Critical role of angiopoietins/Tie-2 in hyperglycemic exacerbation of myocardial infarction and impaired angiogenesis

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Tuo Q, Zeng H, Stinnett A, Yu H, Aschner JL, Liao DF, Chen JX. Critical role of angiopoietins/Tie-2 in hyperglycemic exacerbation of myocardial infarction and impaired angiogenesis. Am J Physiol Heart Circ Physiol 294: H2547–H2557, 2008. First published April 11, 2008; doi:10.1152/ajpheart.01250.2007.—Angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) are the two ligands of the Tie-2 receptor, a receptor tyrosine kinase that is expressed on the endothelium. A balanced angiopoietin/Tie-2 system is critical for the maintenance of vascular integrity. We investigated the potential role of a disrupted angiopoietin/Tie-2 system on hyperglycemic exacerbation of myocardial infarction and impaired angiogenesis. Using streptozotocin (STZ) mice subjected to myocardial ischemia, we examined the effects of shifting the Ang-2-to-Ang-1 ratio on myocardial infarction size, apoptosis, bone marrow (BM) cell-endothelial progenitor cell (EPC) differentiation, and angiogenesis. In control mice, myocardial ischemia increased expression of both Ang-2 and Tie-2. In STZ mice, Ang-2 expression was elevated, whereas Tie-2 expression was reduced, and neither was significantly altered by ischemia. Myocardial infarct size and apoptosis were increased in STZ compared with control mice. Using in vivo administration of an adenosinovirus containing Ang-1 or Ang-2, we found that shifting the Ang-2-to-Ang-1 ratio to favor Ang-1 reduced myocardial apoptosis and infarct size in STZ mice, while shifting the Ang-2-to-Ang-1 ratio to favor Ang-2 resulted in a significant increase in myocardial infarct size and apoptosis in control mice. Myocardial ischemia-stimulated BM cell-EPC differentiation was inhibited and myocardial angiogenesis was reduced in STZ mice. Systemic administration of Ad-Ang-1 restored BM cell-EPC differentiation and increased myocardial VEGF expression and angiogenesis in STZ mice. Our data demonstrate that disturbed angiopoietin/Tie-2 signaling contributes to the hyperglycemic exacerbation of myocardial infarction and impaired angiogenesis. Restoration of the Ang-2-to-Ang-1 ratio may be a novel therapeutic strategy for the treatment of diabetic myocardial ischemic diseases.

hyperglycemia; angiopoietin-1; angiopoietin-2; apoptosis

HYPERGLYCEMIA IS OFTEN OBSERVED in patients with acute myocardial infarction (AMI) and is independently associated with increased post-AMI mortality regardless of whether or not the patient has a history of diabetes mellitus. In animal models, hyperglycemia has been shown to exacerbate AMI and is associated with increased myocardial infarct size and the development of heart failure (10, 11, 26, 30, 48). The increased mortality that is observed in patients with hyperglycemia during AMI might be caused by impaired ischemic preconditioning (14, 19), a larger infarct size due to an increased incidence of the no-reflow phenomenon (17), or a higher incidence of congestive heart failure in diabetic patients (15). While these findings are of great clinical interest, they are largely descriptive and fail to identify potential therapeutic targets that might improve the outcomes for hyperglycemic patients with AMI. To date, very few studies have focused on the identification of factors responsible for AMI exacerbation after ischemic insult under hyperglycemic conditions.

