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Secoisolariciresinol Diglucoside Induces Neovascularization Mediated Cardioprotection against Ischemic-Reperfusion Injury In Hypercholesterolemic Myocardium

Suresh Varma Penumathsa^{1,2}, Srikanth Koneru¹, Lijun Zhan¹, Saji John¹, Venogopal P Menon², Kailash Prasad³, and Nilanjana Maulik¹

¹ Molecular Cardiology and Angiogenesis Laboratory, Department of Surgery, University of Connecticut Health Center, Farmington, CT, USA

² Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar, TN, India

³ Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Abstract

Background—Hypercholesterolemia (HC) induced endothelial cell dysfunction and decreased endothelial nitric oxide formation result in impaired angiogenesis & subsequent cardiovascular disorders. Therapeutic angiogenesis is known to be a novel strategy for treatment of those patients with ischemic heart disease. We have shown that secoisolariciresinol diglucoside (SDG) is angiogenic & cardioprotective against myocardial ischemia. In the present study we examined the efficacy of SDG in a hypercholesterolemic myocardial infarction (MI) model.

Methods—The rats were maintained on a normal and high cholesterol diet (2%) for 8 weeks followed by oral administration of SDG (20mg/kg) for 2 weeks. The rats were divided into 4 groups (n=12 in each): Control (C); SDG control (SDG); HC; & HC + SDG (HSDG). Isolated hearts subjected to 30 min of global ischemia followed by 120 min of reperfusion were used to measure the *cardiac functions*, infarct size & examine the protein expression profile. After treatment MI was induced by ligating the left anterior descending artery. Echocardiographic parameters were examined 30 days after MI.

Results—Significant reduction in total cholesterol, LDL-cholesterol, triglycerides and increase in HDL-cholesterol levels were observed in HSDG as compared to HC. Decreased infarct size was observed in the HSDG group (43%) compared to the HC (54%). Increased phosphorylation of endothelial nitric oxide synthase (p-eNOS) (3.1 fold), Vascular endothelial growth factor (1.9 fold) and Heme Oxygenase-1 (2.3 fold) was observed in the HSDG group as compared to the HC group. Significant improvement in left ventricular functions was also observed in the HSDG group as evidenced by increased ejection fraction (55 vs 45%), fractional shortening (28 vs 22%) & decreased left ventricular inner diameter in systole (8 vs 6 mm) in HSDG compared to HC. Moreover, MI model has shown increased capillary density (2531 vs 1901) and arteriolar density (2.6 vs 1.8) in SDG treated rats as compared to the HC. The increased capillary & arteriolar density along with increased left ventricular functions on SDG treatment might be due to increased HO-1, VEGF and p-eNOS

Address correspondence to: Nilanjana Maulik, Ph.D., FACN, FICA, Molecular Cardiology and Angiogenesis Laboratory, Department of Surgery, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-1110, USA, Phone No: (860) 679-2857; Fax No: (860) 679-2825, Email: E-mail: nmaulik@neuron.uchc.edu.

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expression. **In conclusion**, our study demonstrates for the first time that SDG treatment reduces ventricular remodeling by neovascularization of the infarcted HC myocardium.

Keywords

Hypercholesterolemia; secoisolariciresinol diglucoside; myocardial infarction; capillary density; VEGF; HO-1; neovascularization

Introduction

Hypercholesterolemia is a major risk factor for atherosclerosis, coronary artery disease (CAD) and myocardial infarction (1,2). Epidemiological data have shown that the higher the serum cholesterol level, the higher the incidence of coronary heart disease (CHD) (3) and it has been shown that cholesterol-lowering therapy reduces cardiovascular risk events. Consequently, much attention has been devoted to define the changes in morphology and function of large arteries that result from chronic elevations in blood cholesterol levels. Cells are normally protected from reactive oxygen species (ROS) by the antioxidant defense system. Overproduction of ROS and oxidative stress during hypercholesterolemia plays an important role in vascular dysfunction and atherogenesis (4,5).

