Effects of p38 mitogen-activated protein kinase inhibition on blood pressure, renal hemodynamics, and renal vascular reactivity in normal and diabetic rats

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p38 mitogen-activated protein kinase (p38) has been implicated in mediating vascular smooth muscle and mesangial cell contraction in response to several vasoactive factors, including angiotensin II. Early stages of diabetic nephropathy are associated with renal hemodynamic changes that are, at least in part, attributable to the dysbalance of vasoactive factors that control afferent and efferent arteriolar tone resulting in increased glomerular capillary pressure. Vascular and renal p38 have been found to be activated in diabetes. Therefore, p38 may be involved in the control of systemic and renal hemodynamics in diabetes. To address this issue, mean arterial blood pressure (MAP), glomerular filtration rate (GFR, inulin clearance), renal plasma flow (RPF, PAH clearance), metabolic parameters, and plasma renin concentrations (PRC) were determined in streptozotocin-diabetic rats (DM), and in age-matched non-diabetic controls (C), administered with the p38 inhibitor SB 239063 (SB, 50 mg/bwt, p.o.) or with vehicle.

Furthermore, renal vascular responses to p38 inhibition (SB 202190, 25μM) before and after stimulation with the endothelium-dependent vasodilator acetylcholine (ACh) were studied *in vitro* in tertiary branches of the renal artery from separate groups of DM and C rats, using a fixed support and a force transducer in a myograph system. SB treatment was associated with marked reductions in MAP and GFR in both C and DM rats, whereas RPF remained unchanged, as compared with vehicle-treated animals. Observed differences in MAP and renal hemodynamics were not associated with changes in urinary sodium excretion or PRC. Incubation of KCl-contracted renal arteries from both C and DM rats with the p38 inhibitor resulted in progressive and significant vasorelaxation. Also, vessels from control and diabetic rats treated with the p38 inhibitor exhibited enhancement of ACh-induced vasorelaxation. These data indicate the role of p38 in the control of systemic and renal hemodynamics both in normal and in diabetic rats. The observed effects of p38 inhibition could be mediated at least in part by enhancement of endothelium-dependent vasodilation. (Translational Research 2007;150:343–349)

Abbreviations: ACh = acetylcholine; Ang II = angiotensin II; BG = blood glucose; COX-2 = cyclooxygenase-2; DM = diabetic rats; C = control rats; FF = filtration fraction; GFR = glomerular filtration rate; HBA1c = glycosylated hemoglobin; Hct = hematocrit; HSP = heat shock protein; MAP = mean arterial pressure; MAPK = mitogen-activated protein kinase; MD = macula densa; PAH = para-aminohippurate; PRC = plasma renin concentration; RAS = renin-angiotensin system; RPF = renal plasma flow; RVR = renal vascular resistance; STZ = streptozotocin; VE = vehicle; VSMC = vascular smooth-muscle cell

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p38, a member of the mitogen-activated protein kinase (MAPK) family, is activated by physical and chemical stress factors, inflammatory cytokines, and vasoactive and growth factors that result in growth promotion, apoptosis, and oxidative stress.[1–3] In addition, several lines of evidence suggest that p38 is part of the signaling cascades leading to vascular smooth muscle cell (VSMC) and mesangial cell contraction triggered by such agonists as angiotensin II (Ang II).[4,5] Therefore, p38 may be involved in the control of systemic and renal hemodynamics.

Available evidence suggests that the p38 pathway is activated in rat aortic VSMC,[6] in renal cells cultured in high-glucose media,[7] in isolated glomeruli of streptozotocin (STZ)-diabetic rats,[8] as well as in diabetic renal cortex *in vivo*[9] and in murine models of diabetes.[10] These experimental findings correspond to clinical evidence of increased numbers of phospho-p38-positive cells in various renal cell types in biopsies harvested from patients with type 2 diabetes.[10] Therefore, p38 may be involved in the control of systemic and renal hemodynamics in diabetes. However, the effects of p38 inhibition, in particular, on renal hemodynamics, have not been studied thus far.

