Cho K, Kim H, Rodriguez-Iturbe B, Vaziri ND. Niacin ameliorates oxidative stress, inflammation, proteinuria, and hypertension in rats with chronic renal failure

Am J Physiol Renal Physiol 297: F106–F113, 2009. First published May 6, 2009; doi:10.1152/ajprenal.00126.2009.—Significant reduction of renal mass causes progressive deterioration of renal function and structure which is mediated by systemic and glomerular hypertension, hyperfiltration, oxidative stress, inflammation, and dyslipidemia. Niacin is known to improve lipid metabolism and exert antioxidant/anti-inflammatory actions. Therefore, we considered that niacin supplementation may attenuate oxidative stress, inflammation, and tissue injury in the remnant kidney. To this end, 5⁄6 nephrectomized [chronic kidney disease (CKD)] rats were randomly assigned to niacin-treated (50 mg·kg⁻¹·day⁻¹ in the drinking water for 12 wk) and untreated groups. Sham-operated rats served as controls. The untreated CKD rats exhibited azotemia, hypertension, hypertriglyceridemia, and proteinuria. The present study was designed to test this hypothesis.

Several studies have shown that dyslipidemia promotes (1, 6, 15, 23) and lipid-lowering strategies retard progression of renal disease in humans (4, 17) and in experimental animals (28, 40, 44). Niacin has been widely used in the management of dyslipidemia and atherosclerotic coronary heart disease (22). In addition to its lipid-lowering actions, niacin possesses potent antioxidant and anti-inflammatory properties (8).

Given the role of oxidative stress, inflammation, and dyslipidemia in progression of renal disease and the demonstrated efficacy of niacin in ameliorating these abnormalities, we hypothesized that its long-term supplementation may retard progression of renal disease following subtotal nephrectomy. The present study was designed to test this hypothesis.

METHODS

Study group. Male Sprague-Dawley rats with an average body weight of 225–250 g (Harlan Sprague-Dawley, Indianapolis, IL) were used in this study. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of California (Irvine, CA). Animal were housed in a climate-controlled vivarium with 12:12-h light-dark cycle and fed a standard laboratory diet (Purina Mills, Brentwood, MO) and water ad libitum. The animals were randomly assigned to the CRF and sham-operated control groups. The CRF group underwent 5⁄6 nephrectomy (%Nx) by surgical resection of the upper and lower thirds of the left kidney, followed by a right nephrectomy 7 days later. The control group underwent sham operation. The procedures were carried out under general anesthesia (pentobarbital sodium, 50 mg/kg ip) using strict hemostasis and aseptic techniques. The %Nx animals were randomized to untreated and niacin-treated subgroups. The niacin-treated group was given niacin (50 mg·kg⁻¹·day⁻¹, dissolved in the drinking water) for 12 wk. The untreated group received regular water. Six animals were included in each group.

The animals were placed in individual metabolic cages for a timed urine collection at baseline, week 8, and week 12. Urine protein concentration (Chondrex, Redmond, WA) and specific gravity were determined in 24-h urine collections. Blood pressure was determined by tail-cuff plethysmography (CODA2, Kent Scientific, Torrington, CT). Conscious rats were placed in a restrainer on a warming pad and allowed to rest inside the cage for 15 min before blood pressure
Then overnight in the same buffer containing the given antibodies. The membrane was washed three times for 5 min in 1× TBS, 0.05% Tween 20 before a 2-h incubation in a buffer (1× TBS, 0.05% Tween 20, and 3% nonfat milk) containing horseradish peroxidase-linked anti-rabbit IgG and anti-mouse IgG (Santa Cruz Biotechnology), at 1:1,000 dilution. The membrane was washed four times and developed by autoluminography using ECL chemiluminescent agents (Amersham Life Science). Optical densities of protein bands were determined by a laser densitometer (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Histology. Light microscopy was done in the formalin-fixed sections stained with periodic acid-Schiff and hematoxylin and eosin. Glomerulosclerosis was graded by a score index used in our previous studies (31); glomeruli were graded from 0 to +4 (0 = normal, 1 = <25% involvement of the glomerular tuft, 2 = 25–50% involvement of the glomerular tuft, 3 = 50–75% involvement of the glomerular tuft, and 4 = sclerosis occupying >75% of the glomerular tuft. The glomerulosclerosis score was obtained as follows: ([1 × number of glomeruli with +1] + [2 × number of glomeruli with +2] + [3 × number of glomeruli with +3] + [4 × number of glomeruli with +4]) × 100/total number of glomeruli examined.