Tie-2 is a receptor tyrosine kinase that is expressed in the vascular endothelium; angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) are the two ligands of the Tie-2 receptor (18, 44). Ang-1 is an agonist that binds to Tie-2 and mediates Tie-2 autophosphorylation promoting endothelial cell survival by activation of the protein kinase B/Akt pathway. Ang-2 is an antagonist of the Tie-2 receptor. Systemic elevation of Ang-2 levels has a detrimental effect as evidenced by the embryonic lethal phenotype of transgenic mice with Ang-2 overexpression (18, 28). Recent studies demonstrate that under hyperoxic conditions Ang-2 and its receptor Tie-2 are stimulated, while Ang-1 is inhibited. Increased Ang-2 levels in vivo after exposure to hyperoxia have been implicated in the pathogenesis of oxidant lung injury, cell death, inflammation, vascular leakage, and mortality (2, 3). Although increased expression of Ang-2 in response to myocardial ischemia and ischemia-reperfusion (I/R) has been well documented (37, 38, 43), few published data are available on the functional consequences of increased Ang-2 on myocardial ischemia under hyperglycemic conditions. Recent studies have revealed that Ang-2, but not Ang-1, expression is abnormally elevated in patients with diabetes or those with acute coronary syndrome, and this increase in Ang-2 has been strongly associated with myocardial damage (9, 22–24). In the present study, we hypothesized that hyperglycemia disrupts the angiopoietin/Tie-2 balance in favor of Ang-2. We further hypothesized that Ang-2, which antagonizes the antiapoptotic effects of Ang-1/Tie-2 signaling, would enhance myocardial death pathways and apoptosis and exacerbate myocardial infarction and impaired angiogenesis in diabetes. To test our hypotheses, we characterized the expression of the angiopoietin/Tie-2 system, using the streptozotocin (STZ) hyperglycemic mouse model subjected to acute myocardial ischemia. We examined the effects of shifting the Ang-2-to-Ang-1 ratio on myocardial infarct size, apoptotic activity, bone marrow (BM) cell differentiation into endothelial progenitor cells (EPCs), and the angiogenic response to ischemia.

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MATERIALS AND METHODS

STZ Hyperglycemic Mice

C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). To induce hyperglycemia, the mice were injected intraperitoneally for 5 consecutive days with STZ (60 mg/kg; Sigma). The experimental protocols described below were carried out 4 wk after hyperglycemia (>300 mg/dl) was demonstrated in STZ-treated mice (10, 26, 30). All procedures were in conformance with the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals and were approved by the Vanderbilt University Institutional Animal Care and Use Committee.

Systemic Delivery of Ang-1 and Ang-2 in Mice

Three days before myocardial ischemia, control C57BL/6J or STZ mice received an intravenous injection of Ad-CMV-Ang-1 [1 × 10⁹ plaque-forming units (PFU)] or Ad-β-gal (1 × 10⁹ PFU); control C57BL/6J mice received an intravenous injection of Ad-CMV-Ang-2 (1 × 10⁹ PFU).

Mouse Myocardial Ischemia Model

Experimental mice were anesthetized with ketamine (100–120 mg/kg) plus xylazine (15 mg/kg), intubated, and artificially ventilated with room air. Myocardial ischemia was achieved by ligation of the left anterior descending coronary artery (LAD) (10, 26, 30). The sham-operated control mice underwent the surgery without the LAD ligation. At the end of the experiments, hearts were harvested and the following experimental end points were measured.

Expression of angiopoietins, Tie-2, VEGF, caspase-3, and phospho-Akt. At 24 h after myocardial ischemia, hearts were harvested and homogenized in lysis buffer. The membranes were immunoblotted with anti-Ang-1, -Ang-2, -Tie-2, -VEGF, and -caspase-3 antibodies (1:1,000; Santa Cruz). For Akt phosphorylation, the membrane was immunoblotted with rabbit anti-phospho-Akt (1:1,000; Cell Signaling). Total level of Akt was detected with anti-Akt (1:1,000; Cell Signaling) antibody on the same nitrocellulose blots after stripping.

Assessment of infarct size. Twenty-four hours after ischemia, hearts were excised and sliced into five 1-mm cross sections below the ligature (10, 26, 30). Heart sections were incubated in 1% 2,3,5-triphenyltetrazolium chloride (Sigma) to stain viable myocardium red and the infarct areas pale. The infarct areas and the total left ventricular areas from both sides of each section were measured with Image Pro-express software.

Myocardial cell apoptosis. Serial tissue sections were labeled with terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) according to the manufacturer’s instructions (Promega). Sections were counterstained with DAPI. Apoptosis was quantified by counting TUNEL-positive cells per 100 DAPI cells (×10) in the infarcted area of the left ventricle at 24 h or 14 days after myocardial ischemia.

