Colchicine attenuates renal injury in a model of hypertensive chronic kidney disease

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Guan T, Gao B, Chen G, Chen X, Janssen M, Uttarwar L, Ingram AJ, Krepinsky JC. Colchicine attenuates renal injury in a model of hypertensive chronic kidney disease. Am J Physiol Renal Physiol 305: F1466–F1476, 2013.—Hypertension is a risk factor for chronic kidney disease, particularly when associated with impaired renal autoregulation and thereby increased intraglomerular pressure (Pgc). Elevated Pgc can be modeled in vitro by exposing glomerular mesangial cells to mechanical strain. We previously showed that RhoA mediates strain-induced matrix production. Here, we show that RhoA activation is dependent on an intact microtubule network. Upregulation of the profibrotic cytokine connective tissue growth factor (CTGF) by mechanical strain is dependent on RhoA activation and inhibited by microtubule disruption. We tested the effects of the microtubule depolymerizing agent colchicine in 5/6 nephrectomized rats, a model of chronic kidney disease driven by elevated Pgc. Colchicine inhibited glomerular RhoA activation and attenuated both glomerular sclerosis and interstitial fibrosis without affecting systemic blood pressure. Upregulation of the matrix proteins collagen I and fibronectin, as well as CTGF, was attenuated by colchicine. Activity of the profibrotic cytokine TGF-β, as assessed by Smad3 phosphorylation, was also inhibited by colchicine. Microtubule disruption significantly decreased renal infiltration of lymphocytes and macrophages. Our studies thus indicate that colchicine modifies hypertensive renal fibrosis. Its protective effects are likely mediated by inhibition of RhoA signaling and renal infiltration of inflammatory cells. Already well-established in clinical practice for other indications, prevention of hypertension-associated renal fibrosis may represent a new potential use for colchicine.

hypertensive nephropathy; microtubules; glomerular sclerosis; RhoA; CTGF; TGF-β

HYPERTENSION IS A COMMON CAUSE of chronic kidney disease, marked pathologically by glomerular sclerosis and interstitial fibrosis. Over time, this leads to loss of renal function, ultimately progressing to end-stage renal disease. Elevated glomerular capillary pressure (Pgc) is an important hemodynamic determinant of the initiation and progression of glomerular injury (1, 22, 27). Increased Pgc transmits to mesangial cells (MC), which provide architectural support for the glomerular capillary tuft, as mechanical strain (30). In vitro, cyclic application of vacuum to plates with deformable wells is used to model the effects of strain. MC grown on matrix and subjected to cyclic strain/relaxation increase matrix protein synthesis (31), providing a model system to study mechanical strain-induced signaling in MC.

We showed an important role for the small GTPase RhoA, best known for its effects on the actin cytoskeleton, in strain-induced production of the matrix protein fibronectin in MC (19). Rho-kinase, one of the downstream effectors of RhoA, was required for this profibrotic response. RhoA activation itself, however, can be influenced by the microtubule network. Although microtubule depolymerization has been described to activate RhoA through release of the RhoA activator GEF-H1 (5), several reports have shown that RhoA activation can also be inhibited by microtubule disruption, such as in cytokinesis of embryonic cells (2). Microtubule inhibition has also been shown to inhibit basal RhoA activation located at the basement membrane in epiblast cells (24). We thus assessed the role of microtubules in strain-induced RhoA activation. Our in vitro data showed that the microtubule depolymerizing agents nocodazole and colchicine block RhoA activation as well as RhoA-dependent upregulation of the profibrotic cytokine connective tissue growth factor (CTGF). Whether such agents are effective in vivo in hypertensive renal injury, however, is unknown.

Colchicine is in wide clinical use, most commonly as a therapy for acute gout, but it has also recently been shown to have anti-fibrotic effects in diabetic nephropathy and nephrotoxicity induced by cyclosporine (9, 21). In this study, we investigated the therapeutic potential of colchicine for kidney disease induced by intraglomerular hypertension. We used the 5/6 nephrectomy (remnant kidney) model in our studies. This is a well-established model of chronic kidney disease in which a reduction of nephron mass by uninephrectomy and infarction of 2/3 of the contralateral kidney leads to both systemic and intraglomerular hypertension, proteinuria, glomerular sclerosis, interstitial fibrosis, and kidney dysfunction.