Endothelial dysfunction has been shown in CHD and endothelial-derived nitric oxide plays a major role in regulation of cardiovascular disorders (6,7). After lipid lowering, there is a reversal of coronary artery endothelial dysfunction in hypercholesterolemic patients (8). The loss of endothelium-derived nitric oxide is shown to promote vasospasm, platelet aggregation and inflammation in atherosclerotic coronary arteries resulting in narrowing of the arteries and reduced blood flow leading to symptoms of ischemia (9,10). Recent reports have shown that hypercholesterolemia-induced impairment of angiogenic response may be due to endothelium-derived nitric oxide which is a critical modulator of angiogenesis (11) and *endothelial nitric oxide synthase gene delivery is shown to promote angiogenesis in a rat model of hind limb ischemia* (12). Moreover, angiogenesis induced by vascular endothelial growth factor (a potent angiogenic cytokine) was also found to be attenuated by inhibitors of NO synthase (NOS) (11). In addition, the absence of heme oxygenase-1 (HO-1) is known to exacerbate atherosclerotic lesion formation and vascular remodeling (13). HO-1 is a well known stress response and cytoprotective protein which protects the cells from pathophysiological stress (14). It is a rate limiting enzyme that catalyzes heme to carbon monoxide (CO) and biliverdin which is further reduced to bilirubin (15). CO is shown to have anti-inflammatory, anti-apoptotic properties (16) and bilirubin is shown to be a potent antioxidant (17). Moreover studies demonstrated HO-1 as a mediator of VEGF production and activity (18) and also to facilitate angiogenesis in a rat model of hind limb ischemia (19). There are several studies showed nitric oxide (NO) mediated HO-1 expression and regulatory interactions between the nitric oxide synthase and HO pathways (20,21).

Based on experimental evidence that oxidative stress plays a key role in vascular damage, there has been great interest in developing pharmacological approaches to decrease oxidative stress and improve endothelial function and thus myocardial angiogenic response in hypercholesterolemia. In this context, we have used secoisolariciresinol diglucoside (SDG), a pure compound isolated from flax seed, in hypercholesterolemic animals. Flaxseed is a rich source of omega-3 fatty acids and the richest source of plant lignans. SDG has been isolated from flaxmeal. SDG is effective in retarding the development of type 2 diabetes (22) and cancer prevention (23). SDG has been shown to be effective in lowering serum cholesterol and reducing the development of atherosclerosis in hypercholesterolemic rabbits (5). We have recently reported that SDG is an angiogenic and cardioprotective agent against ischemia-reperfusion injury and this effect is mediated by VEGF/NO (24). The present study is designed

to investigate the effect of SDG on VEGF, eNOS and HO-1 mediated myocardial angiogenesis and cardioprotection in hypercholesterolemic myocardium.

Materials and Methods

Animals

This study was performed in accordance with the principles of laboratory animal care formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (Publication No. 85–23, revised 1985). The experimental protocol was examined and approved by the Institutional Animal Care Committee of the Connecticut Health Center (Farmington, CT).

Experimental design

Male Sprague-Dawley rats weighing 200–250 g (starting body weight) were used for the study. The rats were randomized into four groups (n=12 in each group): 1) Control (C); 2) SDG Control (SDG); 3) Hypercholesterolemia (HC); and 4) Hypercholesterolemia + SDG (HSDG). Rats in HC group were fed with 2% cholesterol diet (CD) for 8 weeks (*High cholesterol diet was purchased from Harlan Teklad Company, Madison, Wisconsin 53744, USA*). Corresponding control rats were fed with normal diet. Eight weeks later the final body weight in the control group was 375–400g and in the HC group was 450–500g. *These rats were used for both ex vivo and in vivo studies. After 8 weeks of high cholesterol diet the rats in experimental groups were treated with SDG (20mg/kg/day) dissolved in drinking water for 2 weeks. SDG was provided as a generous gift by Dr. Kailash Prasad, University of Saskatchewan, Saskatoon, Canada.* The Isolated working hearts subjected to 30 min of global ischemia followed by 120 min reperfusion were used to measure the cardiac fractions, infarct size and protein expression profile. In a separate study, the *in vivo* myocardial infarction model was (subjected to 7 and 30 days of permanent LAD occlusion) used to measure the capillary and arteriolar density respectively. Echocardiogram was used to assess myocardial function after 30 days of myocardial infarction.