BM cell differentiation into EPCs. At 24 h after myocardial ischemia, BM cells were obtained by flushing the tibias and femurs with 10% FBS endothelial growth medium (EGM). Immediately after isolation, 2 × 10⁷ BM-derived mononuclear cells were plated on six-well culture plates coated with fibronectin (50 µg/ml; Sigma). On day 4 after culture, the nonadherent cells were removed and the adherent cells were incubated with FITC-labeled CD31 antibody (1:100; BD Biosciences). Positively stained cells were judged to be EPCs.

Analysis of myocardial capillary and arteriole densities. At 14 days after myocardial ischemia, hearts were harvested and immediately flash frozen. The tissue sections were incubated with fluorescein-labeled antibodies against Griffonia (Bandeiraea) simplicifolia isolecitin B4 (1:200) and smooth muscle actin (SMA, 1:100). SMA-positive neovessels (smooth muscle cells located in vascular walls or coated

Fig. 1. A: Western blot analysis showing increased angiopoietin (Ang)-2 expression in control (Con) mice subjected to myocardial ischemia for 24 h. In streptozotocin (STZ) mice, basal Ang-2 expression is increased without further increase after myocardial ischemia (IS) (n = 5 mice/group; *P < 0.05). B: Western blot analysis showing a significant increase in Tie-2 expression in control mice subjected to myocardial ischemia for 24 h. In STZ mice Tie-2 basal expression was reduced and myocardial ischemia failed to induce Tie-2 expression (n = 3 mice/group). C: the ratio of Ang-2 to Ang-1 protein expression was significantly increased in STZ mice compared with control mice (n = 5 mice/group both under basal and ischemic conditions; *P < 0.05).

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neovessels) were identified and counted. SMA-positive myofibroblasts were identified as spindle shaped and located in extravascular cells. Myocardial capillaries were measured with image analysis software (Image J, NIH). The numbers of capillaries and arterioles were counted and expressed as capillary or arteriole densities per square millimeter.

Statistical Analysis

All results are expressed as means ± SD. Statistical analysis was performed by one-way ANOVA followed by the multiple-comparison test (Student-Newman-Keuls) or unpaired Student’s t-test. A P value <0.05 denotes significance.

RESULTS

Dysregulation of Angiopoietin and Tie-2 Expression in STZ Mice Subjected to Myocardial Ischemia

To address the role of the angiopoietin/Tie-2 system in diabetic myocardial ischemia, we examined the expression of Ang-1, Ang-2, and Tie-2 in control and STZ mice. Western blot analysis demonstrated a significant increase in Ang-2 and Tie-2 protein expression in control hearts subjected to ischemia for 24 h (Fig. 1, A and B). Relative to control mice, hyperglycemic STZ mice had enhanced Ang-2 expression that was not further increased by myocardial ischemia. STZ mice also had reduced expression of Tie-2. Unlike control mice, ischemia failed to induce Tie-2 expression in hyperglycemic STZ mice (Fig. 1, A and B). Despite little difference in the expression of Ang-1 among the five treatment groups, the ratio of Ang-2 to Ang-1 after ischemia was significantly higher in the hearts of diabetic STZ mice compared with control mice (Fig. 1C).

Increased Myocardial Infarct Size and Apoptosis in STZ Mice

Twenty-four hours after myocardial ischemia, the infarction involved 26.1 ± 7.2% of the left ventricular area in control mice. STZ mice had infarcts that were significantly greater in size (Fig. 2A). Representative images and quantification of infarct size of the left ventricle in control and STZ mice after 24 h of ischemia are shown in Fig. 2A. The noninfarcted area appears red and the infarct area appears white after 2,3,5-triphenyltetrazolium chloride (TTC) staining. Myocardial infarct area was significantly increased in STZ mice compared with control mice (Fig. 2A). TUNEL-positive cells in the infarcted area of control and STZ mouse hearts 24 h after sham operation or ischemia were identified by TUNEL staining (green, ×10) and total nuclei by DAPI counterstaining (blue, ×10). Quantitative analysis of apoptotic cells in the infarcted area of control and STZ mice is shown in Fig. 2B. Apoptotic cells were significantly increased in STZ mice compared with control mice (n = 3, *P < 0.05).
size (41.7% ± 5.9%) (Fig. 2A). TUNEL staining and quantita-
tive analysis revealed a significant increase in TUNEL-
positive cells in the infarcted area of the left ventricle of STZ mice compared with control mice subjected to myocardial ischemia (Fig. 2, B and C).

