Secoisolariciresinol Diglucoside: Relevance to Angiogenesis and Cardioprotection against Ischemia-Reperfusion Injury

Suresh Varma Penumathsa, Srikanth Koneru, Mahesh Thirunavukkarasu, Lijun Zhan, Kailash Prasad, and Nilanjana Maulik

Molecular Cardiology and Angiogenesis Laboratory, Department of Surgery, University of Connecticut Health Center, Farmington, Connecticut (S.V.P., S.K., M.T., L.Z., N.M.); and Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada (K.P.)

Received September 21, 2006; accepted November 21, 2006

ABSTRACT

Therapeutic angiogenesis represents a novel approach for the prevention and treatment of ischemic heart disease. This study examined a novel method of stimulating myocardial angiogenesis using secoisolariciresinol diglucoside (SDG), a plant lignan isolated from flaxseed. SDG has been shown to decrease serum cholesterol and reduce the extent of atherosclerosis. In the present study, the angiogenic properties of SDG were investigated in three different models. First, in the in vitro model, human coronary arteriolar endothelial cells (HCAEC) treated with SDG (50 and 100 μM) showed a significant increase in tubular morphogenesis compared with control. Western blot analysis indicated an increased expression of vascular endothelial growth factor (VEGF), kinase insert domain-containing receptor (KDR), Fit-1, angiopeptin-1 (Ang-1), Tie-1, and phosphorylated endothelial nitric oxide synthase (p-eNOS) in the SDG-treated cells. Second, in the ex vivo ischemia/reperfusion model, SDG-treated rats (20 mg/kg b.wt./day for 2 weeks orally) showed an increased level of aortic flow and functional recovery after 2 h of reperfusion following 30 min of ischemia compared with the control group [dP/dt (mm Hg/s) of 2110 ± 35 versus 1752 ± 62]. SDG reduced infarct size compared with the control group by 32% (38 versus 26%) and also decreased cardiomyocyte apoptosis. Increased protein expression of VEGF, Ang-1, and p-eNOS was also observed in the SDG-treated group. Third, in the in vivo myocardial infarction model, SDG increased capillary density and myocardial function as evidenced by increased fractional shortening and ejection fraction. In conclusion, these results suggest that SDG has potent angiogenic and antiapoptotic properties that may contribute to its cardioprotective effect in ischemic models.

Ischemic heart disease develops as a consequence of coronary atherosclerotic lesion formation. Coronary collateral vessels and microvascular angiogenesis develop as adaptive responses to myocardial ischemia, which ameliorates the function of the damaged heart. Modulation of adaptive response to ischemic heart disease has become a major focus of current research. Therapeutic coronary angiogenesis (sprouting of new vessels at the capillary level) and collateralization (opening of pre-existing vessels) have tremendous therapeutic potential as strategies for treatment of patients with ischemic heart disease, along with traditional coronary interventional therapies such as coronary artery bypass graft (CABG) and percutaneous transluminal coronary intervention (PTCI). Angiogenesis is a complex process that requires growth factors such as vascular endothelial growth factor (VEGF) and the angiopeptin (Ang) systems that directly or indirectly affect the endothelial cells and produce proliferation and differentiation. VEGF is one of the critical factors involved in mobilization and induction of mitosis in endothelial progenitor cells (Asahara et al., 1999) and in enhancement of endothelial cell survival (Gerber et al., 1998; Fujio and Walsh, 1999), which are considered to be important in the stimulation of blood vessel growth. Ang-1 contributes to stabilization and maturation of vessels via matrix, pericyte, and endothelial cell interactions. Evidence indicates that new vessel growth and maturation are highly complex and coordinated processes, requiring the sequential activation of ABBREVIATIONS: CABG, coronary artery bypass graft; PTCI, percutaneous transluminal coronary intervention; VEGF, vascular endothelial growth factor; Ang, angiopeptin; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; SDG, secoisolariciresinol diglucoside; HCAEC, human coronary arteriolar endothelial cells; I/R, ischemia/reperfusion; MI, myocardial infarction; LVDP, left ventricular developed pressure; TBST, Tris-buffered saline/Tween 20; LV, left ventricular; LAD, left anterior descending coronary artery; p-eNOS, phosphorylated endothelial nitric oxide synthase; LVPW, LV posterior wall in systole; LVAW, LV anterior wall in systole; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; KDR, kinase insert domain-containing receptor.
After allowing the gel to settle for 30 min at 37°C in a 5% CO2 incubator, the endothelial cells (HCAEC) were serially passaged.

