p38 MAPK Inhibitors Ameliorate Target Organ Damage in Hypertension: Part 2. Improved Renal Function as Assessed by Dynamic Contrast-Enhanced Magnetic Resonance Imaging

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ABSTRACT

Recent evidence suggests p38 mitogen-activated protein kinase (MAPK) signal transduction plays an important role in the pathogenesis of progressive renal disease. Using dynamic contrast enhanced magnetic resonance imaging (MRI), we evaluated chronic treatment with a p38 MAPK inhibitor, trans-1-(4-hydroxycyclohexyl)-4-(4-fluorophenyl-methoxy)pyridimidin-4-yl)imidazole (SB-239063), on renal function in a hypertension model of progressing renal dysfunction. Spontaneously hypertensive-stroke prone rats were placed on a high salt/fat diet model of progressing renal dysfunction. Spontaneously hypertensive-stroke prone; SFD, salt/fat diet; ND, normal diet; GFR, glomerular filtration rate; BUN, blood urea nitrogen; TTP, time to peak.

A variety of cellular stimuli, including stress (osmotic, UV, and oxidative) growth factors, neurohumoral agents, and cytokines, activate p38 mitogen-activated protein kinase (MAPK) by dual phosphorylation of Thr and Tyr in the Thr-Gly-Tyr motif (Tian et al., 2000). Comprehensive evidence indicates activation of the p38 MAPK signal transduction pathway is involved in a variety of inflammation processes, including cytokine generation, proliferation, matrix deposition, and apoptosis (Herlaar and Brown, 1999). As such, inhibition of p38 MAPK is a compelling anti-inflammatory target with broad therapeutic potential. The identification of p38 MAPK inhibitors, highly selective for p38 α/β isoforms, have recently allowed examination of p38 MAPK-dependent processes in a variety of disease models (Barone et al., 2001; Behr et al., 2001; Furuichi et al., 2002).

Recent studies implicate MAPK activation in the pathogenesis of renal disease. Specifically, dietary salt activates renal p38 MAPK and promotes p38 MAPK-dependent renal expression of TGF-β1 and nitric-oxide synthase 3 (Ying and Sanders, 2002). In addition, p38 MAPK inhibitors reduce fibronectin production induced by angiotensin-II and 12(S)-hydroxyeicosatetraenoic acid in renal mesangial cell (Reddy et al., 2002) and activation of p38 MAPK in human proximal tubule cell culture stimulates interleukin-8 and MCP-1 production (Li and Nord, 2002). Evidence also suggests p38 and other MAPKs play a central role in renal damage associated with diabetes and ischemia/reperfusion (Evans et al., 2002; Furuichi et al., 2002). Preliminary evidence also suggests that p38 MAPK plays an important role in renal damage associated with hypertension (Behr et al., 2001).

Part 1 of this study (Ju et al., 2003) illustrates the role of p38 MAPK in endothelial dysfunction associated with a salt/fat-sensitive model of hypertension and the reversal of this
dysfunction after p38 MAPK inhibition. In Part 2 of this study, we examined the role of p38 MAPK inhibition on the renal dysfunction associated with this salt/fat-sensitive model of hypertension. Specifically, the effects of chronic treatment with a p38 MAPK inhibitor, trans-1-(4-hydroxycyclohexyl)-4-(4-fluorophenyl) methoxyppryimidin-4-yl)imidazole (SB-239063), were examined in stroke prone hypertensive rats on a high salt/fat diet with established renal dysfunction (albuminuria). Indices of regional renal function and structure were examined by dynamic contrast enhanced magnetic resonance imaging (MRI) and histopathology, respectively.

