Angiopoietin-1 Mediates Adipose Tissue-Derived Stem Cell-Induced Inhibition of Neointimal Formation in Rat Femoral Artery

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Background: Adipose tissue-derived stem cells (ASC) produce a variety of cytokines that potentially mediate the proangiogenic and antiapoptotic effects of the ASC. We examined whether ASC produced angiopoietin-1 (Ang1) and whether Ang1 functionally mediated ASC-induced suppression of neointimal formation.

Methods and Results: Ang1 production was measured by enzyme-linked immunosorbent assay. Production of endogenous Ang1 by ASC was inhibited with small interfering RNA (siRNA) for Ang1. Overproduction of Ang1 was achieved with an adenovirus that expresses Ang1 (AdAng1). ASC expressing Ang1 siRNA, or AdAng1 were administered around the femoral artery after wire injury, and immunohistochemical analysis was performed to examine their effects on neointimal formation. ASC produced Ang1 in a time-dependent manner, especially when cultured in medium containing growth factors for vascular endothelial cells. When ASC were treated with Ang1 siRNA, the inhibitory effect of ASC on neointimal formation was significantly reduced. Knockdown of Ang1 significantly increased macrophage infiltration in the neointima, and significantly decreased endothelial regeneration. In contrast, forced expression of Ang1 using AdAng1 significantly suppressed neointimal formation and macrophage infiltration, and stimulated reendothelialization.

Conclusions: Ang1 was implicated in ASC-induced suppression of neointimal formation. The results also suggested that Ang1 inhibited neointimal formation via stimulation of reendothelialization and suppression of macrophage infiltration in the neointima. (Circ J 2013; 77: 1574–1584)

Key Words: Adipose tissue-derived stem cells; Angiopoietin-1; Macrophages; Neointimal formation; Reendothelialization

Drug-eluting stents (DES) have been widely used for the treatment of coronary artery disease and are definitely useful for the inhibition of restenosis compared with bare metal stents in the short to mid term. However, recent long-term analysis of DES studies indicates that the use of DES does not always improve the outcome of patients, probably because DES potentially increase the risk of late thrombosis. This complication may come from the suppression of vascular endothelial cell (VEC) proliferation, as well as vascular smooth muscle cell (VSMC) proliferation, by antiproliferative drugs, resulting in delayed reendothelialization of stented vessels and thrombus formation. Because VECs produce several factors, such as prostacyclins and C-type natriuretic peptide, that inhibit the proliferation of VSMCs, stimulation of reendothelialization may be a promising strategy to inhibit restenosis and late thrombosis.

Adipose tissue-derived stem cells (ASC) are mesenchymal stem cells that exist in large numbers in subcutaneous adipose tissue. Although ASC are similar to bone marrow-derived mesenchymal stem cells (BM-MSC) in terms of surface marker expression, ASC and BM-MSC reportedly show different patterns of protein expression, suggesting that they may have different effects on tissue regeneration. Recent studies showed that administration of ASC was useful for the treatment of hindlimb ischemia and acute myocardial infarction, especially in animal models. Furthermore, ASC were used clinically...
to treat patients with critical limb ischemia, and were effective. However, the mechanisms whereby ASC protect those ischemic tissues are still controversial. ASC were engrafted into blood vessels or cardiac myocytes, and stimulated angiogenesis and regeneration of tissues in some studies. However, in other studies the ASC produced several cytokines that protected the ischemic tissues without engraftment into blood vessels or cardiac myocytes. We have recently shown that ASC inhibited neointimal formation in a rat model of wire injury to the femoral artery. ASC administration from the adventitial side also significantly suppressed neointimal formation and stimulated reendothelialization, suggesting that ASC inhibited neointimal formation in a paracrine fashion. Therefore, it is speculated that ASC produce several proangiogenic cytokines that promote proliferation and migration of VECs, and thereby stimulate reendothelialization.

