Rosuvastatin improves vascular function of arteriovenous fistula in a diabetic rat model

Jun-Neng Roan, MD,[a,b] Shih-Yuan Fang, MD,[c] Shih-Wei Chang, BS,[c,d] Chih-Hsin Hsu, MD,[a,e] Chein-Chi Huang, MS,[c] Meng-Hsuan Chiou, MS,[c] Yu-Chuan Tsai, MD,[c] and **Chen-Fuh Lam, MD, PhD,[c]** *Tainan, Taiwan*

Objective: **This study investigates the pathogenesis of arteriovenous (AV) fistula failure in patients with diabetes mellitus (DM) and tests the vascular protective effect of rosuvastatin on the fistulous communication of diabetic rats.**
Methods: **DM was induced in rats by a single injection of streptozotocin. One week later, a fistula was created in the descending aorta and the adjacent inferior vena cava (aortocaval [AC] fistula). Rats were then randomly assigned to receive placebo or rosuvastatin (15 mg/kg/d) in chow for 2 weeks. Blood flow in the aortic segments of the fistula was measured. Circulating CD34+/KDR+ endothelial progenitor cells (EPCs) were determined 2 weeks after creation of the AC fistulas using flow cytometry. Vascular function of the AC fistulas was assessed by isometric force testing. The expression of proinflammatory genes and generation of superoxide anions in the fistulas were examined.**
Results: **The number of EPCs was reduced in diabetic rats, and rosuvastatin significantly increased the number of circulating EPCs. Reduced blood flow and impaired endothelium-dependent relaxation in the AC fistula of animals with diabetes was significantly potentiated after treatment with rosuvastatin. Rosuvastatin also attenuated the expression of inducible nitric oxide synthase and nicotinamide adenine dinucleotide phosphate oxidase and generation of superoxide anions in the fistula tissues isolated from diabetic rats.**
Conclusions: **We provide the first evidence demonstrating that rosuvastatin improves blood flow and endothelial function of AC fistulas in rats with DM by attenuating the activity of proinflammatory genes and generation of superoxide anions in the remodeled vasculature. (J Vasc Surg 2012;56:1381-9.)**

Clinical Relevance: **Arteriovenous (AV) fistula is the most common vascular access for hemodialysis in patients with end-stage renal disease. Studies have shown that blood flow in the AV fistula is significantly reduced in patients with diabetes and the period for maturation of an AV fistula is longer in these patients. The underlying mechanisms of AV fistula failure in diabetes are still poorly understood and there are limited therapeutic approaches that can increase the lifespan of these fistulas. The present study demonstrates that oral administration rosuvastatin improves blood flow and endothelial function of AC fistulas in rats with diabetes, which results from attenuating the activity of proinflammatory genes in the remodeled vasculature, thereby reducing the generation of tissue superoxide anions. Our results may thus enhance our ability to prevent and manage vascular access failure in patients with diabetes with chronic renal disease.**

Hemodialysis is currently the main therapy of renal replacement therapy for patients with end-stage renal disease (ESRD). In Taiwan, diabetic nephropathy is the second most common cause of ESRD, but patients with diabetes engender 12% more expense for care of dialysis than patients who are nondiabetic.[1] Hemodialysis necessitates the creation of an arteriovenous (AV) fistula in native vessels or placement of a synthetic graft or catheter. The procedures for creating the AV fistula and treatment of the related complication account for over 20% of hospitalizations of patients receiving dialysis and cost about $100 million U.S. dollars annually.[2] Two major hurdles in establishing a usable and patent AV fistula for dialysis are primary failure (failure of fistula to mature adequately for dialysis) and long-term survival of the vascular access.[2] It has been shown that reduction of blood flow is an important factor contributing to failure of an AV fistula.[3] Although blood flow in the venous site of an AV fistula is also determined by the surrounding draining veins, blood pumped from the arterial site is the most important factor in maintaining sufficient fistula blood flow. However, very limited studies have reported the effect of arterial blood flow on the function and patency of an AV fistula, particularly in patients with diabetes.

From the Institute of Clinical Medicine,[a] Division of Cardiovascular Surgery,[b] Department of Anesthesiology,[c] and Department of Internal Medicine,[e] National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University; and the Department of Biotechnology, Chia Nan University of Pharmacy and Science.[d]

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Reprint requests: Chen-Fuh Lam, MD, Department of Anesthesiology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, 138 Sheng Li Road, Tainan, Taiwan (e-mail: lamcf@mail.ncku.edu.tw).

