Antioxidants Block Angiotensin II–Induced Increases in Blood Pressure and Endothelin

Maria Clara Ortiz, Melissa C. Manriquez, Juan C. Romero, Luis A. Juncos

Abstract—Chronically infusing a subpressor dose of angiotensin (Ang) II increases blood pressure via poorly defined mechanisms. We found that this hypertensive response is accompanied by increased oxidant stress and is prevented by blocking endothelin (ET) receptors. Thus, we now tested whether blocking oxidant stress decreases both blood pressure and ET levels. We infused Sprague-Dawley rats (via osmotic pumps) with either vehicle (group 1) or Ang II (5 ng · kg⁻¹ · min⁻¹; groups 2 to 4) for 15 days. Groups 3 and 4 also received either tempol in the drinking water (1 mmol/L) or vitamin E (5000 IU/kg diet), respectively, for 15 days. We measured systolic blood pressure (SBP) and urinary nitrite excretion every 3 days, and on day 15 we measured systemic and renal venous plasma levels of ET, isoprostanes, and thiobarbituric acid reactive substances (TBARS). SBP in Group 1 did not change throughout the study, whereas Ang II increased SBP (from 132±5 to 151±7 mm Hg). In addition, Ang II increased the systemic and renal venous levels of isoprostanes, TBARS, and ET and caused a transient decrease in urinary nitrites (that returned to control levels by day 9). Both tempol and vitamin E prevented Ang II–induced hypertension and either prevented or tended to blunt the increase in systemic and renal isoprostanes, TBARS, and ET. Finally, both antioxidants abolished the transient decrease in urinary nitrites. These results together with our previous study suggest that subpressor-dose Ang II increases oxidant stress (and isoprostanes). This in turn increases ET levels, which participate in the hypertensive response to Ang II. (Hypertension. 2001;38[part 2]:655-659.)

Key Words: angiotensin II ▪ renal blood flow ▪ free radicals ▪ oxidative stress ▪ hypertension, arterial ▪ kidney ▪ antioxidants

Chronically infusing a subpressor dose of angiotensin (Ang) II slowly increases blood pressure after several days.¹,² This hypertensive response, known as the slow pressor response to Ang II, occurs even though circulating Ang II concentrations do not reach pressor levels. Thus, blood pressure may be increasing via mechanisms other than Ang II–induced vasoconstriction.¹,² Indeed, Ang II has many effects other than direct vasoconstriction, but which effects are implicated in the slow pressor responses is unknown. One action of Ang II that has received increasing attention is its ability to increase production of substances capable of increasing blood pressure, such as endothelin (ET)³ and superoxide (with the subsequent quenching of NO and formation of isoprostanes).⁴–⁸ Indeed, both of these factors have been implicated in several models of hypertension.¹⁰–¹² However, their role, as well as the sequence of events in Ang II–induced hypertension, remains incompletely understood. We recently found that infusing a subpressor dose of Ang II increased blood pressure and plasma isoprostanes levels.⁵ Blocking either ET or Ang II receptors (with bosentan or losartan, respectively) prevented the hypertension, but only losartan normalized the plasma isoprostanes. This suggests that Ang II increases oxidative stress (isoprostanes), which in turn causes ET-dependent hypertension. Thus, blocking oxidative stress should also prevent Ang II–induced increases in blood pressure and ET levels. In addition, we reasoned that because Ang II accumulates intrarenally¹³ and increases renal ET mRNA,¹⁴ intrarenal levels of oxidant stress and ET might represent Ang II effects more than the systemic levels. Thus, we examined whether antioxidants prevent Ang II–induced hypertension and, if so, whether this is associated with a decrease in the systemic or renal levels of ET and oxidative stress (isoprostanes and thiobarbituric acid reactive substances [TBARS]).