Flaxseed is a rich source of omega-3 fatty acids and the richest source of plant lignans (Hunter, 1990; Kelley et al., 1991). Secoisolariciresinol diglucoside (SDG) has been isolated from flaxseed. Colonic bacteria convert SDG into the major mammalian lignans enterodiol and enterolactone (Rickard et al., 1996). SDG has been shown to be effective in decreasing serum cholesterol and reducing the extent of atherosclerosis in the hypercholesterolemic rabbit (Prasad, 1999). It is also effective in retarding the development of type 2 diabetes (Prasad, 2001) and in cancer prevention (Rickard et al., 2000). Recent clinical trials have shown that flaxseed supplementation reduces the lipid levels in hypercholesterolemic subjects. However, the angiogenic property of SDG is not known. Therefore, the objective of the present study was to determine the angiogenic property of SDG. The effects of SDG on protein expression profiles of the VEGF-Ang system were studied using human coronary arterial endothelial cells (HCAEC) and rat myocardial ischemic models. In conclusion, this study shows for the first time that SDG is a potent angiogenic compound that has significant potential to be used as a clinical drug during myocardial ischemia.

Materials and Methods

The study was conducted in three different models: 1) in vitro HCAEC for tubular morphogenesis; 2) ex vivo I/R myocardial injury; and 3) in vivo MI.

In Vitro HCAEC Tubular Morphogenic Model

Endothelial Cell Culture. HCAEC were obtained from Cambrex Biosciences (Walkersville, MD), and they were serially passaged. Cells were maintained in a culture medium (endothelial growth medium 2) supplemented with growth factors and antibiotics according to company specifications (Cambrex).

SDG Treatment. Confluent HCAEC were plated on plastic 100-mm dishes supplemented with cell media, with specifications as mentioned above, and subjected to different concentrations of SDG (1, 5, 10, 50, and 100 μM) for tube formation for 24 h in a 5% CO2 incubator for determination of optimal concentration. A concentration of 100 μM SDG was selected to determine the protein expression profile.

In vitro tube formation on Matrigel (BD Bioscience, Bedford, MA): 100 μl of ice-cold Matrigel was coated on a 12-well cell culture plate (Corning Life Sciences, Acton, MA) as a base for tube formation. After allowing the gel to settle for 30 min at 37°C in a 5% CO2 incubator, the endothelial cells (5 × 10⁴) were seeded onto the Matrigel and incubated overnight at 37°C in the CO2 incubator. The wells were treated with various concentrations of SDG immediately after the cells were seeded. After 24-h exposure to SDG, the extent of tube formation was recorded by the phase contrast microscope (magnification ×200) with a digital camera (Vidavalar et al., 2006).

I/R Myocardial Injury Model (ex Vivo)

Animals. All the animals used in the study received care in compliance with the principles of laboratory animal care formulated by the National Society for Medical Research and Guide for the Care and Use of Laboratory Animals published by National Institutes of Health.

Experimental Design. The rats were randomized into two groups: control and SDG-treated (20 mg/kg b.wt./day for 2 weeks orally) (n = 6 in each group). For the ex vivo studies, Langendorff’s working perfused rat hearts subjected to 30 min of global ischemia followed by 120 min of reperfusion were used.

