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Treatment of cardiovascular dysfunction associated with the metabolic syndrome and type 2 diabetes

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Abstract

Our previous studies have shown vascular dysfunction in small coronary and mesenteric arteries in Zucker obese rats, a model of the metabolic syndrome, and Zucker Diabetic Fatty (ZDF) rats, a model of type 2 diabetes. Because of their lipid lowering action and antioxidant activity, we predicted that treatment with Rosuvastatin, an HMG-CoA reductase inhibitor (statin) or Enalapril, an angiotensin converting enzyme (ACE) inhibitor would improve vascular dysfunction associated with the metabolic syndrome and type 2 diabetes.
Methods: 20-week-old Zucker obese and 16-week-old ZDF rats were treated with Rosuvastatin (25 mg/kg/day) or Enalapril (20 mg/kg/day) for 12 weeks. We examined metabolic parameters, indices of oxidative stress and vascular dysfunction in ventricular and mesenteric small arteries (75–175 μm intraluminal diameter) from lean, Zucker obese and ZDF rats (untreated and treated).
Results: Endothelial dependent responses were attenuated in coronary vessels from Zucker obese and ZDF rats compared to responses from lean rats. Both drugs improved metabolic parameters, oxidative stress, and vascular dysfunction in Zucker obese rats, however, only partial improvement was observed in ZDF rats, suggesting more aggressive treatment is needed when hyperglycemia is involved.
Conclusion: Vascular dysfunction is improved when Zucker obese and, to a lesser degree, when ZDF rats were treated with Rosuvastatin or Enalapril.
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1. Introduction

The metabolic syndrome is an emerging epidemic characterized by insulin resistance, abdominal obesity, atherogenic dyslipidemia, hypertension, and proinflammatory and prothombotic states, with or without glucose intolerance. Each of these characteristics is a significant risk factor for development of vascular dysfunction and cardiovascular disease. Hyperglycemia accentuates this vascular dysfunction. The metabolic syndrome encompasses Type 2 diabetes, and estimates are that 47 million U.S. residents have the metabolic syndrome (Ford et al. 2002).

Zucker obese and Zucker Diabetic Fatty (ZDF) rat strains have been well characterized as models of the metabolic syndrome and Type 2 diabetes (Frisbee et al, 2002; Oltman et al., 2005; Peterson et al., 1990; Stepp and Frisbee, 2002). In this study, we utilized a) Zucker obese rats which are insulin resistant, hypertensive and dyslipidemic (Peterson et al., 1990, b) ZDF rats which have similar characteristics of the Zucker obese rats and are also hyperglycemic, and c) lean control rats, which are euglycemic, normotensive and have normal lipid metabolism. We have shown that increases in indices of oxidative stress precede development of vascular dysfunction, and diabetes accelerates the progression of vascular dysfunction in these rat models (Oltman et al., 2006).

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Various studies have been conducted with animal models of the metabolic syndrome and type 2 diabetes demonstrating prevention of disease when treatment is initiated early prior to the onset of complications (Erdos et al., 2006; Miller et al., 2004). However, patient treatment of diabetes does not normally begin until after symptoms have been diagnosed and, in many instances, complications are already present. Less information is available on the benefits of drugs in correcting complications once progression of the disease process has begun. The current study was designed to examine the efficacy of a statin or ACE inhibitor in reducing vascular dysfunction following development of complications from the metabolic syndrome or type 2 diabetes.

The goal of treating the metabolic syndrome is prevention of cardiovascular events and development of type 2 diabetes mellitus. Treatment of the metabolic syndrome includes therapeutic lifestyle changes along with pharmacologic therapy. We have chosen two classes of drugs that would be appropriate for treatment of the metabolic syndrome and type 2 diabetes. We have treated Zucker obese and ZDF rats with 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor (Rosuvastatin) or angiotensin converting enzyme (ACE) inhibitor (Enalapril). These therapies allow us to determine the role of lipid metabolism and increased oxidative stress on progression of vascular dysfunction in Zucker obese and ZDF rats.