Methods

Animals
All experiments were performed according to the guidelines of the Care and Use of Laboratory Animals (NIH publication No. 93-23, revised 1985) and approved by the Institutional Animal Care and Use Committee of Mayo Clinic. Male Sprague-Dawley rats (weight, ~300 g; Harlan, Indianapolis, Ind) were housed in separate cages and trained for 7 days before implanting the minipumps (day 0). On day 0 they were anesthetized with ketamine (100 mg IM/kg body weight; Fort Dodge Laboratories) and xylazine (50 mg/kg body weight).
weight; Lloyd Laboratories), and osmotic minipumps were implanted with the minipump catheters inserted into the external jugular vein for a continuous intravenous infusion.8 The animals were randomized into one of 4 experimental groups. Group 1 (n=5) received vehicle (0.9% NaCl), whereas groups 2 through 4 (n=6, 6, and 5, respectively) received 5 ng · kg⁻¹ · min⁻¹ of Ang II for 15 days. In addition to the Ang II, groups 3 and 4 also received, respectively, either 1 mmol/L of tempol (a superoxide dismutase mimetic, Sigma Chemicals) in their drinking water or vitamin E (5000 IU/kg diet of dry vitamin E acetate; Harlan Teklad) in the rat chow. The antioxidants were started on the same day that the minipumps were implanted, and their doses were chosen because we (data not shown) and others12-15 have found that these doses reduce oxidant stress yet do not alter blood pressure or renal hemodynamics in control animals.

Systolic blood pressure (SBP), by tail-cuff plethysmography, and urine collections (to determine nitrite excretion) were taken during on days −1, 3, 6, 9, 12, and 14. On day 15, we anesthetized the rats with 100 mg/kg body weight IP of thiobutabarbitral, (Inactin, BYK-Güdden) and performed acute studies according to our previous protocols.8 We measured renal blood flow (RBF) by an electromagnetic flowmeter and glomerular filtration rate (GFR) as calculated by inulin clearance techniques (measured by colorimetric methods and factored per gram of kidney tissue). After completion of the experiment, we obtained arterial and renal venous plasma samples and stored them at −80 C. From these samples, we measured isoprostanes, TBARS, and ET.

Analytic Determinations
Free isoprostanes in plasma were measured using extraction and enzyme immunoassay (EIA) procedures, following a modification of the methods provided in the isoprostanes measurement kit from Cayman Chemical, as we previously described.6 TBARS were determined by a colorimetric method. Plasma ET was measured, using an ET RIA kit supplied by Peninsula Laboratories, as previously described.6 Twenty-four-hour urinary excretion of nitrites was determined by the Griess reaction.

Statistical Analysis
Data are expressed in mean±SEM, and P<0.05 was considered significant. Difference in values between groups and treatments were tested using 1-way ANOVA of repeated measurements. To examine for differences in individual points a Student’s 2-sample t test with Duncan’s multiple comparison adjustment was used. Comparisons between groups at the end point of the study were performed using a paired Student t test.

Results
SBP and Renal Function in Rats During Chronic Ang II Infusion
Figure 1 shows SBP in the 4 groups of rats. Infusion of vehicle had no effect on SBP throughout the duration of the experiments (119±7 mm Hg on day −1 versus 118±5 mm Hg on day 15). On the other hand, Ang II increased SBP (from 132±5 mm Hg on day −1, to 151±8 mm Hg on day 15), with a maximal SBP at day 9 (157±4 mm Hg). Tempol did not alter basal blood pressure (141±5 versus 140±7 mm Hg on days −1 and 3, respectively) but inhibited Ang II–induced increases in SBP (119±5 mm Hg on day 15). Likewise, vitamin E did not change basal SBP (148±5 versus 143±7 mm Hg on days −1 and 3, respectively) but also blocked Ang II–induced increases in SBP (127±6 mm Hg on day 15).

Ang II infusion decreased RBF (5.4±0.8 versus 9.8±1.9 mL · min⁻¹ · g⁻¹ in controls) but did not alter GFR (1.4±0.2 versus 1.5±0.3 mL · min⁻¹ · g⁻¹, Ang II versus controls, respectively). Tempol restored RBF (to 9.3±0.8 mL · min⁻¹ · g⁻¹) but caused GFR to fall (to 1.0±0.1 mL · min⁻¹ · g⁻¹). Surprisingly, vitamin E’s effect did not mimic that of tempol; it did not significantly alter either RBF or GFR (6.8±1.4 and 1.4±0.3 mL · min⁻¹ · g⁻¹, respectively).

