Antioxidant Treatment Prevents Renal Damage and Dysfunction and Reduces Arterial Pressure in Salt-Sensitive Hypertension

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Abstract—The goal of this study was to test the hypothesis that oxidative stress in Dahl salt-sensitive (SS) rats on a high-sodium intake contributes to the progression of renal damage, the decreases in renal hemodynamics, and the development of hypertension. We specifically studied whether antioxidant therapy, using vitamins C and E, could help prevent renal damage and glomerular filtration rate (GFR) and renal plasma flow reductions and attenuate the increases in arterial pressure. Thirty-three 7- to 8-week old Dahl SS/Rapp strain rats were placed on either a high-sodium (8%) or a low-sodium (0.3%) diet with or without vitamin E (111 IU/d) in the food and 98 mg/d vitamin C in the drinking water for 5 weeks. Rats were equipped with indwelling arterial and venous catheters at day 21. By day 35 in the rats with high-sodium diet, vitamin C and E treatment significantly decreased renal cortical and medullary O2/H2O2 release, mean arterial pressure, urinary protein excretion, glomerular necrosis, and renal tubulointerstitial damage. At this time, GFR significantly decreased in the high-sodium diet group (1.6±0.2 mL/min) when compared with either the high-sodium plus vitamins C and E (2.9±0.2 mL/min) or the low-sodium diet group (2.9±0.3 mL/min). In SS rats on high-sodium diet, renal plasma flow decreased 40%, and this reduced flow was restored by vitamin treatment. In Dahl salt-sensitive hypertension, increased oxidative stress plays an important role in the renal damage, decreases in renal hemodynamics, and increases in arterial pressure that occur. Antioxidant treatment with vitamins C and E improves renal dysfunction, lessens renal injury, and decreases arterial pressure in Dahl salt-sensitive hypertension. (Hypertension. 2005; 45:934-939.)

Key Words: antioxidants ■ hemodynamics ■ hypertension ■ renal disease

Hypertension continues to be a major cardiovascular risk factor and a major contributor to end-stage renal disease (ESRD). In particular, patients with salt-sensitive hypertension are much more likely to experience ESRD compared with salt-insensitive hypertensive patients.1 Recent studies in humans and in animal models of salt-sensitive hypertension indicate that an increase in oxidative stress is associated with a progressive elevation in arterial pressure and a reduction in renal function. However, the mechanisms underlying the progression of hypertension and ESRD in salt-sensitive hypertension are not clear.

A model that closely mimics human salt-sensitive essential hypertension is the Dahl S rat. Common traits shared by salt-sensitive humans and the S rat include progressive increases in arterial pressure and renal damage,23 increased O2/ release,4 and endothelial dysfunction.5 Recently, we have shown that the oxidative stress that occurs in Dahl S rats on high-sodium intake for 3 weeks contributes to the increase in arterial pressure, but renal hemodynamics were unchanged in high-sodium diet rats and renal damage was minor.6 In contrast, renal damage is much more severe in S rats on high-sodium diet for 5 weeks, with progressive declines in glomerular filtration rate (GFR) and renal plasma flow, but the role of oxidative stress in the hypertension decreases in renal hemodynamics and the related renal damage is not clear.

Our goal was to test the hypothesis that oxidative stress in Dahl S rats on a high-sodium intake for 5 weeks contributes to the progression of renal damage, the decreases in GFR and renal plasma flow, and the development of hypertension. We specifically studied whether antioxidant therapy, using vitamins C and E, could help prevent renal damage and decreases in renal hemodynamics and attenuate the increases in arterial pressure.

Methods

Animal Preparation, Experimental Measurements, and Instrumentation

Experiments were conducted in 33 conscious 7- to 8-week-old male Dahl S rats, Rapp strain (Harlan Sprague Dawley; Indianapolis, Ind),
and the local Institutional Animal Committee approved the project. Rats were received when they were 5 to 6 weeks old and were allowed to recover for 1 week before starting 1 of 4 diets. The following 4 groups of Dahl rats were studied: S with high-sodium diet alone (n=9); S with high-sodium diet and vitamins C and E (n=7); S with low-sodium diet alone (n=8); and S with low-sodium diet and vitamins C and E (n=9). During the 5-week experimental period, the high-sodium diet and low-sodium diet alone groups received a high-sodium (8%) or low-sodium (0.3%) diet with tap water, and both diets had an essential 50 IU/kg of vitamin E. The high-sodium plus vitamins C and E and low-sodium plus vitamins C and E groups received a high-sodium (8%) or low-sodium (0.3%) diet that contained vitamin E (5000 IU/kg) and tap water with vitamin C (1 g/L, high-sodium groups; 5g/L, low-sodium groups).