**Shifting Ang-2-to-Ang-1 Ratio to Favor Ang-1 Reduces Infarct Size in STZ Mice**

Next, we sought to determine whether shifting the Ang-2-to-Ang-1 in favor of Ang-1 would minimize the hyperglycemia-induced exacerbation of myocardial infarction and apoptosis. STZ mice pretreated with an adenoviral vector expressing Ang-1 (Ad-Ang-1) 3 days before ischemic surgery showed a significant decrease in the area of infarction 24 h after myocardial ischemia compared with STZ mice that received the control vector (Fig. 3). Consistent with previous studies, overexpression of Ang-1 in mouse hearts also significantly reduced the area of infarction in control mice (Fig. 3).

**Shifting Ang-2-to-Ang-1 Ratio to Favor Ang-1 Alters Markers of Myocardial Apoptosis and Survival in STZ Mice**

Compared with control and STZ mice pretreated with Ad-β-gal, mice pretreated with Ad-Ang-1 had significantly fewer TUNEL-positive cells in the left ventricular infarct area (Fig. 4A).

Caspase-3 expression was increased in STZ mouse hearts relative to control hearts and further increased by myocardial ischemia. Ad-Ang-1 treatment significantly blunted caspase-3 protein expression 24 h after myocardial ischemia in STZ mice (Fig. 4B).

Myocardial ischemia-induced Akt phosphorylation was significantly attenuated in STZ mouse hearts compared with hearts from control mice (Fig. 4C). Treatment of STZ mice with Ad-Ang-1 resulted in a significant increase in Akt phosphorylation 24 h after ischemic injury (Fig. 4D).

**Shifting Ang-2-to-Ang-1 Ratio in Favor of Ang-2 Exacerbates Tissue Damage Following in Vivo Myocardial Ischemia**

To test whether increased Ang-2 exacerbates myocardial infarction in vivo, we examined systemic delivery of Ad-Ang-2 on the infarct size in control mice. Systemic delivery of Ad-Ang-2 to increase circulating Ang-2 significantly increased infarct size (Fig. 5).

**Shifting Ang-2-to-Ang-1 Ratio Modulates BM Cell Differentiation into EPCs in STZ Mice**

EPCs exhibiting features of endothelial cells incorporate into ischemic regions and are thought to contribute to the revascularization and repair of ischemic tissue. Therefore, we examined BM cell differentiation into EPCs in STZ mice after myocardial ischemia. The basal level of BM cell differentiation into EPCs was similar in STZ mice and control mice (Fig. 6, A and B). After myocardial ischemia for 24 h, the number of BM cells that differentiated into EPCs was significantly increased in control mice (Fig. 6, A and C). By comparison, BM cell differentiation into EPCs was impaired in STZ mice in response to myocardial ischemia (Fig. 6, B and C). Systemic delivery of Ad-Ang-1 was associated with a significant increase in the number of BM cells that differentiated into EPCs in STZ mice (Fig. 6, B and C).

**Shifting Ang-2-to-Ang-1 Ratio Alters Early VEGF Expression in STZ Mice**

To test whether shifting the Ang-2/Ang-1 balance has effects on myocardial angiogenesis, myocardial VEGF expression was...
A: representative images and quantitative analysis of apoptotic cells 24 h after surgery in STZ mouse hearts treated with Ad-β-gal or Ad-Ang-1. Systemic delivery of Ad-Ang-1 to STZ mice diminished myocardial apoptosis compared with systemic delivery of Ad-β-gal (n = 3, *P < 0.05).