2. Materials and methods

2.1. Animals and treatments

The investigation conforms to the *Guide of the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Male Zucker obese, Zucker Diabetic Fatty (ZDF), and age matched lean (control) rats were obtained from Charles River Laboratories at 6 weeks of age. Rats were allowed to develop vascular dysfunction prior to treatments. Results of our previous study (Oltman et al., 2006) showed coronary artery dysfunction developed earlier in ZDF rats (~16 weeks of age) compared to Zucker obese rats (~28 weeks of age), thus, in the current study, we chose to start treatment at 16 weeks of age in ZDF rats and at 20 weeks of age in Zucker obese rats. This time frame allowed for vascular studies to be performed in Zucker rats prior to development of insulinopenia and hyperglycemia (Oltman et al., 2006).

Rats were treated with Rosuvastatin, a hydrophilic statin (25 mg/kg/day) or Enalapril (20 mg/kg/day) in the diet for 12 weeks. These doses were based on results of previous studies (Coppey et al., 2006; Oltman et al., in press-a,b).

On the day of the experiment, the rat was anaesthetized and a catheter inserted into the carotid artery to measure blood pressure. Blood samples were collected. The rat was euthanized and tissues were harvested.

2.2. Metabolic measurements

Metabolic parameters were assessed utilizing bioassays including triglycerides (Sigma), and free fatty acids (Roche, Manheim Germany). Insulin was determined using a Luminex 100 system. Cholesterol levels were determined with a kit from BioVision, Inc.

2.3. Determination of oxidative stress

Reactive oxygen species (ROS) were measured in aortas by lucigenin-enhanced chemiluminescence (Coppey et al., 2001). For coronary arteries hydroethidine (2×10^{-6} mol/L) was applied to frozen vessel segments, incubated and images obtained, to evaluate in situ levels of O_2^-(Coppey et al., 2000; 2001).

2.4. Isolated microvessels

Coronary microvessels (75–175 μm intraluminal diameter) and 4th order mesenteric vessels were isolated, cannulated, and allowed to equilibrate for 30 min at 40 mm Hg (Oltman et al., 2003, 2006). To evaluate relaxation responses to acetylcholine (Ach) or sodium nitroprusside (SNP), vessels were preconstricted with the thromboxane analog U46619.

2.5. Statistical analysis

Data are expressed as mean±SEM. Metabolic parameters were assessed with a one-way ANOVA. Concentration response curves were evaluated using two-way repeated measures ANOVA followed by the Fisher LSD correction for multiple comparisons. Differences with $p\leq0.05$ were considered significant.

3. Results

3.1. Metabolic parameters

Body weight of Zucker obese rats was considerably greater than lean rats at 32 weeks of age. At 28 weeks of age, body weight of ZDF rats was slightly less than lean rats. These results are similar to previously published results (Oltman et al., 2006). 12 weeks of treatment with Rosuvastatin or Enalapril did not alter body weight in Zucker obese or ZDF rats (Table 1).

Lean and Zucker obese rats had blood glucose values in the normal range (approximately 100 mg/dl), which was slightly increased in rats treated with Enalapril (Table 1A). ZDF rats had glucose levels that were 3-fold higher than lean rats and were not improved by either Rosuvastatin or Enalapril treatment (Table 1B). Blood glucose values from treated rats, were not significantly different than values from untreated.

Compared to lean rats, mean arterial blood pressure was increased in Zucker obese rats, and treatment with ACE inhibitor, but not statin, lowered blood pressure in Zucker obese rats (Table 1A). Blood pressure was not altered in ZDF rats or in either treatment group (Table 1B).

In Zucker obese rats, plasma insulin levels were increased, and did not improve with Rosuvastatin or Enalapril treatment (Table 1A). In ZDF diabetic rats, insulin levels were slightly lower than lean and in some animals below detectable limits (data not shown).