Vitamin C concentrations in the low-sodium group were adjusted because of lower fluid intake in the low-sodium group. Vitamin E intake averaged 112.5 IU/d and 110 IU/d in the high-sodium and low-sodium vitamins C and E groups, respectively. Vitamin C intake averaged 95.8 mg/d and 100.8 mg/d in the high-sodium and low-sodium vitamins C and E groups, respectively.

After 3 weeks on the various diets, chronic arterial and venous catheters were implanted through the femoral artery and vein using aseptic techniques and isoflurane anesthesia (1%). Pulsatile arterial pressure signals were sent to a digital computer at 500 Hz for 4 seconds of each minute throughout the entire 24-hour period and were used to determine arterial pressure and heart rate. All rats were allowed to recover for 4 days before a 10-day (days 26 to 35) collection of arterial pressure and renal functional data began. GFR and effective renal plasma flow were determined at the end of the 5-week period in conscious rats, as we have performed before,6,7 by measuring the iothalamate and aminohippurate concentrations of a 4-hour fasted plasma sample after a 12-hour period of intravenous infusion of 14C Iothalamate and aminohippurate sodium.

At the end of the 5-week period, the rats were anesthetized with isoflurane, and the kidneys were removed for various analyses. Renal cortical and medullary tissues were homogenized with appropriate buffers and protease inhibitors and chemiluminescence was measured using 5 μmol/L lucigenin, as we have performed before.8 The amounts of protein in renal tissues were quantified with the Lowry assay, and urine protein was measured using the Bradford assay.

Analysis of Glomerular Necrosis, Glomerular Sclerosis, and Tubulointerstitial Injury
A PAS-hematoxylin section from each rat was examined for necrotic and sclerotic glomeruli at a magnification of 200x. Sections were scanned in nonoverlapping microscopic fields using a computer monitor, and necrotic and sclerotic glomeruli were counted as a proportion of all consecutively examined glomerular profiles of any diameter.

Tubulointerstitial injury was analyzed using a Masson trichrome-stained section from each rat. At a magnification of 100x, images were taken of consecutive, nonoverlapping fields of subcapsular to juxtamedullary renal cortex over consecutive, nonoverlapping areas moving laterally along the subcapsular surface. Using Adobe Photoshop 5.5, images were imported into a computer with an 88-point counting grid overlay using Image Pro Plus 4.5 Analytical Imaging Software. Tubulointerstitial injury was measured as the proportion of the number of points overlying increased amounts of blue-stained interstitial tissue, dilated cast containing tubules, or tubules showing acute injury divided by the number of points on the grid overlying nonglomerular and nonvascular cortex.

Statistical Analysis
Comparison of data from animals on low-salt and high-salt diets with or without vitamins C and E were first performed using 2-way analysis of variance for repeated measures or a completely randomized analysis of variance followed by a 1-way analysis of variance for each group and a Fisher least significant difference test for post hoc analysis. Differences were considered to be statistically significant if P<0.05. All data are expressed as mean±SE.

Results
Renal Cortical and Medullary \( O_2^- \) Release Responses to Vitamins C and E
Figure 1 shows that vitamins C and E treatment significantly decreased \( O_2^- \) release from renal cortical and medullary tissue in rats on high-sodium diet. In the renal cortex and medulla, the vitamin treatment decreased the \( O_2^- \) release in the high-sodium groups to levels not significantly different from those of the low-sodium group.

Mean Arterial Pressure, Heart Rate, and Urinary Protein Excretion Responses to Vitamins C and E
Figure 2 shows that vitamins C and E treatment significantly decreased arterial pressure in the Dahl S rats on high-sodium diet. The pressures are shown for the past 10 days of the 5-week experiment. On day 35, mean arterial pressure was 180±3 mm Hg in the group on high-sodium diet and 159±5 mm Hg in the high-sodium diet plus vitamin C and E group (P<0.05). Heart rate decreased significantly on days 31 to 34 only in the high-sodium plus vitamin group when compared with the high-sodium alone group.