However, the effect of proangiogenic factors on the stimulation of reendothelialization is not so straightforward. Vascular endothelial growth factors (VEGF) are essential for vasculogenesis and play a pivotal role in the stimulation of angiogenesis. Among the VEGF family, VEGF-A is the major player in angiogenesis. Although it was first reported that over-expression of VEGF-A inhibited neointimal formation, subsequent studies showed that blockade of the endogenous VEGF family rather than stimulation of VEGF-A expression inhibited neointimal formation. There are several possible mechanisms by which VEGF-A stimulates neointimal formation. First, VEGF-A stimulates the migration of monocytes/macrophages, because monocytes/macrophages express Flt-1, the first VEGF receptor. VEGF-A may also stimulate the migration of monocytes/macrophages by increasing the permeability of blood vessels. Second, VEGF-A stimulates the migration and/or proliferation of VSMC-like cells that reside in the neointimal layer, because these VSMC-like cells reportedly express Flt-1.

Materials and Methods

Reagents

Anti-Tie2 antibody was purchased from Santa-Cruz Biotechnology (Santa-Cruz, CA, USA). Anti-CD31 antibody was obtained from ABR-Affinity BioReagents (Golden, CO, USA). Anti-ED1 antibody was obtained from AbD Serotec (Oxford, UK).

Cell Culture

ASC were cultured from male Wistar rats as previously reported. ASC were first cultured in a 1:1 mixture of Dulbecco’s modified Eagle medium (DMEM) and F12 medium containing 10% fetal bovine serum (FBS), and split several times to expand the cells. Passages 2–4 were used for the experiments. ASC were then cultured on fibronectin-coated dishes in endothelial growth medium-2MV (EGM; Lonza Walkersville, Walkersville, MD, USA). EGM consists of endothelial basal medium-2 (Lonza Walkersville) containing 5% FBS plus growth factors such as epidermal growth factor, hydrocortisone, VEGF-A, basic fibroblast growth factor (bFGF) and insulin-like growth factor-I. ASC were also cultured on fibronectin-coated dishes in endothelial basal medium-2 containing 5% FBS (EBM) as the negative control. In some experiments, ASC were labeled with PKH26 red fluorescent dye (Sigma-Aldrich, St. Louis, MO, USA) according to the protocol provided by the manufacturer in order to trace the fate of ASC administered around the rat femoral artery. NRK-52E cells, a cell line derived from rat renal tubular cells, and HEK293 cells were obtained from ATCC (Manassas, VA, USA) and cultured in DMEM containing 5% FBS.

Wire Injury Model

All procedures involving experimental animals were approved by the Institutional Committee for Animal Research of Tokyo University. Transluminal mechanical injury to rat femoral artery was performed as previously described. Male Wistar rats (8–10 weeks old) were anesthetized with pentobarbital injected intraperitoneally and a groin incision was made under a surgical microscope. A guidewire (0.46-mm diameter) was introduced through a small muscular branch of the femoral artery, proximal to the aortic bifurcation and withdrawn. ASC (10⁶ cells) cultured in EGM for 7 days after adenovirus suspensions (1×10⁸ plaque forming units) were placed around the femoral artery from the adventitial side after wire injury and just before suturing of the incision.

Histochemistry

The femoral arteries were fixed by perfusing them with 4% paraformaldehyde and then they were processed for paraffin embedding. Cross-sections (2 μm) were cut, deparaffinized, rehydrated, and stained with hematoxylin and eosin. For immunohistochemistry, sections were incubated with primary antibodies reactive to CD31, ED1 and Tie2. Sections were then incubated with biotinylated secondary antibody and finally horseradish peroxidase-labeled streptavidin according to the instructions provided by the manufacturer (DAKO, Japan). Some sections were counterstained with hematoxylin.