B: Western blot and densitometric analysis of myocardial caspase-3 protein expression. Caspase-3 protein was markedly enhanced in STZ mice subjected to myocardial ischemia compared with control mice. Systemic delivery of Ad-Ang-1 to STZ mice diminished myocardial ischemia-induced caspase-3 activation compared with STZ mice (n = 4, *P < 0.05).

C: representative Western blot and densitometric analysis of myocardial Akt phosphorylation. Phosphorylated Akt (p-Akt) expression was reduced in STZ mice compared with control mice at baseline and at 24 h after myocardial ischemia (n = 3 mice, *P < 0.05).

D: systemic delivery of Ad-Ang-1 resulted in a significant increase in Akt phosphorylation in STZ mice subjected to myocardial ischemia for 24 h. This increase was not observed in mice treated with Ad-β-gal (n = 4, *P < 0.05).

Fig. 4: A: representative images and quantitative analysis of apoptotic cells 24 h after surgery in STZ mouse hearts treated with Ad-β-gal or Ad-Ang-1. Systemic delivery of Ad-Ang-1 to STZ mice diminished myocardial apoptosis compared with systemic delivery of Ad-β-gal (n = 3, *P < 0.05). B: Western blot and densitometric analysis of myocardial caspase-3 protein expression. Caspase-3 protein was markedly enhanced in STZ mice subjected to myocardial ischemia compared with control mice. Systemic delivery of Ad-Ang-1 to STZ mice diminished myocardial ischemia-induced caspase-3 activation compared with STZ mice (n = 4, *P < 0.05). C: representative Western blot and densitometric analysis of myocardial Akt phosphorylation. Phosphorylated Akt (p-Akt) expression was reduced in STZ mice compared with control mice at baseline and at 24 h after myocardial ischemia (n = 3 mice, *P < 0.05). D: systemic delivery of Ad-Ang-1 resulted in a significant increase in Akt phosphorylation in STZ mice subjected to myocardial ischemia for 24 h. This increase was not observed in mice treated with Ad-β-gal (n = 4, *P < 0.05).
examined 24 h after myocardial ischemia in control mice, STZ mice, and STZ mice pretreated with Ad-Ang-1. Myocardial ischemia led to an increase in VEGF expression in control mice but failed to induce VEGF expression in STZ mice (Fig. 7). Pretreatment of STZ mice with Ad-Ang-1 led to a significant increase in VEGF expression at 24 h (Fig. 7).

**Shifting Ang-2-to-Ang-1 Ratio Alters Myocardial Apoptosis and Angiogenesis in STZ Mice 14 Days After Ischemic Injury**

Fourteen days after ischemia, hearts were harvested for assessment of apoptosis by TUNEL staining. No TUNEL-positive cells were observed in control hearts that underwent sham surgery (data not shown). There were significantly more TUNEL-positive cells in the infarcted area of the left ventricle in STZ mice than in control mice. The number of TUNEL-positive cells was significantly decreased in Ad-Ang-1-treated STZ mice compared with STZ control mice that underwent myocardial ischemia 14 days earlier (Fig. 8A).

We also examined myocardial angiogenesis by measuring capillary and arteriole densities 14 days after ischemia. Myocardial ischemia led to an increase in capillary density in control but not in STZ mice. There was an increase in capillary density 14 days after ischemia in the hearts of STZ mice pretreated with Ad-Ang-1 but not Ad-β-gal (Fig. 8, B and C). Arteriole densities were increased 14 days after ischemia in both control and STZ mice. STZ mice pretreated with Ad-Ang-1 had a greater arteriole density than those pretreated with Ad-β-gal (Fig. 8, D and E).