MATERIALS AND METHODOLOGY

Animals. Experiments were conducted in accordance with McMaster University and Canadian Council on Animal Care guidelines. Protocols were approved by the McMaster Animal Research Ethics Board (Animal Utilization Protocol no. 10-07-54). Male Sprague-Dawley rats (Charles River) weighing 200 g underwent a two-stage 5/6 nephrectomy. In the first stage, two of the three branches of the left renal artery were ligated to induce infarction of 2/3 of the kidney. This was followed 1 wk later by a right nephrectomy with care taken to preserve the adrenal gland. Control rats (n = 7) underwent sham operation, consisting of a laparotomy and manipulation of the renal pedicles. The day after surgery, remnant rats were either injected intraperitoneally with 30 μg/kg of colchicine daily as used previously (21) (Sigma; n = 10) or saline (n = 11). Sham-operated rats also received intraperitoneal saline. Housing was in a temperature-controlled facility with free access to standard chow and water.

Rats were killed 8 wk after surgery. Before death, they were weighed and blood pressure was determined by tail-cuff volume pressure recording (Coda 2, Kent Scientific). A 6-h urine collection...
was obtained using a metabolic cage. Blood was analyzed for serum creatinine and urine for protein and creatinine using an automatic biochemical analyzer.

Cell culture. Sprague-Dawley primary rat MC were cultured in Dulbecco’s modified Eagle’s medium, 5.6 mM glucose, supplemented with 20% fetal calf serum (Life Technologies), streptomycin (100 μg/ml), and penicillin (100 U/ml) at 37°C in 95% air-5% CO₂ and used between passages 6–15. To apply mechanical strain, MC were plated onto six-well plates with flexible bottoms coated with bovine type I collagen (Flexcell International, Hillsborough, NC). At conflu-

![Graphical representation of RhoA activation](image)

Fig. 1. RhoA activation by strain is microtubule-dependent. S, stretch; Con, control. A, B: mesangial cells (MC) pretreated with the microtubule depolymerizing agents nocodazole (Noc) or colchicine (Col) were stretched for 5 min. Both inhibited RhoA activation, assessed as described in MATERIALS AND METHODOLGY (‡P < 0.001 S vs. others, n = 3 for both). C: serum-deprived MC were treated with full medium containing serum, or with nocodazole and colchicine. Neither agent induced basal RhoA activation, compared with the induction by serum. D: activation of Erk in response to platelet-derived growth factor (PDGF), assessed by its phosphorylation, was unaffected by microtubule disruption with nocodazole and colchicine. E: Rho-kinase inhibitor Y-27632 prevented connective tissue growth factor (CTGF) protein upregulation by 3 h of stretch, as assessed by immunoblotting (†P < 0.01 S vs. others, n = 5). F: colchicine inhibited stretch-induced activation of the CTGF promoter luciferase construct (*P < 0.05 S vs. others, n = 7).
ence, cells were serum deprived for 24 h. They were then exposed to continuous cycles of strain/relaxation by vacuum which was controlled by software (Flexercell 4000, Flexcell International). Each cycle constituted 0.5 s of strain (10%) and 0.5 s of relaxation, for a total of 60 cycles/min. Pharmacologic inhibitors were added as follows before stretch: colchicine (75 μM, 3 h; Sigma), nocodazole (20 μM, 3 h; Sigma), and Y-27632 (10 μM, 30 min; Calbiochem). PDGF-BB was used at 10 ng/ml for 10 min (Sigma).

Luciferase assay. MC plated to 80% confluence were transfected with 1 μg of the CTGF promoter luciferase construct pGL3-CTGF (4.5 kb), kindly provided by Dr. M. Goppelt-Struebe (University of Erlangen-Nuremberg, Germany), and 0.1 μg pCMV-β-galactosidase (β-gal; Clontech) using LipofectAMINE (Qiagen) in six-well stretch plates. MC were serum-deprived overnight 24 h after transfection before stretch. Cells were lysed with Reporter Lysis Buffer (Promega, Madison, WI) using one freeze-thaw cycle, and luciferase and β-gal activities were measured on clarified lysate using specific kits (Promega) with a Berthold luminometer and a plate reader (420 nm), respectively. β-Gal activity was used to adjust for transfection efficiency.

