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COMMENTARY

Combined rho kinase and renin–angiotensin system inhibition: a new therapeutic perspective for renal and cardiovascular remodeling

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Asymptomatic organ damage due to progressive kidney damage, cardiac hypertrophy and remodeling put hypertensive patients at high risk of developing heart and renal failure, myocardial infarction and stroke. Current antihypertensive treatment normalizes high blood pressure, partially reverses organ damage, and reduces the incidence of heart and renal failure. Activation of the renin–angiotensin system (RAS) is a primary mechanism of progressive organ damage and, specifically, a major cause of both renal and cardiovascular fibrosis. Inhibition of the RAS system (mainly with acetylcholinesterase (ACE) inhibitors or angiotensin II (Ang II) receptor antagonists) is the most effective antihypertensive strategy for normalizing blood pressure and preventing target organ damage. However, residual organ damage and, consequently, a high risk for cardiovascular events and renal failure still persist. Thus, it is very important to develop new therapeutic strategies, beyond reducing blood pressure, to further reduce target organ damage by acting on pathways that trigger and maintain renal and cardiovascular remodeling.

One novel intracellular mechanism that triggers remodeling is the small guanosine triphosphatase Rho and its target Rho kinase (ROCK). Both of these kinases have important roles in blood pressure regulation, vascular smooth muscle contraction, and cardiovascular and renal remodeling. Rho is activated by the agonists of receptors coupled to the cell membrane G protein, such as Ang II and noradrenaline. Once Rho is activated, it translocates to the cell membrane, where it activates ROCK. Activated ROCK has an important role in mediating various cellular functions, such as vascular smooth muscle cell contraction, actin cytoskeleton organization, adhesion and motility, cytokinesis, and expression of genes involved in cardiovascular and renal remodeling. ROCK also mediates the upregulation of several proinflammatory, thrombogenic and fibrogenic molecules, and the downregulation of endothelial nitric oxide (NO) synthase (eNOS).[1,2] Thus, when ROCK is activated, inflammation, thrombosis and tissue fibrosis are accelerated, whereas endothelial NO production is inhibited. In addition, ROCK activation promotes the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and oxidative stress (Figure 1).

There have been several reports of experimental observations regarding the role of ROCK in kidney and cardiovascular remodeling by using single ROCK inhibition. In experimental hypertension, ROCK inhibition prevented kidney damage.[3–5] Potential mechanisms of renoprotection by ROCK inhibition in these models are inhibition of the extracellular matrix gene expression, reduction of monocyte/macrophage infiltration and oxidative stress, and upregulation of eNOS gene expression.[4] In aldosterone-induced renal injury in rats, ROCK inhibition by fasudil did not alter blood pressure but did ameliorate proteinuria, renal injury and inflammation, and reduced messenger RNA (mRNA) levels of collagen, transforming growth factor-β, connective tissue growth factor (CTGF) and monocyte chemoattractant protein-1 in renal cortical tissue.[6] In diabetic rats, fasudil treatment attenuated renal interstitial fibrosis and decreased ROCK and α-smooth muscle actin expression.[7]

In hypertensive DOCA/salt rats, fasudil ameliorated diastolic dysfunction, cardiomyocyte hypertrophy, cardiac fibrosis, superoxide production and monocyte/macrophage infiltration.[8] In AT1aR knockout mice receiving aldosterone and salt, ROCK inhibition with fasudil reduced cardiac hypertrophy, fibrosis, elevated expression of CTGF, NADPH components p22phox, p47phox and p67phox, and myocardial damage due to oxidative stress.[9] ROCK has an important role in the differentiation of monocytes that mature into cardiac fibroblasts and induce fibrosis in murine ischemia/reperfusion injury.[10]

In this issue of *Hypertension Research*, Takeda *et al.*[11] describe a very elegant study that assessed for the first time the hypothesis that a combination of a ROCK inhibitor (fasudil) and an ACE inhibitor (imidapril) would improve renal interstitial fibrosis more than monotherapy, using an experimental model of normotensive renal fibrosis induced by unilateral ureteral obstruction (UUO). After surgery, mice were randomly assigned to imidapril, fasudil or a combination of the two for 11 days. The results were very neat. The combination of the ROCK inhibitor and the ACE inhibitor was clearly more effective than monotherapy in prevention of kidney interstitial fibrosis, induction/differentiation of interstitial myofibroblasts, inflammation, cytokine production and oxidative stress. Blood pressure reduction was similar across both the groups that received the ACE inhibitor.