**DISCUSSION**

In the present study we demonstrate the critical role of a disturbed angiopoietin/Tie-2 system in hyperglycemic exacerbation of myocardial infarction and impaired angiogenesis. Our data show that 1) Ang-2 expression was significantly increased in the hearts of STZ-hyperglycemic mice with or without myocardial ischemia; 2) Tie-2 expression and Ang-1/Tie-2 signaling were inhibited in STZ mice that exhibited increased myocardial infarct size after ischemia; 3) shifting the Ang-2-to-Ang-1 ratio to favor Ang-1 by administration of Ad-Ang-1 reduced myocardial apoptosis and infarct size, restored BM cell-EPC differentiation, increased VEGF expression, and enhanced angiogenesis; and 4) shifting the Ang-2-to-Ang-1 ratio to favor Ang-2 resulted in a significant increase in myocardial infarct size in response to in vivo ischemia in control mice.

Our findings suggest that hyperglycemia exacerbates myocardial infarction at least in part through a mechanism involving disruption of angiopoietin/Tie-2 signaling. Our studies also suggest that a disturbed Ang-2/Ang-1 balance may contribute to impaired BM cell-EPC differentiation and angiogenesis under hyperglycemic conditions. These data shed light on the mechanisms underlying the detrimental effects of hyperglycemia in the setting of myocardial infarction. Moreover, our results indicate that therapeutic approaches aimed at restoration of the Ang-2/Ang-1 balance may improve post-AMI repair mechanisms and lead to attenuation of myocardial infarction size in diabetes.

Constitutive Ang-1/Tie-2 signaling has been recognized as a critical survival signal for cells (18, 36). Previous studies show that activation of Ang-1/Tie-2 signaling prevents endothelial cell apoptosis via the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/survivin pathway and the inhibition of caspase-3 activation (8, 20, 21, 35). In contrast, profound endothelial dysfunction has been attributed to insufficiency of Ang-1/Tie-2 signaling in congestive heart failure patients (22). Our study revealed that disruption in Ang-1/Tie-2 expression and signaling in STZ mice was accompanied by a decrease in Akt phosphorylation, an increase in myocardial apoptosis, as well as an enlargement in the size of myocardial infarct. The PI3K/Akt pathway has also been shown to be activated by myocardial I/R. Activation of Akt in the heart protects against cardiomyocyte apoptosis after myocardial I/R (12, 13, 32). These findings led to the speculation that attenuation of Ang-1 binding to Tie-2 under hyperglycemic conditions may be mechanistically linked to exacerbation of myocardial infarction after an ischemic insult in diabetic patients. This notion is supported by our data showing that systemic delivery of Ad-Ang-1 to increase Ang-1/Tie-2 signaling led to a significant reduction of infarct size in STZ mice that underwent myocardial ischemia. Furthermore, systemic delivery of Ad-Ang-1 increased myocardial Akt phosphorylation and decreased myocardial caspase-3 activation and apoptosis. Since augmentation of Ang-1/Tie-2 signaling ameliorates the extent of infarction in the hearts of STZ mice, impaired constitutive Ang-1/Tie-2 signaling may explain, at least in part, the detrimental impact of hyperglycemia on post-AMI survival and may represent a promising therapeutic target in the clinical setting of myocardial ischemia in the diabetic patient.

Ang-2 expression is limited to sites of vascular remodeling and is upregulated by hypoxia and I/R (25, 34, 37, 38, 43). Previous studies have shown that increased Ang-2 is associated with endothelial apoptosis in rat cortical injury (33). In addition, increased Ang-2 in vivo after exposure to hypoxia has been implicated in the pathogenesis of lung injury, cell death, inflammation, vascular leakage, and mortality. Furthermore, increasing Ang-2 in vitro enhanced hyperoxia-induced epite-
Our data, for the first time, demonstrate that the hyperglycemic or diabetic exacerbation of myocardial infarction is mediated, at least in part, by an increase in Ang-2 expression. Normally, Ang-1 concentrations dominate and counteract the potentially deleterious effects of Ang-2. Under hyperglycemic conditions, Ang-2 expression is upregulated and leads to a local shift in the Ang-2-to-Ang-1 ratio favoring Ang-2 in the myocardium. Consequently, the interference of Ang-2 with constitutive Ang-1/Tie-2 signaling destabilizes the myocardium and endothelial cells. Overexpression of Ang-2 also results in aberrant myocardial angiogenesis, hemorrhage, and edema in the mouse heart; Ang-1 rescues these abnormalities (49). Furthermore, chronic systemic delivery of Ang-2 in the mouse model results in a significant reduction of myocardial vasculature formation (5). Additionally, upregulation of Ang-2 in the absence of growth-promoting factors such as hypoxia-inducible factor (HIF)-1α and VEGF as found in the diabetic heart (29, 31) might result in myocardial vessel regression and subsequently blunt collateral vessel growth and myocardial repair, ultimately leading to...
exacerbation of myocardial infarction. Together, our data provide a strong rationale for the exploitation of anti-Ang-2 therapies for the treatment of hyperglycemia- or diabetes-associated exacerbation of myocardial infarction after ischemic insult.