Imaging. Formalin-fixed sections (3 μm) were stained by periodic acid-Schiff (PAS) reagent. Glomerular matrix expansion was visually scored by a blinded assessor on 20 random glomeruli from each rat. The following scoring system was used: 0 (none), 1 (<25% glomerular area involved), 2 (25–50%), 3 (50–75%), 4 (75–90%), 5 (>90%). Interstitial fibrosis was assessed by Masson’s trichrome staining and also scored semiquantitatively by a blinded assessor. Ten random fields at ×400 magnification were taken and tubulointerstitial (TI) fibrosis was scored using the following system: 0 (none), 1 (mild; up to 25% of TI area), 2 (moderate; 25–50%), 3 (severe; more than 50%).

For immunohistochemistry (IHC), 4-μm paraffin sections were deparaffinized and heat-induced epitope retrieval was performed. Primary antisera used were rabbit collagen I (1:200, Abcam), mouse fibronectin (1:200, BD Transduction), goat CTGF (1:500, Santa Cruz Biotechnology), rabbit monoclonal phospho-Smad3 S423/S425 (1:100, Cell Signaling), mouse ED-1 (1:100, Serotec), rabbit CD3 (1:200, Dako), and mouse PCNA (1:1,000, Cell Signaling). ED-1-, CD3-, and PCNA-positive cells were counted in 20 random fields at ×400. For other IHC, positive staining was quantified using ImagePro from five different fields at a magnification of ×20.

In situ RhoA activation by immunofluorescence was performed on 4-μm frozen sections cut from OCT-embedded tissue. Sections were fixed in 4% formaldehyde, blocked, permeabilized, and then incubated with 25 μg GST-RBD (Cytoskeleton) for 30 min at 37°C. Some sections were incubated with GST (Santa Cruz Biotechnology) alone.

Fig. 2. Clinical parameters in remnant rats. A: systolic blood pressure was elevated in remnant (5/6) rats and not affected by treatment with colchicine (‡P < 0.001 con vs. others). B: remnant rats did not gain weight as well as controls, and this was not altered by colchicine (‡P < 0.01 con vs. others). C: remnant rats had significantly increased urine protein:creatinine ratio (*P < 0.05 con vs. others). There was a trend toward decrease in the colchicine group, but this was not statistically significant (P = 0.259). To illustrate the variance in the data, they are also represented as a scatter box plot with mean and 95% confidence intervals in D. E: serum creatinine increased in remnant rats (‡P < 0.01 con vs. others). The decrease by colchicine did not reach significance (P = 0.166). Data are shown as a scatter box plot in F.
as a negative control. Samples were then incubated with mouse anti-GST (1:800, Cell Signaling) for 1 h at room temperature followed by donkey anti-mouse AF488 (Invitrogen) for 2 h at room temperature. Slides were mounted with DAPI (Vector Labs) and images were captured using Metamorph software with a fluorescent microscope.

**Real-time PCR.** Snap-frozen kidney cortex was homogenized in TRIZol and total RNA was extracted according to the manufacturer’s instructions (Invitrogen). RT was performed using standard methods and cDNA was analyzed using real-time PCR for fibronectin, collagen Iα1, or CTGF, with values normalized to 18S.

**Protein analysis.** Cells were lysed and protein was extracted as published (19), with tissue additionally being homogenized in lysis buffer. Lysates were centrifuged at 4°C, 14,000 rpm for 10 min. Supernatant (50 μg) was separated by SDS-PAGE, and Western blotting was performed. Antibodies used were monoclonal collagen I (1:1,000, Sigma), goat polyclonal CTGF (1:2,000, Santa Cruz Biotechnology), monoclonal fibronectin (1:500, BD Biosciences), monoclonal tubulin (0.5 μg/ml, Sigma), polyclonal pErk Thr202/Tyr204 (1:1,000, Cell Signaling), and polyclonal total Erk (1:1,000, Cell Signaling).

The RhoA pull-down assay was performed as described previously (19). Briefly, tissue or cells were quickly lysed in hypertonic buffer and GTP-bound RhoA immunoprecipitated from cleared lysate with 30 μg GST-tagged Rhotekin-RBD bound to glutathione-agarose (Cytoskeleton). Beads were washed and the immunoprecipitate was resolved on 15% SDS-PAGE. Membranes were probed with monoclonal anti-RhoA (1:500, Santa Cruz Biotechnology). Lysate was also probed for RhoA to ensure equality across conditions.

