Systemic arterial pressure response to two weeks of Tempol therapy in SHR: involvement of NO, the RAS, and oxidative stress

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Yanes, Licy, Damian Romero, Radu Iliescu, Valeria E. Cucchiarelli, Lourdes A. Fortepiani, Francisco Santacruz, William Bell, Huimin Zhang, and Jane F. Reckelhoff. Systemic arterial pressure response to two weeks of Tempol therapy in SHR: involvement of NO, the RAS, and oxidative stress. Am J Physiol Regul Integr Comp Physiol 288: R000–R000, 2005. First published December 16, 2004; doi:10.1152/ajpregu.00530.2004.—The roles of nitric oxide (NO) and plasma renin activity (PRA) in the depressor response to chronic administration of Tempol in spontaneously hypertensive rats (SHR) are not clear. The present study was done to determine the effect of 2 wk of Tempol treatment on blood pressure [mean arterial pressure (MAP)], oxidative stress, and PRA in the presence or absence of chronic NO synthase inhibition. SHR were divided into four groups: control, Tempol (1 mmol/l) alone, nitro- L-arginine methyl ester (L-NAME, 4.5 mg·kg−1·day−1) alone, and Tempol + L-NAME for 2 wk. With Tempol, MAP decreased by 22%: 191 ± 3 and 162 ± 21 mmHg for control and Tempol, respectively (P < 0.05). L-NAME increased MAP by 16% (222 ± 2 mmHg, P < 0.01), and L-NAME + Tempol abolished the depressor response to Tempol (215 ± 3 mmHg, P < 0.01). PRA was not affected by Tempol but was increased slightly with L-NAME alone and 4.4-fold with L-NAME + Tempol. Urinary nitrate/nitrite increased with Tempol and decreased with L-NAME and L-NAME + Tempol. Tempol significantly reduced oxidative stress in the presence and absence of L-NAME. In conclusion, in SHR, Tempol administration for 2 wk reduces oxidative stress in the presence or absence of NO, but in the absence of NO, Tempol unable to reduce MAP. Therefore, NO, but not changes in PRA, plays a major role in the blood pressure-lowering effects of Tempol. These data suggest that, in hypertensive individuals with endothelial damage and chronic NO deficiency, antioxidants may be able to reduce oxidative stress but not blood pressure.

nitric oxide; superoxide; antioxidant; renin-angiotensin system

SEVERAL LINES OF EVIDENCE have implicated oxidative stress and reactive oxygen species (ROS) in human and experimental hypertension (3, 5, 6, 19, 22, 23). For example, Lacy and colleagues (6) found that hydrogen peroxide was increased in the plasma of hypertensive individuals compared with age-matched normotensive subjects. Similarly, in experimental hypertension, superoxide and hydrogen peroxide released from isolated mesenteric arterioles and aortic strips are increased in spontaneously hypertensive rats (SHR), a model of essential hypertension (3, 22, 23). The major vascular oxygen-derived free radical is superoxide anion. Superoxide is routinely scavenged by superoxide dismutase (SOD). However, superoxide has also been shown in vitro to combine with nitric oxide (NO), which results in quenching of NO and, theoretically, reduces its biological activity (i.e., vasodilation) (12). The role of NO in the response to Tempol in SHR is controversial. Schnackenberg and colleagues (19) reported that acute intravenous infusion of Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl), a stable membrane-permeable SOD mimic, caused a significant reduction in blood pressure that was not produced in Wistar-Kyoto (WKY) rats. These data suggested that oxidative stress plays a role in the increased blood pressure of SHR. In these studies, in which nitro-L-arginine methyl ester (L-NAME), the NO synthase (NOS) inhibitor, was acutely infused, the depressor response to Tempol in SHR was abrogated. However, when Kagiyama and colleagues (4) administered Tempol acutely into the lateral ventricle of the brain of SHR, it did not have an effect on blood pressure, nor was the pressor response to L-NAME affected by Tempol. In acute studies in isolated aortic rings or mesenteric vascular beds from SHR, Shastri and colleagues (20) found that Tempol reduced the maximum tension in response to ANG II, and this effect was lost in aorta or mesenteric vascular beds when L-NAME was present or the endothelium was denuded. Although two of these studies (19, 20) suggested a role for NO in the acute response to Tempol, there are no studies in which the role of chronic NO inhibition was studied in chronic scavenging of superoxide by Tempol in SHR.