Figure 2 also shows that by day 25, the urinary protein excretion, an index of renal damage, significantly decreased in the high-sodium plus vitamin group compared with the high-sodium group. By day 35, urine protein excretion averaged 285±20 mg/d in the high-sodium group and 178±36 mg/d in the high-sodium plus vitamins C and E group (P<0.05).
Renal Hemodynamic Responses to Vitamins C and E

Figure 3 illustrates that GFR significantly decreased in the high-sodium group (1.6 ± 0.2 mL/min) when compared with either the high-sodium plus vitamins C and E (2.9 ± 0.2 mL/min) or the low-sodium group (2.9 ± 0.3 mL/min). The data show that antioxidant treatment with vitamins C and E prevented a decrease in GFR in rats on high-sodium intake. Effective renal plasma flow also significantly decreased in the high-sodium group (6.3 ± 0.6 mL/min) when compared with either the high-sodium plus vitamins C and E group (10.1 ± 0.9 mL/min) or the low-sodium group (11.1 ± 1.0 mL/min).

Histological Analyses of Kidneys

Figure 4 shows representative kidney sections for each group of rats. In the low-sodium group (Figure 4A) and low-sodium plus vitamins C and E group (Figure 4B), the kidneys had a normal appearance with only occasional small globally sclerotic glomeruli in the subcapsular cortex.

In the rats on high-sodium diet (Figure 4C), the kidneys revealed fibrinoid arterial and arteriolar necrosis and fibrinoid glomerular necrosis. Fibrinoid glomerular necrosis was commonly encountered in continuity with necrosis of afferent arterioles. Occasional glomeruli at all levels of the cortex demonstrated global or segmental glomerular sclerosis. The tubules contained degenerate cells, cell debris, and red blood cells with interstitial hemorrhage adjacent to necrotic arterioles and glomeruli.

In the rats on high-sodium plus vitamins C and E diet (Figure 4D), the kidneys had only occasional glomeruli that showed fibrinoid glomerular and arteriolar necrosis. In addition to the small globally sclerotic glomeruli in the subcapsular cortex, which were found in rats on low-sodium diet, glomeruli were found at all levels of the cortex, which demonstrated segmental and global glomerular sclerosis. Tubulointerstitial injury, which was much less prevalent than in the rats on high-sodium diet, consisted of clusters of dilated tubules with thickened basement membranes that were surrounded by increased amounts of interstitial connective tissue.

Average Glomerular and Tubulointerstitial Damage Responses to Vitamins C and E

Figure 5 shows that the average glomerular necrosis significantly increased in the Dahl S rats on high-sodium diet when compared with either the high-sodium plus vitamin group or the low-sodium group. The vitamins C and E treatment significantly decreased the amount of glomerular necrosis in the Dahl S rats on a high-sodium intake. Glomerular sclerosis, although present to a degree in all rats, was not significantly different in any group. Segmental sclerosis was present in the high-sodium group and high-sodium plus vitamins C and E groups, but not to the degree that caused statistical significance for total sclerosis. Figure 5 also shows that the average tubulointerstitial damage averaged 0.19 ± 0.03 (fractional area) in the high-sodium group, and this area decreased to 0.11 ± 0.02 in high-sodium rats treated with vitamins C and E. Data concerning renal SOD activity, food and water intake,
and body and kidney weights can be found in the online supplement (http://hyper.ahajournals.org).

Discussion

Treatment of Dahl salt-sensitive rats with vitamins C and E significantly reduced renal oxidative stress. The vitamin treatment effectively increased GFR and renal plasma flow, markedly decreased renal damage, and decreased arterial pressure. These data suggest that oxidative stress is one of the major contributors to renal dysfunction and damage in salt-sensitive hypertension. The data also indicate that contributing mechanisms to Dahl salt-sensitive hypertension are decreases in GFR and renal plasma flow.

