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Atorvastatin Increases Myocardial Indices of Oxidative Stress in a Porcine Model of Hypercholesterolemia and Chronic Ischemia

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Abstract

Background/Aim—Atorvastatin has previously been shown to reduce the endogenous angiogenic response to chronic ischemia in a porcine model. One possible mechanism for this effect is reduced bioavailability of nitric oxide, a key mediator of angiogenesis, secondary to increased oxygen free radicals. We sought to determine if atorvastatin modulates oxidative stress in myocardial tissue.

Methods—Dietary induction of hypercholesterolemia was performed over 20 weeks in Yucatan swine with treated animals receiving atorvastatin 3 mg/kg/day. Chronic myocardial ischemia was induced via surgical placement of an ameroid constrictor ring around the proximal circumflex artery at age 20 weeks, followed by tissue harvest at age 27 weeks. Myocardial levels of protein, lipid, and DNA biomarkers of oxidative stress, serum levels of 8-isoprostane, nitric oxide (NO) dependent, and independent coronary microvascular reactivity, as well as isotope labeled microsphere myocardial perfusion analysis and histologic analysis for endothelial cell density was performed.

Results—Atorvastatin treatment was associated with elevated levels of myocardial protein oxidation and lipid peroxidation. Conversely, serum oxidant stress biomarkers were not elevated. Atorvastatin treatment improved nitric oxide dependent and independent microvascular reactivity, and was associated with decreased perfusion in the ischemic myocardial territory.

Conclusion—Treatment with atorvastatin was associated with increased levels of myocardial tissue protein and lipid oxidative stress biomarkers and a reduced functional endogenous angiogenic response, but improved coronary microvascular reactivity. Increased oxidative stress in tissues may play a role in the reduced angiogenic response seen with atorvastatin treatment in other studies.

End-stage coronary artery disease (CAD) not amenable to percutaneous or surgical revascularization poses a formidable challenge to clinicians. Attempts to induce angiogenesis in the ischemic myocardium utilizing vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) provided a novel therapeutic strategy and promising results in large animal models.¹⁻⁴ Despite the success in these models, randomized placebo controlled clinical trials failed to demonstrate a similar degree of success.⁵⁻⁹ Multiple reasons may exist for the disparate results seen between animal models and patients, including the presence of comorbidities such as hypercholesterolemia and diabetes, and medication usage, which may alter the milieu of signaling molecules involved in the angiogenic cascade.¹⁰ A key signaling molecule in the angiogenic cascade, nitric oxide (NO), acts as a mediator of the angiogenic response^{11,12} and diminished availability of NO has been implicated in reduced angiogenic

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responses in animal models.^{13,14} In the presence of oxidative stressors, NO is converted to peroxynitrite, thereby possibly limiting its bioavailability to participate in the angiogenic response. Based on these findings, and recent work from our lab, which demonstrated a reduced endogenous angiogenic response to chronic ischemia in swine treated with atorvastatin,¹⁵ we hypothesized atorvastatin may increase oxidative stress in the myocardium, providing a possible mechanism for the reduced endogenous angiogenic seen in earlier studies as described above. We attempt in this study to evaluate the effects of the commonly prescribed 3-Hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitor, atorvastatin, on oxidative stress in the myocardium itself utilizing a porcine model of diet induced hypercholesterolemia in the setting of chronic myocardial ischemia.

MATERIALS AND METHODS

General experimental sequence

Twelve Yucatan miniswine of either sex (Sinclair Research Inc, Columbia, MO, USA) were used for the studies. After weaning at 7 weeks of age, animals were divided into 2 groups. The first group was fed a hypercholesterolemic diet (Purina Modified Mini-Pig Grower Diet 5081, Richmond, IN, USA) consisting of 20% lard, 4% cholesterol, and 1.5% sodium cholate (HC group; n = 6), and the second received a hypercholesterolemic diet with oral atorvastatin (3 mg/kg per day) (HC-ATR group; n = 6) for the duration of the study (total 20 weeks).