Isolated Working Heart Preparation (Langendorff’s Working Heart). The procedure for creating isolated perfused rat hearts was similar to that described previously (Meldik et al., 2000). In brief, rats were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) (Abbott Laboratories, Baxter Health Care Corporation, Deer Field, IL) and heparin sodium (500 IU/kg, i.p.) (Elkins-Sinn Inc., Cherry Hill, NJ). Under anesthesia, a thoracotomy was performed, the heart was rapidly excised, and the aorta was cannulated for perfusion in the retrograde Langendorff mode at 37°C at a constant perfusion pressure of 100 cm of water (10 kPa) for a 10-min washout period. The perfusion buffer used in this study consisted of a modified Krebs-Henseleit bicarbonate buffer (118 mM NaCl, 4.7 mM KCl, 1.7 mM CaCl₂, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, and 10 mM glucose). The Langendorff preparation was switched to the working mode after the washout period as described previously (Engelman et al., 1995). The working mode was introduced by switching the flow to the left atrium from the aortic root with a constant preload of 17 cm of H₂O and an afterload of 100 cm of H₂O. At the end of 10 min, after attaining steady-state cardiac function, baseline functional parameters were recorded. The circuit was then switched back to the retrograde mode, and hearts were perfused for 5 min with Krebs-Henseleit bicarbonate buffer; then the hearts were subjected to global ischemia for 30 min followed by 2 h of reperfusion. The first 10 min of reperfusion was in the retrograde mode to allow for postischemic stabilization and thereafter in the antegrade working mode to allow for assessment of functional parameters, which were recorded at 30, 60, 90, and 120 min of reperfusion.

Cardiac Function. Aortic pressure was measured using a pressure transducer (Micro-Med, Inc., Louisville, KY) connected to a side arm of the aortic cannula, and the signal was amplified using a Heart Performance Analyzer model 400 (Micro-Med, Inc.). Heart rate, left ventricular developed pressure (LVDP), and the first derivative of developed pressure (dP/dt) were all derived or calculated from the continuously obtained pressure signal (Sasaki et al., 1999). Aortic flow was measured using a calibrated flowmeter (Gilmont Instrument Inc., Barrington, IL), and coronary flow was measured by timed collection of the coronary effluent.

Infarct Size Estimation. At the end of the reperfusion, a 1% (w/v) solution of triphenyl tetrazolium in phosphate buffer was infused into the aortic cannula for 1 min at 37°C. The hearts were excised and stored at ~70°C. Sections of frozen heart were fixed in 10% formalin, placed between two coverslips, and digitally imaged using an Epson (Torrance, CA) Perfection 1660 PHOTO Scanner. To quantify the areas of interest in pixels, NIH image 5.1 (a public domain software package) was used. The infarct size was quantified and expressed in pixels (Thirunavukkarasu et al., 2006).

Western Blot Analysis for VEGF, KDR, Flt-1, Ang-1, Tie-1, Tie-2, and Phosphorylated eNOS. Standard SDS-polyacrylamide gel electrophoresis Western blot technique was used to quantify the presence of these proteins. Total protein was isolated from cells by removing the medium, washing the culture plate with phosphate-buffered saline, and scraping the cells with radioimmunoprecipitation assay buffer (Boston Bioproducts, Worcester, MA). After scraping the cells with radioimmunoprecipitation assay buffer, the lysate was passed through a 21-gauge needle syringe to shear the DNA, and the lysate was precipitated with phenylmethylsulfonyl fluoride. Following precipitation, the lysate was centrifuged at 10,000 g for 10 min at 4°C. The supernatant was the total protein lysate.

Rat heart tissues from each group were homogenized and suspended (50 mg/ml) in sample buffer (10 mM HEPES, pH 7.3, sucrose 11.5%, 1 mM EDTA, 1 mM EGTA, diisopropylfluorophosphate, 0.7
mg/ml peptatin A, 10 mg/ml leupeptin, and 2 mg/ml aprotinin). The homogenates were centrifuged at 3500 rpm. The cytosolic fractions were used for protein analysis. The total protein concentration was determined using a bicinchoninic acid protein assay kit (Pierce, Rockville, IL). The total proteins from cells and the cytosolic proteins from tissue were run on polyacrylamide electrophoresis gels (SDS-polyacrylamide gel electrophoresis) typically using a 10% acrylamide/bis ratio. The separated proteins were electrophoretically transferred to Immobilon-P membranes (Millipore Corp., Bedford, MA) using a semidyve transfer system (Bio-Rad, Hercules, CA). Protein standards (Bio-Rad) were run in each gel. The blots were blocked in Tris-buffered saline/Tween 20 (TBST, containing 20 mM Tris base, pH 7.6, 137 mM NaCl, and 0.1% Tween 20) supplemented with 5% (w/v) nonfat dry milk for 1 h. Blots were incubated overnight at 4°C with the various primary antibodies. The antibodies were purchased from Cell Signaling and BD Transduction Laboratories (San Jose, CA) and were used at manufacturer-recommended dilutions. Membranes were washed three times in TBST before incubation for 1 h with horseradish peroxidase-conjugated secondary antibody diluted 1:2000 in TBST and 5% (w/v) nonfat dry milk. After incubation, membranes were washed three times with TBST for 10 min each. Blots were treated with enhanced chemiluminescence (GE Healthcare, Little Chalfont, Buckinghamshire, UK) reagents, and the required proteins were detected by autoradiography for variable lengths of time on X-Omat film (Eastman Kodak, Rochester, NY) (Vidavalur et al., 2006).