Determination of lipid levels

Blood was collected from the jugular vein and transferred into heparinized tubes and the plasma was separated after centrifugation. Cholesterol, triglycerides, and HDL-C levels were measured using *colorimetric* kits for total cholesterol (TC), triglycerides (TC) and HDL-C (*Wako Diagnostics, Richmond, VA 23237, USA*) following the kit protocol. LDL-C was calculated using the Friedwald Equation $LDL-C = TC - (HDL-C + (TG/5))$.

Isolated working heart for cardiac functions and infarct size measurement

Male Sprague Dawley rats weighing 375–400g (groups 1 & 2 in which rats were fed with normal diet for 8 weeks) 450–500g (groups 3 & 4 in which rats were fed with high cholesterol diet for 8 weeks) were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and heparinized (500 U/kg, i.p.). The heart was excised and immediately immersed in ice-cold perfusion buffer. The aorta was cannulated and the heart was perfused with Krebs-Henseleit Bicarbonate (KHB) buffer in the Langendorff mode through the aortic cannula at a perfusion pressure of 65 mm Hg. After 10 min of retrograde perfusion, the heart was switched to antegrade perfusion mode during which KHB buffer entered through the cannulated left atrium at a pressure equivalent to 10 cm of water, and passed to the left ventricle from which it was spontaneously ejected through the aortic cannula against a pressure equivalent to 100 cm of water. Cardiac functions like heart rate, coronary flow, aortic flow, left ventricular developed pressure (LVDP), and derivative of pressure/derivative of time (dp/dt) were recorded at baseline level and after

ischemia during 2 hours of reperfusion at time intervals of 30, 60, 90 & 120 min of reperfusion. The cardiac functions were measured as previously described by Penumathsa et al (25) At the end of reperfusion, a 1% (W/V) solution of triphenyl tetrazolium chloride (TTC) in phosphate buffer was infused into the aortic cannula for 20 min at 37°C. The TTC staining was performed to demarcate the infarcted area in the heart. The hearts were removed and stored at -70°C. Sections of frozen heart were fixed in 4% para-formaldehyde, placed between two glass slides and digitally imaged using an Epson Scanner. To quantitate the areas of infarct in pixels, NIH image software was used. The infarct size was quantified and expressed in pixels (25).

Western Blot analysis for phosphorylated eNOS, VEGF and HO-1

To quantify the p-eNOS, VEGF and HO-1, standard SDS/PAGE Western blot technique was performed. Heart tissues from each treatment group were homogenized and suspended (50 mg/ml) in sample buffer [10 mM HEPES, pH 7.3, sucrose 11.5%, EDTA 1mM, EGTA 1mM, diisopropylfluorophosphate (DFP), pepstatin A 0.7 mg/ml, leupeptin 10 mg/ml, aprotinin 2 mg/ml]. The cytosolic protein was isolated and total protein concentration was determined using a BCA (bicinchoninic acid) protein assay kit (Pierce, Rockville, IL). The cytosolic proteins were run on polyacrylamide electrophoresis gels (SDS-PAGE) typically using 7% for p-eNOS (*the phosphorylation site is specific to serine-1177*) & 10% for VEGF and HO-1 (acrylamide to bis ratios). Standard Western blot technique was done as described previously (25). The antibodies were purchased from p-eNOS, eNOS (Cell Signaling Technology, Danvers, MA) and VEGF, HO-1 from Santa cruz Biotechnology, Santa cruz, CA, USA respectively.

Surgical procedure

Male Sprague Dawley rats weighing 375–400g (groups 1 & 2 in which rats were fed with normal diet for 8 weeks) 450–500g (groups 3 & 4 in which rats were fed with high cholesterol diet for 8 weeks) 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 (4th intercostal space). A 6-0 polypropylene suture was passed with tapered needle under the left anterior descending coronary artery (LAD) just below the tip of the left auricle, and a non-traumatic occluder was applied on the artery. Myocardial infarction was produced by permanent LAD occlusion. After completion of all surgical protocols, the chest wall 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.

