Differential Effects of Selective Cyclooxygenase-2 Inhibitors on Endothelial Function in Salt-Induced Hypertension

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Background—In view of the ongoing controversy about potential differences in cardiovascular safety of selective cyclooxygenase (COX)-2 inhibitors (coxibs), we compared the effects of 2 different coxibs and a traditional NSAID on endothelial dysfunction, a well-established surrogate of cardiovascular disease, in salt-induced hypertension.

Methods and Results—Salt-sensitive (DS) and salt-resistant (DR) Dahl rats were fed a high-sodium diet (4% NaCl) for 36 days. From days 35 to 56, diclofenac (6 mg·kg⁻¹·d⁻¹; DS-diclofenac), rofecoxib (2 mg·kg⁻¹·d⁻¹; DS-rofecoxib), celecoxib (25 mg·kg⁻¹·d⁻¹; DS-celecoxib) or placebo (DS-placebo) was added to the chow. Blood pressure increased with sodium diet in the DS groups, which was more pronounced after diclofenac and rofecoxib treatment (P<0.005 versus DS-placebo) but was slightly decreased by celecoxib (P<0.001 versus DS-placebo). Sodium diet markedly reduced NO-mediated endothelium-dependent relaxations to acetylcholine (10⁻¹⁰, 10⁻⁵ mol/L) in aortic rings of untreated hypertensive rats (P<0.005 versus DR-placebo). Relaxation to acetylcholine improved after celecoxib (P<0.005 versus DS-placebo and DS-rofecoxib) but remained unchanged after rofecoxib and diclofenac treatment. Vasoconstriction after nitric oxide synthase inhibition, indicating basal NO release, with N⁴-nitro-L-arginine methyl ester (10⁻⁴ mol/L) was blunted in DS rats (P<0.05 versus DR-placebo), normalized by celecoxib, but not affected by rofecoxib or diclofenac. Indicators of oxidative stress, 8-isoprostanate levels, were elevated in untreated DS rats on 4% NaCl (6.55±0.58 versus 3.65±1.05 ng/mL, P<0.05) and normalized by celecoxib only (4.29±0.58 ng/mL).

Conclusions—These data show that celecoxib but not rofecoxib or diclofenac improves endothelial dysfunction and reduces oxidative stress, thus pointing to differential effects of coxibs in salt-induced hypertension. (Circulation. 2003;108:2308-2311.)

Key Words: endothelium • drugs, antiinflammatory • hypertension • nitric oxide • stress

There is concern that the gastrointestinal safety of coxibs may come at the cost of increased cardiovascular complications, because selective cyclooxygenase (COX)-2 inhibition reduces prostaglandin I₂ formation without inhibiting platelet derived thromboxane A₂, with a resultant possibility for thrombosis and ischemic events.¹–⁴ The cardiovascular safety of coxibs is an important public health issue, in view of the large number of predominantly elderly patients with osteoarthritis presenting with a relatively high incidence of cardiovascular comorbidity, hypertension in particular.

Novel therapeutical strategies in hypertension aim at reversing endothelial dysfunction, which has been implicated in the pathogenesis and clinical course of hypertension and its cardiovascular complications.⁵ COX-2 inhibition may represent a novel therapeutical approach, because COX-2 expression is increased at sites of inflammatory responses of the vessel wall⁶ and production of vasoconstrictor cyclooxygenase products, particularly reactive oxygen species (ROS), contributes to the development of endothelial dysfunction in hypertension.²⁷

Thus, the present study investigated the effects of 2 different coxibs compared with a traditional NSAID on vascular function and oxidative stress in an experimental model of hypertension.

Methods

Animals
All male salt-sensitive (DS) and salt-resistant (DR) Dahl rats (11 weeks of age, mean weight 330 g; M&B, Ry, Denmark) were fed a high-sodium diet (4% NaCl) for 8 weeks. For the last 3 weeks, all DS...
rats were randomly assigned to receive either diclofenac (6 mg · kg⁻¹ · d⁻¹; DS-diclofenac, n=8), rofecoxib (2 mg · kg⁻¹ · d⁻¹; DS-rofecoxib, n=8), celecoxib (25 mg · kg⁻¹ · d⁻¹; DS-celecoxib, n=6), or placebo (DS-placebo, n=8), whereas DR rats received placebo (DR-placebo, n=8) in addition to the high-sodium diet. In the present study, dosage was calculated based on data from recent publications and took into account the differences in half life between the compounds. Food and drug intake were calculated daily.