**Determination of Cardiomyocyte Apoptosis.** The formaldehyde-fixed left ventricle was embedded in paraffin, cut into transverse sections (4 mm thick), and deparaffinized with a graded series of xylene and ethanol solutions. Immunohistochemical detection of apoptotic cells was carried out using terminal deoxynucleotidyl transferase dUTP nick-end labeling, in which residues of digoxigenin-labeled dUTP are catalytically incorporated into the DNA by terminal deoxynucleotidyl transferase, an enzyme that catalyzes a template-independent addition of nucleotide triphosphate to the 3'-OH ends of double- or single-stranded DNA (Kajstura et al., 1996). The incorporated nucleotide was incubated with a sheep polyclonal antidigoxigenin antibody followed by a fluorescein isothiocyanate-conjugated rabbit anti-sheep IgG as a secondary antibody as described by the manufacturer (Intergen, Purchase, NY). The sections (n = 5) were washed in phosphate-buffered saline three times, blocked with normal rabbit serum, and incubated with mouse monoclonal antibody recognizing α-sarcomeric actin (Sigma Japan, Tokyo, Japan) followed by staining with tetramethylrhodamine B isothiocyanate-conjugated rabbit anti-mouse IgG (200.1 dilution, Dako Japan, Tokyo, Japan) (Maulik et al., 1998). The fluorescence staining was viewed with confocal laser microscopy (Fluoview, Olympus, Tokyo, Japan). For quantitative purposes, the numbers of terminal deoxynucleotidyl transferase dUTP nick-end labeling-positive cardiomyocytes and nonmyocytes (negative for α-sarcomeric actin) were counted on 100 high-power fields (magnification ×600) from the endocardium through the epicardium of the mid-portion of the left ventricular (LV) free wall in five sections from each heart.

**Myocardial Infarction Model (in Vivo)**

**Experimental Design.** For the in vivo study, the MI model was used, where the rats were subjected to 2 weeks of permanent left anterior descending coronary artery (LAD) occlusion. For the MI model, the rats were randomized into four groups: 1) control (surgical procedure without MI) (C); 2) SDG control (SC); 3) MI (MI); and 4) SDG MI (SMI) (n = 6 in each group).

**Surgical Procedure (Permanent LAD Occlusion Model).** Male Sprague-Dawley rats weighing 250 to 300 g were anesthetized with ketamine HCl (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.). Cefazolin (25 mg/kg i.p.) was administered as a preoperative antibiotic cover. After tracheotomy and initiation of ventilation (Harvard Apparatus Rodent Ventilator, model 683), the heart was exposed through a left lateral thoracotomy (fourth intercostal space). A 6-0 polypropylene suture was passed with tapered needle under the LAD just below the tip of the left atrium, and a nontraumatic occluder was applied on the artery. MI was produced by permanent LAD occlusion. On completion of all the surgical procedures, the chest was closed. After application of buprenorphine (0.1 mg/kg s.c.) and weaning from the respirator, the rats were placed on a heating pad while recovering from anesthesia (Kaga et al., 2005a).

**Immunohistochemistry for Capillary Density.** To determine the capillary density (CD-31), the rats were sacrificed 14 days after the surgical procedure. The hearts were removed, and paraffin-embedded sections were made. The sections were stained for capillary density using platelet-endothelial cell adhesion molecule-1 primary antibody (Santa Cruz, CA), and bound antibody was detected with Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and visualized with diaminobenzidine (Sigma) (Kaga et al., 2005b). Using 400× magnification, pictures were obtained for CD-31 counting. For the quantitative measurement, the number of CD-31 was counted on an area at risk, from the endocardium through the epicardium of the mid-portion of the LV free wall. Counts of capillary density per square millimeter were obtained after superimposing a calibrated morphometric grid on each digital image using Adobe (Mountain View, CA) Photoshop Software.