**Statistical analysis.** Statistical analyses were performed with SPSS20 for Windows using one-way ANOVA, with Tukey’s honestly significant difference for post hoc analysis. For IHC analysis, data were analyzed using a linear mixed model, random effects. A P value < 0.05 (2-tailed) was considered significant. Data are presented as means ± SE, and number of repetitions is denoted as “n = .”

**RESULTS**

**RhoA activation by strain is dependent on intact microtubules.** We previously showed that the small GTPase RhoA, through

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**Fig. 3. RhoA is activated in remnant kidneys.** A: in situ RhoA activity assay was performed as described in MATERIALS AND METHODOLGY. Increased RhoA activation was seen in remnant glomeruli and this was prevented by colchicine. Arrows identify glomeruli. B, C: active, GTP-bound RhoA was immunoprecipitated from lysate of kidney cortex. Remnant kidneys had significantly increased RhoA activation and this was inhibited by colchicine (*P < 0.05 remnant vs. others).
its downstream effector Rho-kinase, mediates strain-induced matrix upregulation in MC (19). Rho-kinase was also shown to regulate production of matrix proteins in response to the profibrotic cytokine TGF-β and to regulate increased production of the profibrotic cytokine CTGF (7, 15). It is thus an important central mediator of the fibrotic response. Whether the microtubule cytoskeleton is involved in RhoA activation in MC in response to stretch, however, is unknown. We assessed

Fig. 4. Colchicine attenuates renal fibrosis. A: kidney sections were stained with periodic acid-Schiff (PAS) to highlight glomerular sclerosis and trichrome to identify tubulointerstitial (TI) fibrosis and scoring was performed as outlined in MATERIALS AND METHODS. The arrow identifies an example of tubular injury, pointing to a dilated tubule with flattened epithelial cells. B: remnant rats had increased glomerular sclerosis that was decreased by colchicine (‡P < 0.001 5/6 vs. others). C: similarly, the TI fibrosis seen in remnant rats was also inhibited by colchicine (‡P < 0.001 con vs. 5/6, *P < 0.05 5/6 vs. colchicine).

Fig. 5. Colchicine inhibits extracellular matrix and CTGF gene upregulation. A, B: kidney cortex was analyzed by real-time PCR for transcript levels of the extracellular matrix genes fibronectin and collagen 1α1. Both were increased in the remnant rat and inhibited by colchicine (*P < 0.05 5/6 vs. others). C: similarly, CTGF was also upregulated in remnant rats and this was inhibited by colchicine (*P < 0.05 5/6 vs. others).
the effects of two agents which bind the microtubule subunit tubulin, thereby inhibiting microtubule polymerization, on RhoA activation. Both nocodazole and colchicine prevented strain-induced RhoA activation (Fig. 1A and B). As shown in Fig. 1C, neither agent increased RhoA activation basally, compared with the expected induction with medium containing full serum. To determine whether other signaling responses were intact in the presence of microtubule disruption, MC were treated with platelet-derived growth factor (PDGF) and Erk activation was assessed by immuno blotting for its phosphorylated form. PDGF-induced Erk activation was not affected by either nocodazole or colchicine. Last, to assess whether lower doses of both agents would also be effective, we performed dose- and time-dependent experiments to assess the lowest dose of colchicine and nocodazole which would disrupt microtubules in MC, as assessed by immunofluorescence for tubulin. We found 2 μM nocodazole and 2.5 μM colchicine (2-h incubation) to be effective, and confirmed that both agents at these lower doses inhibited stretch-induced RhoA activation (not shown).

We next assessed whether strain-induced CTGF upregulation is dependent on Rho-kinase signaling, as has been shown in other cell types (6). Figure 1E shows that the Rho-kinase inhibitor Y-27632 blocked strain-induced CTGF upregulation. The CTGF promoter was activated by strain, as shown by increased luciferase activity in Fig. 1F. This was prevented by pretreatment with colchicine, confirming a role for microtubules in CTGF regulation. These data thus support a role for the microtubule network in regulating strain-induced matrix upregulation through its effects on RhoA/Rho-kinase activation.