All animals underwent an identical experimental protocol involving three separate procedures on each animal. All animals received humane care in compliance with the Harvard Medical Area Institutional Animal Care and Use Committee and the National Research Council's *Guide for the Care and Use of Laboratory Animals*, prepared by the Institute of Laboratory Animals and published by the National Institutes of Health (NIH publication No. 5377-3, 1996). Briefly, for all surgical procedures, anesthesia was induced with ketamine (10 mg/kg IM), thiopental (5 to 10 mg/kg IV), and thiopental 2.5% and maintained with a gas mixture of oxygen at 1.5 to 2 L/min and isoflurane at 0.75% to 3.0%. The animals were endotracheally intubated and mechanically ventilated at 12 to 20 breaths per minute with tidal volumes of 600 cc per breath.

The first procedure, performed via small left anterolateral thoracotomy at 20 weeks of age, consisted of the placement of a 1.75-mm ameroid constrictor around the proximal circumflex artery and the injection of 1.5×10^7 gold-labeled microspheres into the left atrium during temporary circumflex coronary occlusion to subsequently allow for identification, by shadow labeling, of the myocardial territory at risk.

The second procedure, also performed via left anterolateral thoracotomy, 3 weeks after ameroid placement, consisted of 1.5×10^7 Lutetium microspheres injected into the left atrium during rest conditions and 1.5×10^7 Europium microspheres injected during rapid atrial pacing (150 bpm) to allow for determination of baseline perfusion after ameroid closure. To document ameroid closure, left coronary angiography was performed through an 8 F sheath (Cordis Corporation, Miami, FL, USA) surgically inserted in the femoral artery, with the use of a catheter with the appropriate distal angulations and high atomic weight contrast (Mallinckrodt Inc, St Louis, MO, USA).

The third procedure was performed with animals at 27 weeks of age (4 weeks after the second procedure and 7 weeks after ameroid placement). Sternotomy was performed, 1.5×10^7 Samarium microspheres were injected into the left atrium during rest conditions, and 1.5×10^7 Lanthanum microspheres were injected during pacing (150 bpm). Euthanasia was then performed with 10 mL/kg of a saturated KCl solution administered intravenously. Left ventricular samples were harvested and snap-frozen for molecular studies and sectioned, weighed, and dried for myocardial microsphere analyses. Ameroid constrictors were resected

along with a segment of circumflex artery and examined under low-power magnification to confirm occlusion. Serum samples were drawn prior to euthanasia and stored at -80 °C prior to analysis.

Assessment of myocardial perfusion

Collateral-dependent myocardial perfusion was assessed to provide a functional measurement of the endogenous angiogenic response in the ischemic territory utilizing isotope-labeled microspheres (ILM; BioPAL, Worcester, MA, USA) during each procedure. ILM, 15 μ m in diameter, of different isotopic mass were used at each experimental stage. Gold-labeled microspheres were injected during temporary LCx arterial occlusion at the time of ameroid placement to identify myocardial samples that originated from the LCx coronary artery distribution (those with the lowest count of gold-labeled microspheres). Lutetium- and Europium-labeled ILM were used during the second procedure to determine baseline blood flow at rest and with atrial pacing at 150 bpm. Samarium- and Lanthanum-labeled ILM were injected at rest and during pacing during the third procedure. Reference blood samples were obtained from the femoral artery during the second and third procedures. After euthanasia, 10 circumferential, transmural left ventricular sections were collected for ILM assays in each animal, weighed, and dried. Each sample was exposed to neutron beams and microsphere densities measured in a gamma counter. Adjusted myocardial blood flow (at rest and with pacing), reflecting changes in lateral myocardial perfusion, was determined from the 2 myocardial samples that showed the lowest count of red microspheres with the use of the following equations:

$$\text{Crude Blood Flow (Tissue Sample)} = [\text{Withdrawal Rate (mL/min)} / \text{Weight (Tissue Sample)}] \times [\text{Isotope Counts (Tissue Sample)} / \text{Isotope Counts (Reference Sample)}]$$

$$\text{Adjusted Blood Flow} = \text{Crude Blood Flow (Third Surgery)} - \text{Crude Blood Flow at Baseline (Second Surgery)}$$