Systolic blood pressure and heart rate were measured by the tail-cuff method. Study design and experimental protocols were approved by the institutional animal care committee (Kommission für Tiererversuche des Kantons Zürich, Switzerland) and are in accordance with the American Heart Association guidelines for research animal use.

Tissue Harvesting and Organ Chamber Experiments
Animals were anesthetized after 8 weeks before 10 mL of blood was drawn to exsanguinate the animals. Aortic rings were used for the assessment of endothelial function by their response to acetylcholine, sodium nitroprusside, superoxide dismutase (SOD), and N²-nitro-L-arginine methyl ester. Some aortic rings were used for Western blot analysis.

Prostanoids and 8-Isoprostane Plasma Levels
Prostaglandins were measured as recently described. Some 8-Isoprostanes were analyzed with enzymeimmunoassay (Cayman Chemical).

Immunoblotting and Cytokine Assay
Western blotting was performed with appropriate antibodies. Cyto-kines were measured by the cytometry-based Luminex system.

Calculations and Statistical Analysis
Data are given as mean±SEM, and n indicates the number of animals. Relaxation, contraction, negative logarithm of the concentration causing half-maximal relaxation (pD₂ value), and area under the curve were determined for each individual dose-response curve by nonlinear regression analysis with MatLab software. For multiple comparisons, ANOVA followed by unpaired Student’s t test was used. A value of P<0.05 was considered significant.

Results
Systolic Blood Pressure and Heart Rate
After 8 weeks of high-sodium diet, the blood pressure increase in DS animals was more pronounced with rofecoxib (P<0.005 versus DS-placebo) and was significantly reduced in the DS-celecoxib group (P<0.05 versus DS-rofecoxib and DS-diclofenac) (Table).

Alterations of the Vascular NO System
In aortic rings of hypertensive DS-placebo rats, acetylcholine-induced relaxation was blunted compared with normotensive DR animals (P<0.005 versus DS-placebo). Endothelium-dependent relaxation was improved by celecoxib (P<0.005 versus DS-placebo and DS-rofecoxib) but remained unchanged after treatment with rofecoxib or diclofenac (Figure).

N²-nitro-L-arginine methyl ester (10⁻⁴ mol/L)–induced vascular constriction, reflecting basal NO release, was diminished in aortic rings of hypertensive animals (P<0.05 for DS-placebo, DS-diclofenac, and DS-rofecoxib versus DR-placebo) and was normalized by celecoxib (Table).

Expression of endothelial nitric oxide synthase protein was decreased in DS-placebo (P<0.05 versus DR-placebo) but tended to increase in the DS-celecoxib group (P=0.088 versus DS-placebo) (Table).

Parameters of Oxidative Stress and Prostaglandins
Vasorelaxation to SOD was attenuated in hypertensive DS-placebo rats (P<0.001 versus DR-placebo) and improved by celecoxib (P<0.05 versus DS-rofecoxib and DS-diclofenac) (Table).

MnSOD and COX-2 protein expression in the aorta was not affected by high-salt diet or any treatment (data not shown).

8-Isoprostane plasma levels were increased in DS-placebo, DS-diclofenac, and DS-rofecoxib (P<0.05 versus DR-placebo) and decreased in the DS-celecoxib group (P<0.05 versus DS-placebo and DS-rofecoxib) (Figure). Prostaglandin E₂, prostaclin, and thromboxane B₂ plasma levels did not change between all groups (Table).

Cytokine Plasma Levels
Interleukin-1β increased in the DS-placebo group (P=0.054 versus DR-placebo) and was significantly reduced in the DS-celecoxib group (P=0.04 versus DS-placebo) (Table).

Discussion
The present head-to-head comparison of selective and non-selective COX inhibitors is, to the best of our knowledge, the first study to show differential effects of coxibs on vascular function. Interestingly, celecoxib but not rofecoxib and diclofenac improved endothelial dysfunction and reduced oxidative stress in this experimental model of hypertension.

