Resveratrol Improves Myocardial Perfusion in a Swine Model of Hypercholesterolemia and Chronic Myocardial Ischemia

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Background—Resveratrol may provide protection against coronary artery disease. We hypothesized that supplemental resveratrol will improve cardiac perfusion in the ischemic territory of swine with hypercholesterolemia and chronic myocardial ischemia.

Methods and Results—Yorkshire swine were fed either a normal diet (control, n=7), a hypercholesterolemic diet (HCC, n=7), or a hypercholesterolemic diet with supplemental resveratrol (100 mg/kg/d orally, HCRV, n=7). Four weeks later, an ameroid constrictor was placed on the left circumflex artery. Animals underwent cardiac MRI and coronary angiography 7 weeks later before euthanasia and tissue harvest. Total cholesterol was lowered about 30% in HCRV animals ($P<0.001$). Regional wall motion analysis demonstrated a significant decrease in inferolateral function from baseline to 7 weeks in HCC swine ($P=0.04$). There was no significant change in regional function in HCRV swine from baseline to 7 weeks ($P=0.32$). Tissue blood flow during stress was 2.8-fold greater in HCRV swine when compared with HCC swine ($P=0.04$). Endothelium-dependent microvascular relaxation response to Substance P was diminished in HCC swine, which was rescued by resveratrol treatment ($P=0.004$). Capillary density (PECAM-1 staining) demonstrated fewer capillaries in both HCC and HCRV swine versus control swine ($P=0.02$). Immunoblot analysis demonstrated significantly greater expression in HCRV versus HCC swine of the following markers of angiogenesis: VEGF ($P=0.002$), peNOS (ser1177) ($P=0.04$), NFkB ($P=0.004$), and pAkt (thr308) ($P=0.001$).

Conclusions—Supplemental resveratrol attenuates regional wall motion abnormalities, improves myocardial perfusion in the collateral dependent region, preserves endothelium-dependent coronary vessel function, and upregulates markers of angiogenesis associated with the VEGF signaling pathway. **(*Circulation*. 2010;122[suppl 1]:S142–S149.)**

Key Words: coronary disease ■ hypercholesterolemia ■ ischemia ■ perfusion ■ angiogenesis

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Resveratrol, a polyphenol found in red wine, has been implicated in protection against coronary artery disease.[1] Potential mechanisms include enhancing angiogenesis, reducing oxidant stress and mimicking effects of caloric restriction. Resveratrol has also been implicated in protection from ischemic injury. The mechanism probably is attributable to the vasorelaxation and antiapoptotic and proangiogenic properties of the supplement.[2]

We previously demonstrated that hypercholesterolemia can significantly blunt the endogenous angiogenic response to chronic myocardial ischemia. In that study, the effects of hypercholesterolemia on the chronically ischemic myocardium resulted in decreased vessel density, increased levels of antiangiogenic mediators, depressed microvascular function, and decreased perfusion in the ischemic territory relative to those animals fed a normal diet.[3] These detrimental changes, along with atherosclerosis, probably contribute significantly to the development of cardiovascular disease.

Resveratrol is thought to activate sirtuins, a family of highly conserved proteins with deacetylase activity.[4] The most well-studied sirtuin is SIRT1, a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase, which acts at the DNA and protein levels to exert its effects.[5] In the cardiovascular system, activation of SIRT1 is thought to lead to

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decreased inflammation, platelet aggregation, and apoptosis.[6,7] Furthermore, activation of SIRT1 may promote native angiogenesis and has been shown to increase VEGF and NO production.[8] However, these findings have not been validated in large animal models, and the role of resveratrol in the setting of chronic myocardial ischemia, hypercholesterolemia and endothelial dysfunction remains to be examined.

Given the considerable potential for cardioprotective and proangiogenic effects of resveratrol, we examined the effects of this agent in a clinically relevant porcine model of hypercholesterolemia and chronic myocardial ischemia. We hypothesized that supplemental resveratrol will improve cardiac perfusion and vascular function in the ischemic territory.

Methods

Animal Model

Yorkshire miniswine (Parsons Research, Amherst, Mass) were fed 1 of 3 diets daily throughout the 11 weeks of the experiment. The first group was given 500 g of a hypercholesterolemic diet daily (HCC, n=7) composed of 4% cholesterol, 17.2% coconut oil, 2.3% corn oil, 1.5% sodium cholate, and 75% regular chow. A second group was fed the same hypercholesterolemic diet supplemented with 100 mg/kg/d resveratrol (HCRV, n=7) (Chromadex, Irvine, Calif). The third group of swine was fed regular chow (control, n=7).

After 4 weeks of dietary modification, all animals underwent functional cardiac MRI (CMR) and ameroid constrictor placement on the proximal left circumflex coronary artery (LCx). For all surgical procedures, anesthesia was induced with ketamine (10 mg/kg IM) and thiopental 2.5%, and maintained with a gas mixture of oxygen at 1.5 to 2 L/min and 3.0% isoflurane. The animals were intubated and mechanically ventilated at 12 to 20 breaths/min.

