Differential Effects of p38 Mitogen-Activated Protein Kinase and Cyclooxygenase 2 Inhibitors in a Model of Cardiovascular Disease

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ABSTRACT
The evidence is compelling for a role of inflammation in cardiovascular diseases; however, the chronic use of anti-inflammatory drugs for these indications has been disappointing. The recent study compares the effects of two anti-inflammatory agents [cyclooxygenase 2 (COX2) and p38 inhibitors] in a model of cardiovascular disease. The vascular, renal, and cardiac effects of 4-(4-methylsulfonylphenyl)-3-phenyl-5H-furan-2-one (rofecoxib; a COX2 inhibitor) and 6-{5-[(cyclopropylamino)carbonyl]-3-fluoro-2-methylphenyl}-N-(2,2-dimethylpropyl)-3-pyridinecarboxamide [GSK-AHAB, a selective p38 mitogen-activated protein kinase (MAPK) inhibitor], were examined in the spontaneously hypertensive stroke-prone rat (SHR-SP). In SHR-SPs receiving a salt-fat diet (SFD), chronic treatment with GSK-AHAB significantly and dose-dependently improved survival, endothelial-dependent and -independent vascular relaxation, and indices of renal function, and it attenuated dyslipidemia, hypertension, cardiac remodeling, plasma renin activity (PRA), aldosterone, and interleukin-1β (IL-1β). In contrast, chronic treatment with a COX2-selective dose of rofecoxib exaggerated the harmful effects of the SFD, i.e., increasing vascular and renal dysfunction, dyslipidemia, hypertension, cardiac hypertrophy, PRA, aldosterone, and IL-1β. The protective effects of a p38 MAPK inhibitor are clearly distinct from the deleterious effects of a selective COX2 inhibitor in the SHR-SP and suggest that anti-inflammatory agents can have differential effects in cardiovascular disease. The results also suggest a method for evaluating long-term cardiovascular efficacy and safety.

A substantial body of evidence suggests that chronic low-grade inflammatory processes contribute to the pathogenesis of cardiovascular disease, e.g., coronary artery disease, heart failure, hypertension, and other components of the metabolic syndrome (Grundy, 2003; Berg and Scherer, 2005; De Tena et al., 2005; Forrester and Libby, 2007; Savoia and Schiffrin, 2007). Manifestations of vascular inflammation are believed to include early pathogenic changes such as endothelial dysfunction and plaque formation and later events related to plaque rupture, thrombosis, and end-organ disease. At a cellular level, vascular inflammation is associated with activation of a variety of intracellular signaling pathways (e.g., p38 MAPK, nuclear factor κB, and eicosanoid) in endothelial, inflammatory, and synthetic smooth muscle cells. These often intersecting and overlapping pathways promote cellular adhesion, infiltration, apoptosis, proliferation, and remodeling of the extracellular matrix (Touyz, 2003). Specific mediators that have been associated with cardiovascular disease include proinflammatory cytokines [interleukin-6, interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), and monocyte chemoattractant protein-1], lipid mediators (oxidized LDL, lipoprotein-associated phospholipase A2, and thromboxane A2), and acute-phase proteins, e.g., C-reactive protein (Willerson and Ridker, 2004). Evidence also sug-

ABBREVIATIONS: MAPK, mitogen-activated protein kinase; SHR-SP, spontaneously hypertensive, stroke-prone rat; SFD, salt-fat diet; PRA, plasma renin activity; COX2, cyclooxygenase 2; GSK-AHAB, 6-{5-[(cyclopropylamino)carbonyl]-3-fluoro-2-methylphenyl}-N-(2,2-dimethylpropyl)-3-pyridinecarboxamide; FENa, fractional excretion of sodium; ND, normal diet; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LV, left ventricular; PBMC, peripheral blood mononuclear cell; SNP, sodium nitroprusside; IL-1β, interleukin-1β; ANOVA, analysis of variance; BP, blood pressure; ELISA, enzyme-linked immunosorbent assay; LPS, lipopolysaccharide; NOX, NADPH oxidase; A23187, calcimycin.
gests that some drugs prescribed to reduce the risk of cardiovascular disease (e.g., statins and angiotensin-converting enzyme inhibitors) reduce biomarkers of inflammation associated with cardiovascular disease (Schieffer et al., 2004; Forrester and Libby, 2007).