We have previously investigated the role of oxidative stress in Dahl S rats after 3 weeks on a high-sodium diet compared with 5 weeks of high-sodium in the present study. The S rats after 3 weeks of high-sodium had elevated blood pressure, but neither GFR nor renal plasma flow decreased, and there was only a small amount of renal damage as assessed by histological methods. In contrast, in the present study we found nearly a 50% reduction in GFR in the high-sodium Dahl S group after 5 weeks on the high-sodium diet when compared with the low-sodium control group (filtration fraction was 26% in both groups). Renal plasma flow also decreased ~40% in the S rats on high-sodium diet, and Figure 4 shows the severity of the pathological damage to the kidneys. ESRD in humans is associated with progressive decreases in GFR as was found in our present study. A recent study gave vitamin E and high-sodium to Dahl S rats for 2 weeks and found decreased systolic pressures (measured by tail-cuff), and both GFR and renal plasma flow, measured under anesthesia, decreased. Filtration fraction in these anesthetized rats ranged from 50% to 63%. In contrast, in a previous study in our laboratory, Dahl S rats on high-sodium diet for 2 or 3 weeks did not have a change in GFR or renal plasma flow, and filtration fraction was 27%. Treatment of the high-sodium S rats in the present study with vitamins C and E prevented the precipitous decreases in GFR and renal plasma flow. This suggests that increases in reactive oxygen species are associated with decreases in renal hemodynamics in salt-sensitive hypertension.

Several factors could have contributed to the decreases in GFR and renal plasma flow. First, it is evident in Figure 4 that a high-sodium diet results in glomerular and vascular necrosis. This could directly cause a decrease in the number of functioning nephrons, with its associated vasculature thus decreasing GFR and renal blood flow. Second, salt-sensitive hypertension has been associated with increases in vasocon-
strioters. In a previous study in our laboratory, an increased sodium diet in Dahl S rats for 3 weeks caused an increase in urinary and plasma F$_2$-isoprostanes, which can cause vasoconstriction. Other studies have shown that a high-sodium diet is associated with increased intrarenal angiotensin II, which also can result in renal vasoconstriction. Third, increased renal O$_2^-$ release inactivates nitric oxide, which will decrease renal vasodilation, resulting in increased vasoconstriction.

Renal damage in the rats on high-sodium diet in the present study was evidenced by proteinuria and renal morphological changes. The decreases in urinary protein excretion and the improvement in renal pathology in vitamin-treated Dahl S rats on high-sodium intake suggest a major role of oxidative stress in the advancement of nephropathy in salt-sensitive hypertension. For many years, it has been believed that renal damage in hypertension is directly caused by exposure of the kidney to high pressure. However, in the present study our data indicate that the improvement in renal dysfunction in the high-sodium plus vitamin group could have been caused by either a decrease in arterial pressure or a reduction in free radicals in the kidney, or a combination of the 2 effects. The decrease in arterial pressure in Dahl S rats on high-sodium and vitamins intake was $\approx$20 mm Hg compared with the high-sodium rats. Thus, the mean arterial pressure of the vitamin-treated rats remained elevated at $\approx$160 mm Hg. However, the renal cortical and medullary O$_2^-$ release significantly decreased in the high-sodium plus vitamins C and E group. Because the decrease in arterial pressure was only moderate in the rats treated with vitamins and high-sodium diet, it is possible that the reduction in O$_2^-$ release played an important role in the improvement in renal dysfunction and damage. However, the decrease in arterial pressure could have also lessened renal damage and dysfunction.

The high-sodium and the high-sodium plus vitamins E and C animals revealed contrasting pathological changes. The high-sodium rats had the renal changes of florid malignant hypertension with acute tubular injury, in which the tubular injury seemed to primarily occur adjacent to necrotic glomeruli in the vascular supply of necrotic and thrombosed arteries and arterioles. The kidneys of the rats on high-sodium diet plus vitamins C and E showed some evidence of malignant hypertension but at a much less intense level than the animals on high-sodium intake alone. In the high-sodium plus vitamin C and E group, the histopathology was shifted toward glomerulosclerosis and arterial intimal hyperplasia rather than glomerular and arterial necrosis, and tubulointerstitial injury appeared more indolent than in animals on high-sodium intake.