During the first procedure (ameroid placement), through a left minithoracotomy, gold-labeled microspheres (Biophysics Assay laboratory, Worcester, Mass) were injected into the left atrium during temporary occlusion of the LCx to determine the exact myocardial territory at risk. Next, a titanium ameroid constrictor (1.75 mm internal diameter) was placed around the proximal LCx.

Seven weeks after ameroid placement, swine were anesthetized and CMR and x-ray coronary angiography were completed. The heart was then exposed and microspheres were injected both at rest and during ventricular pacing (150 beats per minute), followed by euthanasia. The heart was harvested, and two 1-cm-thick transverse slices were cut at the midventricular level and sectioned into 8 segments. Samples were divided and rapidly frozen in liquid nitrogen (molecular studies), placed in 4°C Krebs solution (microvessel reactivity studies), 10% formalin (immunohistochemistry studies), or weighed and dried (microsphere perfusion analyses).

All experiments were approved by the Beth Israel Deaconess Medical Center Animal Care and Use Committee. Animals were cared for in compliance with the Harvard Medical Area Institutional Animal Care and Use Committee and 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* (NIH publication No. 5377–3 1996).

Cardiovascular MRI

Animals in the HCC and HCRV groups underwent a CMR study before placement of the ameroid (pre) and before euthanasia (post) 7 weeks later. All animals were scanned using a 1.5-T Philips Achieva scanner (Philips Healthcare, Best, The Netherlands) with a 5-element cardiac phased-array receiver coil. MR tagging was performed to evaluate dyssynchrony and local myocardial function using 3 short-axis slices with a spiral complementary spatial modulation of magnetization sequence.[9] Images were analyzed using a MATLAB (The Mathworks, Natick, Mass) program.[10] Each myocardial slice was divided into 6 segments according to the American Heart Association/American College of Cardiology 17-segment model, and the circumferential strain of each segment was analyzed. The values obtained represent the amount of regional contraction contributing to the overall ejection fraction. Thus, a more negative number represents greater contraction of the myocardial segment.

X-Ray Coronary Angiography

X-ray coronary angiography was carried out to ensure occlusion of the LCx and assess collateral formation. Recorded images were interpreted by a blinded interventional cardiologist. Angiographic collateral formation was assessed according to the Rentrop grading system of 0 to 3, depending on the presence and extension of the collateral filling of coronary epicardial vessels.[11]

Microvessel Studies

After cardiac harvest, coronary arterioles (80 to 180 μm in diameter) from the ischemic territory were dissected from the surrounding tissue and placed in isolated microvessel chambers as described previously.[12] The microvascular responses to sodium nitroprusside (SNP, 10^{-9} to 10^{-4} mol/L, endothelium-independent cGMP-mediated vasodilator), and substance P (10^{-12} to 10^{-7} mol/L, an endothelium-dependent vasodilator) were evaluated. All drugs were applied extraluminally. Relaxation responses were defined as the percent relaxation of the preconstricted diameter. All reagents were obtained from Sigma-Aldrich (St Louis, Mo).

Myocardial Perfusion Analysis

Myocardial perfusion was determined during each procedure with isotope-labeled microspheres, 15 μm diameter (BioPAL Worcester, Mass) using previously reported methods.[13] Briefly, 1.5×10^7 gold-labeled microspheres were injected during temporary LCx occlusion at the time of ameroid placement to identify the area at risk. Lutetium (rest) and Europium (pace) isotope-labeled microspheres were injected at the final procedure. After euthanasia, 10 transmural left ventricular sections were collected for isotope-labeled microsphere assays. The samples were exposed to neutron beams and microsphere densities were measured using a gamma counter.

Molecular Studies

Western blotting was performed as previously described.[14] Sixty micrograms of total protein was fractionated by 4% to 20% gradient, SDS polyacrylamide gel electrophoresis (Invitrogen, San Diego, Calif), and transferred to PVDF membranes (Millipore, Bedford, Mass). Each membrane was incubated with one of the following specific antibodies: anti-VEGF (Calbiochem, San Diego, Calif), anti-FGF-2 (US Biological, Swampscott, Mass), anti-NFkB, anti-peNOS (ser1177), anti-pAkt (ser473), anti-pAkt (thr473), anti-pERK (thr202/tyr 204) (Cell Signaling, Beverly, Mass), anti-eNOS antibody (BD Biosciences, San Jose, Calif), and antimyeloperoxidase (MPO) antibody (Abcam, Inc, Cambridge, Mass). Immune complexes were visualized with an enhanced chemiluminescence detection system (Amersham, Piscataway, NJ). Bands were quantified by densitometry of autoradiograph films. Ponceau staining was used to ensure equal protein loading.