Histologic assessment of endothelial cell density

Myocardial sections from the ischemic left circumflex (LCx) territory of high cholesterol and high cholesterol + atorvastatin-treated animals were stained with anti-PECAM-1 (CD-31) antibody diluted to 1:600 (BD Biosciences Pharmingen, San Diego, CA, USA) as previously described.¹⁵ The sections were counterstained with methyl green, and examined for capillary endothelial cell density in a triplicate, blinded fashion from 600 $\times$ 440 μ m (0.264 mm²) cross-sectional fields randomly selected from the center of LCx territories of high cholesterol and high cholesterol + atorvastatin-treated animals.

Assessment of myocardial protein oxidation

Oxidized proteins were detected using a commercial kit (Oxyblot, Chemicon International, Temecula, CA, USA). Tissue homogenates were first incubated with 2,4-dinitrophenylhydrazine (DNPH) and the carbonyl groups in the protein side chains were derivatized to 2,4-dinitrophenylhydrazine. The dinitrophenylhydrazine-derivatized protein samples were separated by 10% polyacrylamide gel electrophoresis and transferred to PVDF membranes. The membranes were incubated with primary antibody specific to the dinitrophenylhydrazine moiety of the proteins, followed by incubation with a horseradish peroxidase-linked secondary antibody. Immune complexes were visualized with the enhanced chemiluminescence detection system (Amersham, Piscataway, NJ, USA). Bands were quantified by densitometry of radioautograph films.

ASSESSMENT OF MYOCARDIAL LIPID PEROXIDATION

Measurement of lipid peroxidation was carried out using a commercial kit to assess levels of free malondialdehyde (MDA), a reactive carbonyl compound produced during decomposition

of lipid peroxides (MDA-586, Oxis International, Foster City, CA, USA) employing methods suggested by the manufacturer. Tissues were homogenized in the presence of 5 mM butylated hydroxytoluene to prevent oxidation during homogenization. Homogenates were incubated with N-methyl-2-phenylindole in acetonitrile, probucol, and concentrated hydrochloric acid, followed by incubation at 45 °C for 60 minutes. MDA forms a conjugate with N-methyl-2-phenylindole resulting in the production of a blue chromogenic signal. Samples were centrifuged and the MDA containing supernatant was isolated. Spectral absorbencies were measured at 586 nm and values were analyzed against a standard curve generated from controls provided by the manufacturer. Concentration of MDA was corrected for protein concentration between samples, and blank sample values were subtracted to correct for any variance due to sample turbidity.

Assessment of myocardial DNA oxidation

Levels of 8-Hydroxy-2'-deoxyguanosine (8-OHdG), an excised product during repair of oxidative damage to DNA, were measured using a commercial ELISA kit (Oxford Biomedical Research, Oxford, MI, USA) after extraction of DNA from tissue samples. Equivalent DNA samples were incubated in wells precoated with (8-OHdG), followed by the addition an 8-OHdG antibody. Wells were washed and secondary antibody against the 8-OHdG primary antibody was added. A chromogen specific for the secondary antibody was then added followed by washing and measurement of absorption at 450 nm. Values were obtained against a standard curve.