Immunohistochemistry for capillary and arteriolar density

Capillary density (CD-31) and arteriolar density staining was done following 7 and 30 days of the surgical procedure, respectively. The rats were sacrificed; hearts removed and paraffin-embedded sections were made. Endothelial cells were labeled using mouse monoclonal anti-CD31/PECAM-1 (1:100, Pharmingen, San Diego, CA, USA) followed by a biotinylated horse anti-mouse secondary antibody (1:200 dilution). The reaction product (brown) was visualized with 3,3'-diaminobenzidine (DAB) substrate using the Vector ABC Vectastain Elite Kit (Vectorlabs, Burlingame, CA, USA). On separate slides, vascular smooth muscle cells were labeled using mouse monoclonal anti-smooth muscle actin (1:100, Sigma, St. Louis, MO, USA) followed by a FITC conjugated donkey anti-mouse secondary antibody (1:200 dilution, Jackson ImmunoResearch Inc, West Grove, PA, USA). After incubation the sections were again washed in PBS for 3 times and mounted with coverslip using mounting medium (Vector laboratories, Burlingame, CA). Images were captured and stored in digital tiff file format for later image analysis. Counts of capillary density and arteriolar density per mm² were obtained

after superimposing a calibrated morphometric grid on each digital image using Adobe 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 (UBM) (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 left ventricular (LV) systolic function, LV cavity diameter, wall thickness, diastolic function, and LV end-systolic and end-diastolic volume determination. Myocardial infarcted segments were determined according to the kinetics: hypokinetic (reduction in wall motion), akinesis (no wall motion) and dyskinesis (unsynchronized movement of segment with normal myocardium). 2D 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 left ventricular parameters were measured according to the modified American Society of Echocardiography–recommended guidelines. Ejection fraction (EF) and fractional shortening (FS) were assessed for left ventricular 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 measurements represent the mean of at least 3 consecutive cardiac cycles. Throughout the procedure ECG, respiratory rate, and heart rate were monitored as previously described by Penumathsa et al., (24).

Statistical Analysis

Results are expressed as Mean $\pm$ Standard Deviation (SD). ANOVA followed by Bonferroni's correction was carried out to determine any differences between the mean values of all groups. The results were considered significant if $p < 0.05$.

Results

Effect of SDG on lipid levels

The levels of serum cholesterol, triglycerides and LDL-C increased while the HDL-C level decreased in the HC group as compared to the control group. Treatment with SDG significantly lowered the cholesterol, triglyceride and LDL-C levels in the high cholesterol-fed rats as compared to the nontreated HC group. The HDL-C levels increased with SDG treatment (Table-1). No significant differences in the lipid levels were observed between the control and SDG control groups.

Effect of SDG on cardiac functions

At baseline level no significant difference was observed in cardiac functions like LVDP (mmHg), heart rate (beat/min) and coronary flow (ml/min) between the groups but significant difference in dp/dt (mm Hg/sec) and aortic flow (ml/min) was observed in SDG control group even at the baseline level when compared with HC and control hearts. Following ischemia, upon reperfusion, cardiac function was found to be more significant in control and SDG treated groups when compared to HC group. Significant increase in dp/dt_{max} and aortic flow were observed during reperfusion in SDG treated groups when compared to corresponding control and HC control groups. Following 120 min of reperfusion, post ischemic values of dp/dt_{max} (mm of Hg/sec) were significantly increased in HSDG (1689.32 ± 98.88) group when compared to HC group (1092.74 ± 39.45). Similarly, aortic flow (ml/min) significantly

increased in SDG (12.6 ± 1.85) and in HSDG (4.21 ± 0.84) groups when compared to corresponding control (4.6 ± 0.8) and HC (0.00) groups after 2 hours of reperfusion (Figure 1A–E).

Effect of SDG on infarct size

The infarct size (percent of infarct vs. total area at risk) of hearts subjected to 30 min of global ischemia followed by 2 h reperfusion was greater in the HC group than in the control group. Significant reduction in infarct size was observed in SDG treated groups as compared to corresponding control groups. Infarct size was significantly reduced in the HSDG group (43%) as compared to the HC group (54%) (Figure-2).

