Two-Week Administration of Tempol Attenuates Both Hypertension and Renal Excretion of 8-Iso Prostaglandin F₂α

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Abstract—8-Iso prostaglandin F₂α (8-ISO) is formed nonenzymatically from the attack of superoxide radical on arachidonic acid. Therefore, 8-ISO is a marker of oxidative stress in vivo. We have recently shown that short-term administration of the membrane-permeable, metal-independent superoxide dismutase mimetic tempol (4-hydroxy-2, 2, 6, 6-tetramethyl piperidinoxyl) normalizes blood pressure in spontaneously hypertensive rats (SHR). The present study was designed to test whether prolonged administration of tempol ameliorates oxidative stress and hypertension in SHR. In control SHR (n = 8), mean arterial pressure and heart rate were increased and renal blood flow and glomerular filtration rate were reduced compared with control Wistar-Kyoto rats (WKY) (n = 7). Twenty-four-hour renal excretion of 8-ISO was significantly increased in SHR compared with WKY. Two weeks of tempol administration in the drinking water (1 mmol/L) to SHR (n = 8) decreased mean arterial pressure by 18% (162±8 to 134±6 mm Hg, P < 0.05), increased glomerular filtration rate by 17% (1.6±0.2 to 1.9±0.3 mL/min), and decreased renal excretion of 8-ISO by 39% (9.8±0.7 to 6.0±0.7 ng/24 hours, P < 0.05). In contrast, tempol administration to WKY (n = 6) had no significant effect on mean arterial pressure (115±5 versus 118±8 mm Hg), glomerular filtration rate (3.0±0.4 versus 2.5±0.5 mL/min), or renal excretion of 8-ISO (7.9±0.4 versus 6.8±0.7 ng/24 hours). In conclusion, the SHR is a model of hypertension and renal vasoconstriction associated with oxidative stress. Because long-term administration of a superoxide scavenger reduces blood pressure and oxidative stress in vivo, this study suggests a role for oxygen radicals in the maintenance of hypertension in SHR. (Hypertension. 1999;33[part II]:424-428.)

Key Words: oxidative stress ■ isoprostanes ■ oxygen radicals ■ superoxide dismutase

Reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, and hydrogen peroxide have been implicated in atherosclerosis, diabetes, ischemia/reperfusion injury, and hypertension.1–4 Compared with normotensive individuals, hypertensive patients have higher levels of plasma hydrogen peroxide, superoxide anion, and lipid peroxides, while having lower plasma levels of the antioxidant ascorbic acid.5–7 We and others9–12 have shown in short-term studies that inhibition of ROS reduces blood pressure in the spontaneously hypertensive rat (SHR). However, the importance of ROS in the long-term regulation of blood pressure in SHR has not been determined. Previous studies have shown increased superoxide release in mesenteric arterioles11,12 and cultured aortic endothelial cells,13 and increased superoxide and hydrogen peroxide release from aortic strips of SHR14 compared with the normotensive control Wistar-Kyoto rat (WKY). These studies suggest that specific vascular beds in the SHR have oxidative stress; however, it is not yet clear whether there is a net excess of ROS in SHR in vivo.

F₂-isoprostanes are a family of prostaglandin (PG) F₂-like compounds that are formed from the nonenzymatic reaction of arachidonic acid and oxygen radicals in vivo and in vitro.15 Because of their unique synthesis pathway, F₂-isoprostanes are recently proposed markers of oxidative stress. For example, tissue and plasma levels of F₂-isoprostanes are increased in rats with oxidative stress caused by carbon tetrachloride.16 Among the several PGF₂-like compounds, 8-iso-PGF₂α (8-ISO) is the major urinary metabolite of F₂-isoprostanes17 and is markedly elevated in the urine of rats after renal ischemia/reperfusion.18 Tempol (4-hydroxy-2, 2, 6, 6-tetramethyl piperidinoxyl) is a stable, membrane-permeable, metal-independent superoxide dismutase mimetic. Tempol is a small molecular weight cyclic nitroxide that has been used as a spin trap for superoxide19,20 and reduces superoxide-related injury in ischemia/reperfusion,21 inflammation,22 and radiation.23 We have recently reported that short-term infusion or 7-day intraperitoneal administration of tempol normalizes blood pressure in the SHR.8 The aim of the present study was to assess the oxidative stress of adult SHR from the measurement of the renal excretion of 8-ISO and to determine whether long-term tempol administration ameliorates the hypertension and oxidative stress in SHR. We measured mean arterial pressure and renal hemodynamic and excretory function in WKY and SHR under normal conditions and after 2 weeks of oral tempol administration.
Methods

Animal Preparation

Groups of male SHR and WKY rats (250±10 g) were maintained on tap water and a standard chow (Ralston-Purina Co, sodium content 0.3 g/100 g). Protocols were approved by the Institutional Animal Care and Use Committee of Georgetown University Medical Center and were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health as well as the guidelines of the Animal Welfare Act. Rats were divided into four groups: WKY given vehicle (control, n=7), SHR given vehicle (control, n=8), WKY given tempol (tempol, n=6), and SHR given tempol (tempol, n=8). Tempol is readily soluble in water and was administered in the drinking water (1 mmol/L) for 2 weeks. After either control or tempol administration, rats were maintained in metabolic cages for 24 hours. Urine was collected in containers with 10 μL of 2 mmol/L EDTA to prevent ex vivo production of 8-ISO. Urine was centrifuged at 1000 rpm for 10 minutes at 4°C and stored in aliquots at −80°C until assayed.