Assessment of serum lipid peroxidation

Serum samples obtained at the final surgery (20 week timepoint) were used for determination of 8-isoprostane levels. 8-Isoprostane is a type of eicosanoid produced by oxidation of phospholipids by oxygen-free radicals. Using a commercial ELISA kit (Cayman Chemical, Ann Arbor, MI, USA), serum samples were used in a competitive binding assay with an 8-isoprostane-acetylcholinesterase conjugate against microplates precoated with mouse monoclonal antibody. The addition of a reagent containing substrate for 8-isoprostane-acetylcholinesterase resulted in the production of a yellow color whose intensity was measured spectrophotometrically at 412 nm. The signal intensity was inversely proportional to the quantity of 8-isoprostane contained in the sample and quantified against standards provided by the manufacturer.

Assessment of serum cholesterol levels

Serum cholesterol levels were from serum samples obtained at three time points (first surgery, second surgery, and harvesting) using an enzymatic method (AccelLAB, Montreal, Quebec, Canada).

Assessment of coronary microvascular reactivity

Epicardial coronary arterioles (80-185 μ m diameter, 1-2 mm length) were harvested from left ventricular tissue samples obtained at the final surgical procedure for investigation in to endothelial dysfunction. Arterioles originating from branches of the left anterior descending and circumflex artery were dissected free using a 40 $\times$ dissecting microscope and examined in isolated organ chambers as described previously.¹⁶ Isolated microvessels were precontracted by 20% to 50% of their baseline diameter utilizing U46619 (0.1-1 μ mol/L) a thromboxane A2 analogue. Microvascular relaxation was then determined in the presence of 10^{-6} and 10^{-7} mol/L of adenosine 5-diphosphate (ADP), an endothelium-dependent vasodilator, which acts via bioavailable NO and 10^{-6} and 10^{-7} mol/L of sodium nitroprusside (SNP), an endothelium-independent vasodilator acting through cGMP.

DATA ANALYSIS

Data are reported as mean $\pm$ SEM. Microvascular responses are expressed as percent relaxation of the precontracted diameter. Immunoblots were analyzed after digitization and quantification of band intensity utilizing Image J 1.33 (National Institutes of Health, Bethesda, MD, USA). Sample comparison was performed utilizing a *t*-test (confidence interval 95%), with significance reported if $p < 0.05$. Statistical significance for regression analysis was determined utilizing Pearson's correlation coefficient derived from data plots with significance reported for $p < 0.05$.

RESULTS

Serum cholesterol levels

Total serum cholesterol levels assessed at the 20-week time point were not significantly different between the HC and HC-ATR groups (386 ± 70 vs 490 ± 46 mg/dL; $p = 0.26$).

Myocardial tissue levels of MDA (lipid peroxidation)

Hypercholesterolemic animals receiving atorvastatin demonstrated a 50.2% increase in levels of MDA (a reactive carbonyl compound produced during decomposition of lipid peroxides) [HC 0.0313 ± 0.00017 vs HC-ATR 0.047 ± 0.004 μ M MDA/ μ g protein ($p = 0.0049$)]. Figure 1.

Myocardial tissue levels of dinitrophenylhydrazine-derivatized protein (protein oxidation)

Hypercholesterolemic animals receiving atorvastatin demonstrated a 33.9% increase in levels of protein oxidation [HC 70640 ± 8418 vs HC-ATR 94580 ± 6443 densitometry units ($p = 0.043$)]. Figure 2.

Myocardial tissue levels of 8-OHdG (DNA oxidation)

Hypercholesterolemic animals receiving atorvastatin demonstrated no statistically significant difference in myocardial levels of 8-OHdG in comparison to animals not receiving atorvastatin. (HC 1.434 ± 0.16 vs HC-ATR 2.420 ± 0.6 ng/mL ($p = 0.12$)). Figure 3.

SERUM LEVELS OF 8-ISOPROSTANE

Serum levels of 8-isoprostane in the HC group were 279.6 ± 93.3 vs 259.3 ± 78.82 pg/mL in the HC-ATR group ($p = 0.87$). Figure 4.