Effect of SDG on protein levels of p-eNOS, VEGF and HO-1

Phosphorylation of eNOS decreased in the HC group (0.6 fold) compared to both the normal (1 fold) and SDG (1.6 fold) control. SDG treatment significantly increased the phosphorylation of e-NOS of the HSDG group (4.2 fold) compared to the HC (Figure-3). The non-phosphorylated e-NOS levels were not significantly different between the groups. VEGF expression was significantly reduced in the HC group (0.7 fold) compared to the normal control. SDG treatment significantly increased the expression of VEGF in the HC group (1.9 fold) as compared to the nontreated HC group. Stress inducible protein HO-1 was reduced in the HC (0.72 fold) as compared to control. A significant increase in HO-1 expression was observed in the SDG treated HC group (2.3 fold) as compared to the non-treated HC group (Figure-4).

Effect of SDG on the extent of capillary density (CD-31) and arteriolar density

Immunohistochemical staining of PECAM-1 and anti-smooth muscle actin were used to assess the capillary density (CD-31) and arteriolar density, respectively. At 400X magnification, 8 non-overlapping random fields, each selected from a non-infarcted risk area, were used for counting. Four sections from each heart were examined. Increased capillary density was observed in the HSDG treated group (2531 vs 1901) as compared to the HC group (Figure-5). Similarly, increased arteriolar density was also observed in the HSDG-treated group (2.6 vs 1.8) as compared to the HC group (Figure-6). The results correlated positively with the VEGF expression levels thus supporting the notion that the increased capillary and arteriolar density on SDG treatment might be due to the angiogenic property of SDG.

Effect of SDG on echocardiographic findings after MI

Echocardiography was performed after 30 days of LAD ligation. The left ventricular functional parameters were assessed. The ejection fraction and fractional shortening of the left ventricle significantly decreased in the HC group compared to control. The ejection fraction (55 vs 45%) and fractional shortening (28 vs 22%) increased significantly in the HSDG group compared to the HC group. The SDG control group also showed a significant increase in the ejection fraction and fractional shortening as compared to control MI group. A significant increase in chamber dilation (as assessed by measuring LVIDd and LVIDs) was observed in the HC group as compared to the control group and was found to be reduced on treatment with SDG in the HSDG group. The left ventricular posterior wall (LVPWs) was found to be decreased in the HC group as compared to the control, SDG and HSDG groups. There was a compensatory increase in the posterior (LVPW) and lateral wall systolic thickness in the HSDG group compared to the HC (Figure-7).

Discussion

In this report we demonstrated that hypercholesterolemia-induced myocardial complications such as infarct size, reduced myocardial function and impaired myocardial angiogenesis could be positively modulated by dietary supplementation of SDG isolated from flaxseed. Regarding the extent of infarct size there are some contradictory results in the literature showing in some studies no increase (26) as well as in some other studies significant increase in infarction following ischemia/reperfusion after feeding cholesterol rich diet to rabbits (27,28). According to our experimental results we have observed increased infarct size in hypercholesterolemic rats as compared to control. The best explanation may be due to species difference as well as different ischemic model.

In addition we have observed increased protein expression of activated eNOS, VEGF and HO-1 in SDG-treated HC rats as compared to the untreated HC group.

It is known that cardiovascular risk factors such as hypercholesterolemia, hypertension, and smoking can impair endothelial function (29) even in the absence of atherosclerotic disease (30). Endothelial dysfunction is a hallmark of hypercholesterolemia, and endothelial function improves with cholesterol reduction (31). Improved endothelial function is one of the clinical markers following atherogenic risk factor modification and depends on LDL levels, as LDL is known to be a potent inhibitor of endothelial function (10). Evidence shows that endothelial dysfunction, which is often defined as the decreased synthesis of endothelial-derived nitric oxide (NO), is an important factor leading to atherosclerosis and is supported by the fact that the lack of NO in atherosclerotic vessels contributes to impaired vascular relaxation. In addition, Ziche et al., (31), have shown that biological processes modulated by NO might play a role in angiogenesis. NO released from cultured human endothelial cells as well as isolated vascular strips confirmed that VEGF stimulates endothelial nitric oxide release *in vitro* (33). VEGF treatment elicited dose-dependent increases in the cellular content of eNOS mRNA and protein, which may account for the chronic stimulation of NO production by VEGF. 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 (34) have shown that infarct size was significantly augmented in eNOS deficient mice, and infarct size was reduced in eNOS transgenic mice following ischemia and reperfusion, thus supporting the role of eNOS-derived NO during ischemia-reperfusion injury and cardioprotection (35). Moreover we have documented earlier that SDG is angiogenic and the process is mediated through VEGF/NO (24). In the present study we have observed protein expression of eNOS and VEGF in SDG treated rats and, as expected, increased expression of VEGF and p-eNOS (an index of NO production) in SDG treated rats as compared to the HC group. Our results also demonstrated improved cardiac functions and a reduction in infarct size in the HC group with SDG treatment which might be due to increased protein expression of VEGF/ NO.