Despite the compelling case for a role of inflammation in cardiovascular disease, drugs that specifically target inflammatory pathways have not been approved for the treatment of cardiovascular disease and the use of the current anti-inflammatory drugs in the setting of cardiovascular disease has been disappointing. In fact, high-dose glucocorticoids, targeted TNF-α inhibition, and some COX2 inhibitors have been associated with increased risk of cardiovascular disease (Mann et al., 2004; Zarraga and Schwarz, 2007).

Recent preclinical studies suggest that p38 MAPK inhibitors, novel anti-inflammatory agents, demonstrate efficacy in a variety of atherosclerotic and nonatherosclerotic preclinical models of cardiovascular disease (Behr et al., 2001, 2003; Ju et al., 2002; Olzinski et al., 2005; Bao et al., 2007; Park et al., 2007; Morris et al., 2008). However, preclinical models used to assess chronic cardiovascular safety and efficacy do not always predict clinical outcome. In the case of COX2 inhibitors, preclinical studies failed to predict the elevated risk of thrombotic events, heart failure, and hypertension (not stroke) observed in the clinic use of rofecoxib, a selective COX2 inhibitor, and other nonsteroidal anti-inflammatory drugs (for review, see Zarraga and Schwarz, 2007). Both p38 MAPK and COX2 inhibitors act by inhibiting complex intracellular inflammatory pathways activated by cellular stress, i.e., hypertension, toll-receptor activation, proinflammatory cytokines, neurohormones, and reactive oxygen species (for review, see Schieven, 2005).

In the present study we have conducted a side-by-side evaluation of a novel selective p38 MAPK inhibitor and a prototypic selective COX2 inhibitor, rofecoxib (Chan et al., 1999), in a nonatherosclerotic model of cardiovascular disease. This comparison is important for the following reasons: 1) p38 MAPK and COX2 inhibitors regulate COX2 expression and activity, respectively (Schieven, 2005); 2) direct COX2 inhibitors (i.e., rofecoxib) have cardiovascular liabilities; and 3) p38 MAPK inhibitors are in advanced stages of clinical development. The present results clearly distinguish the efficacious effects of a p38 MAPK inhibitor from the deleterious effects of a COX2 inhibitor and suggest the benefits of broad-spectrum cytokine suppression in the treatment of cardiovascular disease.

Materials and Methods

SHR-SP Preparation. Male SHR-SPs (n = 70) were obtained from Charles River Laboratories, Inc. (Wilmington, MA) and randomly assigned according to body weight into five groups (n = 14 per group): normal diet controls (ND), high salt-fat diet controls (SFD), SFD + GSK-AHAB (1.2 mg/kg/day), and SFD + GSK-AHAB (12 mg/kg/day) and SFD + rofecoxib (18 mg/kg/day). For clarity, normal diet control data are not always presented graphically but are described in the text. All drugs were administered in the diet by mixing them with the food. A subgroup of animals from each group (n = 6 per group) were anesthetized with isoflurane and surgically instrumented with radiotelemetry units (Data Sciences International, St. Paul, MN) for the conscious measurement of mean arterial blood pressure and heart rate. These animals were allowed to recover for at least 7 days before the start of the study. All experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals (National Institutes of Health Publication 85-23), and experimental protocols were reviewed and approved by the GlaxoSmithKline Animal Care and Use Committee.