MYOCARDIAL PERFUSION

Myocardial perfusion at the end of the experimental protocol as determined by signaling from isotope labeled microsphere injection during surgical procedures demonstrated no statistically significant changes in perfusion at rest in the ischemic circumflex territory (HC-ATR group (0.311 ± 0.050 mL/min/g) vs the HC group (0.477 ± 0.076 mL/min/g) ($p = 0.08$). During pacing conditions however, the HC-ATR=group demonstrated a significant reduction in perfusion by 42.9% (0.213 ± 0.025 mL/min/g) vs the HC group (0.373 ± 0.050 mL/min/g) ($p = 0.01$). Figure 5.

Endothelial cell density

The HC group demonstrated a significant decrease in endothelial cell density compared with HC-ATR (201 ± 26 vs 294 ± 24 ; $p = 0.02$). The density of PECAM-1 (CD31) positive capillary endothelial cells (cells per high-power field) in the ischemic territory of pigs from both diet groups 7 weeks after ameroid placement is shown in Figure 6.

Microvascular reactivity

Coronary microvascular reactivity (results summarized in Table 1) in the ischemic circumflex region and the left anterior descending territory demonstrated improved relaxation in the presence of ADP, a NO dependent vasodilator in atorvastatin-treated animals. At 10^{-7} mol/L, ADP-induced relaxation of LAD territory microvessels was improved by $19.29 \pm 4.205\%$ in the HC-ATR group ($p = 0.0013$), and by $16.46 \pm 2.584\%$ in the ischemic circumflex territory microvessels ($p = 0.0001$). At 10^{-6} mol/L ADP, similar results were obtained with LAD and circumflex territory microvessels in atorvastatin-treated groups demonstrating improved relaxation by $30.6 \pm 8.915\%$ ($p = 0.0075$) and $29.47 \pm 3.861\%$ ($p = 0.0001$), respectively. Coronary microvascular reactivity in the presence of SNP 10^{-7} mol/L improved by $13.56 \pm 5.815\%$ in the LAD territory ($p = 0.0446$), and by $28.40 \pm 5.863\%$ in the circumflex territory ($p = 0.0074$) in atorvastatin-treated animals. At 10^{-6} mol/L improved by $24.29 \pm 8.377\%$ ($p = 0.0176$) in the LAD territory and by $27.57 \pm 7.872\%$ ($p = 0.0067$) in the circumflex territory with atorvastatin treatment. Figure 7.

REGRESSION ANALYSIS

Individual sample values of the serum, lipid, protein, and DNA biomarkers analyzed in this study were plotted against their respective (1) myocardial perfusion results, and (2) microvascular relaxation results, and regression analysis using Pearson's correlation coefficient was performed to determine if an association existed between these biomarkers and myocardial perfusion and microvascular reactivity at the end time point of the study. Increased myocardial levels of protein oxidation were found to correlate with decreased myocardial perfusion under pacing conditions in the HC group ($r = 0.923$, $p < 0.05$) and the HC-ATR group ($r = 0.817$, $p < 0.05$). Serum and tissue lipid and DNA biomarkers demonstrated no correlation to myocardial perfusion. Serum levels of 8-isoprostane demonstrated an inverse relationship with coronary microvascular relaxation to ADP at 10^{-7} and 10^{-6} mol/L ($r = 0.809$, $p < 0.05$ and $r = 0.754$, $p < 0.05$, respectively). No relationship was found between tissue levels of protein, lipid, or DNA markers of oxidative stress and microvascular relaxation.

