Rho Kinase Inhibition: A New Approach for Treating Diabetic Nephropathy?

Leon A. Bach

Rho family GTPases have received increasing attention as critical regulators of cell function (1). Initially, they were shown to regulate actin dynamics, thereby modulating development, cell migration, immune responses, and cancer cell invasion and metastasis. More recent studies have shown that they are also involved in cell-cell adhesion and cell cycle progression. RhoA is one of the most widely studied of the 22 mammalian members of the family, and the serine-threonine kinase Rho kinase (ROCK) is a major RhoA effector.

RhoA/ROCK has a number of functions in the kidney. RhoA/ROCK enhances Ca^{2+}-dependent vascular smooth muscle contraction, thereby modulating tone (2–4). The potent vasoconstrictor angiotensin II (AngII) activates ROCK in smooth muscle cells. The ROCK inhibitors fasudil and Y-27632 dilate afferent and efferent arterioles and reverse AngII-dependent arteriolar vasoconstriction. At the cellular level, RhoA/ROCK mediates cytoskeletal rearrangement in renal tubule cells, mesangial cells and podocytes and may contribute to epithelial-mesenchymal transdifferentiation, which may be important in the development of renal fibrosis. ROCK inhibitors have renoprotective effects in a number of models of kidney damage (2). They prevent tubulointerstitial fibrosis following unilateral ureteral obstruction, and decrease structural and functional damage in hypertensive models without affecting blood pressure.

When activated, RhoA translocates from the cytoplasm to the cell membrane. RhoA translocation to the membrane was increased 1.8-fold in the renal cortex of rats made diabetic with streptozotocin (STZ), suggesting that RhoA was activated (5). RhoA translocation has also been observed in mesangial cells exposed to high glucose in vitro (6). Recently, three studies have investigated the utility of fasudil in experimental diabetic nephropathy (7–9). Fasudil is a relatively specific ROCK inhibitor, although it also inhibits other kinases, including protein kinase C–related protein kinase and mitogen- and stress-activated protein kinase 1, with lower potency (10). Kikuchi et al. (7) studied the effects of fasudil in insulin-resistant diabetic rats. When given from the time of development of diabetes, high-dose fasudil (100 mg/kg) improved metabolic parameters and decreased diabetes-induced proteinuria, glomerulosclerosis, interstitial fibrosis, and macrophage infiltration. Despite a lower dose of fasudil (30 mg/kg) having no effect on glycemic control, it significantly reduced interstitial fibrosis and macrophage infiltration but not glomerulosclerosis or proteinuria. When given to rats with established diabetes and early nephropathy, high-dose fasudil improved glycemic control but had no effect on fibrosis or proteinuria.

Gojo et al. (8) studied the effects of fasudil (10 mg/kg) for 30 days in STZ-diabetic rats. This dose of fasudil had no effect on plasma glucose, but it normalized albuminuria and decreased levels of urinary 8-hydroxyguanosine, a marker of oxidative stress. Fasudil prevented diabetes-related increases in mRNA for the fibrogenic growth factors transforming growth factor (TGF)-β and connective tissue growth factor (CTGF) as well as the NOX-4 catalytic subunit of NADPH oxidase, which contributes to diabetes-related reactive oxygen species formation.

In this issue of *Diabetes*, Kolavennu et al. (9) found that fasudil (10 mg/kg) given for 16 weeks to *db/db* mice from the age of 8 weeks had no effect on glycemic control but significantly decreased albuminuria, mesangial expansion, accumulation of glomerular type IV collagen, and glomerular basement membrane thickening. The specificity of the in vivo findings was confirmed in vitro where both Y-27632, another ROCK inhibitor, and dominant-negative RhoA decreased type IV collagen accumulation in mesangial cells exposed to high glucose. This study and that by Gojo et al. (8) clearly demonstrate that the effect of RhoA/ROCK inhibition is independent of glycemic control. This study extends that of Gojo et al. by its longer duration to a time point where nephropathy would be more severe and by showing the protective effects of fasudil on glomerular structure.

Gojo et al. (8) and Kolavennu et al. (9) both studied the effects of HMGCoA reductase inhibitors (also known as statins) on RhoA activation. Clinically, statins are used predominantly as lipid-lowering agents. However, there is considerable interest in pleiotropic effects of statins that are not mediated by lipid lowering, and there is evidence that statins have beneficial effects in kidney disease (11). These drugs inhibit activation of small GTPase proteins, including RhoA, by suppressing their prenylation, which is required for their attachment to cell membranes. The studies by Gojo et al. and Kolevannu et al. confirmed that renal cortical RhoA activity is increased in these models of type 1 and type 2 diabetes (8,9). Both showed that statins prevented the diabetes-induced increase in RhoA activity and had renal structural and functional effects paralleling those of fasudil. These findings raise the possibility that some of the renoprotective effects of statins may be due to RhoA/ROCK inhibition, although more definitive studies are required to confirm this.

Optimization of glycemic control and inhibition of the

From the Monash University Department of Medicine and Department of Endocrinology and Diabetes, Alfred Hospital, Melbourne, Victoria, Australia.

Address correspondence and reprint requests to Prof. Leon Bach, Department of Endocrinology and Diabetes, Alfred Hospital, Commercial Road, Melbourne, 3004, Victoria, Australia. E-mail: leon.bach@med.monash.edu.au.

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AngII, angiotensin II; RAS, renin-angiotensin system; ROCK, Rho kinase.

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renin-angiotensin system (RAS) are the mainstays of management of diabetic nephropathy, but renal damage progresses in many patients despite these measures. Novel approaches to treating nephropathy are therefore required, and these recent publications suggest that RhoA/ROCK inhibition is a promising approach. Differences that emerged among the studies may relate to the animal models, duration of diabetes, and treatment protocols. In a practical sense, it is important to determine the effectiveness of RhoA/ROCK inhibitors in established early nephropathy, such as persistent microabuminuria, since this is a likely clinical setting for its use, and the lack of efficacy in this setting in the study by Kikuchi et al. (7) provides a cautionary note. Given the potential interactions between RhoA/ROCK and the RAS, it also would be interesting to see whether RhoA/ROCK inhibition has effects over and above those of RAS inhibitors, which are in standard clinical use.

Of course, there is a long way to go before these findings can be applied clinically, since not all successful approaches in animal models prove to be effective in humans. In terms of safety, it is noteworthy that fasudil is approved for short-term clinical use in Japan for patients with subarachnoid hemorrhage with few apparent side effects (3,12). However, this does not preclude possible toxicity with long-term use as would be required for managing nephropathy. Nevertheless, these studies provide an exciting preview of an approach that may one day improve the lives of patients with diabetes.

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