Clinical trials have demonstrated beneficial effects of antioxidant therapy in individuals with atherosclerotic heart disease (36) because increased oxidative stress leads to LDL oxidation which further inhibits endothelial derived relaxing factor (EDRF) release.

Hypercholesterolemia stimulates the generation of O₂⁻ from vascular smooth cells, which may cause endothelial dysfunction by scavenging endothelial-derived NO (37). We have observed the expression of HO-1 protein, a stress inducible protein, and its expression was shown to be beneficial for endothelial cell function. Moreover the degradation of heme liberates CO, which like nitric oxide can activate the guanylate cyclase-cGMP pathway, which in turn elicits vasodilation and inhibits platelet activation and smooth muscle cell proliferation (38). We have observed increased expression of HO-1 in SDG treated rats as compared to the nontreated hypercholesterolemic group. It has been reported that delivery of adeno-associated virus HO-1

gene resulted in protection of a rat model acute ischemia-reperfusion injury (39). Thus the SDG-mediated cardioprotection may also be due to increased HO-1 protein expression.

In the present study, the extent of angiogenesis measured by capillary and arteriolar density staining following MI also validated the strong angiogenic response of the SDG-treated group compared to other groups. An increase in capillary density and arteriolar density was observed in SDG group which might be a compensatory response aimed at sustaining myocardial perfusion. Therefore, enhanced neo-vascularization in the infarcted myocardium induced by SDG seems to mediate chronic cardioprotection against ventricular remodeling.

Echocardiography also revealed a significant reduction in ischemic ventricular remodeling and showed an improved diastolic and systolic thickness of the ventricular wall in the SDG-treated group, showing the efficacy of SDG in maintaining the global left ventricular systolic function along with increased ejection fraction and fractional shortening. These findings show that SDG reduces the extent of the infarction and helps in recovery of the heart from ischemic insult. The extent of angiogenesis measured by capillary and arteriolar density in the MI model compared to the hypercholesterolemic rats also validated the strong angiogenic response by SDG. The increased capillary density resulting in increased blood supply in the MI model with SDG treatment might be mediated by VEGF/ NO/ HO-1. It is known that VEGF administration restores normal vascular responsiveness in ischemically injured tissue (40). Furthermore, VEGF administration does not restore normal collateral development in eNOS-deficient mice, indicating that eNOS may be a downstream effector molecule of VEGF (41). Studies show that eNOS is required for endothelial cell proliferation and/or migration, the essential steps required for neovascularization (42). It is also known that administration of NO donors or eNOS gene transfer increases the vascular generation of VEGF (43) which is well known to be responsible for the growth of angiogenesis in animal models and in patients with critical limb ischemia or myocardial ischemia (44,45). In conclusion, the present study shows that SDG treatment increased expression of VEGF/NO/HO-1 that might have resulted in improved cardiac function by increased neovascularization of the ischemic hypercholesterolemic myocardium.

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Figure 1.

Effect of SDG on cardiac functional parameters at baseline (BL) and after 30min of ischemia at 30, 60, 90 and 120min of reperfusion. The results in A represent LVDP, B represents heart rate, C represents dp/dt_{max}, D represents Aortic flow, E represent Coronary flow. Results are shown in six animals per group. *P<0.05 represents control compared with other groups, #P<0.05 represents SDG group compared HC and HSDG groups, †P<0.05 represents HC group compared HSDG group. Where Black bar represents control, Zigzag bar represents SDG control, Blank bar represents HC and Dark upward diagonal bars represents HSDG group.