Experimental Protocol. At the age of 7 to 8 weeks, rats were placed into metabolism cages for 24 h for the collection of baseline urine and blood samples. After baseline measures, the animals were randomly assigned to the five groups. The ND group received a standard chow and tap water; the SFD diet groups received a fat-enriched diet 24.5% fat and 1% NaCl in the drinking water ad libitum (Behr et al., 2001). Urine collections to determine electrolyte and protein excretion rates were repeated on weeks 2, 4, 6, and 8 and blood samples were taken to determine plasma creatinine and plasma sodium levels after each urine collection period to determine creatinine clearance and fractional excretion of sodium (FENA). Blood samples were also analyzed for HDL cholesterol (HDL), LDL cholester (LDL), and triglycerides. All urine and plasma samples were analyzed by use of an Olympus AU640 Chemistry Analyzer (Olympus America Inc., Diagnostic Systems Group, Melville NY) during week 3. Drug plasma levels were determined in a subgroup of animals (n = 4) from samples obtained at 7:00 AM, noon, and 4:00 PM (this represents the peak-to-trough profile after dietary dosing). Animals were considered moribund, and promptly euthanized with pentobarbital (65 mg/kg i.p.) when they exhibited decreased locomotion and seizure and/or persistent loss of body weight.

Thoracic echocardograms (Vingmed System V; GE Health-care, Chalfont St. Giles, UK) were performed, as described previously (Behr et al., 2001), on randomly chosen animals (n = 8/group) at baseline before treatment (week 0) and at week 4 in high-dose and control groups. Inhalation anesthesia was induced with 4 to 5% isoflurane, and maintained at 1.5 to 2.0% during the procedure. The leading-edge method was used to determine left ventricular (LV) thickness and volumes. Relative wall thickness (RWT) was calculated as RWT = (AWd + PWd)/LVDd, where AWd is diastolic anterior wall thickness, PWd is diastolic posterior wall thickness, and LVDd is the left ventricular diameter in diastole. The t/b ratio is the average LV wall thickness (t) divided by the average LV radius (b) in diastole. Left ventricular systolic diameter, stroke volume, and cardiac output were also determined by a modified Simpson’s method.

At the end of the study, after the 8- to 9-week treatment period, surviving rats were anesthetized with 5% isoflurane in O2 and euthanized by exsanguination. Blood samples were obtained for subsequent determination of plasma renin activity (PRA) and aldosterone. Radioimmunoassays were used for determination of PRA (GammaCoat Plasma Renin Activity; DiaSorin, Stillwater, MN), interleukin-1β (R&D Systems, Minneapolis, MN) and aldosterone (Diagnostic Products, Los Angeles, CA). The wet weight of the left and right kidneys and the whole heart were taken and expressed as tissue to body weight ratios.

Vascular Reactivity. After exsanguination, the proximal descending thoracic aortae were removed and prepared for in vitro vascular relaxation studies as described previously (Behn et al., 2002). Vascular ring segments (2–3 mm) were suspended in 10-ml tissue baths containing oxygenated (95% O2, 5% CO2) Krebs’ solution, and allowed to relax to the resting tension. The 60 mM KCl contraction was repeated. Each tissue was then contracted to equilibration with 1 μM phenylephrine, washed with Krebs’ solution, and allowed to relax to the resting tension. After washout,
cumulative concentration-response curves to phenylephrine were obtained by adding 0.5 log unit increments (1 nM–10 μM) and the EC50 for each tissue was determined. After several washes, each vessel was contracted to equilibrium with an EC50 concentration of phenylephrine and tone was relaxed by adding cumulative concentrations of sodium nitroprusside (SNP) at 0.5 log unit intervals (0.01 nM–100 μM). After washout, the tissues were again contracted to equilibrium with an EC50 concentration of phenylephrine and tone was relaxed by adding cumulative concentrations of sodium nitroprusside (SNP) at 0.5 log unit intervals (0.01 nM–100 μM).