Materials and Methods

Animal Model. Young male (10-week-old) age matched spontaneously hypertensive-stroke prone (SHR-SP) rats (Charles River Laboratories, Inc., Wilmington, DE) were placed on a combined high salt (1% NaCl in drinking water) and high fat (24.5% fat; Ziegler Bros., Gardners, PA) diet (SFD) or a normal rat chow (6% fat, with no salt supplementation; Ziegler Bros.) diet (ND). Rats were allowed to drink and feed ad libitum. Urine samples were collected 1 week before the start of the SFD and once weekly for the duration of the study. For an SFD animal to be assigned to a treatment group, one urine sample had to exhibit albuminuria ≥10 mg/day within 4 to 8 weeks of being placed on the SFD. The albuminuria threshold value was based on pilot studies that indicate that albumin excretion ≥10 mg/day was associated with continued increases in both the rate of albumin excretion and mortality. Once the inclusion criteria had been met, SFD animals were randomly assigned to groups receiving treatment with an α/β p38 MAPK inhibitor, SB-239063, at 1200 ppm (SFD + SB-239063 treated; n = 10) or control (SFD control; n = 12) (see Fig. 1 for study design). Previous p38 MAPK inhibition studies in rat glomeruli harvested from high salt-treated rats suggest that 10 μM is sufficient to entirely inhibit active TGF-β1 production (Ying and Sanders, 2002). The study was terminated after the dynamic contrast enhanced MRI experiments at 13 weeks. The average duration of treatment with SB-239063 at the termination of the study was 6 weeks. The ND control group (n = 7) was monitored similar to SFD control and SFD + SB-239063 groups.

Animals were housed and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (Office of Science and Health reports, DRR/NIH, 1985; U.S. Dept. of Health, Education, and Welfare, Bethesda, MD). Procedures using laboratory animals were approved by the Institutional Animal Care and Use subcommittee of GlaxoSmithKline.

MRI. One day before dynamic contrast enhanced MRI, all rats were anesthetized with isoflurane (2.5% in O2 at 1.0 l/min) and an exteriorized catheter was placed in the femoral vein for administration of the Gd-DTPA contrast agent (Magnevist; Berlex Laboratories, Wayne, NJ). The incision was closed with a 4-0 Ethicon suture (Ethicon Inc., Somerville, NJ), and rats were allowed to recover.

Dynamic contrast enhanced MRI was performed on week 13 of the study (Fig. 1). MRI was performed on a Biospec 4.7T/40 cm horizontal bore magnet (Bruker Medical, Billerica, MA) using a 72-mm diameter 1H volume resonator. All rats were anesthetized with 1.8 to 2.0% isoflurane delivered via medical grade air through a facemask. Anesthetized blood pressure measurements were collected immediately before MRI with a tail cuff sphygmomanometer. Spin echo transverse and coronal scout images (TR/TE, 367/15 ms; field of view, 20 × 20 cm; matrix, 128 × 64; slice thickness, 2 mm, radio frequency flip angle, 25°, number of averages, 1) were acquired to determine proper placement of the coronal slice such that its position was through the center of both kidneys. A gradient echo snapshot low-angle pulse sequence was used to acquire the contrast-enhanced images (TR/TE, 8.14/2.33 ms; spectral width, 100 kHz; field of view, 5 × 5 cm; matrix, 128 × 64; slice thickness, 1 mm; flip angle, 25°). Zero filling was applied for image reconstruction (spatial resolution was 390 μm in plane). Motion artifacts were minimized using sufficient signal averaging (number of averages, 10), resulting in a 5-s acquisition time per image. Immediately after the 13th image, 25 μmol of Gd-DTPA contrast agent (or 50 μl rats) was bolus injected into the venous femoral catheter and then flushed with 1 ml of saline. Images were acquired for 2 min postcontrast injection.

MRI Data Analysis. Gd-DTPA is used for MRI purposes as an image contrast agent. The contrast agent is excreted exclusively by the kidneys into the urine and does not undergo tubular reabsorption or secretion (Bennet and Li, 1997). Therefore, Gd-DTPA has previously been used to detect spatially distinct glomerular filtration rate (GFR) indices (Baumann and Rudin, 2000; Laurent et al., 2001). Previous studies have established that the transport rate of tracer from the cortex to the outer medulla is highly correlated with the gold standard inulin measurement of GFR (Laurent et al., 2001). Additionally, the rate of cortical Gd-DTPA uptake has also been used to assess renal perfusion (Vallee et al., 2000; Aumann et al., 2003).