Construction of Lentivirus Expressing Ang1 siRNA

A lentivirus that expresses Ang1 small interfering RNA (siRNA) was constructed using BLOCK-iT Lentiviral Pol II miRNA Expression System (Invitrogen) according to the manufacturer’s instructions. Each of 3 double-stranded oligonucleotides that target the coding region of rat Ang1 to silence its expression was first subcloned into the pcDNA6.2-GW/EmGFPmiR plasmid that also expresses green fluorescence protein (GFP) to facilitate the identification of transfected cells. The DNA sequences of the sense strand of those oligonucleotides were as follows:

ANG1 siRNA1: 5’-TGCTGTTTTAGTACCTGCTCAGAGTTCTGCAGAA-3’
ANG1 siRNA2: 5’-TGCTGTTCAGACAACCTGTGTGGTTTTTGCCACGTACGTGGGAGACAGGTACTAAA-3’
ANG1 siRNA3: 5’-TGCTGTTCAGACAACCTGTGTGGTTTTTGCCACGTACGTGGGAGACAGGTACTAAA-3’
Ang1 siRNA3: 5’-TGCTGTGAAGTGTTAGGCACATTGCGTCTTGCCACTGACGGCAATGTCTA- CACTTTCA-3’.

After confirming the DNA sequences, the 3 double-stranded oligonucleotides were ligated together in the pcDNA6.2-GW/EmGFPmiR plasmid vector so that this plasmid expressed the 3 Ang1 siRNAs simultaneously. The region that encodes GFP and the 3 Ang1 siRNAs in the vector was then subcloned into pLenti7.3/V5-DEST Gateway vector using the clonase reaction as recommended by the manufacturer. The pcDNA6.2-GW/EmGFPmiR-neg control plasmid supplied by the manufacturer and which expresses siRNA that is predicted not to target any known vertebrate gene (NC siRNA) was also subcloned into the pLenti7.3/V5-DEST Gateway vector to produce a negative control lentivirus. These plasmids were then transfected into 293FT cells, together with ViraPower Packaging Mix (Invitrogen) using the calcium phosphate method to produce the lentivirus. Culture medium was changed the following day, and the medium containing lentivirus was collected 2 days later. Lentivirus was concentrated from the culture medium using polyethylene glycol method as previously reported. The titer of lentivirus was determined using 293FT cells with GFP fluorescence as the marker. Rat ASC were infected with lentivirus expressing NC siRNA (Lent_NC siRNA) or Ang1 siRNA (Lent_Ang1 siRNA), and cultured in EGM for 1 week. Under this condition, approximately 80% of cells expressed GFP and significantly suppressed Ang1 production (Figure 2A). These results were compatible with our previous findings. The amount of Ang1 that accumulated in the culture medium did not change significantly when ASC were infected with Lent_Ang1 siRNA compared with when infected with Lent_NC siRNA (Figure 2B), suggesting that the Ang1 siRNA used in this study significantly suppressed Ang1 production (Figure 2A). These results were compatible with our previous findings.

Results

Statistical Analysis

Values are expressed as the mean±SEM. Statistical analyses were performed using analysis of variance followed by the Student-Neumann-Keuls test. Differences with P<0.05 were considered statistically significant.

Confirmation of the Function of Ang1 siRNA and AdAng1

To study the role of endogenous Ang1 secreted from ASC in the inhibition of neointimal formation, we established systems to knock down and overexpress Ang1. Because it is difficult to introduce siRNA into ASC by lipofection, we used lentivirus for this purpose. ASC were infected with lentivirus expressing NC siRNA (Lent_NC siRNA) or Ang1 siRNA (Lent_Ang1 siRNA), and cultured in EGM for 1 week. The amount of Ang1 that accumulated in the culture medium was significantly lower than that in the culture medium of adipose tissue-derived stem cells (ASC) in a time-dependent manner. The ASC were plated in 24-well plates and cultured in EBM or EGM for 1 week. After washing the wells with phosphate-buffered saline, the medium was replaced with serum-free DMEM and incubated for the indicated periods. Ang1 accumulation in the medium was measured with an ELISA kit. *P<0.05 vs. 0 h; **P<0.01 vs. 0 h (n=6 each). DMEM, Dulbecco’s modified Eagle medium; EBM, endothelial basal medium-2 containing 5% FBS; EGM, endothelial growth medium-2MV; ELISA, enzyme-linked immunoabsorbent assay; FBS, fetal bovine serum.