Several investigators have studied the roles of vitamin C and E alone or in combination in preventing renal damage or increases in blood pressure in rats. We used these studies to choose doses of the vitamins in our study with a goal of reducing renal damage and arterial pressure. Vitamin C reduced elevated arterial pressure in Sprague-Dawley rats on a high-sodium diet (100 mg/kg per day of vitamin C) and in SHR on a high-sodium diet (200 mg/d of vitamin C). In aging rats, vitamin E at 5000 IU/kg in food, which increased plasma vitamin E by 50%, increased GFR and decreased F$_2$-isoprostanes in renal tissue. Either vitamin C (1000 mg/d) or vitamin E (1000 IU/d) decreased arterial pressure and increased vascular SOD activity in stroke-prone spontaneously hypertensive rats. Renal injury and serum lipid peroxides decreased, but systolic pressure, measured by tail-cuff, did not change in Dahl S rats fed an 8% sodium diet with 750 IU/kg of vitamin E added over a 4-week period. In contrast, in the present study, arterial pressure, measured continuously, decreased significantly in the S rats treated with vitamins high-sodium diet. In this study, we added vitamin C to the drinking water (average of 98 mg/d = $\approx$280 mg/kg per day) and vitamin E in the food (111 IU/d = $\approx$317 IU/kg per day), which are lower than most of the aforementioned studies, but our objectives were reached in that renal damage and arterial pressure decreased. The experimental studies on animals that showed significant effects of vitamin E used much higher doses than the typical clinical study. One reason is that the basal dietary requirement vitamin E in rats is 7-fold higher than in humans. This basal level of vitamin E in rats is $\approx$3 IU/kg per day, as recommended by the American Institute of Nutrition, and the recommended daily amount of vitamin E in humans is 30 IU/d or 0.43 IU/kg per day. In a new study in hypercholesterolemic patients, significant decreases in plasma isoprostane were realized only when vitamin E intake was increased 25- to 100-fold over the recommended daily amount. In the present study, vitamin E intake was increased $\approx$100-fold over the basal recommended intake in rats.

The major cellular sources of reactive oxygen species are NADH/NADPH oxidases, xanthine oxidase, nitric oxide synthase, cyclooxygenase, lipooxygenase, and the mitochondrial respiratory chain enzymes. Vascular NADPH oxidase activity increased in stroke-prone spontaneous hypertensive rats, and NADPH oxidase activity decreased after treatment of these rats with vitamin E or vitamin C. Renal xanthine oxidase in Dahl S rats increased during high-sodium intake, and inhibition of xanthine oxidase activity with tungsten decreased arterial pressure.

In summary, vitamins C and E supplements to Dahl S rats on a high-sodium diet decreased arterial pressure moderately, decreased renal oxidative stress and renal damage, and increased renal hemodynamics. Renal damage in the high-sodium Dahl S rats included significant renal interstitial damage and glomerular and arterial necrosis with thrombosed arteries and arterioles. The histopathologic changes in the high-sodium Dahl S rats with vitamins C and E supplements showed significant decreases in interstitial, glomerular, and vascular damage and a shift from glomerular necrosis to a more stable glomerulosclerosis. The decreases in GFR and renal plasma flow were prevented with the vitamins C and E supplements. Renal oxidative stress in salt-sensitive hypertension plays a major role in exacerbating nephropathy and decreasing renal hemodynamics.

Perspectives
Several clinical trials have been performed to determine if vitamin treatment can improve cardiovascular disease. Although variable results have been found, some studies have shown that treatment of hypertensive patients with vitamin C lowers blood pressure. Most clinical studies on vitamin E used doses $\leq$400 IU/d, and no reduction in cardiovascular
risk has been noted; however, when 800 IU/d of vitamin E was used, significant decreases in F2-isoprostanes, but doses of 800 IU/d to 3200 IU/d caused significant reductions in F2-isoprostane levels. A second reason why vitamin E was ineffective in some clinical studies is that the patients had pre-existing conditions when they entered the studies. A third reason why vitamin therapy is ineffective in clinical studies is that the patients had pre-existing conditions when they entered the studies.

Acknowledgments
This research was supported by grant HL-51971 from the National Heart, Lung, and Blood Institute.

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Hypertension. 2005;45:934-939; originally published online April 18, 2005;
doi: 10.1161/01.HYP.0000160404.08866.5a
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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