Molecular Mechanisms and Therapeutic Strategies of Chronic Renal Injury: Role of Rho-Kinase in the Development of Renal Injury

Koichi Hayashi\textsuperscript{1,*}, Shu Wakino\textsuperscript{1}, Takeshi Kanda\textsuperscript{1}, Koichiro Homma\textsuperscript{1}, Naoki Sugano\textsuperscript{1}, and Takao Saruta\textsuperscript{1}

\textsuperscript{1}Department of Internal Medicine, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Received September 28; Accepted November 7, 2005

Abstract. Rho/Rho-kinase plays an important role not only in the vasoconstrictor mechanism but also in cellular morphology, motility, adhesion, and proliferation. This pathway also serves to modulate the structure and function of various kidney cells including tubular epithelial cells, mesangial cells, and podocytes. The inhibition of the Rho/Rho-kinase pathway elicits marked increases in renal blood flow in vivo and dilates both afferent and efferent arterioles preconstricted by angiotensin II in vitro. In renal injury, intrarenal angiotensin II is reported to be activated, which subsequently would upregulate the Rho-kinase pathway. A selective Rho-kinase inhibitor, fasudil, has recently been shown to improve renal damage resulting from hypertensive glomerulosclerosis, unilateral ureteral obstruction (for interstitial renal fibrosis) and subtotal nephrectomy. Of interest, fasudil upregulated the expression of p27\textsuperscript{kip1}, a cyclin-dependent kinase inhibitor, and increased the p27\textsuperscript{kip1} immuno-positive cells in both glomeruli and tubulo-interstitium with the use of immunohistochemistry. Collectively, the Rho-kinase pathway is involved in the pathogenesis of renal injury. Clinical application of this type of therapy however awaits further investigations.

Keywords: Rho-kinase, renal hemodynamics, fasudil, renal injury, p27\textsuperscript{kip1}

Introduction

Renal injury relentlessly progresses to end-stage renal disease that requires renal replacement therapy. A number of clinical and experimental studies have been conducted examining the effect of angiotensin II blockade (ACE inhibitors and angiotensin receptor antagonists) on the progression of diabetic as well as non-diabetic renal disease and have demonstrated a substantial benefit by these agents. Nevertheless, no satisfactory modality has been accepted to halt the injury process of renal disease. Recently, Rho/Rho-kinase has been the focus of investigators because of its potent inhibitory action on hemodynamics and vaso-motor tone. Thus, it has been reported that Rho/Rho-kinase inhibits myosin phosphatase by phosphorylating its myosin binding subunit (MBS), favoring accumulation of phosphorylated myosin light chain and enhanced contraction of vascular smooth muscle cells (1). Recently, two Rho-kinase inhibitors, Y-27632 and fasudil, have been developed and used extensively to elucidate the biological roles of the Rho/Rho-kinase pathway. Indeed, Y-27632 is demonstrated to reduce blood pressure in different forms of experimental hypertensive animals (2). Furthermore, it has also been established that Rho/Rho-kinase participates in cell adhesion/migration and proliferation (1, 3) and is involved in various models of cardiovascular disorders, independently of systemic blood pressure (4). Thus, the inhibition of Rho-kinase is reported to suppress the neointimal formation of balloon-injured rat carotid arteries (5) and attenuate the formation of the coronary injury induced by the chronic treatment with MCP-1 and/or LDL (6). Thus, available evidence clearly indicates the important contribution of Rho/Rho-kinase to the development of hypertension and organ injury induced by various disorders including hypertension and atherosclerosis.

In this review article, we attempted to elucidate the
role of Rho/Rho-kinase in the pathogenesis and/or progression of renal disease in various models of renal injury.

Activation of Rho/Rho-kinase

Rho/Rho-kinase have been shown to regulate the phosphorylation level of myosin light chain of myosin II and contribute to agonist-induced Ca\(^{2+}\) sensitization in vascular smooth muscle contraction. Rho exhibits both GDP/GTP-binding activity and functions as a molecular switch, cycling between a GDP-bound inactive state (GDP-Rho) and a GTP-bound active state (GTP-Rho) (1). In the resting state, the GDP-Rho dissociation inhibitor (Rho-GDI) binds to GDP-Rho and extracts GDP-Rho from the membrane to the cytosol. Under the stimulated condition, GDP-Rho is converted to GTP-Rho through the action of guanine nucleotide exchange factors (GEFs). Activated Rho interacts with the downstream effector of Rho, Rho-kinase (ROCK, Rho-associated kinase), which subsequently inhibits the myosin phosphatase activity by phosphorylating this molecule and increases the phosphorylation level of myosin light chain.

Rho/Rho-kinase is activated in a variety of disorders. For example, Wakino et al. (7) have recently demonstrated that Rho kinase activity, as assessed by the phosphorylation of MYPT, is enhanced in the aorta obtained from spontaneously hypertensive rats (Fig. 1A). Of interest, the upregulated Rho-kinase activity is markedly suppressed by pioglitazone, an insulin sensitizing PPAR\(\gamma\) ligand, with a concomitant reduction in systemic blood pressure. This observation suggests a close link between Rho-kinase and the insulin signaling pathway and may further provide a clue to the therapeutic strategy in the treatment of metabolic syndrome.