**Whole-Blood COX1 Activity.** Heparinized whole blood (100 μL) was incubated at 37°C for 1 h in a cell culture incubator. A volume of 0.5 μl of calcium ionophore (A23187) was added to each well (final concentration, 50 μM) and the plate was incubated for an additional 30 min in the incubator. The incubation was stopped by centrifuging the plate at 4°C for 5 min. Plasma was removed and stored at –80°C until assayed for thromboxane B2 by ELISA according to the manufacturer’s instructions (Cayman Chemical, Ann Arbor, MI). Rat plasma was usually diluted 1:300 with ELISA buffer.

**Whole-Blood COX2 Activity.** Heparinized whole blood was incubated for 1 h at 37°C with aspirin (100 μM final) to inactivate COX1. LPS (100 μg/ml, final concentration, 50 μM) and the plate was incubated for an additional 30 min in the incubator. The incubation was stopped by centrifuging the plate at 4°C for 5 min. Plasma was removed and stored at –80°C until assayed for prostaglandin E2 by ELISA according to the manufacturer’s instructions. Rat plasma was diluted 1:100 with ELISA buffer (modified from Patrignani et al., 1994).

**Drugs and Materials.** Rofecoxib, a well characterized, potent, and selective COX2 inhibitor, was obtained from a commercial source (Hanna’s Pharmaceutical Supply, Wilmington, DE) and GSK-AHAB was synthesized at GlaxoSmithKline Pharmaceuticals (King of Prussia, PA). Both compounds were administered by the dietary route and were formulated by blending with the high-fat powdered diet (Zeigler Bros., Gardners, PA). The rofecoxib dose (18 mg/kg/day) was extrapolated from the literature to achieve selective COX2 inhibition (Chan et al., 1999). An 8-week low-dose (4 mg/kg/day) and high-dose (54 mg/kg/day) pilot study was performed with rofecoxib. The low dose had inconsistent effects on COX2 activity and the high dose achieved plasma levels exceeding previously published selectivity limits. All rofecoxib doses had similar insignificant effects on survival. Only the data for the selective 18 mg/kg/day dose was described in this report. High and low doses of GSK-AHAB (1.2 and 12 mg/kg/day) were calculated as a free base and selected to bracket rat whole-blood IC50 values (Fig. 1). The doses of GSK-AHAB used in this study had no significant toxicological or cardiovascular effects in normal rats. Normal diet control subjects were given standard powdered rodent chow (Purina 5001; Purina, St. Louis, MO) and maintained on tap water. All rats receiving the high-fat diet or the high-fat diet containing drug were maintained on 1% NaCl in the drinking water.

**Data Analysis.** Summary data values are reported as mean ± S.E.M. and p < 0.05 was considered significant. Time- and treatment-related effects were compared by use of a two-way ANOVA for repeated measures followed by post-test comparisons with SFD and/or rofecoxib values by use of the Bonferroni test for multiple comparisons, unless stated in the text (GraphPad Software Inc., San Diego, CA). Morbidity results were assessed by Kaplan-Meier survival analysis followed by the Holm-Sidak test for comparisons between treatment groups (GraphPad Software Inc.).

**Results**

**Activity Profile of GSK-AHAB.** GSK-AHAB is a selective, potent, and orally active p38 MAPK inhibitor that acts by competing for the kinase ATP binding site. GSK-AHAB has single-digit nanomolar potency (pKᵢ) in isolated p38α and p38β enzyme assays, submicromolar potency in both rodent and human LPS-stimulated peripheral blood mononuclear cell (PBMC) assays, and no detectable direct effect on COX2 activity (Fig. 1).

Chronic dietary administration of high (12 mg/kg/day) and low (1.2 mg/kg/day) doses of GSK-AHAB provided dose-linear plasma concentrations above and below the rat PBMC IC50 (Fig. 2A). A COX2 selective dose of rofecoxib (18 mg/kg/day), based on literature precedence, was confirmed in the present study (approximately 2 μM plasma concentration) and had no effect on rat whole-blood COX1 activity (Fig. 2). A low-dose rofecoxib group (3.6 mg/kg/day) had only marginal effects on COX2 activity (Supplemental Fig. 1).

