Rosuvastatin treatment reverses impaired coronary artery vasodilation in fructose-fed, insulin-resistant rats

Allison W. Miller, Christina D. Tulbert, and David W. Busija

Department of Physiology and Pharmacology, Wake Forest University Health Sciences, Winston-Salem, North Carolina 27157

Submitted 4 November 2003; accepted in final form 17 March 2004

Rosuvastatin treatment reverses impaired coronary artery vasodilation in fructose-fed, insulin-resistant rats. Am J Physiol Regul Integr Comp Physiol 287: R157–R160, 2004. First published March 25, 2004; 10.1152/ajpregu.00647.2003.—Insulin resistance (IR) impairs vascular responses in coronary arteries, but mechanisms of dysfunction and approaches to treatment remain unclear. We examined the ability of a new 3-hydroxy-methylglutaryl coenzyme A reductase inhibitor, rosuvastatin, to reverse reduced dilator responses in rats made IR by feeding a fructose-rich diet (FF). Sprague-Dawley rats were randomized to control (normal rat diet) or FF. After 1 wk, rats received rosuvastatin (2 mg/kg) or placebo (saline) subcutaneously for 5 wk. Biochemical measurements and in vitro functional studies of small coronary arteries were performed. Fasting insulin and triglyceride (TG) levels were markedly increased in FF-placebo rats compared with other groups. Rosuvastatin treatment of FF rats normalized TG and modestly decreased insulin levels. ACh-induced dilator responses were depressed in arteries from FF-placebo rats. This impairment was due to decreased responses via calcium-dependent K channels (KCa). Rosuvastatin treatment of FF rats completely reversed the response to ACh to normal levels. Moreover, this recovery in function was due to an improvement in vasodilation via KCa. Thus rosuvastatin treatment of IR rats normalizes coronary vascular dilator responses by improving the KCa function.

fructose-fed rat; insulin resistance; coronary arteries; calcium-dependent potassium channels

EPILOGIC AND EXPERIMENTAL studies provide strong evidence linking insulin resistance to the development of cardiovascular disease. Several prospective studies in which fasting insulin concentrations were used as an index of insulin sensitivity found that elevated insulin levels are associated with increased hypertension, ischemic heart disease, and cardiovascular death (7, 13, 26). Moreover, a direct correlation has been described between insulin resistance and carotid intima media thickness (18). Thus these data provide strong evidence that insulin resistance is an important risk factor for the development of cardiovascular disease.

Previous studies have demonstrated that vascular responses to endothelium-dependent vasodilators are markedly impaired in small coronary, cerebral, and mesenteric arteries from fructose-fed, insulin-resistant rats (12, 21, 28). Moreover, it has been shown that this vascular derangement is primarily due to a defect in the vascular smooth muscle KCa channels (8, 28–29). To date, there is no known specific treatment for this vascular dysfunction. The 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are a class of lipid-lowering agents that are known to lower cardiovascular morbidity and mortality in diabetic patients (15, 16). Although the mechanism of this effect is closely related to their ability to lower lipids, there are also thought to be positive nonlipid effects of these agents (4, 22). One specific property is their ability to improve endothelial function. This effect appears to be due to their ability to upregulate nitric oxide synthase (NOS) expression and to decrease free radical production (4, 5, 17, 22, 24). Obviously, these pharmacologic mechanisms may have positive effects on vascular dysfunction in insulin resistance. Thus the purpose of this study is to assess the effect of chronic rosuvastatin treatment on lipids, insulin, and coronary vascular function in fructose-fed, insulin-resistant rats.