Temporal changes in MRI signal intensity in spatially distinct regions of the kidney were translated into local Gd-DTPA concentrations assuming a linear dependence with signal enhancement as described previously (Baumann and Rudin, 2000; Laurent et al., 2001). The experimental design of the study illustrating the age of rats at start of study, respective numbers assigned into the ND control, SFD control, and SFD + SB-239063 groups, inclusion threshold (albuminuria ≥10 mg/day), randomization schedule (4–8 weeks), and terminal MRI time point with numb...
A GFR index defined as the first order rate constant ($K_c$), which describes the clearance of Gd-DTPA from cortex to outer medulla was calculated according to the following equation: 

$$\frac{dC_c}{dt} = K_c C(t),$$

where $C_c$ and $C_m$ are the Gd-DTPA concentrations in the cortex and outer medulla, respectively. It is assumed that Gd-DTPA distribution from the cortex to the outer medulla is dominant during the initial phase of contrast uptake. Therefore, $K_c$ was determined by fitting the observed transfer of Gd-DTPA from the cortex to the outer medulla during the 1st min after the bolus contrast injection. A renal perfusion index was determined by examining the time to peak (TTP) of Gd-DTPA appearance in the cortex (Baumann and Rudin, 2000; Laurent et al., 2001).

Figure 2 illustrates a pre-Gd-DTPA and post-Gd-DTPA image with four cortical and four adjacent medullary regions of interest shown (four of each in each kidney, $7 \times 7$ image pixels, $\sim 1.9$ mm$^2$). The selection of the regions of interest was determined from the first or second image after injection of the Gd-DTPA that displayed good contrast between the cortex and outer medulla. The final multi-regional $K_c$ was determined as an average of the $K_c$ in all eight regions in both kidneys within each animal. The mean $K_c$ per individual animal was then used to calculate the overall mean $K_c$ per group. In the case where the $K_c$ from one region of interest was outside of 3 standard deviations of the mean $K_c$, this region-specific $K_c$ was excluded. Images were analyzed on a Silicon Graphics O2 Workstation (Silicon Graphics, Mountain View, CA) using the Analyze AVW software package (AnalyzeDirect, Lenexa, KS).

**Urinary Creatinine and Protein Excretion.** A 24-h urine collection was obtained weekly from each animal. Blood urea nitrogen (BUN) and serum and urine creatinine were determined using an AU 640 clinical analyzer (Olympus, Melville, NY). Albumin excretion (milligram per day) was determined using an immunoturbidometric assay optimized for the determination of rat albumin in an AutoAnalyzer format (KRA-010/020; Kamiya Biomedical, Seattle, WA). The progression in albumin excretion is presented as the mean increase in urinary albumin excretion per day as determined by the slope of the regression line fit for each animal’s daily urinary albumin excretion.

**Histology.** At study termination, kidneys were collected and fixed in 10% neutral buffered formalin for 18 to 24 h and processed routinely to paraffin. Five-micrometer sections were prepared, stained with hematoxylin and eosin, Masson’s trichrome, and Masson’s pentachrome stain. Sections were examined microscopically.

**Statistical Analysis.** All summary values are expressed as mean ± S.E.M. All multiple comparisons were made by analysis of variance followed by a Tukey’s post hoc test. All statistical tests were performed using Prism software (GraphPad Software Inc., San Diego, CA), and a value of $P < 0.05$ was considered to be significant.

**Results**

**Effects on Albuminuria.** After the introduction of the high salt/fat diet, ~70% of the SHR-SP rats exhibited elevations in albuminuria within 4 to 8 weeks, sufficient for inclusion (>10 mg/day) in the treatment study. Animals that had passed the albuminuria inclusion threshold by week 8 ($n = 22$) were randomized into either SFD control or SFD + SB-239063 groups at this time point to allow a minimum of 5 weeks of treatment on the SB-239063 compound diet. At the initiation of treatment with SB-239063, urinary albumin excretion was similar in the SFD control and SFD + SB-239063 groups (22.9 ± 5.1 versus 28.6 ± 10.5 mg/day, respectively; NS).