Tubulointerstitial damage was graded according the extension (%) of tubular damage (infiltration, fibrosis, tubular dilatation/atrophy) in

measurements were taken. Rat tails were placed inside a tail cuff, and the cuff was inflated and released several times to allow the animal to be conditioned to the procedure. At the end of the experiment, animals were anesthetized (sodium pentobarbital, 50 mg/kg ip) and euthanized by exsanguinations using cardiac puncture. The kidney and heart were removed and weighed. Remnant kidney hypertrophy was estimated assuming that the left kidney mass that remained after 5/6 Nx was equivalent to the combined weight of its excised poles subtracted from the weight of the removed right kidney. A section of the harvested kidney was separated and fixed in 10% formalin, and the remainder was cleaned with phosphate-buffered saline, snap-frozen in liquid nitrogen, and stored at −70°C until processed.

Plasma total cholesterol (Stanbio Laboratory, Boerne, TX), triglyceride (Stanbio Laboratory), HDL cholesterol (Wako Chemicals, Richmond, VA), urea (Bioassay Systems, Hayward, CA), and creatinine (Stanbio Laboratory), HDL cholesterol (Wako Chemicals, Richmond, VA), urea (Bioassay Systems, Hayward, CA), and creatinine (Stanbio Laboratory) were measured by western blot technique. Antibodies against COX-1, COX-2, catalase, NF-κB p65, NF-κB p50, NF-κB p65, phospho-IκB, and NAD(P)H oxidase subunits (NOX-4, gp91phox, p22phox and p47phox) were measured using the specified reagents. Plasma LDL cholesterol concentration was determined by calculation using the following equation: LDL = total cholesterol − HDL cholesterol − (TG/5). Creatinine clearance was calculated using the standard equation. Plasma malondialdehyde (MDA) was measured as described previously (27).

Tissue preparation. Briefly, 100 mg of kidney cortex was separated and homogenized in 0.5 ml of buffer A containing 10 mM HEPES, pH 7.8, 10 mM KCl, 2 mM MgCl2, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, 1 μM peptatin, and 1 mM p-aminobenzamidine using a tissue homogenizer for 20 s. Homogenates were kept on ice for 15 min, 125 μl of a 10% Nonidet P-40 (NP-40) solution was added and mixed for 15 s, and the mixture was centrifuged for 2 min at 12,000 rpm. The supernatant was used for Western blot analyses. Total protein concentration was determined with the use of a Bio-Rad kit (Bio-Rad Laboratories, Hercules, CA).

Western blot analyses. Protein abundance of transforming growth factor (TGF)-β, MCP-1, plasminogen activator inhibitor-1 (PAI-1), COX-1, COX-2, catalase, NF-κB p50, NF-κB p65, phospho-IκB, and NAD(P)H oxidase subunits (NOX-4, gp91phox, p22phox and p47phox) were measured by western blot technique. Antibodies against COX-1, COX-2, gp91phox, p47phox and PAI-1 were obtained from BD Biosciences (San Diego, CA). Antibodies against Catalase were obtained from Calbiochem (Gibbstown, NJ). Polyclonal antibodies against TGF-β, MCP-1, NF-κB p50, NF-κB p65, NOX-4, and p22phox were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The phospho-IκB antibody was purchased from Cell Signaling Technology (Denver, CO).