**Effects of GSK-AHAB and Rofecoxib on Survival and BP.** Introduction of a high SFD induces a progressive and malignant hypertension in the SHR-SP. Untreated animals receiving the SFD had a 50% survival rate at 8 weeks and a continual increase in mean BP throughout the observation period (Fig. 3). In the rofecoxib groups, the effect of the SFD on mean BP was significantly exaggerated and/or accelerated (p < 0.05) and there was a tendency toward greater mortality (Fig. 3). Blood pressure results were similar in a low-dose rofecoxib group (3.6 mg/kg/day; see Supplemental Fig. 2), but
Effects of treatment on survival (A) and mean arterial blood pressure (B) in stroke-prone, SHR-SPs placed on a SFD. After a 3-day run-in period, SFD with or without treatments was begun at time 0 when animals were 12 weeks of age. In A, SFD and rofecoxib groups differed significantly from the GSK-AHAB groups from day 40 to the end of the study on day 56 (Kaplan-Meier survival analysis with 95% confidence intervals, log rank \( p < 0.002 \)). Blood pressure values were significantly greater (#) or less than (*) the SFD group (\( p < 0.05 \)) by two-way ANOVA for repeated measures and Bonferroni’s test for multiple comparisons.

The SFDs also produce a progressive dyslipidemia characterized by a delayed but progressive increase in plasma concentrations of LDL, HDL, and the LDL/HDL ratio (Supplemental Fig. 1) and a more abrupt increase in triglycerides (Fig. 5). At 6 and 8 weeks the increases in HDL, LDL, and triglycerides were greater in the rofecoxib group than in untreated SHR-SPs on the SFD. In contrast, GSK-AHAB treatment dose-dependently reduced LDL, HDL, and triglycerides and the LDL/HDL ratio (Fig. 5; Supplemental Fig. 5).

**Endothelial Function and Vascular Reactivity.** Both endothelium-dependent and nitrate-dependent (sodium nitroprusside) vasorelaxation were attenuated by the SFD (Fig. 6). Treatment with GSK-AHAB dose-dependently improved both endothelium-dependent (Fig. 6A) and endothelium-independent (Fig. 6B) vasorelaxation. In contrast, treatment with rofecoxib markedly attenuated endothelium-dependent vasorelaxation induced by carbachol (Fig. 6A) and had no significant effect on SNP-dependent vasorelaxation (Fig. 6B). Vasorelaxation induced by carbachol and SNP in SHR-SP receiving a normal diet did differ significantly from the GSK-AHAB high-dose group (data not shown).

**Effects on Plasma Renin Activity, Aldosterone, and Interleukin-1β.** Somewhat paradoxically, the chronic SFD did not reduce PRA and aldosterone at 4 weeks (data not shown) and 8 weeks (Fig. 7). Treatment with GSK-AHAB reduced PRA, aldosterone, and IL-1β (Fig. 7) at both time points. In contrast, rofecoxib tended to increase PRA, aldosterone, and IL-1β (Fig. 7). PRA, aldosterone, and IL-1β in the normal diet group did not differ significantly from the SFD group (10.222 ± 3271 pg angiotensin-1/ml/h, 68.0 ± 7.6 pg/ml, 29.1 ± 5.0 pg/ml, respectively).

**Discussion**

In the present study, chronic treatment with two distinct classes of anti-inflammatory agents had markedly different effects in a rodent model of cardiovascular disease. Exemplars of orally active selective inhibitors of COX2 and p38 MAPK (rofecoxib and GSK-AHAB, respectively) were compared in the SHR-SPs fed a SFD. In most cases chronic treatment with a COX2 selective dose of rofecoxib exaggerated the harmful effects of a SFD in the SHR-SP, i.e., increasing vascular and renal dysfunction, hypertension, dyslipidemia, cardiac hypertrophy, PRA, aldosterone, and IL-1β. In contrast, chronic treatment with GSK-AHAB dose-dependently improved survival, endothelial-dependent and -independent vascular relaxation, lipid profiles and indices of renal function, and attenuated hypertension, cardiac remodeling, PRA, aldosterone, and IL-1β. This differentiation is important when considering the advanced phase of clinical development of p38 MAPK inhibitors, the role of p38 MAPK in regulating COX2, and the known cardiovascular liabilities of COX2 inhibitors.