**Echocardiography.** Each rat was sedated using isoflurane (3%, inhaled). When adequately sedated, the rat was secured with tape in the supine position in a custom-built mold designed to maintain the rat’s natural body shape after fixation. The hair on the chest wall was removed with a chemical hair remover. Ultrasound gel was spread over the precordial region, and ultrasound biomicroscopy (Vevo 770, Visual-Sonics Inc., Toronto, ON, Canada) with a 25-MHz transducer was used to visualize the left ventricle. The left ventricle was analyzed in apical, parasternal long axis, and parasternal short axis views for LV systolic function, LV cavity diameter, wall thickness, diastolic function, and LV end-systolic and end-diastolic volume determination. MI segments were determined according to the kinetics: hypokinetic (reduction in wall motion), akinetic (no wall motion), and dyskinetic (unsynchronized movement of segment with normal myocardium). Two-dimensional directed M-mode images of the LV short axis were taken just below the level of the papillary muscles for analyzing ventricular wall thickness and chamber diameter. All the LV parameters were measured according to the modified American Society of Echocardiography–recommended guidelines. Ejection fraction and fractional shortening were assessed for LV systolic function. Diastolic function was assessed by measuring mitral peak flow velocity of the E-wave and A-wave in centimeters per second (cm/s), as was the ratio between the two waves (E/A). All the measurements represent the mean of at least three consecutive cardiac cycles. Throughout the procedure, ECG, respiratory rate, and heart rate were monitored.

**Statistical Analysis.** Results are expressed as mean ± S.E.M. Student’s t test was used to test for differences, and values were considered to be significant at p < 0.05.

**Results**

**Tubular Morphogenesis of HCAEC after SDG Treatment.** SDG-treated HCAEC plated on the surface of Matrigel formed tube-like structures. Various concentrations of SDG (1, 5, 10, 50, 100, and 150 μM) were used in this study. The formation of the capillary network of tubular structure was more prominent when the cells were exposed to 50 and 100 μM SDG. SDG in lower concentrations showed no effect on tubular morphogenesis (Fig. 1). Cells treated with 150 μM SDG have not shown any significant difference compared with 100 μM SDG. Hence, we considered the 100 μM dose for further study to observe the protein expression profile.

**Effect of SDG on VEGF System in Vitro and ex Vivo.** Protein analysis revealed a marked induction of VEGF (1.6-
fold), KDR (1.8-fold), and Flt-1 (2.12-fold) bands in HCAEC with 24 h of 100 μM SDG treatment compared with non-treated cells (Fig. 2). Similar results were observed ex vivo in SDG-treated rats. Increased expression of VEGF (1.8-fold) was found in SDG-treated rats compared with control rats (Fig. 4).

**Effect of SDG on Ang-Tie System in Vitro and ex Vivo.** Western blot analysis showed marked increases in the expression of Ang-1 (1.3-fold) and Tie-1 (1.3-fold) in HCAEC after 24 h of 100 μM SDG treatment compared with nontreated cells (Fig. 3). No significant difference was observed in Tie-2 expression between the treated and nontreated HCAEC. Similar results were observed in SDG-treated rats. Ex vivo SDG-treated rat myocardium showed an increased expression of Ang-1 (2.2-fold) compared with control (Fig. 4).

**Effect of SDG on Phosphorylated eNOS in Vitro and ex Vivo.** Phosphorylation of eNOS was increased in the SDG-treated group compared with the control group. HCAEC treated with SDG showed increased expression of phosphorylated endothelial nitric oxide synthase (p-eNOS) (4-fold) (Fig. 2) compared with nontreated HCAEC. Similar results were also obtained in the ex vivo study. Significant phosphorylation of eNOS in SDG-treated myocardium compared with untreated myocardium was observed. SDG-treated rats also showed an increased expression of p-eNOS (1.4-fold) compared with control rats (Fig. 4); however, no change was observed in eNOS expression.

