Vitamin E ameliorates enhanced renal lipid peroxidation and accumulation of F_2-isoprostanes in aging kidneys

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Departments of 1Physiology and Biophysics and 2Nephrology, University of Mississippi Medical Center, Jackson, Mississippi 39216; 3Department of Pathology, Johns Hopkins University, Baltimore, Maryland 21205; 4Department of Medicine, Surgery and Pathology, Columbia University, College of Physicians and Surgeons, New York, New York 10032; and 5Department of Pharmacology, Vanderbilt University, Nashville, Tennessee 37232

Reckelhoff, J. Anne F., Vijaya Kaji, Lorraine C. Racusen, Ann Marie Schmidt, Shu Du Yan, Jason Morrow, L. Jackson Roberts II, and Abdulla K. Salahudeen. Vitamin E ameliorates enhanced renal lipid peroxidation and accumulation of F_2-isoprostanes in aging kidneys. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R767–R774, 1998.—Aging results in progressive glomerular sclerosis and reductions in glomerular filtration rate (GFR). Oxidative stress may be an important mechanism for the aging process, but to date the role of oxidative stress on renal aging has not been determined. The present study was performed to determine whether age-related alterations in renal hemodynamics and morphology were associated with oxidative stress and whether this could be attenuated by chronic administration of vitamin E. Rats, aged 13 mo, were given either control diet containing vitamin E 50 IU/kg (n = 6) or a high-vitamin E diet (5,000 IU/kg; n = 6) for 9 mo. Another group of rats (3–4 mo old; n = 7) served as young controls. Aging was accompanied by a 60% reduction in GFR, a threefold increase in renal F_2 isoprostanes, newly discovered vasoconstrictive F_2-like prostaglandins generated by free radical-mediated lipid peroxidation. Renal aging was also associated with an increase in oxidant-sensitive heme oxygenase, advanced glycosylation end products (AGE), and the AGE receptor, RAGE. AGE-RAGE interaction has been shown to induce oxidative stress. With high-vitamin E diet, GFR was increased by 50%, F_2 isoprostanes were suppressed, and expression of heme oxygenase and RAGE was attenuated. There was also a tendency for glomerular sclerosis to be attenuated. These data demonstrate that age-related decline in renal function is accompanied by oxidative stress and that administration of antioxidants, such as vitamin E, could attenuate the decline in renal function.

Several indexes of lipid peroxidation can be measured, such as formation of isoprostanes; increases in advanced glycosylation end products (AGEs); an increase in the receptor for AGEs, RAGE; and induction of heme oxygenase. Whether these parameters are changed in the kidney with aging is currently not known.

The formation of isoprostanes, which are prostaglandin-like compounds that are produced nonenzymatically by free radical-catalyzed peroxidation of arachidonyl lipids, has been shown to be a consequence of cellular lipid peroxidation (10–12, 20). Infusion of isoprostanes causes significant renal vasoconstriction (10, 11, 23) and thus could potentially account for the reduction in GFR and renal plasma flow associated with aging.

Aging is also associated with formation of advanced glycosylation end products (AGEs) due to the interaction of glucose with proteins and lipids such that crosslinking occurs (26). Oxidative stress has been suggested to occur as a consequence of the interaction between AGEs and the AGE receptor, RAGE (24). Therefore, an increase in AGEs and their interaction with RAGE could be a potential mechanism for age-related tissue injury, in part through the free radical-mediated mechanism.

Induction of heme oxygenase, the rate-limiting enzyme in the degradation of heme, has been noted to occur in several models of oxidative tissue injury, including the kidney, and has been suggested as one of the cellular defense mechanisms against oxidative tissue injury (1, 14, 22). However, little is known about the induction of heme oxygenase in aging-related renal injury.

The present study, therefore, was performed with the following objectives: 1) to determine whether age-related renal functional and structural changes are associated with evidence for oxidative stress, assessed by induction of oxidant-sensitive heme oxygenase and increased renal isoprostane levels and changes in the renal expression of AGEs and RAGE, and 2) to determine whether long-term dietary administration of the antioxidant vitamin E could attenuate oxidative stress and thereby preserve renal function and structure in the aging rat.

MATERIALS AND METHODS

Rats

Male Sprague-Dawley rats, aged 13 mo, and young rats, aged 3–4 mo, were purchased from Harlan Sprague Dawley

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Indianapolis, IN) and maintained on standard rat diet (Teklad, Harlan SD) in a room with a 12:12-h light-dark cycle.