The opposing effects on vasoregulation were among the most striking, and perhaps revealing, observations. Chronic treatment with GSK-AHAB significantly improved both endothelial-dependent (carbachol) and nitrate-induced vasorelaxation in SFD-SHR-SP. In contrast, rofecoxib treatment markedly attenuated endothelial-dependent vasorelax-
Plasma lipoprotein concentrations, HDL (A), LDL (B), and triglycerides (C), were determined at 2, 4, 6, and 8 weeks of the study in all groups. All groups (n = 6–12 per group) were compared by a two-way ANOVA and Bonferroni's test for multiple comparisons. All groups were compared with the SFD (*, p < 0.05) and rofecoxib (#, p < 0.05). Drug treatments were added to the SFD.

**Table 2:** Echocardiographic analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Week</th>
<th>ND Mean ± S.E.M.</th>
<th>SFD Mean ± S.E.M.</th>
<th>AHAB (12 mg/kg/day) Mean ± S.E.M.</th>
<th>AHAB (12 mg/kg/day) Mean ± S.E.M.</th>
<th>Rofecoxib (18 mg/kg/day) Mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>0</td>
<td>177.5 ± 11.5</td>
<td>12.1 ± 7.8</td>
<td>181.6 ± 11.4</td>
<td>11.4 ± 13.4</td>
<td>166.8 ± 9.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>254.4 ± 6.8</td>
<td>12.6 ± 8.8</td>
<td>265.5 ± 7.6</td>
<td>8.1 ± 14.8</td>
<td>250.8 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>286.7 ± 7.5</td>
<td>12.6 ± 8.8</td>
<td>278.6 ± 5.6</td>
<td>7.6 ± 12.4</td>
<td>278.9 ± 6.1</td>
</tr>
<tr>
<td>Urine flow (ml/day)</td>
<td>0</td>
<td>11.83 ± 0.53</td>
<td>12.1 ± 7.8</td>
<td>10.7 ± 0.7</td>
<td>1.7 ± 13.4</td>
<td>14.92 ± 4.65</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>13.50 ± 1.14</td>
<td>12.1 ± 7.8</td>
<td>52.7 ± 14.8</td>
<td>3.8 ± 18.4</td>
<td>25.69 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>22.33 ± 5.15</td>
<td>12.1 ± 7.8</td>
<td>89.0 ± 15.8</td>
<td>5.2 ± 18.4</td>
<td>33.77 ± 3.7</td>
</tr>
<tr>
<td>FENa (%)</td>
<td>0</td>
<td>0.45 ± 0.02</td>
<td>12.1 ± 7.8</td>
<td>0.50 ± 0.03</td>
<td>0.01 ± 14.8</td>
<td>0.48 ± 0.04</td>
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<tr>
<td></td>
<td>4</td>
<td>0.48 ± 0.04</td>
<td>12.1 ± 7.8</td>
<td>1.36 ± 0.49</td>
<td>2.50 ± 5.3</td>
<td>16.7 ± 10.0</td>
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<tr>
<td></td>
<td>8</td>