NO is the key endothelium-derived relaxing factor that plays a pivotal role in the maintenance of vascular tone and structure. In the present study, endothelial nitric oxide synthase protein expression and basal and stimulated release of NO were blunted in hypertensive animals, thus pointing to decreased NO bioavailability, consistent with previous studies in experimental hypertension. In addition, increased isoprostane plasma levels and the attenuated relaxation to superoxide-scavenging SOD are in line with previous observations that increased oxidative stress accounts in large part for endothelial dysfunction in hypertension. Cyclooxygenases, particularly the COX-2 pathway, may contribute to the production of ROS and vasoconstrictor prostanoids and in turn to the pathogenesis of endothelial dysfunction. However, the net effect of COX-2 inhibition on endothelial dysfunction before this study remained elusive, because coxibs may potentially reduce prostacyclin generation and leave COX-1–mediated thromboxane production unopposed.
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Because interleukin-1 \( \beta \) dose-dependently induces endothelial dysfunction \( ^{17} \) and upregulates COX-2 expression in vascular smooth muscle cells, \( ^{18} \) the results of the present study suggest that celecoxib reduces the production of inflammatory cytokines and ROS, thus contributing to an improvement of endothelial function.

Rofecoxib, however, did not affect endothelial function under the conditions and doses used in the present study, suggesting that COX-2–independent effects may be involved. It should be noted that although both drugs selectively inhibit COX-2, they are chemically different compounds. Celecoxib, as a sulfonamide, is more extensively distributed into tissues than the sulfone rofecoxib. In addition to the differences in half-lives of rofecoxib and celecoxib, this may result in variations of the degree of COX-2 versus COX-1 inhibition or in additional effects unrelated to COX-2 inhibition at the tissue level, in particular the oxidation of membrane proteins. \( ^{2} \) Whether different, in particular higher, doses of rofecoxib and diclofenac would have elicited more beneficial effects under the conditions of the present study remains elusive. However, because 50 mg of rofecoxib was associated with an increased cardiovascular event rate in the TennCare study, \( ^{4} \) this seems unlikely. Although dosing cannot be ruled out as an explanation for some of the apparent discrepancies regarding cardiovascular safety of the currently available coxibs, \( ^{3,4} \) the results of the present study suggest differences within this class of drugs. Whether these differential effects of coxibs are of clinical significance remains to be determined in well-designed large-scale head-to-head comparative clinical trials that will provide the answer as to the net effect of selective COX-2 inhibition on cardiovascular events.