At the end of the study (13 weeks), there was no difference in the duration of treatment in the SFD control and SFD + SB-239063 groups (Table 1). Creatinine clearance was also similar in the SFD + SB-239063 and SFD control groups but was reduced in both these groups compared with the ND control group (Table 1). There were no differences in the level of BUN between the groups (Table 1). The anesthetized indirect systolic arterial blood pressure, measured by the tail cuff technique, was lower in the SFD + SB-239063 group (143 ± 6 mm Hg) versus the SFD control (162 ± 4 mm Hg) or ND control (160 ± 5 mm Hg) groups.

Despite having similar creatinine clearance rates in the SFD control and SFD + SB-239063 groups, the progressive increase in urinary albumin excretion observed in the SFD control group was significantly reduced by SB-239063 treatment (Fig. 3). There was a trend toward increased survival in the SFD + SB-239063 group (80%) versus the SFD control group (50%). However, this study was not powered to observe significant survival benefit.

**Assessment of Renal Function by Dynamic Contrast-Enhanced MRI.** Fig. 4 illustrates representative MR images showing temporal differences in the uptake of Gd-DTPA contrast agent in ND control, SFD control, and SFD + SB-239063 animals. Initial changes in cortical signal intensity in both ND control and SFD + SB-239063 animals were detected within 5 s of the injection of the contrast agent and were quickly followed by changes in the outer medulla. Apparent is the time lag in both cortical and medullary contrast agent uptake in the SFD control versus ND control and SFD.
calculate the GFR index (\(K_{\text{cl}}\)) as well as the first order fit of the cortical to outer medullary SFD
0.03) and normalized to the ND control group (2.95 /H11006 /H11011 concentration (SB-239063 animals (Fig. 5) seemed to be rapid (/H11011 Gd-DTPA washout period in the ND control and SFD
at Fig. 5. The multiregional interest in a ND control, SFD control, and a SFD
these parameters to the model is shown for one region of the kidney, corresponded to a lower \(K_{\text{cl}}\) (average of four regional \(K_{\text{cl}}\)'s within each kidney) in this animal compared with the ND control and SFD + SB-239063 animals (1.86 ± 0.6, 4.03 ± 0.8, and 4.13 ± 0.6 min^{-1}, respectively).

**Assessment of Histopathological Changes in the Kidney.** Fig. 7 illustrates cortical and medullary histological findings from a representative animal in each of the three study groups. In ND control rats, renal changes were minimal and consisted of focal intimal thickening, glomerular or arteriolar hyalinosis, multifocal minimal tubular degeneration/regeneration with fibrosis, and isolated hyaline casts.

In SFD control rats, a spectrum of glomerular, vascular, and tubulointerstitial changes were observed. Moderate to severe segmental to global glomerular hyalinosis and variable glomerular hypertrophy and collapse/atrophy were present. In addition, segmental to global glomerular fibroinoid necrosis with thrombosis, hemorrhage, and early fibroplasia was consistently observed, often effacing glomerular architecture. Moderate to severe vascular changes were predominately noted in interlobular arteries and afferent arterioles. Pronounced concentric intimal thickening and fibroplasia, medial hyalinosis, and fibroinoid necrosis with thrombosis were noted in interlobular arteries and arterioles. Tubulointerstitial changes consisted of moderate to severe multifocal to confluent cortical tubular atrophy with epithelial degeneration/regeneration, tubular basement membrane thickening and minimal interstitial fibrosis. Dilated tubules with hyaline casts were prominent in SFD control rats (Fig. 7).