Immunohistochemistry

Formalin-fixed tissue samples were processed as previously described.[14] Antibodies against PECAM-1 (Santa Cruz Biotechnology, Santa Cruz, Calif) were applied to the sections for 2 hours at room temperature. Detection was obtained using a biotinylated goat anti-mouse secondary antibody and the avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, Calif). Color was developed using diaminobenzidine substrate (1 mg/mL in PBS and 0.03% H_2O_2). Sections were then counterstained with hematoxylin, dehydrated, and mounted. Photomicrographs were taken with a Zeiss Axiolab microscope (Carl Zeiss Inc, Thornwood, NY) equipped with a digital camera (Photodoc, Upland, Calif), and capillaries were counted in a blinded fashion.

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Table. Comparison of Physiological Measures in Animals Fed Either Regular Chow, High Cholesterol Diet Only, or High Cholesterol Diet With Supplemental Resveratrol

	Control	HCC	HCRV	*P* Value
Total cholesterol, mg/dL	79.9±0.1	390±53	261±54	<0.001
Mean HR, beats/min	129±16	122±11	115±33	0.8
MAP, mm Hg	69±7	73±10	65±7	0.03
Mean DBP, mm Hg	46±2.1	71±4.7	53±9	0.01
Blood flow, rest, mL/min/g	0.07±0.03	0.13±0.003	0.08±0.003	0.25

Blood flow indicates the amount of myocardial blood flow to collateral-dependent region at a resting heart rate; HR, heart rate; MAP, mean arterial blood pressure; and DBP, diastolic blood pressure.

Immunofluorescence Double Staining for Dividing Endothelial Cells

Frozen sections (12 μm in thickness) of myocardium from the ischemic territories were formalin fixed for 10 minutes. Antibodies against Ki-67 (Abcam Inc, Cambridge, Mass) and PECAM-1 (CD-31, R&D Systems, Minneapolis, Minn) were simultaneously applied to the sections and incubated overnight at 4°C. Detection was obtained using appropriate secondary antibodies (Jackson ImmunoReaserch, West Grove, Pa). Sections were then mounted in Vectashield with 4′,6-diamidino-2-phenylindole (DAPI, Vector Laboratories, Burlingame, Calif). Photomicrographs were taken with a Zeiss Axiolab microscope (Carl Zeiss Inc, Thornwood, NY) equipped with a digital camera and 200× magnification (Photodoc, Upland, Calif). Dividing endothelial cells were counted in a blinded fashion. Structures that were DAPI positive, Ki-67 positive, and within an endothelial cell (CD-31 positive) were considered dividing endothelial cells. Data are presented as number of Ki-67 positive endothelial cells/mm^2.

Data Analysis

All results are expressed as mean±SEM. Microvessel responses are expressed as percent relaxation of the preconstricted diameter and were analyzed using 2-way, repeated-measures ANOVA with a post hoc Bonferroni test, which was applied to interactions of treatment and dose. Western blots were analyzed after digitalization (ScanJet 4c; Hewlett-Packard, Palo Alto, Calif) and NIH ImageJ 1.33 software (National Institute of Health, Bethesda, Md). For data analysis, levels of phosphorylated proteins were normalized to total expression levels. Comparisons between the 3 groups for blood flow, endothelial proliferation, and Western blot data were analyzed by 1-way ANOVA with Newman-Keuls Multiple Comparison post hoc test, with the exception of the circumferential strain data which was analyzed by 2-tailed *t* test, using GraphPad Prism 4 (GraphPad Software Inc, San Diego, Calif). Probability values less than 0.05 were considered significant.

Results

Experimental Model

There was 1 animal death each in the control and HCRV groups. Necropsy did not identify a clear cause of death but probably was related to ventricular arrhythmia. There was no mortality in the HCC group. There was no difference in mean heart rate. The HCC group had a higher mean arterial pressure and mean diastolic pressure than the control or HCRV group. Total cholesterol levels were elevated in the HCC and HCRV groups, though the levels in the HCRV group were significantly lower than the HCC group (Table).

Cardiac MRI

Comparisons of regional function were made by analyzing the differences in the amount of muscle contraction in each region before placement of the ameroid with the amount of contraction at the end of the experiment within each group. Regional wall motion analysis using CMR circumferential strain demonstrated a significant decrease in inferolateral regional function (region of measurement shown in Figure 1A) from baseline (pre) to 7 weeks of chronic ischemia (post) in HCC swine in the basal myocardial slice (−19.1±4% to −11.1±1%) (Figure 1B). Regional function in HCRV swine from baseline (pre) to 7 weeks chronic ischemia (post) was preserved, without a significant worsening in inferolateral wall motion despite LCx ischemia (−17.8±7% to −14.7%±3%)(Figure 1C).