METHODS

The protocol was approved by the Institutional Animal Care and Use Committee of Wake Forest University School of Medicine. All experiments complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Male Sprague-Dawley rats were obtained at 6 wk of age and randomized into one of the following two groups: 1) normal diet (control; n = 24) or 2) fructose-fed (FF; n = 24) rats. After 1 wk of the respective diets, rats were further randomized to receive either rosuvastatin (2 mg/kg) or placebo (volume-matched saline) subcutaneously for a period of 5 wk. This dose was based on recommendations from AstraZeneca, who have performed complete dose ranging studies during research and development of this agent. The route of administration was chosen based on ease to the investigators; however, we were assured by AstraZeneca, based on their previous studies, that this route maintained comparable bioavailability to oral administration. The animals in the FF group were fed a fructose-rich diet containing 66% fructose, 22% casein, and 12% lard, plus essential vitamins and minerals (Teklad Labs; Madison, WI), whereas control animals received standard rat chow. Previous studies in our laboratory and others have shown that receiving the fructose-rich diet induces a state of insulin resistance, which is characterized by glucose intolerance, hyperinsulinemia, and hypertriglyceridemia (19, 28). We showed that hyperinsulinemia occurs within 7 days of beginning the diet (20).

After completion of the treatment protocol, fasted rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and anticoagulated with heparin (500 units ip). A midline incision was made, and a 1 ml blood sample was withdrawn from the heart for biochemical analysis. Subsequently, the heart was removed and placed in a chilled oxygenated modified Krebs-Ringer bicarbonate solution (millimolar concentration: 118.3 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, 11.1 dextrose). Small coronary arteries (∼150 μm diameter) from the septum and/or the left ventricular free wall were isolated from surrounding perivascular tissue and removed. Intraluminal di-
ameter of pressurized (60 mmHg) arteries was measured as previously described (12, 21, 28). Only one concentration-response experiment was performed per arterial segment; however, several arterial segments were taken from each rat.

Protocol. Coronary arteries were allowed to equilibrate for 30 min in the tissue bath. Subsequently, vessels were preconstricted to 30–50% of their resting diameter with endothelin-1 (~10^{-8} M). Concentration-response studies to ACh (10^{-9} to 3 \times 10^{-5} M), an endothelium-dependent vasodilator, were performed. To evaluate the role of the specific endothelium-derived relaxing factors, several inhibitors were used. To evaluate nitric oxide in the vascular response to ACh, arteries were pretreated for a period of 30 min with N-nitro-l-arginine (l-NNA; 100 \mu M), an inhibitor of NOS. To evaluate the role of prostacyclin, indomethacin (100 \mu M), an inhibitor of cyclooxygenase, was added to the bath for 30 min before the determination of ACh-induced relaxations. Finally, to evaluate the role of endothelium-derived hyperpolarization via K_{ca} in the vascular response to ACh, arteries were pretreated with charpydotoxin (CTX; 0.1 \mu M) and apamin (0.5 \mu M) for a period of 30 min before ACh dose-response experiments. The combination of these two inhibitors was chosen on the basis of evidence showing that they consistently inhibit K_{ca}-mediated relaxation and on the basis of our prior experiments with these agents (2, 9, 12, 28).

Biochemical measurements. Plasma insulin was assayed by using a dextran-coated charcoal immunoassay with rat antibody. Glucose concentrations were measured using a Glucose Trinder Kit (Sigma, St. Louis, MO). Lipid concentrations (including triglycerides and total cholesterol) were measured using a Dimension Clinical Chemistry System (Newark, DE) with specific reagent kits for each lipid determination.

Chemicals. All chemicals used in this study were obtained from Sigma. All agents were dissolved in deionized water and diluted with Krebs buffer. l-NNA was dissolved in water and titrated to a pH of 7.2 with hydrochloric acid for dissolution. The pH was then titrated to physiological level (7.4) with sodium hydroxide.