<td>0.81 ± 0.20</td>
<td>12.1 ± 7.8</td>
<td>5.39 ± 1.21</td>
<td>3.26 ± 0.38</td>
<td>2.51 ± 0.19</td>
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<tr>
<td>Hematocrit (%)</td>
<td>0</td>
<td>43.1 ± 2.7</td>
<td>12.1 ± 7.8</td>
<td>40.7 ± 0.8</td>
<td>44.0 ± 0.8</td>
<td>41.1 ± 1.2</td>
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<tr>
<td>Platelet count (×1000/µl)</td>
<td>0</td>
<td>436.6 ± 74.7</td>
<td>12.1 ± 7.8</td>
<td>285.1 ± 51.7</td>
<td>29.5 ± 11.4</td>
<td>461.3 ± 33.1</td>
</tr>
<tr>
<td>RBC count (×10^12/µl)</td>
<td>0</td>
<td>8.12 ± 0.18</td>
<td>12.1 ± 7.8</td>
<td>7.84 ± 0.64</td>
<td>8.52 ± 0.23</td>
<td>8.35 ± 0.24</td>
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<tr>
<td>HW/BW (mg/g)</td>
<td>0</td>
<td>4.2 ± 0.12</td>
<td>12.1 ± 7.8</td>
<td>0.25 ± 0.8</td>
<td>4.7 ± 0.12</td>
<td>4.5 ± 0.08</td>
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<tr>
<td>Kidney/BW (mg/g)</td>
<td>0</td>
<td>4.4 ± 0.05</td>
<td>12.1 ± 7.8</td>
<td>0.48 ± 0.8</td>
<td>4.74 ± 0.15</td>
<td>4.5 ± 0.05</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>0</td>
<td>79.4 ± 4.0</td>
<td>9</td>
<td>74.5 ± 4.3</td>
<td>3.4 ± 11.4</td>
<td>77.5 ± 2.9</td>
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<tr>
<td>SV(µl)</td>
<td>0</td>
<td>243.6 ± 8.1</td>
<td>9</td>
<td>242.8 ± 10.7</td>
<td>8.1 ± 21.7</td>
<td>251.0 ± 8.9</td>
</tr>
<tr>
<td>EF (%)</td>
<td>0</td>
<td>60.3 ± 3.7</td>
<td>9</td>
<td>62.7 ± 15.7</td>
<td>5.5 ± 11.4</td>
<td>60.2 ± 1.8</td>
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<tr>
<td>LVDd (mm)</td>
<td>0</td>
<td>70.6 ± 0.12</td>
<td>9</td>
<td>6.8 ± 0.14</td>
<td>0.11 ± 13.4</td>
<td>7.01 ± 0.13</td>
</tr>
<tr>
<td>LVDs (mm)</td>
<td>0</td>
<td>8.23 ± 0.22</td>
<td>9</td>
<td>7.41 ± 0.21</td>
<td>0.10 ± 13.4</td>
<td>7.92 ± 0.15</td>
</tr>
<tr>
<td>LVM/BW (mg/g)</td>
<td>0</td>
<td>3.81 ± 0.13</td>
<td>9</td>
<td>3.75 ± 0.17</td>
<td>0.11 ± 13.4</td>
<td>3.78 ± 0.11</td>
</tr>
<tr>
<td>T/B</td>
<td>0</td>
<td>0.369 ± 0.010</td>
<td>9</td>
<td>0.365 ± 0.013</td>
<td>0.358 ± 0.007</td>
<td>0.354 ± 0.008</td>
</tr>
</tbody>
</table>

RBC, red blood cell; HW/BW, heart weight to body weight ratio.

©, p < 0.05; **, p < 0.01; ###, p < 0.001 vs. SFD.

##, p < 0.05; ##, p < 0.01; ###, p < 0.001 vs. rofecoxib.