Renal changes were clearly attenuated in SB-239063-treated rats. In general, there was greater preservation of glomerular architecture. Glomerular hyalinosis, hypertrophy, and atrophy were observed but were more limited in extent and severity. Medial hyalinosis and concentric intimal thickening and fibroplasia were observed in interlobular ar-

### TABLE 1

Physiological parameters in the ND control, SFD control, and SFD + SB-239063 groups measured at week 13 (just before MRI)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ND Control (n = 7)</th>
<th>SFD Control (n = 6)</th>
<th>SFD + SB-239063 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>311 ± 4</td>
<td>307 ± 8</td>
<td>310 ± 7</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>160 ± 5</td>
<td>162 ± 4</td>
<td>143 ± 6*</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>2.4 ± 0.1**</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>15.1 ± 0.6</td>
<td>20.5 ± 5.8</td>
<td>16.4 ± 1.1</td>
</tr>
<tr>
<td>Mean time post-albuminuria (weeks)</td>
<td>NA</td>
<td>5.5 ± 0.2</td>
<td>6.3 ± 0.4</td>
</tr>
</tbody>
</table>

BP, blood pressure; NA, not applicable.

* \(P < 0.05\) versus SFD control and ND control groups; ** \(P < 0.05\) versus SFD control and SFD + SB-239063 groups.

+ SB-239063 animals. A semiquantitative index of renal perfusion was assessed as the time to peak cortical Gd-DTPA uptake (Fig. 5). The TTP cortical uptake of Gd-DTPA was slower in the SFD control group, but there was a trend to a more rapid cortical uptake of the contrast agent in the SFD + SB-239063 group similar to that in the ND control group (14.3 ± 1.2, 10.2 ± 1.3, and 12.6 ± 1.1 s in the SFD control, SFD + SB-239063, and ND control groups, respectively).

The cortical and outer medullary Gd-DTPA concentration as well as the first order fit of the cortical to outer medullary change in Gd-DTPA concentration data are necessary to calculate the GFR index (\(K_{\text{cl}}\)). The time course of cortical and outer medullary Gd-DTPA as well as the least-squares fit of these parameters to the model is shown for one region of interest in a ND control, SFD control, and a SFD + SB-239063 animal, respectively, in Fig. 5. The multiregional \(K_{\text{cl}}\) was higher in the SFD + SB-239063 group (3.12 ± 0.23 min^{-1}) versus the SFD control group (2.25 ± 0.14 min^{-1}, \(P < 0.03\)) and normalized to the ND control group (2.95 ± 0.36 min^{-1}) indicative of a global improvement in kidney filtration after SB-239063 treatment. Additionally, the cortical Gd-DTPA washout period in the ND control and SFD + SB-239063 animals (Fig. 5) seemed to be rapid (\(-1.0–1.5\) mM Gd-DTPA at 20–25 s) versus a sustained high Gd-DTPA concentration (\(-4\) mM) during the 1st min after contrast infusion in the SFD control animal (Fig. 5). This qualitative observation is consistent with the SFD control group having impaired glomerular filtration ability versus ND control and SFD + SB-239063 groups.

Figure 6 illustrates the distribution of signal intensity found in the entire renal cortex only at the time of maximum uptake of the contrast agent within a representative kidney of an animal in each of the three study groups. The SFD control animal had considerably more signal intensity variability compared with the ND control and SFD + SB-239063 animals (SD = 17.98, 8.86, and 9.96 for SFD control, ND control, and SFD + SB-239063 animals, respectively). The cortical signal intensity color map for each signal intensity histogram is shown in Fig. 6, inset. The higher signal intensity distribution observed in the SFD control group corresponds to greater heterogeneity in cortical perfusion in this group. The greater signal intensity variability associated with the SFD animal, within the renal cortex of an individual kidney, corresponded to a lower \(K_{\text{cl}}\) (average of four regional \(K_{\text{cl}}\)'s within each kidney) in this animal compared with the ND control and SFD + SB-239063 animals (1.86 ± 0.6, 4.03 ± 0.8, and 4.13 ± 0.6 min^{-1}, respectively).