It has been demonstrated that Rho-kinase is also upregulated by angiotensin II. We have recently shown that in the aortic wall of angiotensin II-infused mice, aortic medial wall thickness was reduced by the treatment with fasudil, a selective Rho-kinase inhibitor (8). This change is accompanied by the increased expression of p27kip, a marker for cell cycle signaling. Since the increase in p27kip is a factor suppressing cell proliferation, the Rho-kinase would exert its action on the vascular tissue which is modified by p27kip.

Much attention has been focused on the role of T-type calcium channels in a variety of organs. Sugano et al. have shown in a preliminary report a substantial contribution of T-type calcium channels in mediating the angiotensin II-induced Rho kinase activation. Thus, the angiotensin II-enhanced Rho-kinase activity in the rat aorta is partially suppressed by the calcium antagonists that possess T-type calcium channel blocking activity, efonidipine and mibebradil. Furthermore, an R(-)-enantiomer of efonidipine, which is characterized by a more T-channel-specific blocking activity, inhibits the Rho/Rho-kinase pathway more markedly than an S(+) -enantiomer possessing the blocking activity on both T- and L-type calcium channels (9). Since it is established

![Fig. 1. Effects of angiotensin II on Rho-kinase activity. In cultured vascular smooth muscle cells, angiotensin (100 nmol/L) markedly enhanced the Rho-kinase activity, as assessed by MYPT phosphorylation (A, from Ref. 7 with permission from Lippincott Williams & Wilkins ©2004). In the isolated perfused hydronephrotic kidney model, angiotensin II (0.3 nmol/L)-induced vasoconstriction of afferent and efferent arterioles were reversed by both Y-27632 and fasudil (B, from Ref. 10 with permission from S. Karger AG ©2003). *P<0.05 vs angiotensin II.](image-url)
that angiotensin II constitutes a pivotal determinant of the progression of renal injury, these observations would provide a novel therapeutic approach to the treatment of angiotensin II-mediated organ injury.

In addition to smooth muscle contraction, RhoA regulates a wide range of cellular functions in response to a variety of stimuli. Rho regulates the actin cytoskeleton and activation of Rho leads to the assembly of actin-myosin stress fiber filament (1). Rho also is involved in focal adhesion complex formation (1) and in the regulation of cell shape, movement, polarity, and progression of the cell cycle. Collectively, these effects would raise our expectation for the Rho-kinase inhibitor as a therapeutic tool for the treatment of vascular disease, including atherosclerosis and neointimal formation.

**Role of Rho/Rho-kinase in the kidney**

Rho-kinase is constitutionally active in the renal circulation. Thus, recent studies demonstrate that Rho-kinase inhibition by Y-27632 and fasudil dilates basal tone of afferent as well as efferent arterioles in the in vitro (10) and in vivo hydrenephrotic kidney models (11), although the vasodilator response of efferent arterioles is slightly less than that of afferent arterioles. Furthermore, both Y-27632 and fasudil reverse the angiotensin II-induced vasoconstriction of afferent and efferent arterioles (Fig. 1B). Thus, the altered balance of renal pre- and post-glomerular microvascular tone may affect glomerular hemodynamics (12) and subsequently could modify the development of renal disease, although it has not been evaluated whether the inhibition of Rho-kinase alters the glomerular capillary pressure. These mechanisms of the Rho-kinase inhibition may serve in part to exert renal protective action in chronic renal injury.

In addition to a critical role of Rho-kinase in the renal microvasculature, Rho proteins are important endogenous regulators of several types of renal tubular functions, including proliferation, migration, and apoptosis (13). It has been demonstrated that Rho regulates the formation of stress fibers, focal adhesions, and peripheral bundles through reorganization of the actin cytoskeleton in a renal epithelial cell line, Madin-Darby canine kidney cells (MDCK cells) (14). Furthermore, renal epithelial cells are able to transform mesenchymal-like cells (e.g., myofibroblasts) via the process of epithelial mesenchymal transdifferentiation (EMT) (15). These changes have been observed in the process of renal tubulointerstitial fibrosis. For example, human proximal tubular epithelial cells (HK2 cells) undergo EMT by the stimulation with activated peripheral blood monocyte conditioned medium (16).

Mesangial cells are smooth muscle-like cells that reside in the renal glomerulus and produce extracellular matrix (ECM) protein or collagen to form mesangial matrix. Increased accumulation of ECM causes glomerulosclerosis, where TGF-β1 has been implicated as a causative factor (17). Although the effects of TGF-β1 on mesangial collagen I accumulation are mediated by several pathways, the Rho/Rho-kinase mechanism constitutes an important role in mediating the cytoskeletal rearrangement of mesangial cells (18). Furthermore, similar to renal tubular epithelial cells, mesangial cells also employ transdifferentiation into myofibroblasts in various glomerular diseases (19). This transdifferentiation is characterized by the activation of smooth muscle α-actin expression, and Rho-kinase inhibitors, Y-27632 and HA-1077, block this process in cultured renal mesangial cells (20). Finally, in mesangial cells, mechanical stress, which is considered to cause glomerular hypertension and glomerulosclerosis, enhances mitogen-activated protein kinase (MAP kinase) activity, stress fiber formation, and cellular proliferation (21). In this disease process, RhoA plays an essential role, acting as a modulator of MAP kinase and the subsequent cellular impact. Collectively, these data strengthen a significant role of Rho/Rho-kinase pathway in the development of glomerulosclerotic renal disease.