**Colchicine attenuates RhoA activation in hypertensive chronic kidney disease.** Given these in vitro findings, we tested the effects of colchicine in the remnant (5/6 nephrectomized) rat, an in vivo model of chronic kidney disease marked by systemic and intraglomerular hypertension. As shown in Fig. 2A, remnant rats developed significant hypertension with an average systolic blood pressure of 181.3 ± 6.2 mmHg. Colchicine was well-tolerated and showed no blood pressure-reducing effects. Remnant rats had lower weights, and this was not altered by treatment (Fig. 2B). Proteinuria, a clinical marker of renal injury, was significantly increased in remnant rats. Serum creatinine, a marker of renal dysfunction, was also significantly increased. Although a small decrease in these parameters was seen with colchicine, this was not statistically significant (Fig. 2, C–F).

We next assessed whether RhoA was activated in vivo in remnant glomeruli and whether this was attenuated by colchicine. We used a recently developed in situ RhoA activity assay in which only active, GTP-bound RhoA is able to interact with a GST-fused Rho-binding domain from the RhoA effector Rhotekin (3). Active RhoA is localized with an anti-GST antibody. Figure 3A shows that while the sham group had minimal glomerular RhoA activity, remnant glomeruli had clearly increased RhoA activation (arrows) and this was inhibited by colchicine. As a negative control, incubation with GST alone did not produce any immunofluorescence (not shown). We further performed a RhoA activity assay on cortical lysate in which active GTP-bound RhoA is immunoprecipitated with the same GST fusion protein as used for imaging, and immunoprecipitated RhoA was detected by immunoblotting. Remnant kidneys had clearly increased RhoA activation which was attenuated by colchicine (Fig. 3B). Data are shown graphically in Fig. 3C.

**Colchicine protects against fibrosis in hypertensive chronic kidney disease.** We next assessed the effects of colchicine on glomerular and tubulointerstitial fibrosis. The degree of glomerular matrix accumulation was assessed on PAS-stained sections, shown in Fig. 4A, by an observer blinded to treatment group according to the scale outlined in MATERIALS AND METHODOLOGY. As shown graphically in Fig. 4B, significantly increased glomerular sclerosis was seen in remnant rats, and this was attenuated by colchicine. Tubular injury was also present in remnant rats, with significantly dilated tubules and flattened epithelial cells as indicated by the arrow in the trichrome stain in Fig. 4A. The degree of tubulointerstitial fibrosis was assessed on these sections in a blinded fashion according to the scale outlined in MATERIALS AND METHODOLOGY. As shown

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**Fig. 6.** Colchicine inhibits extracellular matrix and CTGF protein upregulation. A, B: kidney cortex was analyzed by immunoblotting for expression of the extracellular matrix genes fibronectin and collagen 1α1. Both were increased in the remnant rat and inhibited by colchicine (*P < 0.05 vs. others for fibronectin, †P < 0.01 5/6 vs. others for collagen). C: similarly, CTGF was also upregulated in remnant rats and this was inhibited by colchicine (‡P < 0.001 5/6 vs. others).
graphically in Fig. 4C, colchicine also attenuated interstitial fibrosis in the remnant model.

The transcript levels of several constituents important for fibrosis were assessed by real-time PCR on kidney cortex. Transcripts of both fibronectin and collagen I were significantly increased in remnant kidneys and this was largely prevented by treatment with colchicine. CTGF upregulation in remnant kidneys was similarly attenuated by colchicine (Fig. 5, A–C). Protein expression of each of these was also assessed. Figure 6 shows that the upregulation of fibronectin, collagen I, and CTGF in remnant kidneys was also inhibited by colchicine. We further assessed matrix upregulation by IHC. Figure 7A shows upregulation of fibronectin, collagen I, and CTGF in glomeruli and to some extent in tubules of remnant rats, with inhibition by colchicine. Quantification of stain intensity is shown in Fig. 7, B–D. The degree of reduction in these proteins is more profound than the observed reduction in PAS and trichrome staining seen in Fig. 4. Since these stains capture overall presence of multiple proteins, a possible explanation for this discrepancy is a differential effect of colchicine on the production or breakdown of other constituents not tested such as collagen III, collagen IV, and heparan sulfate proteoglycan.