Figure 2.

Effect of SDG on infarct size. Graph represents the % of infarct size between the comparative groups following 30min of ischemia and 2hr of reperfusion. Values are mean $\pm$ SD (n = 6). *P<0.05 represents control compared with other groups, #P<0.05 represents SDG group compared HC and HSDG groups, †P<0.05 represents HC group compared HSDG group. Where HC represents high cholesterol diet fed rats, SDG represents SDG control rats, HSDG represent HC rats treated with SDG.

Figure 3.

Representative western blot showing the protein expression of phosphorylated eNOS. Graph represents the quantitative comparison between the groups. eNOS was used as the loading control. * $P < 0.05$ represents control compared with other groups, # $P < 0.05$ represents SDG group compared HC and HSDG groups, † $P < 0.05$ represents HC group compared HSDG group. Where HC represents high cholesterol diet fed rats, SDG represents SDG control rats, HSDG represent HC rats treated with SDG (n=5 repeated experiments).

Figure 4.

A) Representative western blot showing the protein expression of VEGF and HO-1 and GAPDH. B) Graph represents the quantitative comparison of VEGF expression between the groups. C) Graph represents the quantitative comparison of HO-1 expression between the groups. GAPDH was used as the loading control. * $P < 0.05$ represents control compared with other groups, # $P < 0.05$ represents SDG group compared HC and HSDG groups, † $P < 0.05$ represents HC group compared HSDG group. Where HC represents high cholesterol diet fed rats, SDG represents SDG control rats, HSDG represent HC rats treated with SDG (n=5 repeated experiments).

Figure 5.

A) Representative pictures show the effect of SDG on capillary density. B) Graph represents the quantitative analysis of capillary density between the comparative groups in the peri-infarct area after 7 days of myocardial infarction. * $P < 0.05$ represents control compared with other groups, # $P < 0.05$ represents SDG group compared HC and HSDG groups, † $P < 0.05$ represents HC group compared HSDG group. Arrows represent the capillaries staining. Where HC represents high cholesterol diet fed rats, SDG represents SDG control rats, HSDG represent HC rats treated with SDG (n=6 in each group).

Figure 6.

A) Representative pictures show the effect of SDG on arteriolar density. B) Graph represents the quantitative analysis of arteriolar density between the comparative groups in the peri-infarct area after 30 days of myocardial infarction. * $P < 0.05$ represents control compared with other groups, # $P < 0.05$ represents SDG group compared HC and HSDG groups, † $P < 0.05$ represents HC group compared HSDG group. Arrows represent the arteriolar density staining. Where HC represents high cholesterol diet fed rats, SDG represents SDG control rats, HSDG represent HC rats treated with SDG (n=6 in each group).

Figure 7.

Echocardiographic measurements between the comparative groups after 30 days of myocardial infarction. A) Ejection fraction B) Fractional Shortening C) LVPW D) LVIDs(mm) E) Figures representing the LVID(S) in a parasternal short axis view in M-mode. Yellow arrow represents the interventricular septum and white arrow represent posterior wall. Graph represents the quantitative comparison between the groups. * $P < 0.05$ represents control compared with other groups, # $P < 0.05$ represents SDG group compared HC and HSDG groups, † $P < 0.05$ represents HC group compared HSDG group. Where HC represents high cholesterol diet fed rats, SDG represents SDG control rats, HSDG represent HC rats treated with SDG (n=6 in each group).

Table 1

EFFECT OF SDG ON LIPID LEVELS

S. No	Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
1	Control	83.34±5.32	76.42±6.37	39.67±2.92	27.14±2.96
2	SDG	84.29±6.29	77.58±5.97	40.14±3.13	26.38±1.99
3	HC	146.83±6.16 [*]	158.17±8.74 [*]	27.69±2.84 [*]	79.03±5.91 [*]
4	HSDG	98.16±8.29 [#]	96.63±4.78 [#]	33.75±4.26 [#]	43.71±3.79 [#]

Results are expressed as Means ± SD.

^{*} p<0.05, compared with group 1 and 2;

[#] p<0.05, compared with group 3