Curcumin ameliorates renal failure in 5/6 nephrectomized rats: role of inflammation

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Ghosh SS, Massey HD, Krieg R, Fazelbhoy ZA, Ghosh S, Sica DA, Fakhry I, Gehr TW. Curcumin ameliorates renal failure in 5/6 nephrectomized rats: role of inflammation. Am J Physiol Renal Physiol 296: F1146–F1157, 2009. First published February 18, 2009; doi:10.1152/ajprenal.90732.2008.—TNF-α and NF-κB play important roles in the development of inflammation in chronic renal failure (CRF). In hepatic cells, curcumin is shown to antagonize TNF-α-elicted NF-κB activation. In this study, we hypothesized that if inflammation plays a key role in renal failure then curcumin should be effective in improving CRF. The effectiveness of curcumin was compared with enalapril, a compound known to ameliorate human and experimental CRF. Investigation was conducted in Sprague-Dawley rats where CRF was induced by 5/6 nephrectomy (Nx). The Nx animals were divided into untreated (Nx), curcumin-treated (curcumin), and enalapril-treated (enalapril) groups. Sham-operated animals served as a control. Renal dysfunction in the Nx group, as evidenced by elevated blood urea nitrogen, plasma creatinine, proteinuria, segmental sclerosis, and tubular dilatation, was significantly reduced by curcumin and enalapril treatment. However, only enalapril significantly improved blood pressure. Compared with the control, the Nx animals had significantly higher plasma and kidney TNF-α, which was associated with NF-κB activation and macrophage infiltration in the kidney. These changes were effectively antagonized by curcumin and enalapril treatment. The decline in the anti-inflammatory peroxisome proliferator-activated receptor γ (PPARγ) seen in Nx animals was also counteracted by curcumin and enalapril. Studies in mesangial cells were carried out to further establish that the anti-inflammatory effect of curcumin in vivo was mediated essentially by antagonizing TNF-α. Curcumin dose dependently antagonized the TNF-α-mediated decrease in PPARγ and blocked transactivation of NF-κB and repression of PPARγ, indicating that the anti-inflammatory property of curcumin may be responsible for alleviating CRF in Nx animals.

TNF-α; remnant; angiotensin; PPARγ

IT IS WELL ESTABLISHED THAT INFLAMMATION PLAYS AN IMPORTANT ROLE IN PROGRESSIVE KIDNEY DISEASE IN BOTH HUMANS AND ANIMAL MODELS OF RENAL FAILURE (1). Proinflammatory cytokines such as TNF-α are known to increase in patients with chronic kidney disease (52) and animal models of renal failure such as 5/6 nephrectomized (Nx) rats (55). One of the most important downstream signaling targets activated by TNF-α is NF-κB, a well-established transcription factor, which plays a major role in inflammatory renal disease (20). TNF-α is also known to decrease the expression of peroxisome proliferator-activated receptor γ (PPARγ) (29). Inhibition of PPARγ activity by TNF-α is important in the pathogenesis of insulin resistance, atherosclerosis, and inflammation (62). PPARγ agonists have been shown to be effective in the treatment of insulin resistance, atherosclerosis (19, 34), and they ameliorate the progression of glomerulosclerosis in Nx rats (36).

Curcumin is the active ingredient in the traditional herbal remedy and dietary spice turmeric (Curcuma longa) and is undergoing clinical trials for various diseases such as cancer, Alzheimer’s disease, and ulcerative colitis (22). It is possible that many of the beneficial effects of curcumin relate to its ability to suppress acute and chronic inflammation (4). Curcumin was shown to inhibit TNF-α-dependent NF-κB activation in human embryonic kidney cells (2). In hepatocyte cells, activation of PPARγ by curcumin resulted in inhibition of the trans-activating activity of NF-κB (60). In addition, curcumin’s anti-inflammatory property plays a major role in protecting against carbon tetrachloride-induced liver injury in rats (11). There have been some experimental studies investigating the role of curcumin in renal dysfunction. The antioxidant property of curcumin has proven effective in improving renal function in diabetic animals (39, 49) and acute renal failure induced by ischemia-reperfusion injury (6). In Thy-1 nephritic rats, the inflammatory reaction begins hours after induction of disease and is followed by a fibrotic phase with relatively little inflammatory activity (13). Gaedeke et al. (13) have shown that curcumin treatment in these nephritic animals reduced fibrosis by inducing hemeoxygenase-1 (13). In rats with unilateral ureteral obstruction, curcumin significantly inhibited NF-κB activity and reduced influx of macrophages (31). However, to the best of our knowledge, the role of curcumin in chronic renal failure has not been elucidated and it is not clear whether the anti-inflammatory properties of curcumin would have any effect on Nx rats, a model of chronic renal failure.