Thereafter, WKY and SHR were anesthetized with thiobutabarbital (100 mg/kg IP, Inactin, Research Biochemicals International) and maintained at 37°C on a servo-controlled heated rodent operating table. A tracheostomy was performed with polyethylene PE-240 tubing and the left jugular vein and carotid artery were cannulated with PE-50 tubing. A 1% albumin solution in 0.154 mol/L NaCl was infused at 2 mL/h IV, to maintain a euclidean state. A midline incision was made and the left renal artery was isolated. A blood flow probe was placed around the renal artery and connected to a transit-time blood flowmeter (1RB, Transonic Systems, Inc). We have shown previously that this method of measuring real-time changes in renal blood flow (RBF) is valid in the rat.24 Mean arterial pressure (MAP) and heart rate were recorded continuously from the carotid artery using a Statham pressure transducer (model P23, Gould Instruments) and MACLab data acquisition software. Glomerular filtration rate (GFR) was determined from the clearance of [1H]-inulin. After surgery and a 60-minute equilibration period, MAP, heart rate, GFR, and RBF were measured for 30 minutes and the data were averaged.

Determination of 8-ISO Prostaglandin F2α

Urine 8-ISO was extracted, purified, and measured according to methods previously established using an enzyme immunoassay kit (Cayman Chemical). Briefly, urine was spiked with [1H]-8-ISO, treated with ethanol followed by 15% potassium hydroxide, incubated for 1 hour at 40°C, and acidified to pH 4.0 with hydrochloric acid. The sample was extracted using a polyboronic acid column, eluted with ethyl acetate containing 1% methanol, and evaporated under nitrogen. 8-ISO was assayed using competitive binding with mouse anti-rabbit IgG monoclonal antibody in a 96-well plate. Concentration of the reaction product was determined from its absorbency at 412 nm using a standard curve. Samples were assayed in duplicate and corrected for individual recovery of [1H]-8-ISO. The recovery averaged 76% (n=12). The limits of sensitivity of the assay are 1 to 3 pg/mL and the intraassay coefficient of variation is 8% (n=6). Samples were diluted to fall in the middle portion of the linear standard curve (10 to 100 pg/mL). To validate the collection method for measurement of 8-ISO in urine, a second set of urine was collected from control SHR (n=5) and WKY (n=5) in metabolic cages into containers containing 10 μL of 0.01% butylated hydroxytoluene as suggested by Roberts and Morrow13 and assayed as described above. The results showed a similar increase (30%) in renal excretion of 8-ISO in control SHR compared with control WKY as described for the control groups reported below.

Statistics

All values shown are mean ± standard error. Analysis of variance was used to test the overall effect of tempol. Unpaired comparisons using Student’s t test were used to determine significance between specific groups. P<0.05 was considered statistically significant.

Results

Rats maintained on tempol given in the drinking water for 2 weeks had similar dietary consumption as control rats drinking water alone. There was no significant difference between food intake (control: 25±1 versus tempol: 24±1 g/d) or body weight gain (control: 69±4 versus tempol: 66±3 g/14 days), but water intake was increased modestly in the tempol-treated groups: control: 34±2 versus tempol: 43±3 mL/day, P<0.05). Water intake was increased similarly in tempol-treated WKY and SHR.

MAP in WKY and SHR is represented in Figure 1. Under normal conditions, MAP in SHR was increased by 41% compared with WKY (SHR: 162±8 versus WKY: 115±5 mm Hg, P<0.001). After 2 weeks of tempol administration, MAP was reduced in SHR to a value that was not significantly different from WKY (SHR: 134±6 versus WKY: 118±7 mm Hg). MAP in SHR given tempol was significantly lower by 18% compared with normal SHR. Analysis of variance showed that tempol specifically and significantly (P<0.05) decreased MAP in SHR. Heart rate was significantly (P<0.001) elevated in SHR (420±6 bpm) compared with WKY (374±9 bpm) during control conditions and was not changed by tempol (SHR: 414±9 versus WKY: 373±8 bpm).