TGF-β is an important pathologic factor in the development of renal fibrosis in this model (20). Smads are the canonical downstream mediators of TGF-β signaling, with Smad3 being required for both CTGF upregulation and fibrosis in models of

Fig. 7. Immunohistochemistry shows attenuation of extracellular matrix protein and CTGF upregulation by colchicine. A: immunohistochemistry was performed on cortical sections for fibronectin, collagen I, and CTGF. All were increased in both glomeruli as well as some surrounding tubules. Quantification of positive areas was performed and is shown in B–D. *P < 0.001 5/6 vs. others for CTGF and collagen, †P < 0.05 5/6 vs. others for fibronectin.
chronic kidney disease (7, 10). Smad3 phosphorylation at its COOH-terminus S423/S425, representative of its activation, was assessed by IHC in cortical kidney sections. As seen in Fig. 8A, Smad3 phosphorylation was significantly increased in remnant glomeruli and this was prevented by treatment with colchicine. Quantification of stain intensity is shown in Fig. 8B.

Colchicine attenuates proliferation and protects against inflammatory cell infiltration. Cell proliferation is an early event, observed before the development of glomerulosclerosis in the remnant model (11). Both resident glomerular and tubular cells, as well as infiltrating inflammatory cells, may undergo proliferation, and increased proliferation has been associated with worsened renal function and fibrosis (26, 36). Since colchicine inhibits cell division through its effects on microtubules (33), we assessed whether it altered proliferation in our model. As shown in Fig. 9A, IHC performed for PCNA showed significantly increased proliferation in remnant rats. Quantification of the number of PCNA-positive cells in both total sections and specifically in glomeruli shows significant attenuation by colchicine (Fig. 9, B–C).

Renal inflammatory cell infiltration has been shown to contribute to injury in the remnant model (12, 18, 32). Colchicine is well-known as an anti-inflammatory agent, affecting the mobility and function of cells (21, 38). We thus assessed the effects of colchicine on the degree of inflammatory cell infiltration in our model. IHC was performed on sections for ED-1 and CD3, markers of macrophages and T-cells, respectively. As seen in Fig. 10, very few inflammatory cells were present in sham kidneys. ED-1 and CD3 were both significantly higher in remnant rats, with more ED-1-positive than CD3-positive cells observed. The presence of both cell types was significantly lower in colchicine-treated rats. This is shown quantitatively in Fig. 10, B–C. Taken together, these results clearly show that colchicine attenuates both renal fibrosis and inflammatory cell infiltration in this model of kidney disease driven by intraglomerular hypertension.

DISCUSSION

Hypertension is a major risk factor for the development or progression of chronic kidney disease, particularly in the setting of renal mass reduction of any etiology. Reduced renal mass is associated with impaired autoregulatory capacity, allowing transmission of elevated blood pressure to glomeruli (13). Early experimental studies showed that correction of intraglomerular hypertension was critical to protection against the development of renal fibrosis (1), providing insight into the significant role of intraglomerular hypertension as a risk factor for renal failure. The effectiveness of angiotensin II signaling blockade lies at least in part in its ability to reduce intraglomerular hypertension (1). However, angiotensin II blockade is not fully protective against renal functional decline and is not tolerated by all patients. Thus, there is a need to identify alternate therapies that target the renal fibrotic process.

Intraglomerular hypertension can be modeled in vitro by mechanical stretch of mesangial cells, which lie centrally within the glomerulus and are a major source of extracellular matrix production in the glomerular sclerotic process (8). We and others showed that RhoA/Rho-kinase signaling is an important mediator of matrix upregulation and extracellular organization in response to various stimuli (14, 15, 40). In our initial in vitro studies, the microtubule disrupting agents colchicine and nocodazole inhibited stretch-induced activation of RhoA. Since, in vivo, Rho-kinase inhibition decreased renal fibrosis in models of hypertensive kidney disease (25), we hypothesized that colchicine may be similarly protective. Indeed, in our model of chronic kidney disease characterized by both systemic and intraglomerular hypertension, colchicine inhibited RhoA activation in remnant kidneys. This was associated with significant attenuation of glomerular and interstitial fibrosis and a decrease in upregulation or activation of two key profibrotic cytokines, CTGF and TGF-β, in the absence of any effects on systemic blood pressure. These studies indicate that colchicine modifies hypertensive renal fibrosis, effects that are likely mediated at least in part by inhibition of RhoA signaling.