Experimental Design

Rats were divided into three groups: group 1, young rats, aged 3–4 mo (n = 7), maintained on standard rat chow containing a normal essential amount of vitamin E (50 IU/kg diet); group 2, middle-aged rats, aged 13 mo, given standard rat chow containing a normal essential amount of vitamin E (50 IU/kg diet, n = 6); and group 3, middle-aged rats maintained on rat chow containing a high amount of vitamin E (5,000 IU/kg diet, n = 6). Rats in groups 2 and 3 received the diets for 9 mo, until 22 mo of age.

Before the beginning of the high-vitamin E diet and at 2, 4, 6, and 9 mo during the dietary study, rats were placed in metabolism cages and urine was collected for 24 h for measurement of urinary protein excretion. After the last urine collection was made, rats were allowed to recover for 24 h before renal function was measured.

Protein Assays

Urinary protein was measured using a commercially available dye reagent according to the Bradford method (Bio-Rad, Richmond, CA) and bovine serum albumin as the standard (15). Data are expressed as milligrams protein excreted per 24 h.

Renal Function Studies

Renal function was measured in rats in groups 2 and 3 at 22 mo of age and in rats in group 1 at 3–4 mo of age. Rats were anesthetized by intraperitoneal injection of the thio-barbiturate Inactin (100–110 mg/kg body wt; A. Lockwood, Sturtevant, WI) and placed on a temperature-regulated surgery table to maintain rectal temperature at 36–38°C. Catheters were placed in the femoral artery (for continuous monitoring of blood pressure and for blood sampling) and in the femoral vein for infusion of isoncotic artificial rat plasma (2.5 g/dl bovine immunoglobulin, 2.5 g/dl bovine serum albumin in Ringer solution) at 12.5 ml·kg⁻¹·h⁻¹ for 45 min during the preparatory surgery and thereafter at 1.5 ml·kg⁻¹·h⁻¹ throughout the experimental period to maintain an euolemic preparation (5, 17). A catheter was placed in the left jugular vein for infusion of 0.9% saline with or without [³H]inulin (15–20 µCi/ml, 0.9% saline; New England Nuclear, Wilmington, DE) at 1 ml/h. A tracheostomy was performed. A midline abdominal incision was then made, and a catheter was placed in the left ureter for collections of urine samples into oil in graduated glass tubes. The left renal vein was cannulated in the retrograde position with a 23-gauge needle connected to PE-50 tubing to be used for blood sampling.

Following a 40-min equilibration period for [³H]inulin infusion, two 30-min urine collections were made and midpoint arterial and renal venous blood samples were also taken. After the experiment, the left kidney was removed, weighed, and placed in 10% buffered Formalin for morphological studies. The right kidney was snap frozen in liquid nitrogen for determination of malondialdehyde and F₂ isoprostane levels.

Analytic methods and calculations. Samples of urine (1 µl) and femoral arterial and renal venous plasma (5 µl) were counted by liquid scintillation counting (Beckman LS 6500). These measurements allowed for the calculation of GFR, renal plasma flow, and renal vascular resistance (RVR), using standard equations (16).

Measurement of Plasma Vitamin E Concentration

Vitamin E levels were measured by Tsien's modification of Emmerie-Engel method based on a colorimetric reaction (21). Briefly, tocopherol was extracted from the plasma with n-heptane in the presence of ethanol. Bathophenanthroline ferric chloride was added, followed by orthophosphoric acid. Absorbance was read at 534 nm. α-Tocopherol was used as standard.

Measurement of Kidney and Plasma Malondialdehyde

Evidence for lipid peroxidation in the renal cortical homogenate was assessed by measuring malondialdehyde in the supernatant as thiobarbituric acid-reactive substances by colorimetric thiobarbituric acid reaction (19). Reaction was carried in the presence of EDTA (3 mM) and antioxidant butylated hydroxytoluene (BHT; 2.5 µl of 2%/ml), and the absorbance was measured at 532 nm. Plasma samples were treated first with sodium dodecyl sulfate and acetic acid followed by the thiobarbituric acid reaction in the presence of EDTA and BHT.