We hypothesized that if inflammation plays a key role in renal failure, then curcumin should be effective in alleviating chronic renal failure. In this study, we used enalapril as a positive control because ANG II is a known inflammatory mediator and angiotensin-converting enzyme inhibitors (ACEI) are known to ameliorate both experimental and human renal failure (43, 56).

MATERIALS AND METHODS

Curcumin was purchased from Biomol. All antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) unless otherwise stated.

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committees of Virginia Commonwealth University.
Rats were divided into four groups with six animals in each group. The control group underwent sham surgery, and three other groups underwent 5/6 nephrectomy by the procedure described below.

Surgery. Chronic renal failure was induced in rats by the method published previously by Ghosh et al. (16) with modification for adult rats. Briefly, 5/6 nephrectomy of rats weighing between 150 and 200 g was performed using sterile techniques. All operations were carried out under isoflurane anesthesia. A left flank incision was made, and the left kidney was exposed. The renal artery was temporarily occluded, and the upper and lower thirds of the kidney were ligated and excised. Bleeding was controlled by compression, until it stopped. Thus one-third of the mass of the left kidney remained. The muscle and skin incisions were sutured with polypropylene suture. The animals were returned to the vivarium to recover. One week later, a right flank incision was made, and the right kidney was exposed. The renal artery was made, and the right kidney was exposed. The renal artery was temporarily occluded, and the upper and lower thirds of the kidney were ligated and excised. Bleeding was controlled by compression, until it stopped. Thus one-third of the mass of the left kidney remained. The muscle and skin incisions were sutured with polypropylene suture. The animals were returned to the vivarium to recover. One week later, a right flank incision was made, the renal vessels and ureter were tied, and the right kidney was excised. Animals were returned to the vivarium to recover, and treatment was started after 7 days. The animals were studied for 8 wk, and on week 9 they were killed. Suspension of curcumin and enalapril were made in 0.5% carboxymethylcellulose (CMC). Curcumin is nonpolar and must be suspended in CMC. Due to the instability of curcumin in an aqueous system, the compound was made fresh and given within 10 min of the preparation. The Nx animals were divided into three groups. The curcumin-treated group were gavaged with 75 mg/kg curcumin daily (curcumin group). The enalapril group received 10 mg/kg oral enalapril suspended in CMC daily (enalapril group). The untreated Nx animals (Nx group) received vehicle, which was 0.5% CMC, daily. The control group had sham operation, where the capsule was removed. On week 9, the animals were killed, and blood and tissue were collected for analyses.

Blood Urea Nitrogen and Creatinine and Serum TNF-α

Blood urea nitrogen (BUN) and creatinine were measured by a NOVA16 autoanalyzer (NOVA Biomedical, Waltham, MA). A rat TNF-α ELISA kit was used for the quantitative determination of TNF-α in rat serum (ALPCO, Salem, NH). Serum was processed for ELISA according to the manufacturer’s instructions.

Longitudinal Measurement of Arterial Pressure by Tail Plethysmography

Arterial pressure (BP) was determined by tail plethysmography as previously described (16) using the CODA 2 system (Kent Scientific, Torrington, CT). CODA 2 utilizes volume pressure recording sensor technology to measure rat tail blood pressure. This is a computerized, noninvasive tail-cuff acquisition system which can simultaneously measure systolic, diastolic, and mean arterial pressure without operator intervention. Before surgery, rats were trained for 3 days and were kept in a restraining holder for a 5- to 10-min period. On the fourth day, BP was recorded (week 0). During this period, 25 sequential readings were obtained. Readings within a range of 10 mmHg were averaged. Two weeks after the second surgery, the animals were retrained and BP was recorded (week 2). Similar BP recording was done on weeks 5 and 8 postsurgery. In this study, we report the mean systolic BP of each group.

Homogenization

Kidney homogenate. The kidney was cut and immediately frozen in liquid nitrogen and kept at −70°C until use. The frozen kidney was ground to a powder and then mixed in ice-cold HEPES buffer (10 mM HEPES, 0.2% Triton X-100, 50 mM NaCl, 0.5 mM sucrose, 0.1 mM EDTA, protease, and phosphatase inhibitors) and homogenized with an ice-chilled dounce homogenizer at 4°C. An aliquot of the homogenate was stored and the rest was used to make cytosolic and nuclear extracts. This was spun at 10,000 rpm for 10 min, and the supernatant was aliquotted and stored at −70°C as the cytosolic extract. The pellet was suspended in ice-cold buffer (10 mM HEPES, 500 mM NaCl, 10% glycerol, 0.1 mM EDTA, 0.1 mM EGTA, 0.1% IGEPA, and protease and phosphatase inhibitors) and vortexed at 4°C for 15 min and centrifuged for 10 min at 14,000 rpm. The resulting supernatant was aliquoted and stored as the nuclear extract at −70°C. The absence of cross-reactivity with β-actin in Western blots confirmed the purity of nuclear extracts. A small aliquot of kidney homogenate, cytosolic, and nuclear extract was kept at 4°C for protein estimation.