Rat Ang1 antisense primer:
5’-TCAGAAATCCAGGCGGAT-3’.

A recombinant adenovirus that expresses GFP (AdGFP) was obtained from Quantum Biotechnologies (Montreal, Canada).

Enzyme-Linked Immunosorbent Assay

Rat Ang1 in culture medium was measured with an enzyme-linked immunosorbent assay (ELISA) kit (Kamiya Biomedical Company, Seattle, WA, USA) according to the manufacturer’s method. Fluorescence was measured (excitation wave length: 320 nm, emission wave length: 460 nm) with a Fluoroskan Ascent FL fluorescent microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

ASC Produce Ang1

Using real-time PCR analysis and Western blot analysis, we previously showed that ASC produce Ang1, especially when cultured in EGM compared with EBM. We first measured the time course of Ang1 accumulation in culture medium by ELISA. Culture medium collected from EGM-cultured ASC contained Ang1, and the amount of Ang1 increased significantly in a time-dependent manner, whereas that in the culture medium collected from EBM-cultured ASC did not increase significantly (Figure 1). These results were compatible with our previous findings.

Figure 1. Angiopoietin-1 (Ang1) accumulates in the culture medium of adipose tissue-derived stem cells (ASC) in a time-dependent manner. The ASC were plated in 24-well plates and cultured in EBM or EGM for 1 week. After washing the wells with phosphate-buffered saline, the medium was replaced with serum-free DMEM and incubated for the indicated periods. Ang1 accumulation in the medium was measured with an ELISA kit. *P<0.05 vs. 0 h; **P<0.01 vs. 0 h (n=6 each). DMEM, Dulbecco’s modified Eagle medium; EBM, endothelial basal medium-2 containing 5% FBS; EGM, endothelial growth medium-2MV; ELISA, enzyme-linked immunoabsorbent assay; FBS, fetal bovine serum.
Ang1, we used AdAng1. We infected NRK52E cells with AdGFP or AdAng1, because ASC produce endogenous Ang1. The amount of Ang1 in the culture medium increased significantly when NRK52E cells were infected with AdAng1 compared with AdGFP (Figure 2C).

**Role of Ang1 in Neointimal Formation**

We have reported that EGM-cultured ASC significantly suppress neointimal formation compared with EBM-cultured ASC. To examine the role of endogenous Ang1 produced by ASC in neointimal formation, ASC were infected with Lent_NC siRNA or Lent_Ang1 siRNA, cultured in EGM for 1 week, and then used in the rat femoral artery wire injury model. Because we preliminarily confirmed that the extent of neointimal formation in the AdGFP-infected group was similar to that observed in the wire-injured femoral artery without adenoviral infection or ASC administration (data not shown), we compared the extent of neointimal formation in ASC-administered groups with that in the AdGFP-infected group. EGM-cultured ASC and EGM-cultured ASC preinfected with Lent_NC siRNA significantly inhibited neointimal formation compared with AdGFP infection when they were seeded around the femoral artery (Figure 3). In contrast, the inhibitory effect of ASC on neointimal formation was weakened significantly when EGM-cultured ASC were preinfected with Lent_Ang1 siRNA, suggesting that endogenous Ang1 produced by ASC is implicated in ASC-induced suppression of neointimal formation. We also infected AdAng1 from the adventitial side of the femoral artery, which significantly suppressed neointimal formation compared with AdGFP infection. This result further suggested that Ang1 has the potential to inhibit neointimal formation. We also examined the fate of ASC administered around the femoral artery. For this purpose, we labeled ASC cultured in EGM with PKH26 and seeded the cells around the femoral artery. ASC remained in situ until 7 days after their administration, but were barely detected around the femoral artery 14 days after administration (Figure 4A). This result further suggested ASC inhibited neointimal formation via secretion of several cytokines rather than ASC being engrafted around the femoral artery. We also examined whether adenoviruses administered around the femoral artery had infected the tissues surrounding the vessel. For this purpose, we dripped a suspension of AdGFP around the femoral artery and 7 days after infection the fluorescence of GFP was detected in the tissues around the femoral artery, confirming that adenoviruses had infected the tissues around the femoral artery and were expressing the proteins that they encoded (Figure 4B).

