 407±121/mm²; sFlt-1: 287±75.7 mm²) (Figure 6F).

Figure 7. Mechanisms underlying the angiogenic effect of PlGF. EPCs (Flk-1+Sca-1+ cells) were mobilized into the peripheral circulation in MI mice (A). The percentage of EPCs was increased in MI mice, and was further increased in rhPlGF-treated MI mice. Values are means±SD; *P<0.05, †P<0.05 vs PBS. The number of GFP-positive cells in the infarct area in bone marrow transplant mice (B). The number of infiltrated monocytes (CD68-positive cells) in the infarct area was also increased by rhPlGF treatment compared with PBS treatment (C). Values are means±SD; *P<0.05 vs PBS. Bone-marrow-derived cells were examined by immunohistochemistry. GFP-positive (green) cells were observed in both PBS- (D) and rhPlGF- (E) treated mice hearts (×400). GFP+CD31+ cells were observed in the vessels of the rhPlGF group (F: GFP, G: CD31, H: merge of F and G; ×1,200). Green represents GFP and red represents CD31. See text and Figures 1–4 for abbreviations.
positive monocytes were also increased in the infarct areas in the rhPlGF group relative to the PBS group (2.878 ± 567/mm² vs 2.346 ± 398/mm²; P<0.01; Figure 7C). Immunofluorescent histological analysis revealed a few GFP and CD31 double-positive cells or BMCs directly differentiated into endothelial cells existing in the infarct area in both rhPlGF and PBS groups (Figures 7D,E). However, the number of these cells was very small and there was no significant difference between the 2 groups (rhPlGF: 6–8 per 10 high-power fields; PBS: 1–2 per 10 high-power fields) (Figures 7F–H).

Discussion

The present study demonstrates that exogenous administration of rhPlGF enhances angiogenesis and arteriogenesis, reduces infarct size, and improves cardiac function and survival rate following AMI. These beneficial effects were blocked by co-administration of rhsFlt-1, which traps rhPlGF in the circulation and thus inhibits its action. These findings suggest a possible use of PI GF as adjunctive therapy in AMI.

Exogenous PlGF Improves the Prognosis of MI Via Angiogenesis and Arteriogenesis

Earlier experimental studies have shown that several growth factors and cytokines, including basic fibroblast growth factor,5 G-CSF,10 erythropoietin,20 angiopoietin-121 and VEGF,22 improve cardiac function after AMI by promoting angiogenesis. However, no molecule has been clinically proved to be useful as an adjunctive therapy in AMI. In the present study we assessed the therapeutic use of PI GF in several reasons. Firstly, PI GF is a member of the VEGF family and induces not only angiogenesis but also arteriogenesis in vivo.10 Secondly, PI GF is able to mobilize EPCs from bone marrow to the peripheral circulation,17 and finally, we recently observed that PI GF mRNA expression is substantially upregulated in the endothelium of the coronary arteries and interstitial cells in the infarct regions, suggesting participation of PI GF in the pathology of AMI.13 Some previous investigations revealed that transfer of the PI GF gene or direct injection of PI GF protein induced angiogenesis in ischemic hindlimb and myocardium.9,10 Moreover, Autiero et al reported that intraperitoneal injection of a VEGF/PI GF heterodimer enhanced angiogenesis 2 weeks after AMI.23 However, those studies focused primarily on the local angiogenic effect of PI GF and did not examine its broader role; for instance, its influence on cardiac function and survival rate, which are important in terms of the molecule’s clinical application. The present study demonstrates that 3-day intraperitoneal administration of rhPI GF reduces infarct size, preserves EF and reduces mortality. Considering that PI GF mRNA expression was augmented in the infarct region and that the plasma PI GF level peaked on the 3rd day after the onset of AMI in our human study,13 injection of exogenous PI GF would enhance the beneficial effect of endogenous PI GF.