X-Ray Coronary Angiography

All animals were found to have ≥99% occlusion of the proximal LCx by the ameroid constrictors. Rentrop collateral scores were not significantly different among the groups

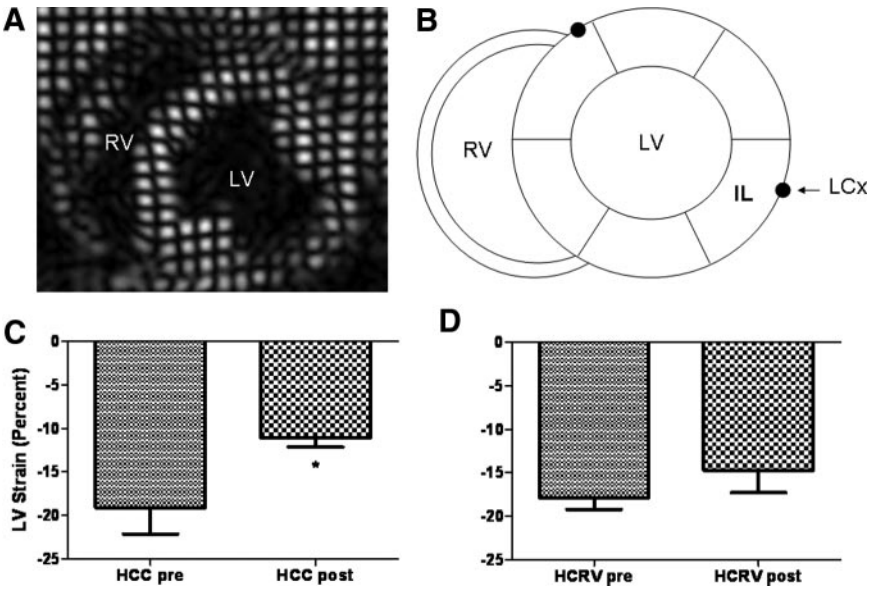

Figure 1. Coronary MRI analysis. A, Axial CMR circumferential strain image. B, Schematic cross-sectional representation of the heart depicting the proximity of the LCx to the inferolateral (IL) wall of the left ventricle (LV). RV indicates right ventricle. C, Regional wall motion analysis using circumferential strain demonstrates a significant decrease in IL wall function in the HCC group post (*P=0.04) (D) and no change in the HCRV group (P=0.32).

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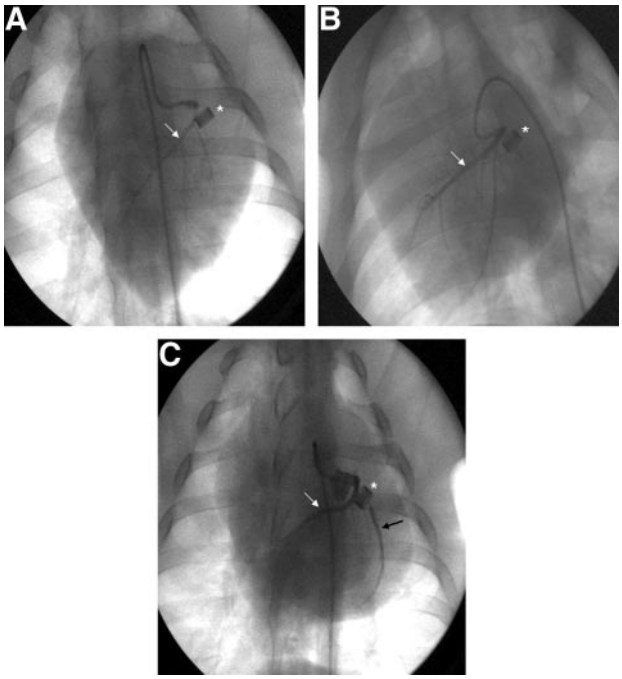

Figure 2. X-ray coronary angiograms demonstrating presence of flow in the LCx in the control (A), the HCC (B), and the HCRV (C) groups. The HCRV animal demonstrates brisk filling of the LCx through proximal collaterals from the left anterior descending artery (LAD). *Ameroid constrictor; white arrow denotes the LAD, black arrow depicts antegrade flow in the LCx.

(control, 0.9±0.3; HCC, 1.3±0.3; HCRV, 1.3±0.4; $P=0.68$) (Figure 2A through 2C).

Microvessel Analysis

Microvascular response to the endothelial independent drug SNP demonstrated no differences between groups (control, 93.9±5.7%; HCRV, 97.4±3.4%; HCC, 93.9±5.7% at 10^{-4} M) (Figure 3A). Endothelium-dependent microvascular relaxation to Substance P ($10^{-}7$ M) was diminished in HCC swine but was normalized in the HCRV group (HCC 38.5±6.7%, HCRV 65.1±10.2%, control 69.6±9.4%) (Figure 3B).

Myocardial Flow Analysis

At 7 weeks after ameroid placement, myocardial perfusion at rest was similar between groups (control, 0.07±0.03 mL/min/g; HCC, 0.13±0.03 mL/min/g; HCRV, 0.08±0.03 mL/min/g; $P=0.25$). When the heart was stressed with ventricular pacing at 150 bpm, there was a significantly greater increase in baseline-adjusted myocardial flow in the ischemic territory of the HCRV group (control, 155±27%; HCC, 89±14%; HCRV, 247±124%) (Figure 4).