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![Fig. 3. Progression of albuminuria. Once the threshold value of 10 mg/ day was passed and subsequent treatment with SB-239063 was initiated, individual animal's progression in albumin excretion was determined. The SB-239063-treated group showed an overall lower mean albumin excretion progression once treatment was initiated compared with the SFD control group. The normal diet control group experienced essentially no increase in albumin excretion over the duration of the study. Data are mean ± S.E.M. * P < 0.05 versus SFD control group.](image-url)
teries and afferent arterioles but also reduced in severity and distribution compared with SFD control rats. Fibrinoid necrosis and thrombosis involving glomeruli, arteries, and arterioles were infrequently noted. Disease modulation was best appreciated by the apparent preservation of cortical tubules. In most SFD + SB-239063 rats, tubular atrophy with epithelial degeneration, regeneration, and interstitial fibrosis was limited to small scattered foci in the cortex, separated by larger areas of morphologically normal tubules. Dilated tubules with hyaline casts were still present but reduced compared with SFD control rats (Fig. 7).

**Discussion**

We evaluated the effects of chronic treatment with a p38 MAPK inhibitor, SB-239063, on renal function in a hypertensive animal model with established progressive renal dysfunction and known activation of p38 MAPK in the kidney (Ying and Sanders, 2002). Using dynamic contrast enhanced MRI, we demonstrate, for the first time, that treatment with the p38 MAPK inhibitor SB-239063 improved indices of regional glomerular filtration and renal perfusion in these animals. Additionally, treatment with SB-239063 in these animals also attenuated albuminuria and histopathological changes in renal glomeruli, tubules, and arteries.

This study was designed to determine whether chronic p38 MAPK inhibition could ameliorate the progression of renal dysfunction once established. Our pilot studies in the high salt/fat diet SHR-SP model indicate that albuminuria and the mortality rate escalate rapidly over 3 to 4 weeks when the p38 MAPK inhibitor reduced degeneration and sclerotic changes in glomeruli, interstitium, and tubules as well as reducing proteinaceous tubular casts and hypertrophic/fibrinoid changes in renal arteriole. Therefore, creatinine clearance is generally an overestimation of GFR, and inhibition of tubular creatinine secretion would result in a truer estimation of GFR. The extent to which SB-239063 blocks tubular creatinine secretion is not known.

Attenuation of histopathological change in the kidney seems to underlie the improved renal function associated with SB-239063 treatment. Specifically, chronic treatment with the p38 MAPK inhibitor reduced degeneration and sclerotic changes in glomeruli, interstitium, and tubules as well as reducing proteinaceous tubular casts and hypertrophic/fibrinoid changes in renal arteriole. However, quantitative assessment and correlation of renal pathological changes with MRI results were not performed because the study was not designed for stereological quantitation. Consistent with the histological changes observed in the kidney, albuminuria was significantly reduced in the SFD + SB-239063 group. It is noteworthy, that the beneficial effects of p38 MAPK inhibition may have been underestimated in this study in that 80% of SFD + SB-239063 animals survived the protocol compared with only 50% in the SFD control group (similar results as in Part 1 of this study; Ju et al., 2003). Thus we were unable to assess the severe renal dysfunction that is known to accompany morbidity in the SFD control group.

Recent evidence suggests that p38 MAPK plays an important role in renal physiology, development/malformation, and nephropathy. For example, p38β MAPK activation influences Na⁺ transport in cells from the inner medullary collecting duct by modulating the osmotic regulation of natriuretic peptide receptor-A (Chen and Gardiner, 2002). In addition, p38 MAPK is up-regulated in models of diabetic nephropathy and has been proposed as a glucose transducer in diabetic complications (Tomlinson, 1999). Perhaps most
importantly, p38 MAPK plays an integral role in renal inflammation through the transduction and production of cytokines (Li et al., 2001; Park et al., 2001; Wada et al., 2001). In a nephrotoxic model of crescentic glomerulonephritis, treatment with a p38 MAPK inhibitor reduced markers of inflammation, glomerulosclerosis, interstitial fibrosis, and preserved renal function (Wada et al., 2001). In an elegant study by Park et al. (2001), it was suggested p38 MAPK mediates microvascular inflammation and vascular congestion in the outer medulla after ischemia and reperfusion of the kidney and that the down-regulation of p38 MAPK activation was associated with the protective effects of ischemic preconditioning. Evidence also suggests p38 MAPK may play a more direct role in renal fibrosis through transduction of TGFβ-mediated collagen expression (Li et al., 2001). Consistent with the ubiquitous and promiscuous nature of p38 MAPK, these studies suggest a fundamental role in response to stress and tissue repair in the kidney.