It has been well established that factors related to impaired blood circulation in the kidney initiate a cascade of events that ultimately contribute to kidney failure in susceptible patients with diabetes and in animal models of the disease.[11] Renal hemodynamic changes early in the course of clinical and experimental diabetes are characterized by increases in glomerular filtration rate (GFR). Micropuncture studies have determined that on the single-nephron level, increases in GFR in diabetes are caused by imbalance of afferent and efferent arteriolar tone with a disproportionate decrease in afferent arteriolar resistance, and a relatively higher efferent arteriolar tone, resulting in increased glomerular capillary pressure.[12] Acting in concert with other factors, Ang II is a major vasoactive peptide implicated in the pathophysiology of these renal hemodynamic alterations in diabetes.[11] Although acting on both afferent and efferent arterioles, Ang II is considered to be a predominant efferent constrictor.[13] Antagonizing Ang II actions in the kidney leads to amelioration of diabetic hyperfiltration, and it has been a major component of nephroprotective actions of agents that interfere with the rennin–angiotenisn system.[14] Since p38 has been shown to be involved in mediating Ang II-induced constriction of the VSMC,[4,5] inhibition of p38 could have an impact on renal hemodynamics in diabetes.

In addition, p38 also has been implicated in reactive oxygen species signaling involving Ang II,[3,15] as well as in signaling leading to increased oxidative stress in the kidney.[16] Diabetes is considered to be a state of increased oxidative stress and reduced bioavailability of endothelial nitric oxide.[17] Therefore, inhibition of p38 could enhance endothelium-dependent vasodilation in diabetes.

To determine whether p38 inhibition affects the known changes observed in the regulation blood pressure and renal hemodynamics with diabetes, we examined the acute effects of the p38 inhibitor SB239063 in STZ-diabetic rats, an experimental model of type 1 diabetes, as compared with age-matched nondiabetic animals. Furthermore, we studied the effects of p38 inhibition on renal vascular reactivity *in vitro* in preparations harvested from control and diabetic rats.

METHODS

The diabetic rat model. Studies were conducted in adult male Sprague-Dawley rats with initial weights of approximately 300 g. The rats were made diabetic by intraperitoneal injection of STZ (Sigma Chemical Co., St. Louis, Mo), 65-mg/kg bodyweight. Three days later, induction of diabetes was confirmed by measurements of tail blood glucose (BG) level using a reflectance meter (One Touch II; Lifescan, Milpetas, Calif). Diabetic rats received daily evening injections of ultralente insulin (Iletin II; Eli Lilly and Co., Indianapolis, Ind) in doses individually adjusted to maintain BG levels between 200 and 300 mg/dL (11–17 mmol/L). Age-matched (renal hemodynamic studies) or weight-matched (renal vascular reactivity studies), nondiabetic Sprague-Dawley rats served as controls. All rats were fed standard rat chow (Rodent Laboratory Chow 5001; Ralston Purina, Richmond, Ind) ad libitum. These studies were approved by the Portland Veteran Affairs Institutional Animal Care and Use Subcommittee.

Design of hemodynamic studies. After 4 weeks of duration of diabetes, the rats and age-matched controls were divided into the following treatment groups: C-VE [control rats treated with vehicle (VE; 0.5% tragacanth in water)]; C-SB [control rats treated with the cell-permeable p38 inhibitor SB 239063 (50-mg/kg gavage; dissolved in VE; Calbiochem, San Diego, Calif)]; DM-VE (moderately hyperglycemic diabetic rats treated with VE); and DM-SB (moderately hyperglycemic diabetic rats treated with SB 239063). SB 239063 or VE were administered by gavage 1 h preceding the renal hemodynamic measurements. The dose of SB was chosen based on previously reported long-term studies suggesting beneficial effects of SB 239063 on end-organ damage in experimental models of hypertension.[18,19] BG levels were measured preceding the completion of the experiments. After the surgical preparation described below and after 90 min of equilibration, all rats underwent measurements of mean arterial pressure (MAP), GFR, renal plasma flow (RPF), renal vascular resistance (RVR), and urinary sodium excretion.

Surgical preparation and functional studies. Rats were anesthetized with Inactin (100 mg/kg i.p.) and placed on a temperature-regulated table. The left femoral artery was catheterized, and a baseline blood collection was obtained for