Briefly, aliquots containing 50 μg protein were fractionated on 8% and 4–20% Tris-glycine gel (Novex, San Diego, CA) at 120 V for 2 h and transferred to Hybond-ECL membrane (Amersham Life Science, Arlington Heights, IL). The membrane was incubated for 1 h in blocking buffer (1× TBS, 0.05% Tween 20, and 5% nonfat milk) and then overnight in the same buffer containing the given antibodies. The membrane was washed three times for 5 min in 1× TBS, 0.05% Tween 20 before a 2-h incubation in a buffer (1× TBS, 0.05% Tween 20, and 3% nonfat milk) containing horseradish peroxidase-linked anti-rabbit IgG and anti-mouse IgG (Santa Cruz Biotechnology), at 1:1,000 dilution. The membrane was washed four times and developed by autoluminography using ECL chemiluminescent agents (Amersham Life Science). Optical densities of protein bands were determined by a laser densitometer (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

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Tubulointerstitial damage was graded according the extension (%) of tubular damage (infiltration, fibrosis, tubular dilatation/atrophy) in

Table 1. General data from % nephrectomized (CRF), niacin-treated CRF, and control rats

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<th>CTL</th>
<th>CRF</th>
<th>CRF+Niacin</th>
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<tbody>
<tr>
<td>Body wt 12 wk, g</td>
<td>459.80±21.51</td>
<td>411.74±55.36</td>
<td>418.92±30.52</td>
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<tr>
<td>Left kidney wt, 12 wk, g</td>
<td>1.42±0.03</td>
<td>2.20±0.51</td>
<td>2.03±0.47</td>
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<tr>
<td>Left ventricle wt, 12 wk, g</td>
<td>1.27±0.08</td>
<td>1.50±0.10</td>
<td>1.33±0.05</td>
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<tr>
<td>Hematocrit, %</td>
<td>48.91±4.43</td>
<td>38.12±9.64</td>
<td>43.14±5.40</td>
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successively evaluated fields in the renal cortex. To this end, successive fields were examined at \( \times 20 \) magnification in the entire cortical and juxtamedullary areas of each specimen, and areas with tubulointerstitial damage were identified and related to the total area under examination using computer-assisted image analysis. The Olympus BX51 system and DP70 microscope, and a digital camera with Sigma Pro (Leesburg, VA) image-analysis software were used as previously described (31, 32, 36). All histological evaluations were done in a blinded fashion.

**Statistical analysis.** Statistical analyses were done by ANOVA and post hoc Tukey tests (SPSS 13.0, Chicago, IL). Values are presented as means \( \pm \) SD, and \( P \) values <0.05 were considered significant.

**RESULTS**

**General data.** Data are summarized in Table 1. As expected, the CRF animals had a significant elevation of plasma creatinine and urea nitrogen concentrations. Although mean plasma creatinine and urea concentrations were lower and creatinine clearance was higher in the niacin-treated CRF group than in the untreated CRF group, the differences did not reach statistical significance. As shown in Fig. 1, the CRF group exhibited a significant rise in systolic and diastolic arterial pressure and a marked increase in left ventricular weight (Table 1). Long-term niacin administration resulted in significant reduction of systolic and diastolic blood pressure (Fig. 1) as well as left ventricular mass.

Urinary protein excretion was markedly elevated in the untreated CRF animals and was significantly lowered by niacin supplementation (Fig. 2). Similarly, the CKD animals exhibited marked polyuria and reduced urine-specific gravity, which significantly improved by long-term niacin administration (Fig. 2).

**Histological findings.** The untreated CRF group exhibited significant glomerulosclerosis, tubulointerstitial injury, and heavy interstitial mononuclear leukocyte infiltration. Niacin supplementation reduced glomerulosclerosis, attenuated tubulointerstitial injury, and lowered leukocyte infiltration (Fig. 3). In addition, niacin therapy decreased the magnitude of remnant kidney hypertrophy by \( \sim 30\% \).

**Plasma lipid data.** Data are shown in Table 2. The CRF group exhibited increased plasma triglyceride, total cholesterol, and LDL cholesterol concentrations and decreased plasma HDL cholesterol-to-total cholesterol ratio. Niacin ad-
ministration resulted in a mild reduction of plasma triglyceride, total cholesterol, and LDL cholesterol concentrations and an increase in HDL cholesterol-to-total cholesterol ratio.