CONCLUSIONS

In this clinically relevant model of chronic myocardial ischemia, we found that treatment with atorvastatin resulted in increased levels of biomarkers of myocardial lipid and protein oxidative stress at the tissue level, but no changes in the degree of DNA oxidation biomarkers. These increased levels of protein and lipid oxidative biomarkers were accompanied by a reduced endogenous functional angiogenic response in the ischemic circumflex territory under pacing conditions. However, histologic analysis of the angiogenic response (as per CD 31 staining) demonstrated improved endothelial cell density with atorvastatin therapy. In the coronary micro-circulation, atorvastatin treatment improved NO mediated and NO independent coronary microvascular reactivity in the left anterior descending and circumflex territory. Myocardial levels of protein oxidation were found to correlate with end perfusion under pacing conditions, while our serum analysis of oxidant stress demonstrated no correlation. Conversely, serum levels of 8-isoprostane did however show some correlation (reduced 8-isoprostane was associated with improved relaxation) to microvascular relaxation in response to ADP, an endothelium dependent vasodilator mediated by nitric oxide, whereas tissue levels of oxidative stress were found to have no correlation with coronary microvascular relaxation. Serum levels of total cholesterol were not significantly different between atorvastatin treated and untreated groups. This is an expected finding given atorvastatin acts only on endogenous cholesterol, in the setting of a dietary-induced model of hypercholesterolemia.

Statins have been widely reported on for their antioxidant properties based on assessment of oxidative stress biomarkers in readily accessible fluids such as blood,¹⁷⁻¹⁹ plasma,^{20,21}

serum,²² and urine.^{23,24} Although thought to reflect generalized oxidative stress status, a study investigating the specific relationship between peripheral (fluid) and tissue levels of oxidative stress demonstrated peripheral levels of oxidative stress biomarkers do not correlate with tissue levels and may not reflect the local oxidative stress status of individual organs.²⁵ These findings are consistent with those in our model where tissue levels of protein and lipid oxidation were elevated with atorvastatin treatment, but conversely, an elevation of oxidative stress biomarkers was not seen in the serum samples of atorvastatin-treated animals. The lack of elevation of oxidative stress biomarkers in the serum is also consistent with the improved microvascular reactivity seen with atorvastatin treatment. This study has not assessed the molecular mechanism through which atorvastatin may induce increases in myocardial levels of oxidative stress biomarkers, but a potential mechanism relating to oxidative stress may be through diminished levels of reducing agents available in tissue such as coenzyme Q10 whose production is also dependent upon HMG CoA reductase,²⁶ leading to impaired redox conditions.

In vitro²⁷ and in vivo models²⁸ have shown statins can have a dose-dependent antiangiogenic effect. The currently proposed mechanism behind this effect involves reductions in farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GPP), two isoprenoids produced from L-mevalonate, the product of HMG CoA reduction. These isoprenoids are involved in post-translational modification of multiple proteins including Rho, which modulates the activity of VEGF receptor-2 (VEGFR-2), a key receptor in the angiogenic signaling cascade.^{29,30} Interestingly, in our study we found therapy with atorvastatin improved the angiogenic response when assessed histologically, but actually was associated with an impaired response in comparison with control animals when assessed functionally under pacing conditions. This functional response had a strong negative correlation with the degree of protein oxidation, which was elevated in the atorvastatin group. These seemingly discordant results may be due to differences in the techniques of measurement—histologic assessment of the angiogenic response is limited in that some immature or dysfunctional vessels may be identified.³¹ Use of labeled microspheres for perfusion-based assessment of the functional angiogenic response eliminates quantification of nonfunctioning vessels, and has been described as the gold standard for assessing perfusion and collateral development.³¹⁻³⁴ In addition to the reductions in pyrophosphate levels with statin usage as described above, we hypothesize the increased protein oxidation associated with statin therapy in this study may result in dysfunctional signaling or structural proteins, subsequently leading to the formation of dysfunctional collaterals.

Statin therapy has been shown to improve endothelial dysfunction³⁵⁻³⁸ and studies involving the peripheral vasculature have shown this may be related in part to reductions in oxidative stress in the blood.³⁹ Our studies of the coronary microvasculature are consistent with these prior reports and demonstrate a significant negative correlation between circulating levels of the oxidative stress biomarker 8-isoprostane and the degree of endothelium-dependent/NO mediated vasorelaxation providing further support that this beneficial effects associated with atorvastatin therapy may be related to the drugs effects on the degree of oxidative stress in the circulation.