**Effect of SDG on Functional Parameters in the I/R Model.** There were no significant differences in heart functions [LVDP (mm Hg), heart rate (beats/min), dP/dt (mm Hg/s)], coronary flow (ml/min), and aortic flow (ml/min) before the induction of ischemia in the treatment group compared with the control group. Following reperfusion after ischemia, the values of all the functional parameters were lower in the control group compared with the SDG-treated group. Significant increases in dP/dt_max and aortic flow were observed during reperfusion in the SDG-treated group compared with the control group. After 120 min of postsischemic reperfusion, the values of dP/dt_max (2109.6 ± 35.23 mm Hg/s) were significantly higher in the SDG-treated group compared with the control group (1751.5 ± 62.25 mm Hg/s). Likewise, aortic flow was significantly higher in the SDG-treated group compared with the control group. The cardioprotective effects of SDG were shown by a significant recovery of postsischemic myocardial function (Fig. 5, A–E).

**Effect of SDG on Infarct Size in the I/R Model.** Hearts subjected to 2 h of reperfusion after 30 min of ischemia were used to measure the infarct size (percent of infarct area versus total area at risk). The SDG-treated group had significantly reduced regions of MI compared with the control group.
group (26% versus 38%) (Fig. 6). The SDG treatment reduced the development of MI by approximately 32%.

Effect of SDG on Extent of Cardiomyocyte Apoptosis in the I/R Model. Double antibody staining has revealed a significant decrease in the extent of cardiomyocyte apoptosis in the SDG-treated group (38%) compared with the untreated group (53%). The decrease in apoptosis with SDG treatment might be because of increased expression of p-eNOS (Fig. 7).

Effect of SDG on the Extent of Capillary Density (CD-31) in the MI Model. At 400× magnification, eight nonoverlapping random fields each selected from noninfarcted risk areas were used for CD-31 counting. The rats were sacrificed after 2 weeks of permanent LAD occlusion, and samples were removed for paraffin-embedded sectioning. Four sections from each heart were examined. Increased capillary density was found in the SDG-treated group.
compared with the control MI group. The LV chamber was dilated in the control MI group compared with the SDG-treated MI group as assessed by measuring LV internal diameter in diastole and LV internal diameter in systole. There was a compensatory increase in the posterior (LVPW) and lateral wall systolic thickness in the SDG-treated MI group compared with the control MI group (Fig. 9). No significant difference was observed in the left ventricular anterior wall (LVAW) between the SDG-treated and control groups. LV end-systolic volume was significantly reduced in the SDG-treated MI group compared with the control group. There was an increase in the E/A ratio in control rats (3.52 ± 0.28) compared with SDG-treated rats (1.43 ± 0.3) (Fig. 10).

Discussion

The present study shows that myocardial ischemic complications such as infarct size, extent of apoptosis, and impaired angiogenesis could be controlled aggressively with dietary supplementation of SDG isolated from flaxseed. SDG produced a significant increase in the VEGF/Ang system along with increased p-eNOS in HCAEC, as well as in treated rats, which suggests the angiogenic property of SDG. The increased tubular morphogenesis on Matrigel by SDG treatment might be because of increased expression of angiogenic proteins such as VEGF and Ang-1. Reports indicate that VEGF promotes angiogenesis in three-dimensional in vitro models, inducing confluent microvascular endothelial cells to invade collagen gels and form capillary-like structures (Pepper et al., 1992, 1994). Increased expression of receptors for VEGF and Ang-1 (KDR, Flt-1, and Tie-1) was observed in HCAEC with SDG treatment, showing the angiogenic property of SDG. VEGFR-1 and VEGFR-2 gene targeting studies have shown the essential role of these molecules during embryogenesis and developmental angiogenesis. The key role of Flt-1 in embryogenesis and KDR in developmental angiogenesis and hematopoiesis was evidenced by the lack of vasculogenesis and the failure to develop blood islands and organized blood vessels in Flt-1 and Flk-1 null mice, resulting in death in utero between day 8.5 and day 9.5 (Fong et al., 1995; Shalaby et al., 1995). Nitric oxide is one of the most important nonpeptide endothelium-derived vasoactive factors (Sessa, 1994; Hood et al., 1998). Endothelium-derived NO was initially identified as a main molecule representing the endothelial-derived relaxing factors. Studies have shown that biological processes modulated by NO might include
angiogenesis as evidenced by Ziche et al. (1997), who were the first to show that NO might play a role in angiogenesis. NO released from cultured human endothelial cells and isolated vascular strips confirmed that VEGF stimulates endothelial NO release in vitro (van der Zee et al., 1997). VEGF treatment elicited a dose-dependent increase in the cellular content of eNOS mRNA and protein, which may account for the chronic stimulation of NO production by VEGF. SDG treatment in the present study has increased the expression of eNOS in HCAEC, as well as in SDG-treated rats. SDG-treated rats have shown increased functional parameters, decreased infarct size, and decreased cardiomyocyte apoptosis after I/R injury, which might be because of up-regulation of the eNOS-NO and VEGF/Ang-1 systems by SDG. VEGF was shown to prevent the endothelial apoptosis induced by serum starvation in vitro and also acts as a survival factor for endothelial cells both in vitro and in vivo (Alon et al., 1995; Gerber et al., 1998). Evidence suggests that Ang-1 induces cell endothelial survival and inhibits endothelial apoptosis through several pathways, including phosphatidylinositol 3-kinase/Akt activation and up-regulation of survivin protein (Papapetropoulos et al., 2000; Harfouche et al., 2002). Zhao et al. (2002) have shown that impaired angiogenesis in the myocardium may contribute to myocardial apoptosis, heart failure, and high mortality in neonatal eNOS−/− mice, suggesting the possible role of NO production from eNOS in myocardial angiogenesis. Several other studies (Jones et al., 1999) showed that in eNOS-deficient mice infarct size was significantly augmented and in eNOS transgenic mice infarct size was reduced after ischemia and reperfusion, supporting the role of eNOS-derived NO during I/R injury and cardioprotection (Jones et al., 2004).