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Hyperglycemia has been known to increase vascular oxidative stress and causes endothelial dysfunction.[4] There are also extensive data indicating that hyperglycemia enhances release of proinflammatory cytokines, which may exacerbate inflammatory reaction in the vasculature of fistulas. Some studies showed that blood flow in the AV fistula is significantly reduced in patients with diabetes mellitus (DM).[5,6] Patients with diabetes also require a significantly longer period of time for the maturation of AV fistulas and have higher complication rates than patients who are nondiabetic.[7,8]

The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statins) are the most commonly prescribed lipid-lowering agents, and they also mediate important pleiotropic effects, such as improvement of vascular endothelial dysfunction, attenuation of inflammatory responses, stabilization of atherosclerotic plaques, inhibition of vascular smooth muscle proliferation, modulation of procoagulant activity, and mobilization of endothelial progenitor cells (EPCs).[9,10]

Although the beneficial pleiotropisms by statins are well documented in a variety of endothelial dysfunctional disorders, their potential therapeutic effects in maintaining a healthy, usable AV fistula in patients with DM have not been previously demonstrated. In the present study, we hypothesized that the endothelial protective effect of statins may potentiate the vascular function and flow rate in the AV fistula of rats with diabetes.

METHODS

Animal models and experiments. For expanded methods, please see the Supplementary Methods (online only). All procedures were performed in accordance with the guidelines of the Animal Care and Use Committee of National Cheng Kung University, Taiwan. The detailed procedures of creating an aortocaval (AC) fistula have been described in the previous reports by other research groups[11,12] and modified in our laboratory.[13] In brief, Sprague-Dawley rats (weight, 200-250 g) were anesthetized with inhaled isoflurane (2%-3% v/v in oxygen). After a midline abdominal incision, the inferior vena cava (IVC) and aorta were exposed. The aorta was punctured with a 20G disposable needle at the level below the renal vessels. The needle was gradually introduced across the aorta and penetrated the neighboring wall of the IVC. The needle was then withdrawn and the puncturing point at the aorta was closed by a purse-string suture. A single dose of streptozotocin (65 mg/kg i.p.) was injected to induce type I diabetes in rats. Hyperglycemia was defined as a random blood glucose level of more than 200 mg/dL.

Treatment protocol. Rats were randomly allocated into three groups before the creation of AC fistulas (control, DM, and DM + rosuvastatin group). Animals in the rosuvastatin group were fed with rosuvastatin-containing chow (15 mg/kg/d)[14] 3 days before the operation and continued up to 2 weeks after creation of the AC fistulas. The other experimental animals received only plain chow throughout the study period. Plain chow was purchased from Harlan Laboratories (Indianapolis, Ind).

Hemodynamic measurements and vasomotor function assessment. Transthoracic echocardiography (Sonos S500, Ultrasound System with a 12-MHz probe; Agilent Technologies, Santa Clara, Calif) was performed in anesthetized rats before death to evaluate the global myocardial function in animals with or with diabetes and determine the effect of rosuvastatin on cardiac performance. Arterial blood pressure in the carotid artery was directed measured by connecting to a fluid-filled pressure transducing system. Blood flow in the arterial site of an AC fistula was determined using an ultrasonic flow probe (Transonic System, Ithaca, NY) after exploratory laparotomy.[15] All hemodynamic measurements were carried out by investigators who were blinded to the treatment groups. Segments of aorta were mounted in organ chambers containing Krebs-Ringer bicarbonate. Changes in tension were recorded continuously using an isometric force-displacement transducer. After a 45-minute equilibration period, the rings were contracted by addition of KCl (40 mM) and cumulative addition of phenylephrine (10^{-9}–10^{-5} M). Concentration-response curves were obtained by cumulative addition of acetylcholine during precontracting the rings with a median effective concentration (EC_{50}) of phenylephrine. In some experiments, aortic preparations were preincubated with a cell-permeable superoxide dismutase mimetic (Mn–III-tetrakis-4-benzoic acid-porphyrin; MnTBAP; 10^{-5} M) before each contraction.[16]

Measurement of circulating endothelial progenitor cells and blood chemistry. Blood was collected immediately after euthanasia. Blood samples (100 μL) were stained with fluorescent conjugated antibodies CD34 (phenylephrine-conjugated; 1:50 dilution, NOVUS Biologicals, Littleton, Colo) and vascular endothelial growth factor receptor-2/KDR (DyLight 488-conjugated; 1:100 dilution; NOVUS Biologicals). Cell fluorescence was measured immediately under a flow cytometry (BD FACS Calibur; BD Biosciences, San Jose, Calif).[17] Blood glucose, levels of plasma cholesterol, high-density lipoprotein (HDL), and triglyceride were analyzed.

Determinations of superoxide anions, cGMP, and proinflammatory gene expressions. The generation of superoxide anions in the blood vessels was measured using the chemiluminescence and diethedium assays as previously described.[16,17] Tissue levels of cyclic guanosine monophosphate (cGMP; the second messenger of nitric oxide [NO]) were quantified using an enzyme-linked immunosorbent assay (R&D Systems, Xindian City, Taipei, Taiwan). The expressions of inducible NO synthase (iNOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the isolated aorta were determined by Western blotting.[16]

Histologic sections. Isolated arterial tissue was immersed in 4% formaldehyde for 24 hours. Paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin (H&E) and Verhoeff stains.[13]

Statistical analysis. Results are presented as the mean ± SD. Data were compared by analysis of variance. Statistical significance was accepted at a level of $P < .05$.