Histology

Animals from each group were studied for histological changes in the kidney. A portion of the kidney was cut and fixed in 10% buffered formalin for light microscopy. The basic scoring system, described previously (14), quantified the fraction of total glomeruli showing global and/or segmental sclerosis, the percentage of tubules showing dilatation, and epithelial hyperplasia. A minimum of 100 glomeruli were scored per animal by an observer blinded to the origin of the tissue. Sections were cut at 2-μm thickness and stained with periodic acid-Schiff.

Immunohistochemistry

F4/80 antibody has been used to detect macrophages in rats and mice (61, 64). Therefore, to identify the presence of infiltrating macrophages, kidney sections were immunostained overnight at 4°C with rabbit anti-rat F4/80 antibody to macrophages as described by Zhao et al. (64). After being washed with PBS, they were treated with diluted biotinylated secondary anti-rabbit antibody (1:50; Vector Labs, Burlingame, CA) for 60 min at room temperature, followed by incubation with neutravidin 594 conjugate (Pierce, Rockford, IL). Images were acquired using an Olympus inverted microscope fitted with a digital camera, and analyzed with Image-Pro Plus software (Media Cybernetics). The single color channel of an image was extracted to produce a 256-level gray scale image. Using a histogram-based segmentation model, background was subtracted, and the relative fluorescence intensities for each image were measured. Quantitative assessments for F4/80-positive cells were performed, and the number of positive cells in four randomly selected fields of view for each animal was averaged and expressed as total integrated optical density (35). Results are depicted as means ± SD.

Immunoblotting

Kidney homogenates, cytosol (75–100 μg total protein), and nuclear extracts (50 μg total protein) were separated on a 4–20% SDS-PAGE gel, and proteins were transferred to a polyvinylidene difluoride membrane as described before (16). After being briefly washed in phosphate-buffered saline containing 1% Tween-20 (PBS-T) and blocked in 5% nonfat dry milk, blots were incubated with appropriate antibodies in 5% nonfat dry milk overnight at 4°C. After being washed three to five times in PBS-T, blots were subsequently incubated with secondary antibody appropriately diluted in 5% nonfat dry milk for 1 h at room temperature. After being washed three to five times in TBS, blots were developed using Lightning Chemiluminescence Reagent Plus and exposed to X-ray film.

Cell Culture

Rat mesangial cells were obtained from American Type Culture Collection (Manassas, VA) and were grown as previously described (15) in Ham’s F12 and DMEM with 15% FBS. For transfection studies, cells were plated in 24-well plates; for other studies, they were plated in 35-mm plates.
Mesangial cells (~90–95% confluent) were treated with varying concentrations of curcumin (1.25–20 μM) for 24 h. Various concentrations of curcumin (1.25 μM–20 μM) were dissolved in 15 μl ethanol. In some experiments, TNF-α (10 ng/ml) was added 3 h after the curcumin. Cells were harvested for protein in ice-cold lysis buffer (PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) with protease and phosphatase inhibitors 24 h after the addition of curcumin. RNA was isolated using an RNeasy mini kit (Qiagen, Valencia, CA) and processed as described below.

Transfection of NF-κB- and PPAR Response Element-Containing Reporter Vector

pNF-κB-Luc, designed for monitoring activation of the NF-κB signal transduction pathway, was purchased from Clontech (BD Biosciences Clontech, Palo Alto, CA). PPAR reporter vector, pPPRE(AOx)3-Luc, where PPRE is PPAR response element, was kindly donated by Dr. C. Glass and Dr. M. Ricotte. Mammalian reporter vector pCMVβ-galactosidase (β-gal) was used as an internal control. Mesangial cells were transiently transfected with plasmid containing NF-κB or PPRE luciferase and β-gal vectors (200-ng plasmids). Transfections were performed using Effectene (Promege, Madison, WI) according to the manufacturer’s instructions and as described earlier(15). The control cells received the empty vector. Twenty-four hours after transfection, the cells were treated with various concentrations of curcumin (2.5–20 μM) dissolved in 15 μl ethanol. TNF-α (10 ng/ml) was added 3 h after curcumin treatment. Eighteen hours later, the cells were harvested for luciferase and β-gal activity. Luciferase activity was measured using Promega Kits, and NF-κB and PPRE activity was expressed as a ratio to the internal standard β-gal activity. The results are expressed as fold-changes from the control.