Statin therapy has been shown in multiple studies to reduce mortality⁴⁰ and remains an essential therapy for the treatment of cardiovascular disease. The findings of this study are meant to provide insight in to possible molecular mechanisms underlying the diminished angiogenic response seen with proangiogenic therapy in clinical trials. An enhanced understanding of these mechanisms may augment future strategies to improve the success of proangiogenic interventions in the clinical setting.

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Figure 1. Myocardial levels of malondialdehyde (MDA), a reactive carbonyl compound produced during lipid peroxidation. Atorvastatin-treated animals demonstrated a 50.2% increase in tissue levels of MDA ($p = 0.0049$)*.

Figure 2.

Myocardial levels of 2-4 DNPH-derivatized proteins. Atorvastatin-treated animals demonstrated a 33.9% increase in levels of 2-4 DNPH derivatized proteins ($p = 0.043$)*. DU = densitometry units.

Myocardial Levels of DNA Oxidation

Figure 3. Myocardial levels of 8-OHdG. No differences were observed for levels of DNA oxidation between control (HC) animals and animals receiving atorvastatin (HC + ATR).

Figure 4. Serum levels of 8-isoprostane. No significant difference was seen in serum levels of 8-isoprostane.

Figure 5.

Myocardial perfusion in the ischemic circumflex territory (Cx). The atorvastatin (HC + AT) group demonstrates reduced perfusion by 42.9% during pacing conditions ($p = 0.01$)* relative to the control hypercholesterolemic group (HC). R = rest conditions, P = pacing conditions.

Figure 6.

Endothelial cell density. CD31-positive endothelial cell density per high-power field (0.267 mm²) in the ischemic territory. (A) HC animals, (B) HC + ATR animals, (C) endothelial cell counts per highpower field. The HC animals had reduced endothelial cell density compared with HC + ATR animals (201 ± 26 vs 294 ± 24; $p = 0.02^*$).

Coronary Microvascular Reactivity

Figure 7.

Microvascular relaxation in response to ADP 10^{-7} mol/L, ADP 10^{-6} mol/L, Sodium Nitroprusside (SNP) 10^{-7} mol/L, and SNP 10^{-6} mol/L in the left anterior descending (LAD) and Circumflex (Cx) territory. HC = hypercholesterolemic, HC A = hypercholesterolemic + atorvastatin. (*indicates $p < 0.05$).

TABLE 1
Nitric Oxide-Dependent (ADP) and Independent (SNP) Coronary Microvascular Relaxation

	HC (% Relaxation)	HC-ATR (% Relaxation)	% Difference	P value
ADP(10^{-7})				
LAD	10.13 $\pm$ 2.649	29.42 $\pm$ 3.342	19.29 $\pm$ 4.205	0.0013
Cx	6.383 $\pm$ 1.450	22.84 $\pm$ 2.245	16.46 $\pm$ 2.584	0.0001
ADP(10^{-6})				
LAD	21.28 $\pm$ 6.274	51.88 $\pm$ 6.205	30.6 $\pm$ 8.915	0.0075
Cx	10.95 $\pm$ 1.113	40.42 $\pm$ 4.405	29.47 $\pm$ 3.861	0.0001
SNP(10^{-7})				
LAD	12.50 $\pm$ 5.073	26.06 $\pm$ 1.699	13.56 $\pm$ 5.815	0.0446
Cx	8.767 $\pm$ 1.840	25.90 $\pm$ 5.039	28.40 $\pm$ 5.863	0.0074
SNP(10^{-6})				
LAD	25.25 $\pm$ 6.933	49.54 $\pm$ 3.745	24.29 $\pm$ 8.377	0.0176
Cx	18.03 $\pm$ 3.485	45.60 $\pm$ 7.605	27.57 $\pm$ 7.872	0.0067