Systemic and Renal Vein Levels of Isoprostanes, TBARS, and ET
The Ang II group had higher systemic plasma levels of isoprostanes, TBARS (Figure 2), and ET (Figure 3A) compared with those of controls. Isoprostanes were 122±15 versus 193±17 pg/mL; TBARS were 0.8±0.1 versus 1.7±0.1 nmol/mL; and ET was 32±3 versus 55±8 pg/mL (controls versus Ang II, respectively). As shown in Figure 2, tempol and vitamin E abolished the increase in systemic isoprostanes (122±13 and 122±9 pg/mL, respectively) and mitigated the increase in TBARS (1.2±0.2 and 1.3±0.1 nmol/mL, respectively). Tempol was more effective than
vitamin E at preventing the rise in systemic ET levels (39 ± 5 pg/mL, \( P < 0.05 \) versus vehicle; 39 ± 5 pg/mL, \( P < 0.05 \) versus Ang II; 49 ± 4 pg/mL, respectively; Figure 3A).

Renal venous levels of isoprostanes, TBARS (Figure 2), and ET (Figure 3A) were higher in the Ang II group compared with controls. Isoprostanes were 242 ± 18 versus 353 ± 55 pg/mL; TBARS were 0.7 ± 0.1 versus 1.9 ± 0.3 nmol/mL; and ET was 38 ± 4 versus 58 ± 4 pg/mL (controls versus Ang II, respectively). Tempol blocked the increase in renal venous isoprostanes (202 ± 23 pg/mL), and vitamin E tended to do the same, but the values (279 ± 11 pg/mL) did not reach statistical significance versus Ang II or controls. Both tempol and vitamin E prevented the increase in TBARS (1.1 ± 0.2 and 1.1 ± 0.1 nmol/mL, respectively) and ET (46 ± 4 and 44 ± 4 pg/mL, respectively).

**Urinary Nitrite Excretion**

Daily urinary excretion rates of nitrites are depicted in Figure 3B. Basal urinary nitrite excretion rates were not different in any of the experimental groups and remained stable in the control group throughout the 15-day experimental period. Ang II caused a transient decrease in urinary nitrite excretion that returned to normal by day 9. Both tempol and vitamin E prevented this transient decrease in urinary nitrites, but neither significantly increased it from control values.

**Discussion**

We recently reported that a low dose of Ang II via its angiotensin type 1 (AT1)-receptor increases isoprostanes and causes ET-dependent hypertension. Our present study extends these findings and shows that administering antioxidants (either tempol or vitamin E) prevents the Ang II–induced increase in oxidative stress (thus decreasing isoprostanes) and the hypertensive response. We also found that levels of ET in both the systemic and renal venous plasma were increased by the Ang II infusion and that this increase was prevented by the antioxidants. Finally, we show that urinary nitrite excretion is decreased transiently by the infusion of Ang II and that the concurrent administration of antioxidants inhibits the transient fall in nitrite excretion.

Infusing 5 ng · kg⁻¹ · min⁻¹ of Ang II causes no acute effects on blood pressure nor exhibits any immediate vasoconstrictor activity. However, despite the lack of acute effects, maintaining this infusion chronically causes blood pressure to gradually increase over several days. This hypertensive response is accompanied by an increase in oxidative stress as measured as plasma isoprostanes,6 – 8,11,12 consequently suggesting that oxidative stress may be contributing to the hypertension. Indeed, oxidative stress per se can induce hypertension,10,16 and antioxidants can decrease the blood pressure in some models of hypertension.4,11,12,16 But the effectiveness of antioxidants is not universal to all forms of hypertension,17,18 suggesting that the role of oxidative stress in the different models of hypertension is variable. Because of these studies and the fact that we see an increase in oxidant stress during low-dose Ang II, we tested whether quenching oxidative stress prevents hypertension in this model. We found that both tempol and vitamin E completely prevented the hypertensive response to Ang II, thus extending our previous knowledge to include a role for oxidative stress in the hypertensive response to low-dose Ang II.