Immunohistochemistry

Staining of fixed sections from the ischemic territory for PECAM-1 (CD31) demonstrated that the HCC and HCRV groups had significantly fewer capillaries (PECAM-1 positive vessels/200× field) than control (control, 96±17.1; HCC, 61±5.4; HCRV, 65±5.4; $P=0.02$). Double staining for endothelium (CD31) and cellular proliferation (Ki-67) demonstrated fewer dividing endothelial cells in the HCC and HCRV groups as compared with control (Figure 5).

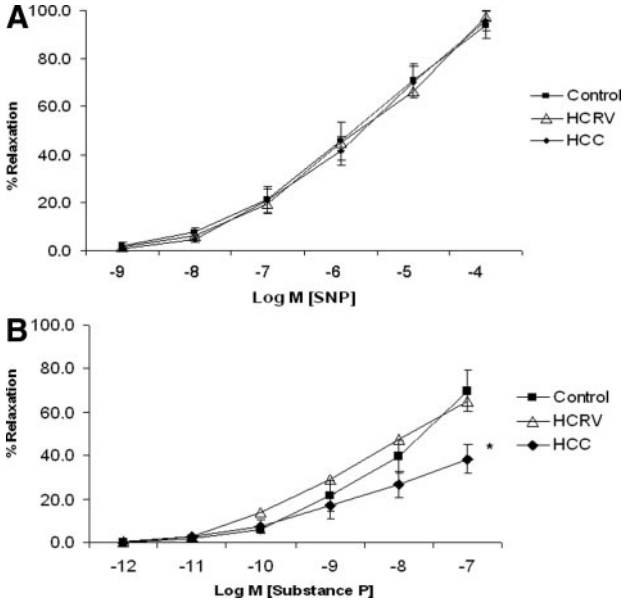

Figure 3. Coronary microvascular responses in vitro. A, Microvessel response to SNP, an endothelium-independent vasodilator ($P=0.95$). B, Microvessel response to Substance P, an endothelium-dependent vasodilator (*$P=0.004$).

Protein Expression

Immunoblot analysis of proteins associated with angiogenesis demonstrated significant increases in angiogenic protein levels in the ischemic territory of the HCRV group. VEGF expression increased 1.45-fold in the HCRV group versus control and 1.26-fold versus HCC (Figure 6A). Phospho-eNOS (ser1177) increased 3.33-fold in the HCRV group versus control and 2.18-fold versus HCC (Figure 6B). Protein expression of NFkB showed a 2.4-fold increase in the HCRV group versus control and 1.3-fold versus HCC (Figure 6C). The expression of MPO was not different between the groups ($P=0.80$). There was a trend toward increased phospho-ERK (thr202/tyr 204) in the HCRV group; however, this was not statistically significant (2.36-fold in HCRV versus control and 2.35-fold versus HCC) (Figure 6D). There was increased

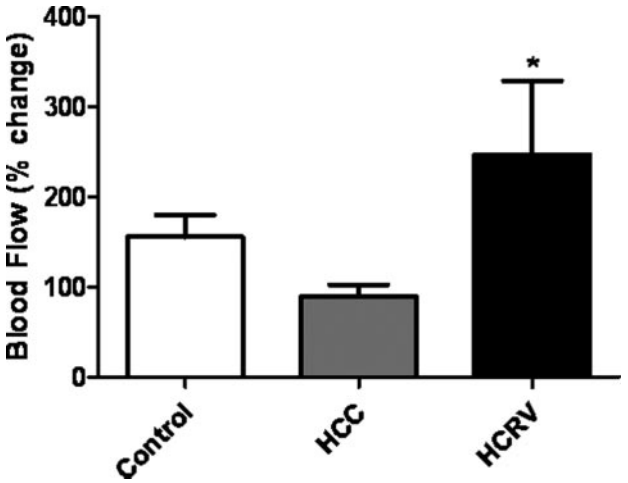

Figure 4. Myocardial blood flow analysis. Percentage change in myocardial blood flow from resting state to a stress state was significantly increased in the HCRV group (*$P=0.04$).