Quantitative Real-Time RT-PCR Analysis

Total RNA was extracted from kidney and mesangial cells with the RNeasy Mini Kit as described before (16). Briefly, two micrograms of total RNA were reverse transcribed with the ThermoScript RT-PCR System (Invitrogen), and first-strand cDNA was used to perform real-time PCR using the Stratagene Mx3000p real-time PCR system with TaqMan Gene Expression Assays for TNF-α, PPARγ, and β-actin obtained from Applied Biosystems (Foster City, CA). The amount of mRNA was calculated by the ΔΔCT method and normalized to β-actin.

Statistical Analysis

Statistical comparisons among groups were performed using ANOVA followed by Tukey’s multiple comparison test. Groups were considered to be significantly different with \( P \leq 0.05 \).

RESULTS

Effect of Curcumin and Enalapril on BP, Proteinuria, Plasma Creatinine, and BUN

Subtotal nephrectomy resulted in renal dysfunction, as evidenced by a gradual increase in proteinuria (Fig. 1A) and elevated BUN and plasma creatinine at the end of 8 wk (Fig. 1, B and C, respectively). Proteinuria was measured on weeks 2, 5, and 8 following nephrectomy. Nx animals demonstrated a progressive increase in proteinuria. As shown in Fig. 1A, curcumin and enalapril treatment significantly reduced the proteinuria by 40–50%. Proteinuria in curcumin and enalapril cohorts was not significantly different. BUN and plasma creatinine measurements made at the end of 8 wk revealed that these biochemical markers in Nx animals were 3.5- and 4-fold higher, respectively, than the vehicle-treated control. Curcumin was as effective as enalapril in reducing both BUN and creatinine (\( P < 0.01 \)). Systolic BP did not change during the first 2 wk but steadily rose at later time points (Fig. 1D). At 4, 5, and 8 wk, the systolic BP of Nx animals was significantly higher than the control (\( P < 0.01 \)). Enalapril treatment effectively curtailed the increase in systolic BP. Between 4 and 8 wk, the average systolic BP of enalapril-treated animals was significantly lower than the Nx animals (\( P < 0.01 \)). In the curcumin group, the BP was similar to Nx until week 8, when it was significantly reduced (\( P < 0.05 \)), although not to the extent of enalapril-treated animals.
Histology

Histological examination of the kidney further confirmed that Nx animals had renal dysfunction which was significantly improved by curcumin and enalapril. Formalin-fixed kidneys were blocked in paraffin wax and stained with periodic acid-Schiff. A minimum of 100 glomeruli were counted in blinded fashion to assess glomerular pathology. Figure 2 shows a representative example of histological changes in each group, and the results are summarized in the table (bottom). In the untreated Nx group, ~75% of the glomeruli were segmentally sclerosed, whereas the curcumin- and enalapril-treated animals developed 11.2 (P < 0.001 compared with Nx) and 17.5% (P < 0.001 compared with Nx) segmental sclerosis, respectively. Segmental sclerosis between the curcumin and enalapril groups was not significantly different. We found only 8% global sclerosis in Nx animals and <1% in other groups. In addition to pronounced glomerular sclerosis, severe tubulointerstitial changes were observed in the untreated Nx rats.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control (A)</th>
<th>Nx (B)</th>
<th>Curcumin (C)</th>
<th>Enalapril (D)</th>
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<tbody>
<tr>
<td>% Segmental Sclerosis</td>
<td>7.1±0.58</td>
<td>72.5±11.09</td>
<td>11.2±1.25***</td>
<td>17.5±4.79***</td>
</tr>
<tr>
<td>% Tubular dilatation</td>
<td>0.8±0.08</td>
<td>48.2±6.04</td>
<td>3.1±0.54 ***</td>
<td>10.7±1.10***</td>
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Effect of Curcumin and Enalapril on Macrophage Infiltration

Deterioration of renal function and marked inflammation in Nx animals are associated with significant macrophage infiltration in the kidney (41). Figure 3A displays representative photomicrographs of macrophage infiltration for each experimental group, and the results are summarized in Fig. 3B. Kidneys from control animals did not show significant macrophages, whereas untreated Nx rats exhibited a significant increase in macrophage infiltration. Curcumin and enalapril treatment led to a significant reduction in macrophage infiltration compared to the untreated Nx group.*** P < 0.001 compared with Nx.
phage infiltration, but Nx animals demonstrated prominent macrophage (F4/80-positive cells) infiltration in the glomerulus and tubulointerstitium (Fig. 3, A and B). Nx animals treated with curcumin and enalapril showed a reduction of macrophage influx by 53 and 43%, respectively ($P < 0.01$). Although enalapril-treated animals showed a greater reduction in macrophage infiltration than curcumin-treated animals, the difference is not statistically significant.

