Cerebrovasculoprotective effects of azilsartan medoxomil in diabetes

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We have shown that Goto-Kakizaki (GK) rats, a lean model of type 2 diabetes, develop significant cerebrovascular remodeling by the age of 18 weeks, which is characterized by increased media thickness and matrix deposition. Although early glycemic control prevents diabetes-mediated remodeling of the cerebrovasculature, whether the remodeling can be reversed is unknown. Given that angiotensin II type 1 receptor blockers reverse pathologic vascular remodeling and function independent of changes in blood pressure in other vascular beds, we hypothesized that azilsartan medoxomil, a new angiotensin II type 1 receptor blocker, is vasculoprotective by preventing and reversing cerebrovascular remodeling in diabetes. Control Wistar and diabetic GK rats (n = 6–8 per group) were treated with vehicle (water) or azilsartan medoxomil (3 mg/kg/d) from the age of 14 to 18 or 18 to 22 weeks before or after vascular remodeling is established, respectively. Blood glucose and blood pressure were monitored and middle cerebral artery structure and function were evaluated using pressurized arteriography. Blood glucose was higher in GK rats compared with Wistar rats. Azilsartan treatment lowered blood glucose in diabetic animals with no effect on blood pressure. Diabetic animals exhibited lower myogenic tone, increased wall thickness, and cross-sectional area compared with control group animals, which were corrected by azilsartan treatment when started at the onset of diabetes or later after vascular remodeling is established. Azilsartan medoxomil offers preventive and therapeutic vasculoprotection in diabetes-induced cerebrovascular remodeling and myogenic dysfunction and this is independent of blood pressure. (Translational Research 2014;164:424–432)

Abbreviations: ACE = Angiotensin converting enzyme; ARBs = Angiotensin II type 1 receptor blockers; AT1R = Angiotensin II type 1 receptor; Azil = Azilsartan medoxomil; Ca2+ = Calcium; CSA = Cross-sectional area; ET-1 = Endothelin-1; GK = Goto-Kakizaki rats; LD = Lumen diameter; M = Molar; MT = Media thickness; MCAs = Middle cerebral arteries; OD = Outer Diameter; PPAR-γ = peroxisome proliferator–activated receptor γ; Wis = Wistar rats; σ = Circumferential stress; ε = Circumferential strain

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INTRODUCTION

Type 2 diabetes, a disease that affects more than 20 million Americans, increases the risk and worsens outcomes of cerebrovascular disease and stroke.[1] Mounting evidence suggests that inhibition of the renin-angiotensin-aldosterone system reduces cardiovascular and cerebral complications including the primary and secondary stroke risk, and angiotensin II type 1 receptor (AT1R) blockers (ARBs) are as effective as angiotensin converting enzyme (ACE)

inhibitors.[2-4] Regulation of cerebrovascular function and structure is critical for the maintenance of cerebral blood flow, which ultimately is the most important determinant of stroke.[5] Several studies suggested that in experimental models of hypertension, a common confounding factor in patients with diabetes and stroke, ARBs reverse pathologic vascular remodeling and function independent of changes in blood pressure.[6-9] The effect of ARBs on cerebrovascular function and structure in diabetes remains unknown. We have recently demonstrated hypertrophic cerebrovascular remodeling in Goto-Kakizaki (GK) rats, a nonobese and nonhypertensive model of type 2 diabetes.[10] We also showed that inhibition of vascular remodeling reduces neurovascular injury if stroke is induced in this model.[11] Given that ARBs are one of the most commonly prescribed drugs in patients with diabetes, we used both preventive and therapeutic approaches to test the overall hypothesis that azilsartan medoxomil, a new ARB, is vasculoprotective in diabetes by improving vascular function and structure.

METHODS

Animals. All experiments were performed using male Wistar rats (Harlan, Indianapolis, IN) and age-matched diabetic GK rats (in-house bred, derived from the Tampa colony or purchased from the Tampa colony [Taconic, Hudson, NY]). The animals were housed at the Georgia Regents University animal care facility that is approved by the American Association for Accreditation of Laboratory Animal Care. All protocols were approved by the institutional animal care and use committee. Animals were fed standard rat chow and tap water ad libitum. Body weights and blood glucose measurements were taken biweekly. Blood glucose measurements were taken from tail vein samples using a commercially available glucometer (Freestyle; Abbott Diabetes Care, Inc, Alameda, CA). Mean arterial blood pressure (measured in millimeters of mercury) was measured using the tail-cuff method. All animals were anesthetized with pentobarbital sodium (Fatal-Plus; Vortech Pharmaceuticals Ltd, Dearborn, MI), exsanguinated via cardiac puncture, and decapitated to extract the brain. Middle cerebral arteries (MCAs) were isolated for structure and function studies as described subsequently.

Experiment 1. To determine whether angiotensin II antagonisms prevent the progression or reverse established cerebrovascular remodeling and dysfunction, 4 groups were included: (1) control vehicle, (2) control + azilsartan medoxomil, (3) GK vehicle, and (4) GK + azilsartan medoxomil. Starting at the age of 18 weeks after the development of diabetes-induced cerebrovascular remodeling, rats were treated with azilsartan medoxomil (3 mg/kg/d by gavage) for 4 weeks.

Experiment 2. To determine whether angiotensin II antagonisms prevent the development of cerebrovascular remodeling and dysfunction, 4 groups were included: (1) control vehicle, (2) control + azilsartan medoxomil, (3) GK vehicle, and (4) GK + azilsartan medoxomil. Starting at the age of 14 weeks before the development of diabetes-induced cerebrovascular remodeling, rats were treated with azilsartan medoxomil (3 mg/kg/d by gavage) for 4 weeks. GK rats showed elevated blood glucose levels (experiment 1, 242 ± 21 and experiment 2, 195 ± 15) at the onset of treatment. Metabolic parameters including body weight, blood glucose, and blood pressure at the end of treatment are given in Table I.

MCA function and morphometry. MCAs were quickly excised and used within 45 minutes of isolation to ensure viability of the vessels. A pressure arteriograph system (Living Systems, Burlington, VT) was used to evaluate MCA structure, myogenic tone, and mechanical properties. For these studies, MCA segments approximately 200–250 μm in diameter and proximal to the junction between the MCA and the inferior cerebral vein were used exclusively. The vessels were first mounted onto glass cannulas in an arteriograph chamber and (N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid bicarbonate buffer (in mM: 130 NaCl, 4 KCl, 1.2 $MgSO_4$, 4 $NaHCO_3$, 10 (N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1.18 KH_2PO_4, 5.5 glucose, and 1.8 $CaCl_2$) was circulated and maintained at 37 ± 0.5°C. The MCA segments were then pressurized at 60 mm Hg for 45 minutes to generate spontaneous tone. A video dimension analyzer connected to the arteriograph system was used to measure media thickness (MT) and lumen diameter (LD) at pressures ranging from 0 to 180 mm Hg in 20 mm Hg increments. The first measurement was taken at 5 mm Hg because negative pressure is generated at 0 mm Hg, causing the vessel to collapse. All vessels were exposed to each pressure point for 5 minutes before readings were recorded. Pressure-diameter curves were obtained, first in the presence of Ca^{2+} to observe the vessels' contractile properties, and then in Ca^{2+}-free buffer with the addition of 10^{-7} M papaverine hydrochloride to evaluate the vessels' passive properties.

Data calculations. Using the MT and LD measurements obtained in active conditions (in the presence of Ca^{2+}) and in passive conditions (in the absence of Ca^{2+}), the following parameters related to MCA structure, myogenic tone, and mechanical properties