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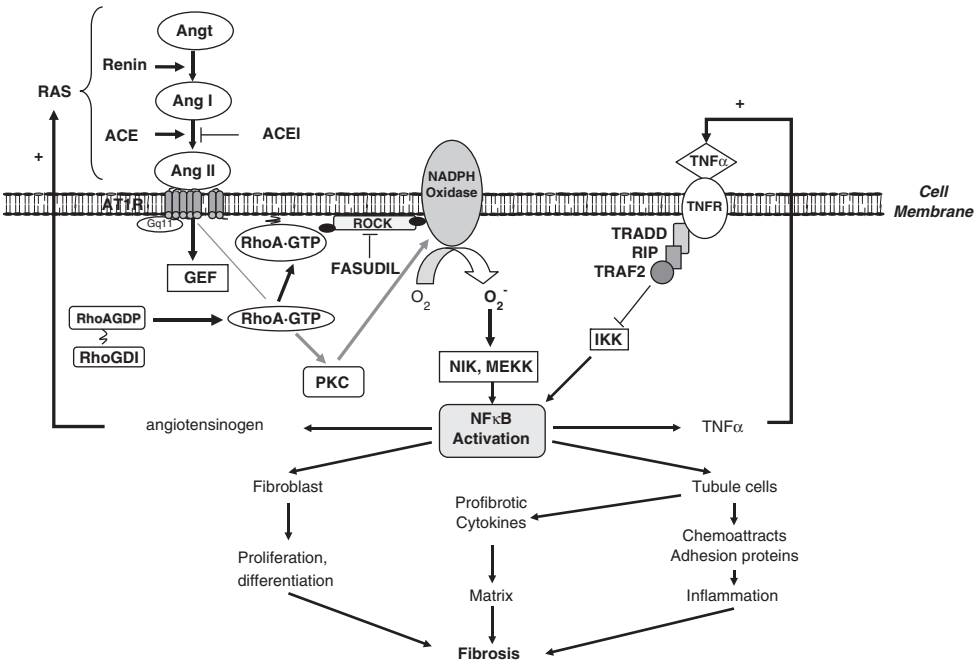

Figure 1 The potential mechanisms involved in inflammation and fibrosis in unilateral ureteral obstruction (UUO). UUO induces interleukin-1β and tumor necrosis factor (TNFα) expression, leading to nuclear factor kappa B (NF-κB) activation. UUO also induces oxidative stress and increases angiotensin II (Ang II) levels. The regulation by Ang II of gene expression occurs through activated transmembrane receptors that directly activate intracellular signaling proteins, specifically G-proteins (Gq/11), which promote activation of RhoA/ROCK and protein kinase C (PKC) cascades. The activation of these signaling cascades modulates the nicotinamide adenine dinucleotide phosphate activity and the reactive oxygen species levels, which promote the activation of mitogen-activated protein kinase (MAPK) cascades: MAPK kinase (MAPKK). The activation of these signaling cascades also modulates the NF-κB family of transcription factors. NF-κB translocates to the nucleus, where it regulates the genes encoding pro-inflammatory cytokines, adhesion molecules and inducible nitric oxide synthase. NF-κB stimulates two other autocrine loops that amplify Ang II by regulating angiotensinogen expression and tumor necrosis factor (TNFα) formation. TNFα activates NF-κB by binding TNFα receptor (TNFR) and I-κB (IKK) inhibition (partially modified from Klahr and Morrissey[12] and Grande *et al.*[13]). ACEI, angiotensin I converting enzyme inhibitor; GTP, guanosine triphosphate; IKK, IκB kinase; MEKK, mitogen-activated protein kinase kinase kinase 1; RIP, receptor-interacting protein kinase; TRADD, TNFRSF1A-associated via death domain; TRAF2, TNF receptor-associated factor 2.

Interestingly, increased levels of kidney NADPH mRNA subunits were reduced only with the combination therapy, which led to a further reduction in renal superoxide anion generation.[11] By generating reactive oxygen species (ROS), NADPH oxidases contribute to oxidative stress and consequent cardiovascular and renal injury. ROS inuence diverse signal transduction pathways via oxidation of reactive cysteine residues on specic target molecules. ROS activate ion channels (Ca^{2+} and K^+ channels), redox-sensitive kinases (Src Akt, protein kinase C, mitogen-activated protein kinases and ROCK) and transcription factors, such as the nuclear factor-κ-light chain enhancer of activated B cells (NF-κB). Through these effects, ROS inuence cell growth, inammation, fibrosis (Figure 1), apoptosis, senescence, secretion, migration, contraction/dilation and permeability, and thus have a major impact on maintaining cardiovascular and kidney integrity. In addition, via the AT1 receptor, Ang II stimulates oxidative stress mainly by O_2^- production. This process is induced by stimulation of NADPH oxidase activity through activation of signaling pathways involving c-Src p21Ras, protein kinase C, and phospholipases D and A2. Ang II also inuences NADPH oxidase activation through transcriptional regulation of its oxidase subunits.

Increased oxidative stress is involved in renal inflammatory damage after UUO, which is related to increased NADPH oxidase activity, which is a major source of superoxide production.[12] In addition, obstructive nephropathy leads to activation of the intrarenal RAS,[13] and Ang II has a central role in the initiation and progression of obstructive nephropathy, by stimulating the production of molecules that contribute to renal injury (such as NF-κB activation and the subsequent increased expression of proinflammatory genes). ACE inhibitors reduce monocyte/macrophage infiltration in the obstructed kidney, but this reduction has been observed only in short-term UUO, possibly because in late-stage UUO infiltration is dependent on cytokine formation that is independent of Ang II.[13]

Before this strategy can be validated, the real impact, the effects of newer ROCK inhibitors and the optimal treatment combination for a specific renal or cardiovascular disease, related or unrelated to hypertension, need to be determined. Takeda *et al.*'s[12] new approach to prevention of renal and cardiovascular remodeling, using the combination of ROCK inhibition and RAS blockade, appears to be a promising therapeutic strategy.

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