**Effect of Curcumin and Enalapril on NF-κB**

It is well documented that NF-κB proteins play an important role in inflammation. Under normal conditions, NF-κB dimers such as p50/RelA are maintained in the cytoplasm by IκB proteins such as IκBα. In response to an inflammatory stimulus, IκBα is degraded, resulting in translocation of p50 to the nucleus and the binding of p50 to cognate DNA binding sites.
In our study (Fig. 4A), we observed that kidney cytosolic IkBα in Nx animals was significantly lower than in the control ($P < 0.01$) and curcumin and enalapril treatment significantly reduced IkBα degradation ($P < 0.01$). Although we did not see a significant change in the cytosolic p50 (Fig. 4B), we found that nuclear p50 in the Nx group (Fig. 4C) was 1.5-fold higher than in the control ($P < 0.01$). Compared with the Nx group, curcumin- and enalapril-treated animals had significantly lower p50 protein in the nucleus ($P < 0.01$). The effects of curcumin and enalapril on IkBα and p50 were not significantly different.

Effect of Curcumin and Enalapril on TNF-α and PPARγ

PPARγ belongs to the nuclear hormone superfamily of ligand-dependent transcription factors which have a significant anti-inflammatory effect (19, 34), and proinflammatory TNF-α is known to downregulate PPARγ (62). Serum TNF-α concentrations and kidney TNF-α mRNA in Nx animals were 2- and 3.6-fold higher, respectively, than in the control ($P < 0.01$; Fig. 5, A and B). Compared with the Nx group, curcumin- and enalapril-treated animals had significantly lower serum TNF-α ($P < 0.05$ and $P < 0.01$, respectively). Curcumin- and enalapril-treated animals had 2- and 2.5-fold lower TNF-α mRNA, respectively, than the Nx group ($P < 0.01$). Although TNF-α in curcumin-treated animals was significantly lower than in Nx animals, it was still 1.6-fold higher ($P < 0.05$) than in the control group. Serum concentrations of TNF-α in enalapril-treated animals were 1.4-fold higher than in the control but were not significantly different. Kidney TNF-α mRNA in curcumin- and enalapril-treated animals was not significantly higher than in the control. Differences in serum and kidney TNF-α concentrations among the curcumin- and enalapril-treated groups were not significant.

As shown in Fig. 5, C and D, kidney PPARγ mRNA and protein in the Nx animals were almost 30% lower than in the controls ($P < 0.01$). Compared with Nx animals, curcumin- and enalapril-treated animals had almost 5 ($P < 0.01$)- and 3.5-fold ($P < 0.05$) higher PPARγ mRNA, respectively (Fig. 5C). The PPARγ protein expression in the curcumin and enalapril groups were more than twofold higher ($P < 0.01$) than in the Nx animals (Fig. 5D).

In Vitro Experiments with Mesangial Cells

In vivo experiments have shown that curcumin counteracts the elevation of TNF-α and NF-κB and the downregulation of PPARγ in Nx animals. Although it is enough to suggest that curcumin has an anti-inflammatory role, it is does not convincingly demonstrate the mechanism of the antagonistic effect of curcumin on TNF-α. The questions, such as whether curcumin directly antagonized TNF-α-mediated downregulation of PPARγ or upregulated PPARγ, resulting in a physiological antagonism...
and/or both, could be only answered by in vitro experiments. Therefore, we used mesangial cells to further evaluate and validate the role of curcumin in this study.

**Effect of Curcumin on TNF-α and PPARγ in Mesangial Cells**

TNF-α has been known to decrease expression of PPARγ (62), and this can further augment inflammation. To evaluate whether TNF-α can contribute to the decrease in PPARγ observed in the Nx kidney, mesangial cells were incubated with TNF-α. As seen in Fig. 6, A and B, mesangial cells treated with TNF-α had significantly decreased expression of PPARγ mRNA and protein (P < 0.01). Curcumin (2.5 μM) reversed the TNF-α-mediated decrease in PPARγ mRNA (P < 0.05) but had no effect on protein levels. However, higher concentrations of curcumin (5–20 μM) significantly antagonized (P < 0.05 to P < 0.01) TNF-α-mediated decreases in both PPARγ mRNA and protein expression (Fig. 6, A and B). We also observed that even in the presence of TNF-α, PPARγ mRNA and protein expression following 20 μM curcumin was significantly higher than in the control (P < 0.05). Therefore, it is possible that curcumin per se can stimulate PPARγ expression.