In addition to its vasodilator activity, endothelium-derived NO has been shown to cause a reduction in renin release (18, 26). In SHR, we showed that blockade of the renin-angiotensin system (RAS) with converting enzyme inhibitors normalizes the blood pressure (15), which suggests that the RAS plays an important role in mediating the hypertension in SHR. Thus Tempol could cause a reduction in blood pressure in SHR by chronically increasing bioavailability of NO, which in turn could reduce renin release and plasma renin activity (PRA). Whether changes in PRA play a role in the depressor response to chronic Tempol in SHR has also not been studied.

Therefore, the present studies were performed to determine the role of NO and changes in renin release in the response to chronic Tempol in SHR. Consistent with the concept that the kidney plays an important role in control of arterial pressure, we also evaluated the effect of Tempol on markers of oxidative stress in this organ. The following questions were addressed: 1) Does Tempol given for 2 wk reduce blood pressure in SHR by affecting the NO system? 2) If so, does an increase in bioavailability of NO associated with Tempol treatment subsequently affect the NO system? 3) Does Tempol affect PRA in SHR? 4) Do the effects of Tempol on PRA depend on the presence or absence of L-NAME? 5) Does Tempol affect L-NAME? 6) Does Tempol affect PRA when the endothelium is denuded? 7) Does Tempol affect PRA when L-NAME is present? 8) Does Tempol affect PRA when L-NAME is present and the endothelium is denuded?

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reduce renin release as measured by PRA and contribute to the Tempol-mediated reduction in blood pressure?

METHODS

Experimental protocol. Male SHR (Taconic Farms) were divided into four groups (n = 6–8 rats per group): Controls (group 1) received tap water throughout the experimental protocol. Group 2 was treated with Tempol (1 mmol/l) in drinking water for 2 wk. Group 3 was treated with L-NAME (4.5 mg·kg\(^{-1}\)·day\(^{-1}\)) in drinking water for 2 wk. Group 4 was treated with Tempol (1 mmol/l) + L-NAME (4.5 mg·kg\(^{-1}\)·day\(^{-1}\)) in drinking water for 2 wk. Tempol and L-NAME are readily soluble in water and were prepared fresh daily. Consumption of water was measured daily in all experimental groups. The dose of Tempol was shown in preliminary studies by us and by Schnackenberg and colleagues (19) to reduce blood pressure in male SHR when given chronically for 2 or 6 wk. This low dose of L-NAME was also used in normotensive rats and found to effectively block NOS (14). All protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

Measurement of blood pressure. On day 13 of treatment, rats were anesthetized by isoflurane gas anesthesia, and a catheter was placed in the femoral artery and exteriorized at the back of the neck, as we previously described (16), for mean arterial pressure (MAP) monitoring. On the next day, animals were placed in restraining cages, and MAP was measured in conscious rats. Rats had been habituated to the restraining cages before catheter placement. MAP was recorded with a pressure transducer connected to a recorder (model 7B-chart, Grass Instrument). After a 60-min stabilization period, results of two 30-min recordings were averaged.

Measurement of urinary nitrate/nitrite. To avoid the contribution of nitrate/nitrite (NOx) in food to NOx excretion, rats were placed on a low-NOx diet throughout the treatment period. NOx was measured daily in 24-h urine specimens by the Griess reagent method, with Escherichia coli used to convert nitrate to nitrite, as we described previously (13). The daily data were averaged for the 14 days and are presented as NOx excreted per 24 h per kilogram body weight.

Measurement of superoxide in kidney homogenates by lucigenin luminescence. In isoflurane-anesthetized rats, kidneys were perfused clear of blood with saline containing 2% heparin. Kidneys were removed and separated into cortex and medulla and homogenized (1:8 wt/vol) in RIPA buffer (PBS, 1% Nonidet P-40 or Igepal CA-630, 0.5% sodium deoxycholate, 0.1% SDS, and a cocktail of protease inhibitors; Sigma Chemical) with a Polytron (model PT10-35). The samples were centrifuged at 12,000 g for 20 min at 4°C. The supernatant was used for measurement of superoxide with lucigenin at a final concentration of 5 μM. The samples were allowed to equilibrate for 3 min in the dark, and luminescence was measured every second for 5 min with a luminometer (Berthold). Luminescence was recorded as relative light units (RLU) per 5 min. An assay blank with no homogenate but containing lucigenin was subtracted from the readings before transformation of the data. Protein concentrations in the kidney homogenates were determined by the method of Lowry et al. (9). The data are expressed as RLU per milligram protein.