The Table depicts renal hemodynamic and excretory function during control conditions and after 2 weeks of tempol administration in the drinking water. Under control conditions, the RBF of SHR was decreased by 34% (SHR: 5.8±0.6 versus WKY: 8.8±0.7 mL/min, P<0.001), the GFR was decreased by 47% (SHR: 1.6±0.2 versus WKY: 3.0±0.4 mL/min, P<0.005), and the renal vascular resistance was increased by 117% (SHR: 29.4±2.7 versus WKY: 13.5±1.0 mm Hg · mL−1 · min−1, P<0.001). After 2 weeks of tempol administration there were no significant changes in renal hemodynamics in SHR, although there were tendencies toward a fall in renal vascular resistance (16%) and a rise in GFR (17%), such that there was no longer a significant difference in GFR between SHR and WKY. Tempol had no marked effects on renal hemodynamics in WKY. Renal excretory function was not significantly different between WKY and SHR during control conditions or tempol admin-
Cyclooxygenase, nitric oxide synthase, and xanthine oxidase, NADPH oxidase, glucose oxidase, and catalase, ROS can oxidize and destroy proteins, membrane lipids, and nucleic acids, diminish the biological half-life of nitric oxide, and generate new vasoconstrictors such as 8-ISO.

One of the stable products when ROS attack lipids is 8-ISO. 8-ISO is generated from arachidonic acid in phospholipids and subsequently released in free form. Investigators have shown that 8-ISO is formed both in vitro and in vivo. Because 8-ISO is a direct, enzyme-independent, stable product of ROS, measurement of 8-ISO has been used as a marker of oxidative stress in vivo. Elevated renal excretion of 8-ISO has been reported in humans with scleroderma and preeclamptic toxemia of pregnancy and in rats with cyclosporin-induced nephrotoxicity, rhabdomyolysis, bile duct ligation, and renal ischemia/reperfusion injury.

Measurement of the rate of excretion of 8-ISO for assessing total endogenous 8-ISO is advantageous over measurement of plasma 8-ISO for two reasons. First, this eliminates the problem of ex vivo generation of 8-ISO because the amount of lipid in urine is negligible. Second, 24-hour urinary measurement of 8-ISO presumably provides an integrated assessment of 8-ISO production with time.

The rate of excretion of 8-ISO in conscious WKY was similar to that previously reported for conscious Sprague Dawley rats. The finding that the SHR has an elevated rate of renal excretion of 8-ISO indicates that it is a model of oxidative stress in vivo. Prolonged tempol administration reduced renal excretion of 8-ISO significantly, consistent with reports that antioxidant therapy in rat models of oxidative stress associated with cyclosporin nephrotoxicity and bile duct ligation reduces renal excretion of 8-ISO. In the present study, the marked decrease in renal excretion of 8-ISO could not be attributed to a decrease in renal function because tempol had no significant effect on either renal hemodynamic or excretory function. In fact, 2-week tempol administration tended to improve GFR in SHR.

Previous in vivo and in vitro studies suggest that systemic vessels of SHR have increased oxidative stress, although the source of ROS remains unclear. The only previous in vivo studies in SHR show that mesenteric vessels generate oxygen radicals through xanthine oxidase. Earlier studies established that glomeruli generate hydrogen peroxide under normal conditions and can upregulate the activities of superoxide dismutase and catalase during oxidative stress. Overproduction of an ROS or dysregulation of antioxidants in...
glomeruli, other vascular beds, or tissues of SHR could contribute to the oxidative stress in this hypertensive model. We and others have shown that renal nitric oxide synthase (NOS) gene and protein expression is higher in SHR compared with WKY, but that nitric oxide generation in blood vessels is limited, perhaps because of a deficiency in the NOS cofactor tetrahydrobiopterin. Tetrahydrobiopterin deficiency enhances the formation of superoxide from NOS. In fact, addition of tetrahydrobiopterin or superoxide dismutase to the isolated aorta of SHR simultaneously reduces superoxide and increases nitric oxide production. These data suggest that NOS may be an important source of ROS in SHR.

Finally, 8-ISO is not only a marker of oxidative stress in vivo, but is also a vasoconstrictor. Receptors for 8-ISO have been located in rat aortic smooth muscle cells, retinal vascular smooth muscle cells, and renal arterial smooth muscle cells. Intrarenal infusion of 8-ISO reduces GFR and RBF in rats, in part, through activation of thromboxane A2 receptors. Whether elevated levels of 8-ISO in the SHR contribute to the hypertension and renal vasoconstriction remains to be determined fully. The present data show, however, that 2 weeks of antioxidant treatment with tempol decreased renal excretion of 8-ISO and blood pressure significantly but did not improve renal hemodynamics significantly, suggesting that 8-ISO may not be the primary mediator of the hypertension and renal vasoconstriction in SHR.

In conclusion, this study provides evidence that the SHR is a model of oxidative stress in vivo. The finding that a superoxide dismutase mimetic reduces blood pressure and oxidative stress in vivo suggests that oxygen radicals may be important in the long-term regulation of blood pressure in SHR. Although there are limitations in extrapolating data from the anesthetized state to the conscious state, this study provides a rational basis for a novel form of antihypertensive therapy based on tempol or similar agents. Such therapy may correct the complications created by hypertension associated with oxidative stress.

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References


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