To examine the mechanisms by which Ang1 inhibited neointimal formation, we examined macrophage infiltration and reendothelialization in the injured artery, ED-1 positive macrophages were detected in the neointimal layer (Figure 5A). EGM-cultured ASC and EGM-cultured ASC preinfected with Lent_NC siRNA significantly inhibited macrophage infiltration compared with EGM-cultured ASC preinfected with Lent_Ang1 siRNA. AdAng1 infection also significantly inhibited macrophage infiltration compared with AdGFP infection, suggesting that Ang1 inhibits macrophage infiltration in the neointimal layer. Adenoviral infection potentially induces tissue inflammation. We examined whether adenoviral infection around the femoral artery increased macrophage infiltration (Figure 5D). ED-1-positive macrophages were detected around the wire-injured femoral artery without adenoviral infection and around that infected with AdGFP, and the number of macrophages was similar between the 2 groups. However, macrophage infiltration was significantly reduced around the wire-
well as in the neointima. We next examined the extent of re-endothelialization by staining CD31-positive endothelial cells as Figure 6. Administration of EGM-cultured ASC preinfected with Lent_Ang1 siRNA had a significantly weaker effect on injured femoral artery infected with AdAng1, suggesting that adenoviral infection did not significantly induce tissue inflammation at the site of infection and that AdAng1 significantly inhibited macrophage infiltration around the femoral artery as well as in the neointima. We next examined the extent of re-endothelialization by staining CD31-positive endothelial cells (Figure 6). Administration of EGM-cultured ASC preinfected with Lent_Ang1 siRNA had a significantly weaker effect on

Figure 3. (A) Effect of knockdown of endogenous angiopoietin-1 (Ang1) produced by adipose tissue-derived stem cells (ASC) and forced expression of Ang1 on neointimal formation. ASC were cultured in 100-mm dishes and infected with LV_NC siRNA (NCsiRNA) or LV_Ang1 siRNA (Ang1siRNA). ASC not infected with LV were used as the positive control (ASC). ASC were then cultured in EGM for 1 week. ASC (10⁶ cells) were seeded around the femoral artery from the adventitial side immediately after wire injury. AdGFP or AdAng1 was also applied to the femoral artery from the adventitial side after wire injury. The femoral arteries were harvested 14 days after the injury for histological analysis. Bars=100μm. (B) Intima:media (I/M) ratio was compared among the groups (n=8 each). *P<0.05 vs. LV_NC siRNA infection, †P<0.01 vs. AdGFP infection. EGM, endothelial growth medium-2MV; LV, lentivirus; siRNA, small interfering RNA.
Ang1 inhibits neointimal formation

It has been reported that induction of adventitial angiogenesis by some proangiogenic factors further stimulates neointimal formation.\(^{27}\) We, therefore, examined whether ASC administration and AdAng1 infection induced adventitial neoangiogenesis (Figure 7). Although VECs forming the luminal surface of the femoral artery and VECs of blood vessels running around the femoral artery were CD31 positive, CD31-positive neoangiogenesis was not observed in or in the vicinity of the adventitial layer. These results suggested that cytokines produced by ASC and Ang1 did not promote adventitial angiogenesis.