**Effect of Curcumin on PPARγ in Mesangial Cells**

To investigate whether curcumin can directly affect PPARγ expression, rat mesangial cells were incubated with curcumin concentrations ranging from 1.25 to 20 μM. Figure 7, A and B, shows that curcumin dose dependently increased the PPARγ mRNA and protein in a dose-dependent manner. A significant increase in PPARγ protein expression was observed with curcumin concentrations of 5 μM and above, suggesting that curcumin mediates its effect by increasing PPARγ protein and thereby negating the downregulation of PPARγ brought about by TNF-α. As shown earlier (Fig. 6, A and B), curcumin (5 μM) significantly antagonized the TNF-mediated decrease in PPARγ. In the absence of TNF-α, we expected lower concentrations of curcumin (<5 μM) to increase expression of PPARγ. However, reduced concentrations of curcumin did not affect PPARγ protein expression significantly.

**Effect of Curcumin on PPRE and NF-κB Response**

In the earlier experiment, we observed that curcumin directly upregulated PPARγ expression causing a physiological antagonism. However, curcumin might also abrogate the effect TNF-α on PPARγ, by interfering at the transcriptional level. The promoters of PPARγ target genes consist of PPRE, a direct repeat of two hexanucleotides (AGGTCA) spaced by one nucleotide (DR-1), through which PPARγ can bind to and activate target gene expression (27). Transcription factor PPARγ binds to PPRE of the target gene promoters in nucleus, leading to regulation of gene expression (19). To determine whether TNF-α affects PPARγ-mediated transactivation of PPRE, mesangial cells were transiently transfected with a PPRE-luciferase reporter construct. Twenty-four hours later, the cells were treated with TNF-α in both the presence and absence of curcumin. As shown in Fig. 8A, TNF-α decreased PPRE activity by 50%, an effect which curcumin reversed in a dose dependent manner.

TNF-α is known to activate a number of signaling pathways, including NF-κB (20) and curcumin, is known to repress NF-κB in hepatic stellate cells (60). Activation of the NF-κB pathway seen in Nx animals was reversed by curcumin. Therefore, we investigated whether TNF-α and curcumin could play a role in modulating NF-κB. Mesangial cells transfected with the NF-κB-luciferase promoter construct were treated with curcumin, and TNF-α was added 2 h after curcumin. NF-κB promoter activity increased >2.5 fold by TNF-α treatment (P < 0.001) and was dose dependently counteracted by curcumin (Fig. 8B). Therefore, it is likely that TNF-α is responsible for the kidney inflammation seen in this Nx model, and curcumin, by antagonizing TNF-α and upregulating PPARγ, abrogates progressive kidney damage seen in Nx animals.
DISCUSSION

Proteinuria, glomerulosclerosis, and tubulointerstitial injury leading to chronic renal failure are the consequences of reduction of renal mass by subtotal nephrectomy, and inflammation is one of the major factors influencing these events (12). Our studies show that curcumin significantly reduces proteinuria, glomerulosclerosis, and tubulointerstitial injury, and subsequently renal failure, by controlling inflammation mediated by TNF-α. ANG II is an inflammatory molecule that can activate TNF-α-mediated decrease in PPARγ expression and protein. Mesangial cells were incubated with increasing concentrations of curcumin dissolved in ethanol (15 μl) for 24 h in the presence and absence of TNF-α (10 ng/ml). The control cells (grey bars) and cells treated with TNF-α (hatched bars) received 15 μl ethanol. TNF-α was added 3 h after the addition of curcumin. PPARγ mRNA was normalized to β-actin and was compared with control (100%). A: TNF-α treatment significantly reduced PPARγ mRNA in untreated cells, and curcumin dose dependently reversed the effect. B, top: representative Western blot of 4 experiments. PPARγ protein immunoblots were normalized to β-actin. TNF-α-mediated decrease in protein expression was reversed by curcumin concentration of 5 μM and above. The results were compared with TNF-α-treated cells. *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. 6. Curcumin reverses TNF-α-mediated decrease in PPARγ mRNA and protein. Mesangial cells were incubated with increasing concentrations of curcumin dissolved in ethanol (15 μl) for 24 h in the presence and absence of TNF-α (10 ng/ml). The control cells (grey bars) and cells treated with TNF-α (hatched bars) received 15 μl ethanol. TNF-α was added 3 h after the addition of curcumin. PPARγ mRNA was normalized to β-actin and was compared with control (100%). A: TNF-α treatment significantly reduced PPARγ mRNA in untreated cells, and curcumin dose dependently reversed the effect. B, top: representative Western blot of 4 experiments. PPARγ protein immunoblots were normalized to β-actin. TNF-α-mediated decrease in protein expression was reversed by curcumin concentration of 5 μM and above. The results were compared with TNF-α-treated cells. *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. 7. Curcumin increases PPARγ mRNA and protein in mesangial cells. To investigate the effect of curcumin on PPARγ, mesangial cells were incubated with increasing concentrations of curcumin dissolved in ethanol (15 μl) for 24 h. The control cells (grey bars) received the vehicle. Cells were treated with increasing concentrations of curcumin. PPARγ mRNA was normalized to β-actin and was compared with control (100%). A: curcumin dose dependently increased PPARγ mRNA. B, top: representative Western blot of 4 experiments. PPARγ protein immunoblots were normalized to β-actin. The results were compared with control cells. *P < 0.05, **P < 0.01.
markers such as TNF-α, to the extent that macrophage numbers in renal biopsy predict renal survival in patients with chronic renal disease (1). By decreasing macrophage infiltration of the kidney, curcumin and enalapril not only reduce the presence of inflammatory markers such as TNF-α but also may improve kidney survival in renal failure.