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### Differences Among Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DR-Placebo (n=8)</th>
<th>DS-Placebo (n=8)</th>
<th>DS-Celecoxib (n=6)</th>
<th>DS-Diclofenac (n=8)</th>
<th>DS-Rofecoxib (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>162.3±3.5</td>
<td>181.1±5.6</td>
<td>179.1±2.4</td>
<td>176.7±5.4</td>
<td>182.8±6.7</td>
</tr>
<tr>
<td>Week 8</td>
<td>158.2±4.5*</td>
<td>222.4±2.9</td>
<td>213.2±2†</td>
<td>225.5±2.4‡</td>
<td>225.4±1.6‡</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>278±12.3</td>
<td>283±9.8</td>
<td>279±14.7</td>
<td>273±15.4</td>
<td>284±13.9</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>393±6.5*</td>
<td>433±5.7</td>
<td>440±4.1</td>
<td>441±2.4</td>
<td>472±5.2</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Area under the curve, AU</td>
<td>291±9§</td>
<td>196±8.1</td>
<td>228±8.2§</td>
<td>152±10</td>
<td>177±10</td>
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<tr>
<td>pD2 (mol/L),</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log</td>
<td>7.92±0.1§</td>
<td>7.16±0.04</td>
<td>7.32±0.04</td>
<td></td>
<td>7.15±0.1</td>
</tr>
<tr>
<td>Maximum, %</td>
<td>98.6±0.58§</td>
<td>77.4±3.75</td>
<td>98.1±0.84§</td>
<td>64.3±3.15‡</td>
<td>66.9±1.84‡</td>
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<tr>
<td>Sodium nitroprusside</td>
<td></td>
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<tr>
<td>Area under the curve, AU</td>
<td>376±21</td>
<td>334±19</td>
<td>366±17</td>
<td>339±21</td>
<td>331±13</td>
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<td>pD2 (mol/L),</td>
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<tr>
<td>log</td>
<td>9.09±0.3</td>
<td>8.76±0.27</td>
<td>8.94±0.25</td>
<td>8.57±0.21</td>
<td>8.37±0.16</td>
</tr>
<tr>
<td>Maximum, %</td>
<td>100.9±0.9</td>
<td>99.3±0.8</td>
<td>100.3±0.8</td>
<td>98.3±0.9</td>
<td>99.9±0.6</td>
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<tr>
<td>SOD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Area under the curve, AU</td>
<td>267±13§</td>
<td>180±13</td>
<td>224±19#</td>
<td>148±13</td>
<td>131±13</td>
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<tr>
<td>Maximum, %</td>
<td>91.9±2.65**</td>
<td>89.1±4.83</td>
<td>96.9±1.1**</td>
<td>95.4±3.0**</td>
<td>78.5±4.6</td>
</tr>
<tr>
<td>( \text{N}^{\circ} )-nitro-( \text{L} )-arginine methyl ester</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Maximum, %</td>
<td>3.185±0.763</td>
<td>1.061±0.499††</td>
<td>2.506±0.125</td>
<td>0.828±0.490‡‡</td>
<td>0.500±0.500††</td>
</tr>
<tr>
<td>Endothelial NO synthase protein, %</td>
<td>100.0</td>
<td>54.8±2.8††</td>
<td>97.8±25.6</td>
<td>73.0±12.4</td>
<td>67.3±13.8</td>
</tr>
<tr>
<td>Prostaglandin ( \text{E}_{2} ), ng/mL</td>
<td>8.91±0.90</td>
<td>10.09±0.56</td>
<td>10.72±0.48</td>
<td>11.15±0.58</td>
<td>9.94±0.94</td>
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<tr>
<td>Prostacyclin, pg/mL</td>
<td>62.86±6.60</td>
<td>58.75±5.15</td>
<td>63.33±14.53</td>
<td>54.27±7.83</td>
<td>46.25±8.44</td>
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<tr>
<td>Thromboxane ( \text{B}_{2} ), ng/mL</td>
<td>10.74±1.75</td>
<td>12.03±0.9</td>
<td>12.25±0.67</td>
<td>13.46±0.78</td>
<td>12.11±0.93</td>
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<tr>
<td>Interleukin-1( \beta ), pg/mL</td>
<td>13.33±5.17</td>
<td>34.54±9.77</td>
<td>3.93±2.30</td>
<td></td>
<td>15.23±6.38</td>
</tr>
</tbody>
</table>

*\( P<0.001 \) vs DS-placebo, DS-celecoxib, DS-diclofenac, DS-rofecoxib.
†\( P<0.001 \) vs DR-placebo, DS-placebo, DS-diclofenac, DS-rofecoxib.
‡\( P<0.005 \) vs DS-placebo.
§\( P<0.005 \) vs DS-placebo, DS-diclofenac, DS-rofecoxib.
||\( P<0.05 \) vs DS-placebo, DS-rofecoxib.
¶\( P<0.01 \) vs DS-placebo, DS-diclofenac, DS-rofecoxib.
#\( P<0.05 \) vs DS-diclofenac, DS-rofecoxib.
\( **P<0.05 \) vs DS-rofecoxib.
††\( P<0.05 \) vs DR-placebo.
A. Endothelium-dependent acetylcholine-induced vasorelaxation. Acetylcholine-induced relaxation was blunted compared with normotensive DR animals (P < 0.005 vs DR-placebo). Endothelium-dependent relaxation was improved by celecoxib (P < 0.005 vs DS-placebo and DS-rofecoxib) but remained unchanged after treatment with rofecoxib or diclofenac (n = 6 to 8 animals in all groups). NE indicates norepinephrine. B, 8-Isoprostane plasma levels were increased in DS-placebo, celecoxib decreased 8-isoprostane levels (†P < 0.05 vs DS-placebo and DS-rofecoxib) but remained unchanged after treatment with rofecoxib or diclofenac (n = 6 to 8 animals in all groups).

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References
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