Measurement of total antioxidant status. Plasma total antioxidant status (TAS) was measured using a commercially available kit (Calbiochem-Novabiochem, San Diego, CA), according to the manufacturer’s instructions. Data are presented as millimoles per liter plasma. The TAS assay is based on the ability of antioxidants in the plasma to inhibit the absorbance of the radical cation ABTS\(^+\) [2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonate)], which has a long-wavelength absorbance, to an extent and on a time scale dependent on the antioxidant capacity of the plasma (17). The TAS assay measures plasma antioxidant levels. The antioxidants determined are not specific and include (among others) selenium, flavonoids, β-carotene, carotenoids, vitamins C and E, and thiols.

Measurement of PRA. In a separate set of animals (n = 5 per treatment group), rats were untreated or treated with Tempol, L-NAME, or Tempol + L-NAME for 2 wk, as described above, and decapitated for collection of blood into EDTA-containing tubes. PRA in samples of rat plasma was measured by radioimmunoassay as previously described (8).

Measurement of 8-iso-PGF\(_{2\alpha}\) (8-isoprostane). Urine was purified by affinity column, and 8-isoprostane was quantified by a competitive ELISA (Cayman Chemical) according to the manufacturer’s instructions. Results are expressed as nanograms of 8-isoprostane per milligram of creatinine. Creatinine was measured by commercially available kit (Sigma).

Statistics. Values are means ± SE. Comparisons among groups were made by ANOVA followed by the Fisher’s test. P < 0.05 was considered statistically significant.

RESULTS

Effects of Tempol and L-NAME on MAP in SHR. After 2 wk of Tempol administration, MAP was reduced by 22% compared with the control group: 191 ± 3.3 vs. 162 ± 5 mmHg (P < 0.01; Fig. 1). As previously shown (7, 14), chronic L-NAME alone increased MAP by 16% (221 ± 1.9 mmHg) compared with controls (P < 0.01). When the rats were given Tempol + L-NAME (215 ± 3.1 mmHg), the depressor effect of Tempol was abolished, and blood pressure was similar to that of the rats treated with L-NAME alone.

Effects of Tempol and L-NAME on urinary NOx excretion in SHR fed low-NOx food. Treatment with Tempol alone for 2 wk increased NOx compared with the control group (12.3 ± 0.38 vs. 15 ± 0.57 μmol·kg\(^{-1}\)·day\(^{-1}\), P < 0.05), whereas chronic L-NAME (7.1 ± 0.96 μmol·kg\(^{-1}\)·day\(^{-1}\)) and L-NAME + Tempol (6.9 ± 0.81 μmol/l) treatment reduced NOx (Fig. 2).

Effects of Tempol and L-NAME on oxidative stress: superoxide anion. In kidney cortex (Fig. 3), Tempol treatment alone or in combination with L-NAME decreased superoxide generation by 22% compared with controls: 11,889 vs. 15,744 (Tempol, P < 0.05), and 9,506 ± 672 RLU/mg protein (Tempol + L-NAME, P < 0.05). L-NAME alone had no effect (10,423 ± 778 RLUs/mg) on superoxide generation in kidney cortex. In the kidney medulla (Fig. 4), Tempol tended to decrease superoxide, but the result did not reach statistical significance.
significance (6,413 ± 262 and 5,944 ± 628 RLU/mg for control and Tempol, respectively), whereas l-NAME significantly increased (7,422 ± 374 RLU/mg) superoxide generation. When Tempol was given in combination with l-NAME (5,248 ± 313 RLU/mg, \( P < 0.01 \)), superoxide generation was significantly attenuated compared with control rats.

TAS. Tempol treatment alone or in combination with l-NAME was associated with a significant increase in plasma TAS: 1.2 ± 0.08, 1.57 ± 0.05, and 1.46 ± 0.06 nmol/l for control, Tempol, and Tempol + l-NAME, respectively (\( P < 0.05 \); Fig. 5). l-NAME alone had no effect (1.25 ± 0.03 nmol/l) on TAS.