The precise cellular substrates and mechanisms by which p38 MAPK inhibitors convey renal protection in the high salt/fat fed animals are unclear, but likely to be multifarious. Pathogenic mechanisms that have been strongly linked to p38 MAPK activation in the high salt/fat fed rats include inflammatory cytokine production and subsequent endothelial dysfunction (Behr et al., 2001). Based on the results presented in Part 1 of this study (Ju et al., 2003), it seems plausible to suggest inhibition of p38 MAPK restores dimin-
An inherent advantage of the dynamic contrast enhanced MRI technique over established methods for assessing GFR (e.g., inulin technique) is the ability to distinguish regional renal function deficits, which is not possible with existing methods. With relatively high spatial resolution, specific regions within the kidney may be interrogated for regional filtration and/or perfusion, which is a significant advantage in conducting preclinical research. The ability to add in vivo dynamic functional data to existing histopathological and urinary protein data provides a more complete view of renal function. For example, our analysis of the signal intensity variability within the renal cortex at the time of maximal contrast uptake revealed an inverse relationship between the signal intensity variability and the filtration rate constants ($K_f$) in that kidney (Fig. 6). The heterogeneity in cortical Gd-DTPA uptake observed in the SFD control group supports the histopathological results where "patchy" deficit regions exhibiting proteinaceous casts and glomerulosclerosis within distinct regions of the renal cortex was observed.

In conclusion, our data demonstrate that treatment with the p38 MAPK inhibitor SB-239063 in the SHR-SP rat fed a high salt/fat diet results in reduced progression of albuminuria and improved renal perfusion and multi-regional GFR as assessed by dynamic contrast enhanced MRI. These results suggest that the inhibition of p38 MAPK may play a clinically relevant role in reducing the harmful effects of tubulointerstitial inflammation, glomerular scarring, and renal fibrosis, which often lead to end stage renal disease in patients with severe proteinuria. Therefore, pharmacological inhibition of p38 MAPK activity may ultimately be beneficial in ameliorating progressive renal disease.

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References


Furuchi K, Wada T, Iwata Y, Sakai N, Yoshimoto K, Kobayashi K, Mukaida N, Matsushima K, and Yokoyama H (2002) Administration of FR-167653, a new somewhat surprising. It is quite likely that normal diet fed, older SHR-SP rats (23–25 weeks old at end of study) have similar blood pressures as in the salt/fat diet fed SHR-SP rats, although the elevation of blood pressure most likely occurs at different progression rates (see Part 1 of this study; Ju et al., 2003).

Similarly to Part 1 of this study (Ju et al., 2003), the present study also shows that p38 MAPK inhibition is associated with a reduction in blood pressure. This finding is unlikely to represent a direct vascular smooth muscle action of p38 MAPK inhibition, because SB-239063 does not alter vascular reactivity in isolated blood vessels nor does it reduce blood pressure when administered acutely in SHR rats (see Part 1 of this study; Ju et al., 2003). The improved indices of renal function resulting from chronic SB-239063 treatment may in part be due to the beneficial lowering of blood pressure in this group. Although renal pathology and function are improved after p38 MAPK inhibition, the direct versus indirect mechanisms of action on kidney are yet to be elucidated. Further studies controlling for blood pressure (i.e., imaging the SFD group at an earlier time point) need to be performed to differentiate direct versus indirect mechanisms of action. The fact that the blood pressures in the ND control and SFD control group were the same at the end of the study was


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