Fig. 1. Body weights (A) and urinary protein excretion (B) in male Sprague-Dawley rats, aged 13 to 22 mo, while on control diet (solid bars) or high-vitamin E diet (open bars). Rats began the diets at 13 mo of age and were treated for 9 mo. *P < 0.05 compared with old untreated rats at 13 mo of age; §P < 0.05 compared with old rats at 13 mo of age before diet was started.
Table 1. Body weights, kidney weights, and systemic and renal hemodynamics in untreated young rats, aged 3–4 mo, and in untreated old control rats, aged 22 mo, or old rats treated with high-vitamin E diet for 9 mo

<table>
<thead>
<tr>
<th></th>
<th>Body Wt, g</th>
<th>Kidney Wt, g</th>
<th>Hct, g%</th>
<th>MAP, mmHg</th>
<th>GFR, ml/min</th>
<th>GFR/g Kidney Wt, ml·min⁻¹·g⁻¹</th>
<th>RPF, ml/min</th>
<th>RPF/g Kidney Wt, ml·min⁻¹·g⁻¹</th>
<th>FF</th>
<th>RVR, mmHg·min⁻¹·ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (n = 7)</td>
<td>379 ± 6</td>
<td>1.3 ± 0.1</td>
<td>49 ± 1</td>
<td>103 ± 3</td>
<td>1.27 ± 0.09</td>
<td>1.00 ± 0.07</td>
<td>5.03 ± 0.86</td>
<td>3.94 ± 0.67</td>
<td>0.27 ± 0.03</td>
<td>10.99 ± 1.03</td>
</tr>
<tr>
<td>Old controls (n = 6)</td>
<td>536 ± 20*</td>
<td>2.1 ± 0.1*</td>
<td>44 ± 1*</td>
<td>123 ± 6*</td>
<td>0.94 ± 0.13</td>
<td>0.44 ± 0.05*</td>
<td>4.21 ± 0.73</td>
<td>1.97 ± 0.31</td>
<td>0.24 ± 0.02</td>
<td>19.28 ± 4.25</td>
</tr>
<tr>
<td>Old + vitamin E (n = 6)</td>
<td>536 ± 10*</td>
<td>2.2 ± 0.1*</td>
<td>44 ± 1*</td>
<td>117 ± 4*</td>
<td>1.37 ± 0.14*</td>
<td>0.64 ± 0.06*</td>
<td>5.96 ± 0.91</td>
<td>2.74 ± 0.36</td>
<td>0.25 ± 0.04</td>
<td>12.33 ± 2.13</td>
</tr>
</tbody>
</table>

Values are means ± SE. Hct, hematocrit; MAP, mean arterial pressure; GFR, glomerular filtration rate; GFR/g, GFR factored for kidney wt; RPF, renal plasma flow; RPF/g, RPF factored for kidney wt; FF, filtration fraction; RVR, renal vascular resistance. *P < 0.05 compared with value in young rats; †P < 0.05 compared with old untreated rats.

Measurement of Renal F₂ Isoprostanes

Kidneys were snap frozen in liquid nitrogen and kept frozen at −70°C for determination of F₂ isoprostane levels. Levels of F₂ isoprostanes esterified to kidney lipids were measured as free F₂ isoprostanes after extraction of lipids from a kidney homogenate, saponification, purification, and derivatization using gas chromatography/negative ion-chemical ionization-mass spectrometry with [2H₄]-prostaglandin F₂ as the internal standard, as described previously (13).

Morphological Studies

Morphology was performed on kidneys from old rats. Kidneys were fixed by placing them in 10% buffered Formalin. For light microscopy, kidneys were sectioned and embedded in paraffin. Paraffin blocks were serially sectioned into 3-μm sections and placed on slides. Slides were stained with periodic acid Schiff reagent. More than 200 glomeruli per section were evaluated by a blinded observer for the presence of sclerosis and graded according to the percentage of each glomerulus undergoing sclerosis (0, 1–25%, 26–50%, 51–75%, 76–100%). The percentage of glomeruli undergoing the levels of sclerosis were determined and averaged for a group. Differences between vitamin E-treated and untreated groups were determined by t-test.