Data analysis. Statistical analysis of the concentration-response experiments was performed using a two-factor ANOVA with repeated measures. Statistical comparisons for baseline diameter and biochemical experiments was performed using a two-factor ANOVA with repeated measures. To evaluate the role of the specific endothelium-derived relaxing factors, several inhibitors were used. To evaluate nitric oxide in the vascular response to ACh, arteries were pretreated for a period of 30 min with N-nitro-l-arginine (l-NNA; 100 \mu M), an inhibitor of NOS. To evaluate the role of prostacyclin, indomethacin (100 \mu M), an inhibitor of cyclooxygenase, was added to the bath for 30 min before the determination of ACh-induced relaxations. Finally, to evaluate the role of endothelium-derived hyperpolarization via K_{ca} in the vascular response to ACh, arteries were pretreated with charpydotoxin (CTX; 0.1 \mu M) and apamin (0.5 \mu M) for a period of 30 min before ACh dose-response experiments. The combination of these two inhibitors was chosen on the basis of evidence showing that they consistently inhibit K_{ca}-mediated relaxation and on the basis of our prior experiments with these agents (2, 9, 12, 28).

**RESULTS**

Biochemical results. Fasting insulin, glucose, total cholesterol, triglycerides, and body weights for all experimental groups are shown in Table 1. Fasting, total cholesterol, and body weight were not significantly different in any of the groups. Both fasting insulin and triglycerides were significantly increased in the FF-placebo group. Treatment of the FF rats with rosuvastatin reversed the triglyceride levels to normal, whereas the insulin concentrations, although significantly reduced, remained markedly elevated.

![Fig. 1. Cumulative concentration-response experiments to acetylcholine in small coronary arteries from control and fructose-fed (FF) rats treated with placebo or rosuvastatin. *Statistically significant reduction (P < 0.05) in vasodilation compared with the remaining groups.](http://ajpregu.physiology.org/)

Table 1. Biochemical results

<table>
<thead>
<tr>
<th>Groups</th>
<th>Wt, g</th>
<th>Glucose, mg/dl</th>
<th>Insulin, pg/ml</th>
<th>Total Cholesterol, mg/dl</th>
<th>Triglycerides, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-saline</td>
<td>359±6</td>
<td>101±3</td>
<td>771±140</td>
<td>66±6</td>
<td>13±2</td>
</tr>
<tr>
<td>Control-rosuvastatin</td>
<td>358±6</td>
<td>93±3</td>
<td>810±95</td>
<td>64±2</td>
<td>12±1</td>
</tr>
<tr>
<td>FF-saline</td>
<td>362±6</td>
<td>107±7</td>
<td>2,279±153*</td>
<td>63±4</td>
<td>106±13†</td>
</tr>
<tr>
<td>FF-rosuvastatin</td>
<td>351±6</td>
<td>93±5</td>
<td>1,889±194†</td>
<td>67±3</td>
<td>16±2</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.01 vs. control-saline group; †P < 0.01 vs. fructose-fed (FF)-saline group; ‡P < 0.01 vs FF-rosuvastatin group.
had little effect on ACh-induced relaxation (maximal relaxation: $55 \pm 9\%$ in the presence of CTX-apamin and $54 \pm 7\%$ for ACh alone, NS; Fig. 3 compared with Fig. 1). Pretreatment with indomethacin had no effect on ACh-induced relaxation in either the control or FF groups (data not shown).

DISCUSSION

The purpose of the present study was to evaluate the effectiveness of chronic treatment with rosuvastatin on coronary vascular dysfunction, lipids, and insulin concentrations in the FF rat model of insulin resistance. We showed that after 5 wk of treatment with rosuvastatin, triglyceride concentrations were normalized in the FF, insulin-resistant rats. Moreover, hyperinsulinemia, a marker for insulin resistance, was modestly improved after rosuvastatin therapy, whereas normal total cholesterol levels were unchanged. Regarding vascular reactivity, we showed that vasodilation to ACh was normalized in coronary arteries from rosuvastatin-treated, FF rats. Furthermore, the current data suggest that rosuvastatin treatment improves vascular responsiveness through its ability to restore KCa channel function to normal levels.