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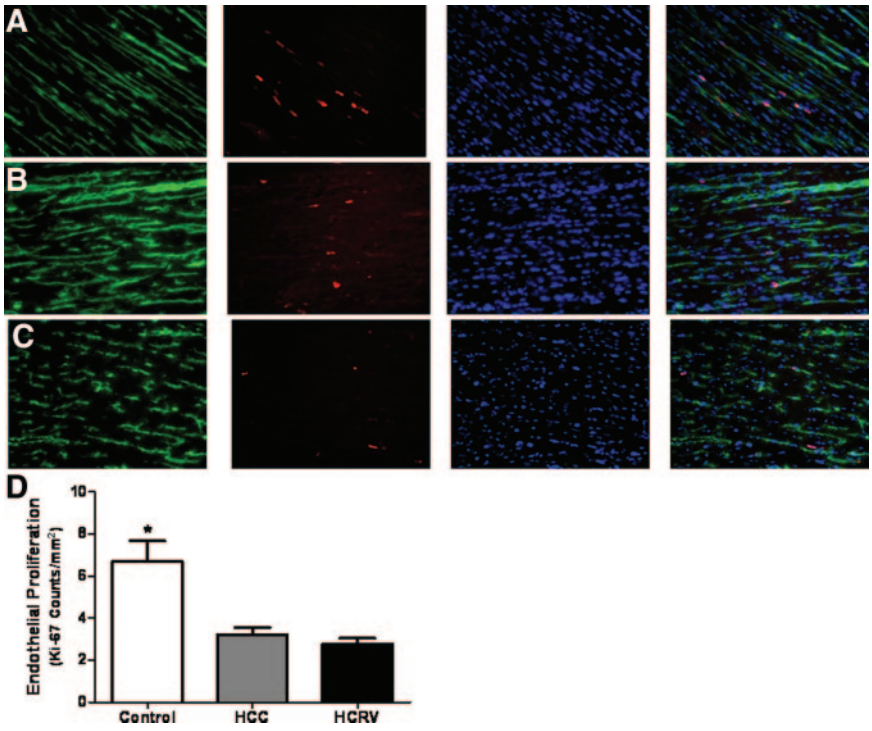

Figure 5. Endothelial cell proliferation. Immunofluorescence double staining for endothelium (CD-31, green) and dividing cells (Ki-67, red) with DAPI (blue) allow visualization of dividing endothelial cells in the ischemic territory, which appear pink in the combined image. This demonstrates significantly fewer vessels in the HCC and HCRV groups as compared with the control group: A, control; B, HCC; and C, HCRV. Quantification of dividing endothelial cells further shows fewer dividing endothelial cells in the HCC and HCRV groups (*P=0.02) (magnification ×200).

phosphorylation of Akt on both threonine 308 (2.8-fold versus control and 1.7-fold versus HCC) and serine 473 (Figure 6E and 6F). There was no significant difference between groups in the expression of total eNOS (P=0.3) and FGF-2 (P=0.2). Additionally, there was no difference in the expression of MPO between the groups (P=0.22).

Relationship Between Total Cholesterol and Phospho-eNOS (ser1177)

Linear regression was used to explore a correlation between total cholesterol and phospho-eNOS (ser1177). There was a significant inverse relationship between total cholesterol and phospho-eNOS (ser1177) levels (R^2=0.42, P=0.02) (Table).

Discussion

The principal findings of the current study demonstrate that administration of supplemental resveratrol improves myocardial and coronary vascular function but has limited effect on vessel density in the setting of chronic ischemia coupled with hypercholesterolemia.

Evidence for improved perfusion in resveratrol treated hypercholesterolemic swine includes (1) preserved regional wall motion in the ischemic territory of HCRV swine that was similar to baseline measurements obtained prior to induction of ischemia, (2) flow augmentation to the ischemic territory during ventricular pacing (myocardial stress). Resveratrol increased collateral dependent blood flow compared with that in the HCC and control groups, and (3) a direct increase in endothelial-dependent microvascular relaxation, facilitating vasodilation in the ischemic myocardium. Supplemental resveratrol decreased the cholesterol level by about 30% but improved the endothelial response to substance P by 94%. Linear regression demonstrated a significant inverse relationship between total cholesterol and phospho-eNOS (ser1177) levels.

Resveratrol also increased expression of phospho-eNOS (ser1177), which probably provides more endothelial NO. Increased NO would facilitate a more robust vasorelaxation response to increased oxygen demand during pacing and may explain why the HCRV group had a marked increase in myocardial blood flow. Previous studies have demonstrated improved microvascular relaxation with resveratrol. In a study by Mattagajasingh et al,[15] endothelial relaxation responses in control rat aortas were improved compared with dominant negative SIRT1 mutant rats. SIRT1 was found to colocalize with eNOS in the endothelium. The result is increased concentrations of NO in the endothelium.[15] A second study demonstrated that eNOS also activates SIRT1 creating a positive feedback loop.[8] In addition, in isolated in vitro swine coronary arteries, resveratrol induced an endothelium-dependent relaxation response. This response was attenuated in the presence of L-NNA, an inhibitor of NO synthesis.[16] Collectively, these reports support our findings that resveratrol may improve endothelium dependent relaxation at least in part through increased activation of eNOS. The increased expression of eNOS and improvement in endothelial dependent relaxation probably contributed to the preservation of regional myocardial function seen in the HCRV group. Additionally, the HCRV animals displayed large increases in VEGF expression. VEGF is not only involved with angiogenesis but is also a potent vasodilator and its expression may also drive increased myocardial blood flow in vivo.[12]