Podocytes are located in the renal glomerulus and are highly differentiated cells with a complex cellular morphology composed of F-actin (22). Cytoskeleton F-actin rearrangement is closely associated with podocyte shape changes and dysfunction in various renal disease (23). A growing body of evidence has been accumulated that the Rho-kinase plays an important role in the aggravation of renal injury. In a study using cultured renal podocytes, a Rho-kinase inhibitor (Y-27632) is reported to inhibit mechanical stress-induced reorganization of cytoskeleton. Thus, Endlich et al. (24) found that Y-27632 inhibited the reorganization of cytoskeleton induced by mechanical stress in cultured renal podocytes. Furthermore, the inhibition of Rho-kinase activity is reported to prevent the transforming growth factor-β1-induced increase in connective tissue growth factor accumulation in cultured human renal fibroblast cells (25).

Although these in vitro observations strongly suggest substantial roles of Rho-kinase in mediating the progression of renal injury, only a couple of studies have been conducted that provided direct in vivo evidence for the contribution of Rho-kinase to the development of renal disease. Since Rho/Rho-kinase regulates glomerular hemodynamics and have substantial effects
on mesangial cell proliferation, matrix production, and contraction, Rho-kinase inhibitors can be candidates for therapeutic tools to treat glomerulosclerotic disease. Thus, it has been demonstrated that Y-27632 and fasudil prevent tubulointerstitial fibrosis in the model of unilateral ureteral obstruction (26, 27). Kanda et al. (28) investigated the role of the Rho/Rho-kinase pathway in the development of renal insufficiency induced by 5/6 nephrectomy in spontaneously hypertensive rats (SHR), a model for hypertensive glomerulosclerosis. They demonstrated that the Rho/Rho-kinase pathway was activated in subtotal nephrectomized SHR (Fig. 2) and suggested an involvement of the Rho/Rho-kinase pathway in the progression of hypertensive glomerulosclerosis. The treatment with fasudil decreases urinary protein excretion, improved glomerular and tubulo-interstitial injury score, and reduced the infiltration of ED-1 positive cells and proliferating cell nuclear antigen positive cells in the kidney of SHR with subtotal nephrectomy. In this study, fasudil did not significantly reduce the systemic blood pressure, suggesting blood pressure independent effects. Alternatively, the mechanisms for the renal protective effects of fasudil were considered to be through the downregulation of p27kip1, a cyclin-dependent kinase inhibitor, and the subsequent inhibition of cell proliferation and macrophage recruitment. Similarly, beneficial results of fasudil were reported in Dahl salt-sensitive rats (29). Chronic treatment with fasudil improved renal function, proteinuria, and histological findings without changes in blood pressure in Dahl salt-sensitive rats. The renoprotective effects of fasudil are mediated probably through the decreased expression of TGF-β, collagen-I, and collagen-III mRNA in the renal cortex. These two studies therefore imply that the Rho/Rho-kinase pathway can be a new strategy for the treatment of hypertensive nephrosclerosis.

Multiple factors contribute to the development of chronic renal injury. As already established, systemic hypertension and the subsequent mechanical stress constitute deteriorating factors of renal injury (30). Furthermore, angiotensin II is demonstrated to contribute to renal impairment in chronic renal disease, even though systemic blood pressure is unaltered (31). The latter observation is consistent with previous reports that non-hemodynamic actions of angiotensin II may be an aggravating factor (32). In this regard, it has been reported that angiotensin II (33) and mechanical stretch (34) activate Rho-kinase in vascular smooth muscle cells. Moreover, enhanced Rho-kinase activity is involved in the pathogenesis of neointimal formation (6). Therefore, it can be extrapolated that the role of Rho-kinase is exaggerated in pathophysiological conditions, such as chronic renal injury. In subtotal nephrectomy, alterations in glomerular capillary pressure (35) may also play a role in the enhancement of Rho/Rho-kinase activity through the mechanical stretch (34). It is possible therefore that the Rho-kinase inhibition confers beneficial action on renal injury through blood pressure-dependent and -independent mechanisms.

Concluding remarks

Evidence has been accumulated regarding important roles of Rho/Rho-kinase in the development and initiation of renal injury. In addition to the authentic action to enhance calcium sensitivity in vascular smooth muscles, the Rho/Rho-kinase pathway is characterized as acting on various cellular functions, including cell migration, proliferation, and fibrosis. Collectively, blocking these actions may constitute a promising tool for the treatment of various types of renal disease that usually manifest hypertension.

References

4 Shimokawa H: Rho-kinase as a novel therapeutic target in