The anatomicopathological study of the heart performed with the help of echocardiography also revealed a significant reduction in ischemic ventricular remodeling and showed an improved diastolic and systolic thickness of ventricular wall in the SDG-treated group, showing the efficacy of SDG in maintaining the global LV systolic function. These findings are attributable to the effect of SDG in reducing the extent of the infarcted area and aiding the heart in recovery from ischemic insult. The extent of angiogenesis as measured by CD31 staining in the MI model compared with the control rats also validated the strong angiogenic response by SDG. The increased capillary density resulting in increased blood

Fig. 10. Echocardiographic measurements in control and SDG-treated rats at baseline and after 2 weeks of MI. A, ejection fraction (%). B, fractional shortening (%). C, LVIDs (mm). D, E/A ratio. E, LVPW. F, LVAW. Group 1 represents control rats; group 2 represents SDG control rats; group 3 represents control rats following 2 weeks of MI; and group 4 represents SDG-treated rats following 2 weeks of MI. *, p < 0.05 represents significant difference between SDG-treated and control rats. LVAW represents LV anterior wall in systole; LVPW represents LV posterior wall in systole; LVIDs represents LV internal diameter in systole. n = 6 in each group.
supply in the MI model with SDG treatment may be medi-
erated by several angiogenic protein candidates such as VEGF, Ang-1, and eNOS. Theoretically, increased capillary density may generally lead to increased arteriolar density. However, in this report, we have not examined the extent of arteriogen-

### references


Hussain SN (2002) Mechanisms which mediate the antiapoptotic effects of angio-


doxin-1, hemeoxygenase-1 and vascular endothelial growth factor. *J Mol Cell Cardiol* 38:813–822.


doxin-1, hemeoxygenase-1 and vascular endothelial growth factor. *J Mol Cell Cardiol* 38:813–822.


doxin-1, hemeoxygenase-1 and vascular endothelial growth factor. *J Mol Cell Cardiol* 38:813–822.


doxin-1, hemeoxygenase-1 and vascular endothelial growth factor. *J Mol Cell Cardiol* 38:813–822.


doxin-1, hemeoxygenase-1 and vascular endothelial growth factor. *J Mol Cell Cardiol* 38:813–822.


doxin-1, hemeoxygenase-1 and vascular endothelial growth factor. *J Mol Cell Cardiol* 38:813–822.


doxin-1, hemeoxygenase-1 and vascular endothelial growth factor. *J Mol Cell Cardiol* 38:813–822.
cells through the expression of thioredoxin, hemeoxygenase and vascular endothelial growth factor. *Vasc Pharmacol* **45:**91–95.

**Address correspondence to:** Nilanjana Maulik, Molecular Cardiology and Angiogenesis Laboratory, Department of Surgery, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-1110. E-mail: nmaulik@neuron.uchc.edu