Numerous studies in vitro and in small animals have demonstrated the angiogenic properties of resveratrol,[17,18] though few have been done in large animals. In the present study, we noted increased molecular markers associated with

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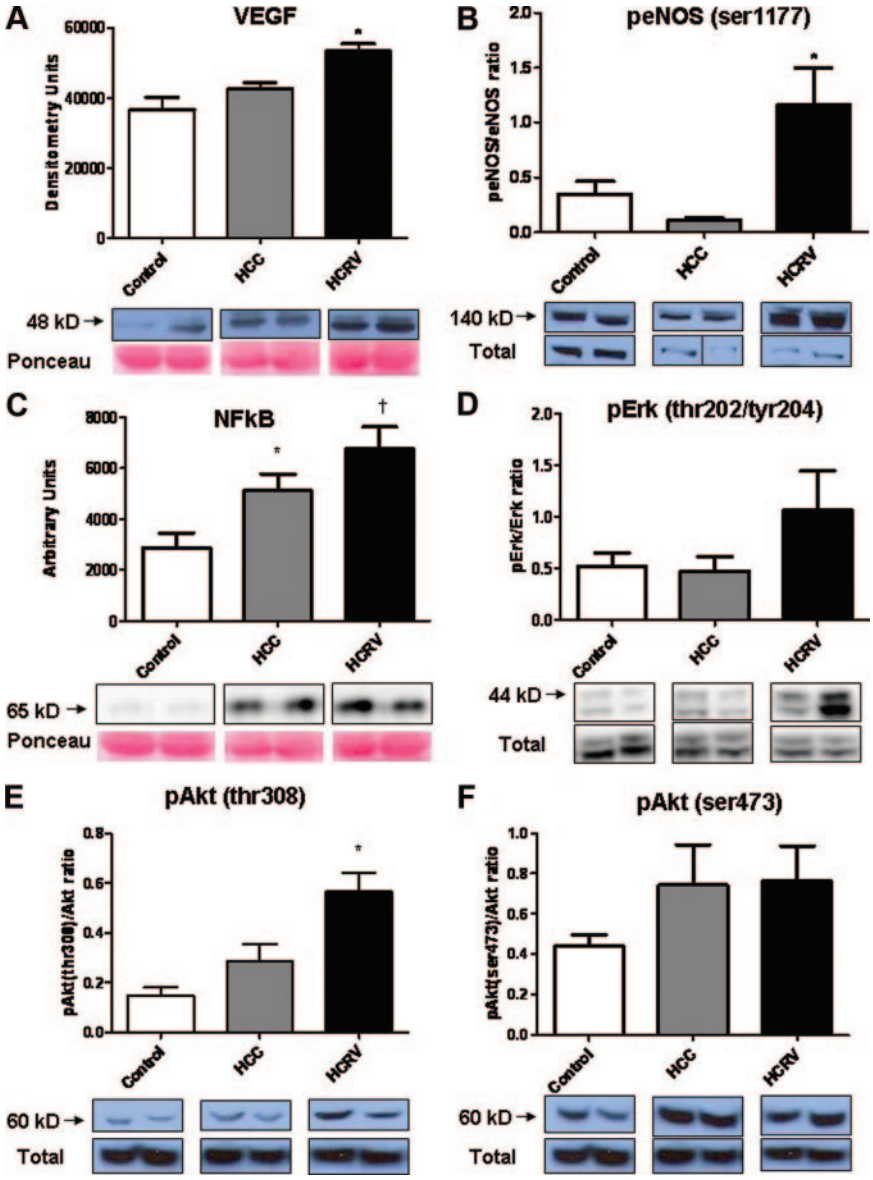

Figure 6. Increased expression of angiogenic signaling proteins and mediators in the HCRV group. A, VEGF (*P=0.002); B, peNOS (ser1177) (*P=0.04); C, NFkB expression is increased in the HCC group and to a greater extent in the HCRV group (*P<0.05 versus control, †P<0.05 versus HCC); D, Phospho-ERK (thr202/tyr 204) (P=0.15); E, Phospho-Akt (thr308) (*P<0.05); F, Phospho-Akt (ser473) (P=0.29). All blots were normalized to total protein stain with Ponceau.

the VEGF pathway but did not demonstrate an increase in FGF-2 expression or the quantity of vessels. It may be that the 7 weeks after ameroid placement did not allow sufficient time to visualize vessel formation in a hypercholesterolemic model. Another possible explanation for this lack of neovascularization may be the presence of unmeasured inhibitors of angiogenesis such as endostatin or angiostatin. Endostatin inhibits endothelial cell proliferation and migration and decreases vascular tube formation[19] (Figure 7). Previous work from our laboratory and others has demonstrated that inhibitors of the angiogenic pathway are increased in the setting of chronic illnesses such as hypercholesterolemia and diabetes.[14] A recent study from our group examining the effects of atorvastatin on angiogenesis in a porcine model of hypercholesterolemia and chronic myocardial ischemia with exogenously administered VEGF demonstrated no increase in new vessel formation, and this was associated with a significantly elevated expression of endostatin in the myocardial tissue.[20] A prolonged increase in expression of phospho-Akt (ser473), observed in the present study, has also been implicated in the inhibition of angiogenesis.[21] Therefore, we think that whereas resveratrol may mediate increased vascular function, possibly via direct vasodilatory effects of VEGF and eNOS, it is unable to rescue the hypercholesterolemia-induced impaired angiogenic response even in the face of elevated VEGF.