**Fig. 5:** Plasma lipoprotein concentrations, HDL (A), LDL (B), and triglycerides (C), were determined at 2, 4, 6, and 8 weeks of the study in all groups. All groups (n = 6–12 per group) were compared by a two-way ANOVA and Bonferroni’s test for multiple comparisons. All groups were compared with the SFD (*, p < 0.05) and rofecoxib (#, p < 0.05). Drug treatments were added to the SFD.
These results are consistent with previous reports of endothelial protection after chronic treatment with p38 MAPK inhibitors and are also consistent with reports of impaired endothelial function after rofecoxib treatment (Ju et al., 2003; Li et al., 2004, 2008; Widder et al., 2004; Bao et al., 2007). The mechanism underlying these differences seems to involve opposing effects on the generation of reactive oxygen species (ROS) that may be related to the regulation of NADPH oxidase (NOX). In the case of p38 MAPK inhibitors, several reports have shown that p38 MAPK inhibitors reduce NOX/subunit expression and ROS generation (Bao et al., 2007; Li et al., 2008). These effects in the vasculature would be expected to increase the bioavailability of nitric oxide and reduce the nitrate-insensitive form of oxidized soluble guanylate cyclase, thereby improving endothelial-dependent and nitrate-dependent vasorelaxation. However, COX2 inhibitors and nonsteroidal anti-inflammatory drugs have recently been shown to up-regulate NOX(is) and increase ROS in blood vessel and heart (Li et al., 2008). Despite this explanation of the results, it should be noted that the precise mechanism(s) for regulation of NOX by either agent is not well understood and that the clinical effect of COX2 inhibitors on endothelial function is controversial and may depend on the study population (Chenevard et al., 2003; Widlansky et al., 2003; Wong et al., 2007).

The role of blood pressure in the protective effects of p38 MAPK inhibitors was investigated by assessing the effects of a blood pressure neutral low dose and a higher dose that abolished the hypertension induced by the SFD. Although the antihypertensive effects may contribute to the end-organ protection observed at the high doses, it was clear that significant improvements in renal function, lipid profiles, and survival were also observed at a blood pressure neutral dose of GSK-AHAB. However, the antihypertensive effects of the high dose of GSK-AHAB were associated with improved endothelial-dependent and nitrate-dependent vasorelaxation and were not observed in the low-dose treatment group. It is tempting to suggest that improved vasoregulation underlies the antihypertensive effects of GSK-AHAB but the reduced plasma renin activity and aldosterone concentrations observed at this dose probably also play an important role. In addition, the augmented hypertension observed in the rofecoxib group probably contributes to the exaggerated end-organ damage, but the single-dose evaluation precludes any further speculation. Modest elevations in blood pressure associated with rofecoxib treatment have also been reported in clinical studies (Aw et al., 2005).

Given the elevated risk of cardiovascular events associated with the use of rofecoxib in the clinic and in the SHR-SP it is tempting to suggest that the SHR-SP is a translational model for predicting cardiovascular liabilities. Although the model possesses vascular, coagulation, metabolic, cardiac, and renal impairments commonly associated with cardiovascular disease it is important to note that it does not exhibit coronary atheroma or myocardial infarction, also important features of human cardiovascular disease.

The present study suggests that the effects of modulating COX2 in the context of the pleiotropic actions of p38 MAPK
inhibition are very different from the effects of selective enzyme inhibitors specifically targeting COX2. However, the study has several limitations that could be addressed in future studies. First, further examination of the rofecoxib dose-response may help to define the correlation of deleterious effects with COX2 inhibition. Second, rofecoxib comparisons with other anti-inflammatory agents, i.e., selective COX2 inhibitors and nonselective COX inhibitors, are needed to determine the validity of using rofecoxib as a prototype COX2 inhibitor and the SHR-SP as a translational model to assess cardiovascular risk. Finally, additional mechanistic studies directed at determining the cellular and molecular events underlying damage and protection in the various end organs, e.g., generation and inhibition of ROS, would enhance our understanding of beneficial and harmful anti-inflammatory drug targets.

In conclusion, the protective effects of a p38 MAPK inhibitor are clearly distinct from the deleterious effects of a selective COX2 inhibitor in SHR-SP on SFD and suggest that anti-inflammatory agents can be differentiated in cardiovascular disease. The results also suggest a model for evaluating long-term cardiovascular efficacy and safety.

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References


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