Several lines of evidence suggest a role of glomerular ultrafiltrate of plasma proteins or protein-associated factors in glomerular and/or chronic tubulointerstitial damage. Activation of the NF-κB system by proteinuria results in renal damage (1). TNF-α is known to be responsible for degradation of IκBα and activation of the NF-κB system (20). In the Nx model, severe and progressive renal injury was associated with increased activity of NF-κB, whereas amelioration of proteinuria and renal structural damage were associated with decreased activity of the NF-κB system (12, 57). In rats with a remnant kidney, hepatocyte growth factor attenuated inflammation and improved kidney function by suppressing NF-κB reporter gene activity which was induced by TNF-α (18). In our study, the Nx animals had a significant increase in serum TNF-α and kidney TNF-α mRNA, and this was associated with increased 1kBα degradation and movement of p50 to the nucleus. Quiroz et al. (41) have shown that the number of NF-κB-positive cells was increased in the kidney of untreated Nx rats and melatonin, a compound with considerable anti-inflammatory and antioxidant properties, not only reduced the number of NF-κB-positive cells but also ameliorated renal failure. Curcumin, which also has antioxidant properties (53), reduced TNF-α in both serum and kidney tissue and prevented the degradation of 1kBα and NF-κB activation. This suggests that TNF-α might be responsible for NF-κB activation, and curcumin and enalapril treatments prevented the inflammatory response. Our experiments in mesangial cells substantiate that TNF-α was responsible for NF-κB activation in vivo. In these experiments, TNF-α stimulated NF-κB promoter activity, which could be effectively blocked by curcumin. ANG II induces the transcription of various cytokines including TNF-α, leading to target organ injury, and these responses to ANG II are caused primarily by NF-κB signaling (33, 58). Enalapril might therefore inhibit NF-κB by decreasing ANG II.

The role of PPARγ in inflammation has been well documented, and PPARγ agonists have been effective in the treatment of renal dysfunction in diabetic and nondiabetic patients (30, 47). Increased proliferation of mesangial cells can lead to glomerular injury (56), and we have shown earlier that growth factors such as PDGF can cause mesangial cell proliferation that can be abrogated by the PPARγ agonist ciglitazone (15). The PPARγ agonist rosiglitazone prevented glomerular and tubular damage as well as proteinuria in obese Zucker rats (5). Glomerulosclerosis in Nx rats was significantly improved by the PPARγ agonist troglitazone (36). In the present study, we have seen that Nx animals had a significant decrease in PPARγ expression in the kidney, and glomerular and tubular damage in these animals were reduced by both curcumin and enalapril. TNF-α is known to inhibit PPARγ activity (54, 62), and natural and synthetic ligands of PPARγ inhibited the proinflammatory actions of TNF-α (24) in human monocytes. Therefore, the increase in TNF-α in Nx rat might have contributed to the decreased expression of PPARγ in the kidney, and both curcumin and enalapril increased the expression of PPARγ. A similar effect of curcumin on PPARγ has been noted in liver fibrosis (11) and hepatic stellate cells (60). However, the effect of enalapril on PPARγ in these experiments was unexpected. AT1 receptor agonists telmisartan and to some extent irbesartan have PPARγ agonistic activity (10), and although enalapril has been shown to elevate PPARγ in ApoE knockout mice (7), this effect has not been reported with other ACE inhibitors or AT1 receptor blockers. However, ANG II is known to induce TNF-α (28, 58) and by blocking the generation of ANG II, enalapril could have prevented the TNF-α-mediated decrease in PPARγ.