Immunohistochemical Detection of Heme Oxygenase-1, AGEs, and RAGE

Immunohistological studies were performed on paraffin sections of Formalin-fixed kidneys as indicated above. Detection of heme oxygenase-1 was performed with anti-heme oxygenase-1 immune serum (1:100; Stress Gen Biotechnologies, Victoria, BC, Canada) (28). Detection of AGEs was performed using affinity-purified anti-AGE immunoglobulin G (IgG), and detection of RAGE was performed using a polyclonal, monospecific anti-RAGE IgG, as previously described (3). We have previously shown that normal bovine glomerular capillary does not stain positive for RAGE. However, mesangial cells weakly stain (3). Sites of binding of primary antibodies against heme oxygenase, AGE, and RAGE were visualized using the avidin-biotin peroxidase method according to the manufacturer’s instructions (3) (Sigma, St. Louis, MO).

Statistical Analyses

The data were analyzed by analyses of variance, as appropriate, using Statview 512 software for the Macintosh. Significance was defined as P < 0.05. All data values are expressed as means ± SE.
lower in the vitamin E-treated old rats than in the untreated old rats, although the difference was not statistically significant.

Absolute GFR was not different between old and young rats, but when factored for kidney weight, GFR in old untreated rats was 50% lower than in young rats (Table 1). In old rats given the high-vitamin E diet, absolute GFR was increased by 47% compared with old untreated rats, levels that were not different from young rats. When factored for kidney weight, GFR
increased by 50% compared with old untreated rats, but not to the level found in young rats.

Absolute renal plasma flow tended to be reduced in the old untreated rats compared with the young rats (Table 1); however, when factored for kidney weight, renal plasma flow was significantly reduced. In old rats fed the high-vitamin E diet, absolute renal plasma flow and renal plasma flow factored for kidney weight tended to be higher than in old rats, but not significantly so, and were similar to values in young rats.

RVR (Table 1) tended to be increased in the old rats fed a normal diet, and vitamin E reduced RVR to the same level as in the young control rats. However, none of the values for the three groups was statistically different from the others.

**Effect of Vitamin E on the Parameters of Oxidative Stress**

As shown in Fig. 2, aging of the kidney was associated with a threefold increase in levels of F2 isoprostanes esterified in the kidney, and long-term vitamin E administration reduced F2 isoprostanes to levels found in young rats.

Consistent with the effects of vitamin E on F2 isoprostanes, renal tissue malondialdehyde levels were attenu-

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**Fig. 3.** Immunohistochemical detection of advanced glycosylation end products (AGEs), AGE receptor (RAGE), and heme oxygenase in kidneys and vascular sections from young rats, aged 3–4 mo, and from old rats, aged 22 mo, given either a control diet or a high-vitamin E diet for 9 mo. A–I are representative glomerular sections; J–R are representative vascular sections. Sections from young rats are in the first panel of each group (A, D, G, J, M, and P). Sections from old untreated rats are in the second panel in each group (B, E, H, K, N, and Q). Last panels in each group are sections from old rats that received high-vitamin E diet (C, F, I, L, O, and R). Immunohistochemical detection of AGEs in glomerular sections is shown in A, B, and C and in vascular sections, J, K, and L. Immunohistochemical detection of RAGE in glomerular sections is shown in D, E, and F and in vascular sections, M, N, and O. Immunohistochemical detection of heme oxygenase in glomerular sections is shown in G, H, and I and in vascular sections, P, Q, and R. Bar, 18 µm.
ated with high-vitamin E administration (old: 3.00 ± 0.26; old + vitamin E: 1.92 ± 0.14 nmol malondialdehyde/mg protein).

As shown in Fig. 3, AGEs increased in the kidney with aging and were only minimally affected by long-term antioxidant treatment. On the other hand, aging was also strongly associated with increased immunostaining for RAGE, which was suppressed in glomerular and vascular sections from old rats given the high-vitamin E diet. Heme oxygenase was clearly induced with aging and was confined mainly to the tubulointerstitial region. Vitamin E treatment attenuated heme oxygenase staining in both glomeruli and vascular sections.

Effect of Long-Term Vitamin E on Age-Related Glomerular Sclerosis

High-vitamin E diet tended to reduce the number of glomeruli exhibiting age-related sclerotic injury, but the improvement was not statistically significant (Fig. 4).