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These findings are in contrast to a study from 1996 in which colchicine did not affect any parameters of experimental renal fibrosis in the 2/3 nephrectomy model in Wistar rats (39). Since this was published as a letter, limited information was provided. However, colchicine therapy was delayed, given 30 days after surgery for a total of 60 days. It is likely that delayed treatment in the setting of already existing significant injury is insufficient to reverse established fibrosis.

In addition to potential effects of microtubule depolymerizing agents on fibrosis, they have also been shown to inhibit inflammatory cell migration. Indeed, colchicine is best known for its use as an anti-inflammatory agent, effective in the treatment of the inflammatory response of numerous conditions including gout, familial Mediterranean fever, and Behcet’s disease. A role for immune cell infiltration, including that of monocytes/macrophages and T-cells, has been shown in mediating hypertensive renal damage through their release of cytokines such as TNF-α, IL-1, and TGF-β (12, 18). Agents that modulate the inflammatory response such as mycophenolate mofetil (MMF) and the leukocyte migration inhibitor FTY720 were shown to decrease not only renal infiltration by inflammatory cells, but also proteinuria and fibrosis (18, 35). We observed a significant reduction in renal infiltration of macrophages and lymphocytes by colchicine, which may also have contributed to its protective effects. Interestingly, Rho-kinase signaling also mediates inflammatory cell migration, and renal inflammation was attenuated by Rho-kinase inhibitors in hypertensive and fibrotic models (25, 34). The inhibitory effects of colchicine on RhoA activation may thus help mediate the observed reduction in inflammatory cell infiltration. Alternately, given that inflammatory cells secrete cytokines such as TNF-α and TGF-β which have been shown to activate RhoA (28, 37), it is also possible that direct effects of colchicine on reduction of inflammatory cells may have led to the decreased RhoA activation.

Animal model data have shown that correcting systemic hypertension, but not elevated intraglomerular pressures, does not lead to improvement in renal function or fibrosis (1, 22). Although colchicine had no effect on systemic hypertension, it is unknown whether it has any effect on intracapillary pressures. Interestingly, the anti-inflammatory agent MMF does reduce intracapillary hypertension. Higher afferent arteriolar resistance was seen in rats treated with MMF, suggesting preservation of the functional capacity of preglomerular vessels. Histologic analysis showed that MMF attenuated afferent arteriolar constriction, although the mechanism of action for this protective effect is yet to be determined (35). Effects of colchicine on reducing elevated intracapillary pressures thus cannot be excluded. Indeed, colchicine has been shown to cause endothelial-independent vasoconstriction in isolated arterioles (29). However, its inhibitory effects on RhoA/Rho-kinase activation in this model are also relevant. Several studies have shown that Rho-kinase inhibition blunts afferent arteriolar constriction, inhibiting its autoregulatory ability (4, 16, 23). This would be expected to lead to increased intraglomerular pressures. However, Rho-kinase inhibition was effective in a hypertensive remnant model in ameliorating renal fibrosis and inflammation (17). This suggests that Rho-kinase...
inhibition, and by extension colchicine, may limit renal injury through pressure-independent effects.

Colchicine has also been shown to protect against renal fibrosis in other models including early diabetic nephropathy and cyclosporin nephrotoxicity (9, 21). However, although protective in our model, colchicine did not fully reverse renal fibrosis, and its effects on renal function were limited. The magnitude of these findings is similar to other studies demonstrating the attenuation of hypertensive renal disease by anti-inflammatory agents (18, 35). The lack of significant effect on proteinuria may reflect the continued presence of intraglomerular hypertension and/or podocyte injury. Assessing changes in creatinine may also not be sufficiently sensitive to detect smaller changes in renal function. Alternatively, given the severity of this model, treatment before severe reduction in renal function occurs may be important for greater protective effect. This would require evaluation in less severe models of hypertensive renal failure. Indeed, blockade of the renin-angiotensin system slows, but does not halt progression of renal insufficiency, particularly when therapy is started late.

Our studies indicate that colchicine modifies hypertensive renal fibrosis. The advantage of colchicine lies in long clinical experience with its use. However, the chronic use of colchicine in more advanced renal failure carries greater potential for toxicity. Whether colchicine has even greater efficacy in hypertensive models with less severe reduction of renal mass in preventing the development of fibrosis merits study. Additionally, evaluation of colchicine in conjunction with other agents that attenuate intraglomerular and systemic hypertension, which may provide greater clinical benefit, would be of significant interest.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