sFlt-1 Cancels the Beneficial Effect of PI GF

Co-administration of rhsFlt-1 with rhPlGF completely inhibited the PI GF-induced reduction of infarct size and improvement of survival rate, which confirms the beneficial effects of PI GF in AMI. However, sole administration of rhsFlt-1, which reduced the plasma level of endogenous PI GF by approximately 50%, did not affect the survival rate after AMI, though it increased infarct size. Considering that the plasma level of rhPI GF during administration was approximately 1,200 pg/ml and the peak plasma level of endogenous PI GF in AMI was approximately 300 pg/ml, augmentation of endogenous PI GF gene expression may not be sufficient to improve mortality. This supports the idea of using supplemental PI GF as an adjunctive therapy in AMI. We also found there was a discrepancy in that rhsFlt-1 administration increased infarct area without suppressing angiogenesis compared with PBS treatment. This result suggests that endogenous PI GF production in the injured myocardium does not have potent stimulatory effects on angiogenesis by itself, but rather it may have direct protective effects on the ischemic myocardium by a mediating pathway except for angiogenesis. It is well recognized that the loss of ischemic cardiomyocytes after MI induces compensatory hypertrophy of the non-ischemic remote myocardium. Roncal et al reported that PI GF induces not only enlargement of vessel size, but also compensatory hypertrophy of cardiomyocyte in remote non-infarcted myocardium.24 We assume this may be one of the mechanisms by which sFlt-1 exacerbates cardiac remodeling via suppressing endogenous PI GF.

Mechanisms of PI GF’s Effect on Ischemic Myocardium

The current study could not elucidate the mechanism by which PI GF reduces infarct size and ultimately improves survival rate. Given previous evidence that VEGF did not improve cardiac function and mortality in either animal or human studies, despite its strong angiogenic properties, it is likely that the dual properties of PI GF, angiogenesis and arteriogenesis, are closely related to its reducing both infarct size and mortality. PI GF is known to induce new vascular formation by 2 mechanisms: stimulation of local proliferation of endothelial cells,10,25–27 as well as recruitment of BMCs to target tissue.17 In particular, PI GF exerts its biological activities by binding specifically to Flt-1 and causing its autophosphorylation.28 Flt-1 is expressed not only on endothelial cells, but also on macrophages, hematopoietic progenitor cells, and EPCs.29,30 Li et al revealed that PI GF enhances EPC recruitment from the bone marrow into peripheral tissues. In this context, we have also investigated whether EPCs and Flt-1-positive monocytes contribute to PI GF-induced angiogenesis in the infarct regions. The present study showed that EPCs and monocytes were mobilized into the peripheral blood by rhPI GF; however, direct transdifferentiation of EPCs into the blood vessels was rarely observed. These findings suggest that angiogenesis induced by PI GF is not caused primarily by differentiation of the progenitor cells, but is probably related to angiogenic properties of rhPI GF itself and to other cytokines that are released by PI GF stimulation. Moreover, PI GF enhances both vessel maturation and collateral growth by inducing monocyte infiltration. Sholz et al reported that collateral growth in a hindlimb ischemia model was delayed in PI GF knock-out mice.32 In our results, the number of monocytes in the infarct area increased with rhPI GF administration, which may result in augmentation of arteriogenesis.33 Earlier studies reported that mobilized monocytes produce MCP-1, bFGF, and TNF-α,34 so it is possible that cytokines other than PI GF released by recruited monocytes indirectly stimulate angiogenesis and collateral artery growth in our study model. Furthermore, improvement of cardiac function by rhPI GF treatment might relate to mechanisms other than angiogenesis, such as inhibition of apoptosis.
In the present study, the infarct area of the rhPIGF group was significantly larger than that of the PBS group because of preservation of viable myocardium and suppression of fibrin deposition in the infarct area. The reason why rhPIGF treatment suppressed fibrin deposition in the infarct area in the chronic phase remains unknown, but preservation of functional blood supply to the infarct area by the PIGF-mediated arteriogenesis plays an important role in ameliorating tissue hypoxia, which stimulates extracellular matrix production by interstitial cells in the infarct area. Further studies are necessary to elucidate the mechanisms underlying the angiogenesis and arteriogenesis induced by the PIGF–Flt-1 pathway.

References