Interestingly, we also demonstrated an increase in NFkB expression in the HCC group and an even greater increase in the HCRV group. Immunoblotting for myeloperoxidase, a marker of neutrophil infiltration, demonstrated no difference between the groups. This suggests there is an alternate stimulus for NFkB expression. Hypoxia is known to induce local inflammation (TNFα, IL-8, and NFkB), and NFkB is known to upregulate VEGF. This local inflammatory response is thought to be involved not only in the initiation of angiogenesis but also with the remodeling necessary for formation of new vasculature. Mediators of inflammation lead to vascular permeability and increase factors involved with extracellular matrix remodeling.[22]

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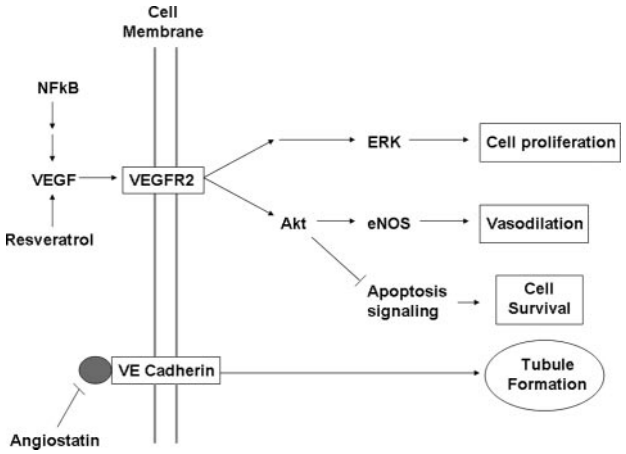

Figure 7. Schematic drawing demonstrating the relevant proteins of the angiogenic pathway influenced by resveratrol and a potential site of inhibition by angiostatin.

There are limitations to the current study. Because of the paucity of large-animal resveratrol studies, there are few data available on the optimum dosage in large animals and humans. There have been concerns about the bioavailability of orally delivered resveratrol, and some studies have shown low systemic concentrations.[23] Early studies in animal models and humans demonstrated no adverse effects at doses up to 5 g.[24] Thus, we chose a higher dose in an attempt to achieve adequate cardiac tissue levels. Though the current study was not designed to examine side effects of resveratrol, no adverse events were found.

Although the observed changes were sufficient to allow determination of statistical significance, the number of animals in each group was relatively small and therefore our findings should be interpreted in this context. Also, in addition to coronary microvessels, it would be interesting to explore the effects of resveratrol on larger coronary vessels as well as peripheral arteries. In addition, molecular markers were examined at only a single time point, and changes in molecular signaling are likely to take place over time. Finally, although a number of significant changes were found in the HCRV group, we did not demonstrate that these changes were wholly or in part secondary to SIRT1 activation.

Conclusion

Supplemental resveratrol in the setting of hypercholesterolemia and chronic myocardial ischemia attenuates regional wall motion abnormalities, improves collateral dependent perfusion, preserves endothelial dependent coronary vessel function, and upregulates markers of angiogenesis associated with the VEGF signaling pathway. This may represent a possible agent for study in humans with risk factors for ischemic heart disease and may result in clinically significant primary prevention or protection during an acute ischemic myocardial event.

Clinical Significance Statement

Currently, resveratrol and its analogs are being developed and evaluated as therapeutic agents for treatment of a wide number of illnesses, such as diabetes mellitus, hypercholesterolemia, and inflammatory bowel disease. In the current study, we demonstrated numerous beneficial effects of oral resveratrol treatment in swine fed a hypercholesterolemic diet. These include decreasing total cholesterol, lowering systemic blood pressures, improved endothelial microvascular function, increased blood flow to ischemic myocardium during stress, and preservation of regional ventricular function. Improved perfusion to the ischemic territory was not associated with an improvement in angiogenesis or increased vascular density but was caused by improved vascular reactivity and elevations in myocardial levels of the vasoactive proteins VEGF and phospho-eNOS. Although resveratrol may provide a number of benefits, it is important to fully characterize the pleotropic effects of this supplement before it reaches large scale clinical trials in humans. These findings are significant as millions of patients in the United States suffer from the effects of illnesses that resveratrol may benefit. Although there appear to be numerous potential benefits of resveratrol, extensive study in clinically relevant large-animal models and human clinical trials will be required to fully evaluate the potential effects, both detrimental and beneficial.

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Disclosures

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