**Figure 4.** (A) Traces of adipose tissue-derived stem cells (ASC) seeded around the femoral artery. ASC cultured in EGM for 1 week were labeled with PKH26 and seeded around the femoral artery. The femoral artery and the surrounding tissue were harvested 1, 3, 7 and 14 days after ASC seeding and fluorescence of PKH26 was analyzed under a fluorescent microscope. Arrows indicate PKH26-positive cells, and arrowheads indicate the lumen of the femoral artery. Bars=100\(\mu\)m. (B) Expression of adenoviruses around the femoral artery. A suspension of AdGFP was dripped around the femoral artery and the fluorescence of GFP was detected 7 days later under a fluorescent microscope. The femoral artery that was not infected with AdGFP was used as the negative control (Control). Arrows indicated GFP-positive areas, and arrowheads indicate the internal elastic lamina. Bars=100\(\mu\)m. GFP, green fluorescence protein.
Figure 5. Macrophage infiltration in the neointima and around the femoral artery. (A) Effect of knockdown of endogenous angiopoietin-1 (Ang1) produced by adipose tissue-derived stem cells (ASC) and forced expression of Ang1 on macrophage infiltration in the neointimal layer. Experiments were performed in the same way as described in Figure 3. ED1-positive cells in the neointima were stained using an anti-ED1 antibody. Bars=50 μm. (B) The number of ED1-positive cells in the neointima was compared among the groups (n=8). *P<0.05 vs. LV_NC siRNA infection; †P<0.01 vs. AdGFP infection. (C) The number of ED1-positive cells around the femoral artery was compared among the groups (n=8). †P<0.01 vs. AdGFP infection. (D) ED1-positive cells around the femoral artery were analyzed. Wire-injured femoral arteries without adenoviral infection and those infected with AdGFP or AdAng1 were used for this analysis. Arrowheads indicate ED1-positive cells. Bars=50 μm. GFP, green fluorescence protein.
Ang1 Inhibits Neointimal Formation

Discussion

In this study, Ang1 was implicated in ASC-induced suppression of neointimal formation after wire injury of the rat femoral artery. The results suggested that Tie2-expressing cells did not contribute to the neointimal formation, even when Ang1 was overproduced.

Tie2 Expression in the Neointima

Because some classes of bone marrow-derived progenitor cells and monocytes/macrophages reportedly express Tie2,28,29 and the neointimal layer contains cells derived from bone marrow,30 we examined whether the neointimal layer contained Tie2-expressing cells, but they were barely detected in the neointima of the femoral artery infected with AdGFP or AdAng1, even though Tie2 was expressed in the regenerated VECs of the AdAng1-infected femoral artery (Figure 8). These results suggested that Tie2-expressing cells did not contribute to the neointimal formation, even when Ang1 was overproduced.

Figure 6.  (A) Effect of knockdown of endogenous angiopoietin-1 (Ang1) produced by adipose tissue-derived stem cells (ASC) and forced expression of Ang1 on reendothelialization. Experiments were performed as described in Figure 3. Vascular endothelial cells (VECs) were stained with an anti-CD31 antibody and the percentage of the CD31-positive area was calculated. Bars=50 μm. (B) The percentage of the CD31-positive area was compared among the groups (n=8). *P<0.05 vs. LV_NC siRNA infection, †P<0.05 vs. AdGFP infection. GFP, green fluorescence protein.
Overexpression of VEGF-A appears to promote neo-intimal formation through stimulation of the extravasation of inflammatory cells, recruitment of monocytes/macrophages via Flt1 expressed on those cells and migration of VSMCs in the neointima via Flt1.

It was also reported that VEGF-A is implicated in the exacerbation of atherosclerotic plaque expansion and immune-mediated vascular remodeling. Thus, it appears that inappropriate overproduction of VEGF-A induces inflammation, although an appropriate amount of VEGF-A is necessary for angiogenesis. In contrast, Ang1 stimulated reendothelialization and inhibited neointimal formation without stimulation of monocytes/macrophages infiltration in the neointima. Thus, Ang1 seems to have the potential to stimulate reendothelialization without stimulating an inflammatory response. The organ-protective and antiinflammatory effects of Ang1 may be mainly mediated by its angiopoietin-like effects on endothelial cells.