8-Isoprostane. After 2 wk of treatment with Tempol in the presence or absence of l-NAME, urinary 8-isoprostane excretion was decreased significantly compared with control: 2.21 ± 0.13 (control), 1.53 ± 0.05 (Tempol, \( P < 0.01 \)), and 1.32 ± 0.10 ng/mg creatinine (Tempol + l-NAME, \( P < 0.001 \); Fig. 6). l-NAME alone (1.89 ± 0.04 ng/mg creatinine, \( P = 0.093 \)) had no effect on urinary 8-isoprostane excretion.

Effects of Tempol and l-NAME on PRA. Tempol treatment for 2 wk had no effect on PRA compared with control: 6.8 ± 0.7 and 9.0 ± 1.0 ng ANG I·ml\(^{-1}·h^{-1}\) for control and Tempol, respectively (\( P = 0.13 \); Fig. 7). l-NAME alone caused a small but significant increase in PRA compared with controls (10.8 ± 0.4 ng·ml\(^{-1}·min^{-1}\), \( P < 0.05 \)). However, in the presence of Tempol and l-NAME, PRA was significantly increased (43.7 ± 1.6 ng·ml\(^{-1}·min^{-1}\), \( P < 0.001 \)).

DISCUSSION

In the present study, we found that chronic Tempol treatment reduced oxidative stress in the presence and absence of NOS inhibition. However, in the presence of chronic NOS inhibition, Tempol was unable to reduce blood pressure in conscious SHR. This is the first study in SHR to show that 2
was abrogated in the presence of acute infusion of L-NAME vs. control. †

Zhou and colleagues (30) reported that angiotensin AT1 receptor blockade in Dahl salt-sensitive (DS) rats fed a high-salt diet prevented the decrease in endothelial NOS (eNOS) activity and the increase in superoxide production and improved endothelial function in response to endothelin and modestly reduced blood pressure (31). These rats were also protected from increased left ventricular hypertrophy and proteinuria. Zhou et al. suggested that protection of vascular eNOS and inhibition of oxidative stress contributed to protection against end-organ damage usually found in DS rats fed a high-salt diet.

We previously showed that blockade of the RAS reduces blood pressure in SHR (15). In the present study, we hypothesized that one mechanism by which Tempol could reduce blood pressure is a reduction in renin release and PRA caused by a chronic increase in NO. As early as 1988, Vidal and colleagues (26) reported that NO could inhibit renin release. Schnackenberg and colleagues (18) reported later that NO caused a reduction in renin release via a macula densa mechanism. However, in our present studies, Tempol treatment and the subsequent increase in systemic NO (as measured by increased urinary NOx) had no effect on PRA; therefore, a reduction in renin release due to increased NO played no role in mediating the depressor response to Tempol.

With these data in mind, we studied the role of chronic NO inhibition on the depressor response to chronic antioxidants in conscious, chronically catheterized SHR. To support a role for NO in the depressor response to Tempol, we measured urinary NOx excretion in rats fed a low-NOx diet and found that Tempol modestly, but significantly, increased NOx. As expected, L-NAME reduced NOx and abolished the effect of Tempol on NOx. Our data are supported by previous vascular reactivity studies by Shastri et al. (20), who showed that L-NAME treatment of aortae or mesenteries from SHR blocked the Tempol-attenuated response to agonists, ANG II, endothelin, and phenylephrine. Cuzzocrea and colleagues (2) reported similar findings, i.e., that the antihypertensive effect of a superoxide scavenger (M-40403) was blocked by L-NAME in SHR and that M-40403 improved the aortic vascular response to acetylcholine in the presence, but not absence, of the endothelium. In addition, Maffei and colleagues (10) found that aortic rings and mesentery from SHR exhibited higher basal NO production but lower responsiveness to agonist-induced NO release. Ascorbic acid improved the NO release. Taken together, these data and our present data support the hypothesis that there is a balance between NO and ROS that is important in maintaining endothelial function and vascular tone.

Studies using other hypertensive animal models also support the interaction between NO and oxidative stress in maintaining endothelial function. For example, Zhou and colleagues (30) reported that angiotensin AT1 receptor blockade in Dahl salt-sensitive (DS) rats fed a high-salt diet, a model of reduced NO production and bioavailability, was able to reduce vascular tone.