Our studies with mesangial cell cultures demonstrate that TNF-α decreases PPARγ expression, confirming that TNF-α played a role in downregulating PPARγ in Nx animals. To understand the mechanism by which curcumin antagonized the effect of TNF-α, we incubated mesangial cells with curcumin and found that curcumin dose dependently increases PPARγ message and protein. We also found that curcumin increases PPARγ expression. This indicates that curcumin may nullify
the downregulating effect of TNF-α on PPARγ by upregulating PPARγ protein. Constitutive expression of PPARγ attenuates the inhibitory effect of TNF-α (63), and induction of PPARγ protein and mRNA expression by curcumin has also been reported in hepatic stellate cells (29, 60). Therefore, the interaction between TNF-α and curcumin might be considered a physiological antagonism. Furthermore, TNF-α has been shown to repress PPARγ at the transcriptional level (60), while increased PPARγ expression (54) and PPARγ ligands (3) are known to activate PPRE. Since curcumin is reported to act as a PPARγ ligand (9), we speculated that it can also antagonize TNF-α-mediated repression of PPARE-luciferase reporter activity. Curcumin may therefore antagonize the effect of TNF-α on PPARγ not only by increasing the expression of PPARγ but also by modulating the repression of PPARE. Transpression, which is primarily responsible for the anti-inflammatory effects of PPARs, involves interference with other signal transduction pathways, including the NF-κB pathway (34); however, further investigation is necessary to determine whether NF-κB inhibition by curcumin occurs by an PPARγ-dependent or PPARγ-independent pathway.

PPARγ agonists lower BP in diabetic patients and animal models, at least partially independent of their insulin-sensitizing effects, although this BP-lowering effect is much more moderate in human patients than in animal models (8). Therefore, it was disappointing to observe that BP was not significantly affected by curcumin. However, it has been shown in obese Zucker and Nx rats that a PPARγ agonist can significantly improve renal function without affecting BP (5, 36). It is known that PPARs partner with liganded retinoid X receptors (RXRs) to form active transcription factors capable of activating target genes with PPARE (19). Although we did not examine curcumin’s effect on RXR, it is possible that RXR might also be involved in the curcumin-mediated anti-inflammatory effect.

Poor bioavailability of curcumin presents a dosing problem that can be circumvented by increasing its dose since it has very low toxicity and is remarkably well tolerated (4, 22, 48). After an oral dose in rats (10, 80, and 400 mg/rat), the absorption of radioactive curcumin ranged from 60 to 66%, suggesting the absorption is not dose dependent and the total absorption of radioactive curcumin ranged from 60 to 66%, although only 34% of curcumin was excreted unchanged. In mice, after intraperitoneal administration of 100 μg/kg curcumin, the peak plasma concentration was reached within 15 min. When the animals were killed after 1 h, the plasma concentration was 0.6 μg/ml and the concentration of curcumin in the kidney, liver, and brain was 7.5, 2.69, and 0.4 μg/g tissue, respectively (40). Tetrahydrocurcumin, a major reduction metabolite, inhibits NF-κB, cyclooxygenase-1, and 5-lipoxygenase, albeit less effectively than curcumin (23, 46). Various conjugated metabolites of curcumin are reported to have mild anti-inflammatory activity (26). If, however, conjugates are deconjugated to free curcumin, as occurs in inflammation (50, 51), one can speculate that the curcumin conjugate concentrations detected would be sufficient to modulate key curcumin-associated intracellular targets. Doses up to 3,600–8,000 mg daily for 4 mo has been used in phase I clinical studies with curcumin (25), which translates to 50–114 mg/kg for a 70-kg human. Li et al. (32) evaluated three different doses of curcumin (50, 75, and 100 mg·kg⁻¹·day⁻¹ in mice) for 1 wk and then subjected the mice to chronic pressure overload generated by aortic banding (AB) and found that curcumin blocked AB-induced inflammation and fibrosis. They found that maximal efficacy was achieved at a curcumin dose of 75 mg·kg⁻¹·day⁻¹ (32). In another study, 50 mg·kg⁻¹·day⁻¹ curcumin ameliorated heart failure in two different models: hypertensive heart failure in salt-sensitive Dahl rats and surgically induced myocardial infarction in Sprague-Dawley rats (38). Due to its low toxicity and poor bioavailability, we chose a dose of 75 mg·kg⁻¹·day⁻¹; however, a lower dose may be also be effective in preventing renal failure.

In summary, we have shown that the anti-inflammatory property of curcumin can significantly improve kidney function in animals with chronic renal failure. Although curcumin has also been shown to cause apoptosis in cancer cell lines (22), it is not certain how this property will affect normal nonneoplastic cells or cells involved with renal failure. Curcumin is remarkably well tolerated in both humans and animals. Its low toxicity in humans and animals make this compound suitable to be used alone or with other agents to improve renal function. Curcumin is a multifaceted compound which can affect other proinflammatory cytokines, adhesion molecules, transforming growth factor-β and is also a potent antioxidant (4, 22). Collectively these pharmacological properties can help to slow renal failure, but in this study we show that curcumin improves chronic renal failure by antagonizing the effect of TNF-α on NF-κB and PPARγ.

GRANTS

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REFERENCES

CURCUMIN AMELIORATES RENAL FAILURE