Several cytokines, such as hepatocyte growth factor and bFGF, that have been identified in ASC are secreted and implicated in the proangiogenic and/or antiapoptotic effect of ASC. However, the expression of these cytokines did not increase when ASC were cultured in EGM compared with EBM, as we previously reported.

It is easy to speculate that proangiogenic factors potentially suppress neointimal formation, because those factors can stimulate reendothelialization, resulting in the inhibition of neointimal growth. However, the scenario is not so straightforward. Overexpression of VEGF-A appears to promote neointimal formation through stimulation of the extravasation of inflammatory cells, recruitment of monocytes/macrophages via Flt1 expressed on those cells and migration of VSMCs in the neointima via Flt1. It was also reported that VEGF-A is implicated in the exacerbation of atherosclerotic plaque expansion and immune-mediated vascular remodeling. Thus, it appears that inappropriate overproduction of VEGF-A induces inflammation, although an appropriate amount of VEGF-A is necessary for angiogenesis. In contrast, Ang1 stimulated reendothelialization and inhibited neointimal formation without stimulation of monocytes/macrophages infiltration in the neointima. Thus, Ang1 seems to have the potential to stimulate reendothelialization without stimulating an inflammatory response. The organ-protective and antiinflammatory effects of Ang1 may be mainly mediated by its angiopoietin-like effects on endothelial cells.
of Ang1 have been reported in several models, such as acute lung injury, acute kidney injury and unilateral ureteral obstruction, in which macrophage infiltration was reduced. Ang1 reportedly has the potential to inhibit the expressions of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, and thereby inhibit macrophage infiltration. Ang1 also appears to have the capability of suppressing the activation of macrophages. Thus, these antiinflammatory activities of Ang1 may be involved in its organ-protective effect.

We also found that Tie2-expressing cells were barely detected in the neointima even after forced expression of Ang1. Recent reports suggest that Tie2 is expressed not only on VECs but also on bone marrow-derived hematopoietic progenitor cells, such as hemangiocytes, and on Tie2-expressing monocytes. Thus, it is possible that overproduction of Ang1 stimulates neointimal formation via recruitment of the bone marrow-derived progenitor cells or macrophages in the neointimal layer. However, our results suggested that Ang1 did not play a major role in the recruitment of bone marrow-derived cells and/or monocytes/macrophages in the neointimal layer and that some other factors, such as stromal cell-derived factor 1, Flt-1, vascular endothelial growth factor receptor 1, is a novel cell receptor such as CXCR4 and Flt1. A major role in the recruitment of bone marrow-derived cells. However, our results suggested that Ang1 did not play a major role in the recruitment of bone marrow-derived cells and/or monocytes/macrophages in the neointimal layer and that some other factors, such as stromal cell-derived factor 1 and VEGF-A, play a major role in that aspect via their cognate receptors such as CXCR4 and Flt1. Thus, it appears that Ang1 has the potential to stimulate reendothelialization and inhibit neointimal formation without inducing any notable inflammatory response.

Study Limitations

The precise mechanism by which Ang1 produced by ASC stimulated reendothelialization and inhibited neointimal formation from the adventitial side remains unclear. An indirect mechanism is possible. Because Ang1 inhibited macrophage infiltration in the adventitia and neointima, it may exert its effects via suppression of macrophage infiltration and activation. Future studies will be necessary to address the issue.

Conclusions

Although DES have been widely used in the field of coronary intervention, they inhibit reendothelialization, as well as neointimal growth. It is therefore preferable to use proangiogenic factors either alone or in combination with drugs that inhibit neointimal growth. Ang1 is a candidate proangiogenic factor to be used in the treatment of coronary artery disease.

Acknowledgments

This research was supported in parts by a Grant-in-Aid for Specially Promoted Research 22000006 to T.N. and Y.H. and a Grant-in-Aid for Scientific Research (C) 22590822 to Y.H. by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Circulation Journal Vol.77, June 2013