Increasing evidence suggests that BM-derived EPCs home in on sites of ischemia and contribute to the healing process in ischemic tissue (16, 51). Intriguing experimental data and clinical studies demonstrate that treatment of AMI with EPCs results in a significant reduction in infarct size (4, 42). Interestingly, BM cell differentiation into EPCs is impaired in patients with coronary artery disease and in diabetic animals (6, 7, 27, 45–47, 52). Our present data show that the number of BM cells that differentiate into EPCs was significantly increased in control mice that underwent myocardial ischemia, whereas ischemia failed to stimulate a similar degree of BM cell differentiation into EPCs in STZ mice to control levels, suggesting that a disturbed Ang-2/Ang-1 balance may contribute to the impairment of BM cell-EPC differentiation under hyperglycemic conditions.

Myocardial angiogenesis and collateral blood vessel growth are adaptive responses to hypoxia or ischemia. Myocardial ischemia has been shown to cause a significant increase in myocardial angiogenic signaling and angiogenesis. These changes correlate with improvement of myocardial function (1, 37, 39–41, 43). In the human heart during ischemia, the expression of HIF-1α and VEGF increases significantly and may contribute to the limitation of ischemic injury by promoting angiogenesis (50). However, the expressions of both HIF-1α and VEGF are significantly decreased in diabetic patients (29, 31). Reduced levels of myocardial angiogenic growth factors may fail to appropriately augment myocardial capillary density and thereby result in poor collateral formation in diabetes. These abnormalities will cause impaired oxygen delivery and ultimately lead to increased myocardial injury under hyperglycemic conditions. Our present data demonstrate that myocardial ischemia-induced VEGF expression is significantly impaired, and this is accompanied by greater myocardial infarction size in STZ mice. Interestingly, these changes are reversed by shifting the Ang-2-to-Ang-1 ratio to favor Ang-1, indicating a critical role for a balanced angiopoietin/Tie-2 system in the impairment of angiogenesis and the exacerbation of myocardial injury. Our present studies also demonstrate that systemic administration of Ad-Ang-1 increases capillary density more than arteriole formation in STZ mice, further implicating the profound protective effects of Ang-1 on endothelial cell survival.

In summary, our present study provides a novel mechanistic explanation for how diabetes might contribute to higher mortality in patients with myocardial infarction. Hyperglycemia results in an imbalanced angiopoietin/Tie-2 system in favor of Ang-2 as well as disturbed Ang-1/Tie-2 signaling in the heart leading to an increase in myocardial apoptosis, a greater infarct size, a reduction in BM cell differentiation into EPCs, and an impairment in myocardial angiogenesis. Given that disruption of the angiopoietin/Tie-2 system is an important contributor to the hyperglycemic exacerbation of myocardial infarction and impairment of angiogenesis, pharmacological or genetic manipulation of the Ang-2-to-Ang-1 ratio should be considered as a novel therapeutic strategy for the treatment of diabetic exacerbation of myocardial infarction. We further speculate that Ang-2 may represent an independent prognostic factor in AMI and a useful tool in the risk-adapted management of AMI in diabetes.

**REFERENCES**


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HYPERGLYCEMIC EXACERBATION OF MYOCARDIAL ISCHEMIC INJURY