DISCUSSION

The results demonstrate that aging-related changes in the rat kidney are accompanied by oxidative stress and lipid peroxidation and that these changes can be partly prevented with the administration of vitamin E. The occurrence of oxidative stress in the aging kidney is supported by the findings of significantly increased renal levels of four macromolecules associated with, or the consequence of, lipid peroxidation: isoprostanes, heme oxygenase, AGE, and RAGE. Because renal function was improved by antioxidants, the data also suggest that the oxidative stress and the attendant lipid peroxidation may contribute to the process of aging-induced renal injury. The effect of vitamin E to inhibit lipid peroxidation affected renal function more than structural changes, which may suggest an important role for the vasoactive products of lipid peroxidation, such as isoprostanes, in the age-related reduction in renal function.

When infused into the kidney, the isoprostanes, 8-iso-prostaglandin F$_2$ and 8-iso-prostaglandin E$_2$, increase preglomerular resistance and decrease GFR (23). The receptor mediating the vasoconstriction was thought to be the thromboxane receptor (23, 29), but may in fact be novel (29). In the present study we hypothesized that if isoprostanes were increased in the kidney, this may be a mechanism to explain the decreased GFR associated with aging. However, despite the fact that vitamin E reduced renal F$_2$ isoprostane levels to those found in young rats, GFR was only improved by 50% and glomerular injury was not affected significantly. Thus, although the vasoconstrictor isoprostanes may play a role in mediating aging glomerular injury, other mechanisms, either other vasoconstrictors or other cellular mechanisms, must be involved in addition. Future studies will be necessary to determine the exact role the vasoconstrictor effects of isoprostanes have in mediating aging glomerular injury by assessing whether isoprostane receptor antagonists, when available, would be protective against aging injury.

AGE levels are reported to be modestly elevated in aging tissues and are considered to be associated with a variety of toxic effects, including crosslinking of long-lived proteins; induction of genes for growth factors, extracellular matrix proteins, and inflammatory cytokines; and quenching nitric oxide (25). The cellular and molecular mechanisms responsible for some of the age-mediated cellular effects may involve the interaction of AGEs with their best-characterized receptor, RAGE. Our new observation that aging is accompanied by an increase in renal RAGE expression is consistent with a role for AGE-RAGE interaction in the aging process, and this notion is further strengthened by the reduction in the overexpression of RAGE with vitamin E. This finding suggests that the cellular redox state may play a regulatory role in the expression of RAGE.

When factored for GFR, urinary protein excretion was lower in the high vitamin E-treated old rats than in the untreated old group. These data suggest that vitamin E may have had an effect to reduce glomerular pressure. The mean arterial pressures tended to be

![Fig. 4. Number of glomeruli examined that exhibited sclerosis (A) and the graded progressive glomerular sclerosis (B) in kidneys from old rats and old rats given high-vitamin E diet for 9 mo. *P < 0.05, old + vitamin E compared with old untreated rats.]
lower in the vitamin E-treated rats than in the old controls, but the differences were not statistically signif-icant. However, the fact that morphological changes in the kidneys were similar, as were the filtration fractions, between the aging groups tends to disprove this hypothesis. Thus we have no data to verify the hypothesis that the proteinuria was low in the high-vitamin E diet rats because of lower glomerular pres-sure. It is possible, however, that vitamin E could have reduced proteinuria by affecting other mecha-nisms, such as by changing the ionic charge of the glomerular extracellular matrix, which would change protein trafficking. Studies by Baylis and colleagues (2) have shown that the proteinuria of aging is mediated by increases in glomerular permeability and by loss of fixed glomerular polyanion. Thus future studies will be necessary to determine the exact role that antioxidants may play in reducing proteinuria with aging.

It is surprising that long-term antioxidant treatment did not protect the glomerulus from injury, as evidenced by our morphological data. We had predicted that if renal function was protected, the mechanism would be via protection against aging injury. The data we have accumulated in these studies reinforce that with aging there is a complex set of mechanisms that must be intervened on to completely prevent the aging injury and loss of renal function.

In summary, F2 isoprostanes and RAGE expression increased in the male rat kidney with aging, and long-term dietary supplementation with the antioxi-dant vitamin E attenuated the increase. GFR was improved by 50% with vitamin E, and RVR was returned to the value found in young rats. Renal mor-phology was only marginally affected by vitamin E. These data suggest that, in aging, the renal vasoconstriction is partly mediated by the increase in free radical-mediated lipid peroxidation product, F2 isoprostanes, and that the AGE-RAGE pathway may be active.

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