Because both tempol and vitamin E have effects that are independent of their oxidant scavenging properties, it is possible that nonantioxidant properties may be responsible for the results observed. However, we consider this improbable because these compounds are unrelated, and to the best
of our knowledge, the only property that they have in common is their antioxidant effect. Indeed, we evaluated their efficacy in reducing oxidative stress by measuring 2 markers of oxidative stress in both the systemic and renal circulation. In the present study, Ang II increased oxidative stress, as suggested by the increase in circulating isoprostanes (as in our previous study) and also by increased TBARS (another marker of oxidant stress). These markers were also elevated in the renal circulation. Tempol and vitamin E were equally effective in their antioxidant properties. In fact, they both prevented the increase in isoprostanes, and ameliorated the increase in TBARS in the systemic and renal circulation. Thus, their effectiveness in lowering blood pressure was associated with decreased oxidant stress.

It is interesting to note that the levels of isoprostanes (but not of TBARS) were higher in the renal than in the systemic circulation, even under control conditions. Despite the puzzling discrepancy between the isoprostanes and TBARS, increased intrarenal isoprostanes has been reported before. Furthermore, certain conditions such as a selenium- and vitamin E–deficient diet and Ang II infusion (as shown in the present study) increase these levels even more. Whether the increased isoprostanes are a result of the high levels of Ang II or whether this suggests that kidneys are more susceptible to oxidative damage remains unknown. However, it is tempting to speculate that because isoprostanes stimulate formation of ET, high renal levels of II isoprostanes during Ang II infusion contribute to the increased renal ET mRNA.

There are several mechanisms by which Ang II–induced oxidative stress may increase blood pressure. First, Ang II induces vascular production of superoxide anion (O2−) via an NADH/NADPH oxidase, and this O2− itself can exert a direct vasoconstrictor effect. Second, O2− can react with NO, thus decreasing the availability of NO. Third, the subsequent lipid peroxidation of arachidonic acid and the formation of F2-isoprostanes, which are not only markers of oxidative stress but also potent vasoconstrictors. Finally, both Ang II and isoprostanes can stimulate the formation of ET and sympathetic nervous system release of norepinephrine, thus promoting a hypertensive effect. Because our previous study found that blocking ET receptors prevented the increase in blood pressure, we currently focused mainly on the possibility that Ang II–induced oxidative stress increased ET, which in turn may contribute to the increased blood pressure. This notion is supported by the fact that Ang II increases renal ET mRNA expression. We found that Ang II elevated the systemic and renal venous plasma levels of ET. The consistent increase in systemic ET observed in the current study was not observed in our previous report. The reason for this disparity remains unclear but may be related to inherent variability between the different sets of rats, or differences in surgical technique (note that the blood samples were collected after the acute experiments, thus the rats were subjected to anesthesia and surgical manipulations). In any event, both tempol and vitamin E blocked the increase in renal ET and had a strong tendency to decrease it in the systemic circulation. Taken together, our results suggest a role for ET in this model of hypertension.

A final important observation of the present study was the transient reduction of urinary nitrite excretion. This occurred before the increase in blood pressure and returned to normal by day 9 (when hypertension was present). The mechanisms for this biphasic response are unknown, but the decrease in nitrite excretion may have been caused by quenching of NO by Ang II–induced O2−, whereas the normalization could be attributed to upregulation of NO synthase either by Ang II or oxidative stress. Regardless of the mechanism, the transient decrease was prevented by either antioxidant. Thus, the net result in the groups treated with tempol or vitamin E is decreased ET levels with preserved NO, perhaps helping preserve the decreased blood pressure.

In summary, subpressor doses of Ang II increases blood pressure, as well as oxidative stress and ET in the systemic and renal circulation. Ang II also causes a transient decrease in urinary nitrite excretion. Antioxidant therapy prevents the increase in ET and blood pressure, and the transient fall in urinary nitrates. Taken together with our previous results, these findings suggest that subpressor dose Ang II increases oxidant stress via its AT1 receptor. The increased oxidative stress may quench NO and increase isoprostane formation, which in turn increases the production of ET, thus leading to the endothelin-dependent hypertension.

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References


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