Our studies follow up on previous investigations of Schnackenberg and colleagues (19) in which Tempol was given acutely by intravenous infusion in graded doses to anesthetized SHR. Tempol (180 μmol·kg⁻¹·h⁻¹) was able to reduce blood pressure in the SHR by ~22%, and this was abrogated in the presence of acute infusion of L-NAME (11 μmol·kg⁻¹·min⁻¹). These data suggested that acute NO deficiency interfered with the depressor response to Tempol in SHR.

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creased TGF significantly, and this would cause a further increase in renin release compared with NO inhibition alone and could account for the increase in PRA. However, we cannot rule out an effect of Tempol on other components of the RAS in renal tissue. For example, Tempol could have had an effect on renal tissue ANG II or AT1 receptor levels, leading to a reduction in blood pressure. These changes would not have been evident in measurements of PRA. However, to our knowledge, there have been no studies in which the effect of Tempol on the intrarenal RAS components in SHR has been examined. We also measured the effect of Tempol and L-NAME on oxidative stress. As expected, Tempol reduced urinary 8-isoprostanate, decreased renal cortical superoxide, and increased plasma TAS. In contrast, Tempol had no effect on medullary superoxide production. L-NAME alone, on the other hand, had no effect on urinary 8-isoprostanes, cortical superoxide production, or TAS compared with controls. L-NAME significantly decreased medullary superoxide production. NO concentration is high in the medulla compared with the cortex. Therefore, inhibition of NO production with L-NAME would leave superoxide unquenched in the medulla, leading to high levels of superoxide. However, in the presence of Tempol and L-NAME, medullary superoxide levels were reduced to control.

Although we are satisfied that the mechanism by which Tempol reduces blood pressure in the SHR is linked to increases in NO and reductions in oxidative stress, other investigators have suggested that Tempol may reduce blood pressure by inhibiting sympathetic nervous system activity. Shohogi and colleagues (21) reported that acute infusion of Tempol and another superoxide scavenger, diethyldithiocarbamic acid, reduced renal sympathetic nerve activity (RSNA) in SHR and WKY rats but reduced blood pressure in SHR only. Intracerebroventricular infusion had no effect on the RSNA or blood pressure. These investigators suggested that superoxide may stimulate RSNA in SHR. The SHR is a model of increased RSNA, and it is possible that Tempol is capable of reducing blood pressure via this mechanism. However, these findings are not consistent in other models of hypertension. For example, Xu and colleagues (29) reported that Tempol, but no other antioxidant, including apocynin, polyethylene glycol-SOD, or SOD alone, reduced RSNA in deoxycorticosterone acetate-salt-treated rats. However, despite the effect on RSNA, Tempol had no effect on blood pressure or measurements of oxidative stress in aorta or vena cava, suggesting that Tempol reduced RSNA independent of an effect on oxidative stress. Both of these studies, in which RSNA was measured, were performed with acute infusion of Tempol at high doses in anesthetized animals (21, 29). Furthermore, ROS may play a more important role in mediating hypertension in SHR than in other models, because, in contrast to the deoxycorticosterone acetate-salt-treated rats, we have preliminary data that apocynin decreases blood pressure in male SHR (unpublished results). Therefore, future studies are needed to determine whether RSNA is indeed modulated chronically by oxidative stress.

Despite many studies that have shown that oxidative stress is elevated in hypertensive individuals compared with normotensive controls (5, 6, 25, 28), few studies have shown that a reduction in oxidative stress was associated with a reduction in blood pressure and cardiovascular disease risk in hypertensive humans. For example, Ascherio and colleagues (1) reported no effect of vitamin E and C supplementation on stroke in 40- to 75-yr-old men. Similarly, in the Heart Outcomes Prevention Evaluation (HOPE) study, treatment with antioxidants in hypertensive patients in whom blood pressure was controlled by other medications did not result in further reductions in blood pressure (11). Our present studies shed light on why antioxidants may not have been efficacious in hypertensive humans. Hypertension is a disease associated with chronic endothelial dysfunction and, therefore, chronically reduced NO. If chronic endothelial injury reduces NO independent of oxidative stress, antioxidants will not be able to reduce blood pressure and protect against cardiovascular disease risk. Therefore, future studies are necessary to determine whether increasing NO in the presence of antioxidants would have a better effect than antioxidants alone in lowering blood pressure in hypertensive individuals.

GRANTS

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REFERENCES