Oxidative stress and inflammatory mediators. The untreated CRF group showed a significant elevation of plasma lipid peroxidation product, MDA, pointing to the presence of systemic oxidative stress (Table 2). This was associated with upregulations of NOX-4, gp91phox, p22phox, and p47phox subunits of the ROS-generating enzyme NAD(P)H oxidase. Niacin supplementation lowered plasma MDA and reversed or markedly attenuated upregulation of NOX-4, p22phox, and p47phox but did not affect gp91phox expression (Fig. 4).

The remnant kidney showed a marked increase in COX-1, COX-2, MCP-1, PAI-1, and TGF-β abundance in the untreated CRF group. This was associated with a marked increase in phospho-IκB, pointing to activation of NF-κB, the general transcription factor for numerous proinflammatory molecules including MCP-1. Niacin supplementation attenuated NF-κB activation and lowered COX-1, MCP-1, PAI-1, and TGF-β abundance in the remnant kidney (Figs. 5–7).

DISCUSSION

In confirmation of earlier studies (29, 36), renal mass reduction by 5/6 Nx resulted in progressive proteinuria, hypertension, glomerulosclerosis, tubulointerstitial injury, and mononuclear leukocyte infiltration in animals employed in the present study. This was associated with upregulation of ROS-producing and proinflammatory molecules in the remnant kidney. It is well known that CKD causes oxidative stress (14, 37), which can, in turn, accelerate progression of renal injury by exerting direct cytotoxicity and by promoting inflammation (14, 30, 36).

Table 2. Plasma concentrations of total cholesterol, LDL cholesterol, triglyceride, MDA, and HDL/total cholesterol ratio in CRF, niacin-treated CRF, and control rats

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<tr>
<td>Plasma total cholesterol, mg/dl</td>
<td>71.19±9.80</td>
<td>221.19±20.53†</td>
<td>197.22±56.93</td>
</tr>
<tr>
<td>Plasma LDL cholesterol, mg/dl</td>
<td>18.16±5.29</td>
<td>95.88±14.07†</td>
<td>77.27±25.21*</td>
</tr>
<tr>
<td>Plasma HDL cholesterol/total cholesterol</td>
<td>0.62±0.09</td>
<td>0.39±0.16*</td>
<td>0.53±0.05</td>
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<tr>
<td>Plasma triglyceride, mg/dl</td>
<td>45.85±18.30</td>
<td>99.72±3.57*</td>
<td>92.69±40.16*</td>
</tr>
<tr>
<td>Plasma MDA, nM/ml</td>
<td>0.44±0.26</td>
<td>2.04±0.39*</td>
<td>1.44±0.19†</td>
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Values are means ± SD; n = 6/group. MDA, malondialdehyde. *P < 0.05, †P < 0.001 vs. control. ‡P < 0.05 versus CRF group.
Given their role in promoting renal injury, strategies aimed at mitigating oxidative stress and inflammation may be effective in retarding the progression of CKD.

The CRF animals employed in the present study exhibited increased plasma triglyceride, total cholesterol, and LDL cholesterol concentrations and decreased plasma HDL cholesterol-to-total cholesterol ratio. This was associated with a marked increase in plasma MDA, which is a byproduct of peroxidation of lipids/lipoproteins. Oxidative modification of LDL and phospholipids markedly increases their proinflammatory activities while oxidative modification of HDL significantly reduces its anti-inflammatory properties (2, 24). These events result in heavy lipid accumulation in the remnant kidney (18) and aorta (25) and contribute to progressive glomerulosclerosis, tubulo-
interstitial injury, and atherosclerosis in this model. Niacin administration resulted in a mild reduction of plasma triglyceride, total cholesterol, and LDL cholesterol concentrations and significant improvement of HDL cholesterol-to-total cholesterol ratio. Although the effect of niacin supplementation on plasma lipid concentrations was small, its efficacy was magnified by the ability to reduce oxidative modification of lipids/lipoproteins. In this context, niacin has been shown to have potent antioxidant and anti-inflammatory properties (8). As a precursor for synthesis of \( \text{NAD}^+ \), niacin increases cellular concentration of \( \text{NAD}^+ \) and upregulates expression of glucose-6-phosphate dehydrogenase, which is the rate-limiting enzyme in the pentose phosphate pathway and a major source of cellular reduced \( \text{NAD(P)}H \) (42). Repletion of cellular \( \text{NAD(P)}H \) contents can lower ROS production by raising cellular redox capacity, maintaining antioxidant enzymes (such as catalase and glutathione reductase) in their active forms, and inhibiting ROS-generating oxidases (3, 11).