Rosuvastatin is a new HMG-CoA reductase inhibitor. This class of agents, commonly referred to as “statins,” has been reported to have both direct and indirect effects on vascular function (24). Indirectly, statins have been shown to improve vascular function and cardiovascular outcomes through their ability to improve lipid profiles, including the reduction of triglycerides (14, 25). In addition, there is a plethora of literature suggesting that the statins have many direct vascular effects. These activities include increased upregulation or activation of endothelial NOS, reduced oxidative stress, and decreased inflammatory responses (4, 5, 17, 22, 24). It is unclear from the current data whether the normalization of vascular function observed is due to direct vascular effects of rosuvastatin or indirect effects due to the fact that it normalized triglyceride concentrations.

Hypertriglyceridemia is a commonly occurring dyslipidemia that often accompanies insulin resistance and type 2 diabetes mellitus and is an obvious metabolic derangement in the current model. Previous studies have shown that in patients with isolated hypertriglyceridemia or in hypertriglyceridemia associated with type 2 diabetes mellitus, lowering triglycerides improves endothelium-dependent vasodilation (3, 6). The mechanism of this improvement in vascular function is purportedly due to a decrease in oxidative stress, although other mechanisms may be operating (23). Therefore, the reduction of triglycerides seen in the current study may be directly related to the improvement in vascular function.

Using pharmacologic tools, we previously demonstrated in small coronary, mesenteric, and cerebral arteries from FF, insulin-resistant rats that vasodilation via KCa channels is nearly abolished (28, 21, 7). Moreover, using patch-clamp techniques, we showed that in vascular smooth muscle cells from mesenteric arteries of FF rats, large-conductance KCa (BKCa) channel activity is markedly reduced (8). In the current study, treatment with rosuvastatin was able to normalize ACh-mediated vasodilation via the KCa channel. Because the underlying mechanism of KCa channel dysfunction in this model is unknown, it is difficult to speculate on the mechanism of rosuvastatin’s effect; however, on the basis of data from others, it may be through the reduction of oxidative stress. Previous data demonstrated that vascular dysfunction of aorta from FF, insulin-resistant rats results from increased free radical production (30, 31). Moreover, it has been shown that the functional activity of the BKCa channel is suppressed by increased oxidative stress (27, 32). Thus it is possible, although not studied, that rosuvastatin improves vascular function by reducing oxidative stress, because this is a known direct effect of statin agents and of normalizing triglycerides.

There have been two previous studies to assess the role of statin treatment on vascular function in insulin-resistant animal models [FF rat and spontaneously hypertensive rat (1, 10)]. Both studies demonstrated that endothelium-dependent vaso-
dilation is improved in large-conduit arteries (carotid and aorta, respectively) after statin therapy. However, these studies differ from our own in that their data suggest that endothelium-dependent vasodilation was restored through increased production of NO. This mechanism is not surprising, because several statins have been shown to directly increase NO production/ activity and because conduit arteries are primarily dependent on NO, as opposed to KCₐ channels, for endothelium-dependent vasodilation. The current data differ from the above studies by the fact that they demonstrate the normalization of triglyceride levels with rosvastatin therapy (neither of the previous studies measured this factor) and that the mechanism of this effect is directed toward improved function of KCₐ channels. This difference in mechanism may be a reflection on the size or type of artery studied.

In conclusion, rosvastatin treatment of FF, insulin-resistant rats completely reverses both hypertriglyceridemia and coronary vascular dysfunction. Moreover, it appears that rosvastatin may normalize coronary vascular responsiveness through its effects on KCₐ channel function. These are the first data to demonstrate this effect by a statin and may prove to be an important clinical feature of rosvastatin in the future.

DISCLOSURES

AstraZeneca, makers of rosvastatin, awarded a grant to Wake Forest University to perform this study.

REFERENCES

5. Delhose S, Morena M, Djouad F, Ledoucen Descomps B, and Cristol JM. Phosphorylation of the potassium channel subunits INK1 and INK2 by 10.220.33.4 on June 28, 2017 http://ajpregu.physiology.org/ Downloaded from