In a recent study, Ganji et al. (8) demonstrated that niacin inhibits vascular inflammation by lowering endothelial ROS production, LDL oxidation, VCAM-1, and MCP-1 expression, resulting in decreased monocyte and macrophage adhesion and accumulation, key events involved in the initiation of atherogenesis. These observations have documented the antioxidant and anti-inflammatory properties of niacin in the vascular tissue. The present study was undertaken to explore the potential effect of niacin in mitigating inflammation, oxidative stress, and progressive deterioration of renal function and structure following subtotal nephrectomy. The study showed that long-term niacin administration attenuates oxidative stress, hypertension, and proteinuria and suppresses inflammatory mediators in rats with renal mass reduction.

In confirmation of earlier studies (7, 32, 33), the CKD animals employed in the present study exhibited increased phospho-IkB, pointing to activation of NF-kB in the remnant kidney. NF-kB is the general transcription factor for numerous proinflammatory cytokines, chemokines, and adhesion molecules that are essential for activation and tissue infiltration of immune cells. Activation of NF-kB in the remnant kidneys of the untreated animals was accompanied by intense interstitial mononuclear leukocyte infiltration and significant upregulation of MCP-1, which is essential for monocyte/macrophage recruitment. Similarly, tubulointerstitial fibrosis and glomerulosclerosis in the CRF rats was accompanied by marked upregulation of TGF-\( \beta \) and PAI-1. The observed upregulation of TGF-\( \beta \), the principal mediator of matrix protein production, and elevation of PAI-1, the potent pericellular metalloproteinase inhibitor, can contribute to glomerulosclerosis and interstitial fibrosis in the remnant kidney.

Administration of niacin resulted in the attenuation of NF-kB activation, reduction in immune cell infiltration, and partial reversal of upregulation of the above proinflammatory profibrotic factors. These findings confirm the anti-inflammatory properties of niacin as shown by Ganji and associates (8) in vascular tissue.

The prototypical phagocytic NADPH oxidase (NOX-II) and its tissue-specific isotypes (NOX-1, NOX-IV, and NOX-V) are a major source of ROS generation in endothelial cells, vascular smooth muscle cells, and cellular constituents of the kidney (5, 19, 34, 39). Proinflammatory cytokines, angiotensin II, and mechanical stress acutely raise ROS production by activating NAD(P)H oxidase. In addition, chronic exposure to these stimuli leads to upregulation of constitutively active isoforms of the enzyme and, thereby, sustained increase in ROS production (21). NAD(P)H oxidase-derived superoxide in the
kidney and vascular tissues plays a major part in the pathogenesis of hypertension (9, 41). Niacin administration resulted in significant attenuation of hypertension in the CKD animals employed in the present study. Amelioration of hypertension with niacin supplementation in the study animals was accompanied by a significant reduction of plasma MDA and partial or complete reversal of CKD-induced upregulation of NAD(P)H oxidase. Therefore, reversal of CKD-induced upregulation of NAD(P)H oxidase in the remnant kidney with niacin administration shown here may have contributed, in part, to the observed attenuation of hypertension in our CKD rats. In addition, niacin is known to promote vasodilatation via production of prostaglandin D2 and E2 (26), which is responsible for the cutaneous flushing, a well-known side effect of niacin therapy (16, 26). The associated vasodilatory action of niacin may have partly contributed to its antihypertensive effect by lowering systemic vascular resistance.

In conclusion, long-term niacin supplementation resulted in partial amelioration of proteinuria, glomerulosclerosis, and tubulointerstitial injury and fibrosis in rats with renal mass reduction. This was associated with and likely mediated by amelioration of hypertension and partial reversal of upregulation of oxidative, inflammatory, and profibrotic mediators in the remnant kidney.

REFERENCES


