Thromboxane Synthesis Inhibition Increases Renal Prostacyclin and Prevents Renal Disease Progression in Rats with Remnant Kidney


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ABSTRACT

Previous studies have demonstrated that inhibition of thromboxane A2-dependent platelet aggregation by the thromboxane A2 synthase inhibitor, OKY 1581, ameliorated the progressive kidney disease of rats with subtotal renal ablation. OKY 1581 also decreased the excessive renal thromboxane A2 synthesis and lowered systemic blood pressure. In the same model, a low dose aspirin and a specific thromboxane A2 receptor antagonist failed to influence proteinuria, glomerulosclerosis, and hypertension, thus excluding a role for either platelet or renal thromboxane A2 in renal disease progression. The aims of this study were to establish (1) whether a thromboxane A2 synthase inhibitor different from OKY 1581 could retard the progression of glomerular disease in rats with remnant kidney and (2) whether this effect was associated with an increase in renal synthesis of the vasodilatory prostacyclin. Treatment of rats with renal mass ablation with FCE 22178 (100 mg/kg by gavage and 200 mg/kg in the drinking water) for 35 days starting 10 days after surgical ablation was associated with an improvement in renal function in comparison with rats receiving the vehicle alone. Proteinuria was significantly lower, and rats were partially protected from the development of glomerulosclerosis. Systolic blood pressure was significantly lower than in animals given the vehicle. Urinary thromboxane B2 excretion was significantly decreased, and urinary 6-keto-prostaglandin F1α increased in respect to vehicle-treated rats. We conclude that FCE 22178 limits glomerular injury in rats with remnant kidney.

Key Words: Proteinuria, glomerular filtration rate, renal plasma flow, systemic hypertension, glomerulosclerosis

Renal mass reduction (RMR) in the rat leads to systemic hypertension, proteinuria, and progressive glomerulosclerosis. This model has contributed in recent years to the identification of factors potentially responsible for the progression of renal disease. These include changes in glomerular hemodynamics with increased glomerular pressure and flow, systemic hypertension, proteinuria, hyperlipidemia, glomerular hypertrophy, and activation of coagulation processes in glomerular microvessels (1–5). Focusing on the latter, Purkerson et al. (6) suggested that intraglomerular thrombosis and platelet aggregation may play a role in the progression of the disease after a critical number of nephrons has been reduced. Their suggestions were based on the observation that inhibition of thromboxane (TX) A2-dependent platelet aggregation by chronic treatment with the TXA2 synthase inhibitor, OKY 1581, ameliorated the progressive renal disease in rats with remnant kidney, as documented by decreased proteinuria and improved renal histology in comparison with animals receiving vehicle alone. The amelioration of the disease occurred despite an increase in renal plasma flow (RPF) and glomerular filtration rate (GFR). In this study, the TXA2 synthase inhibitor, besides inhibiting TXA2-dependent platelet aggregation, also decreased the excessive renal TXA2 synthesis and lowered systemic blood pressure. In order to clarify which was the mechanism responsible for the beneficial effect of the drug, low-dose aspirin (ASA) was administered to rats with RMR with the aim to selectively inhibit platelet TXA2 and TXA2-dependent aggregation without inhibiting renal cyclooxygenase and lowering systemic blood pressure (7). Rats with RMR on a low dose of acetylsalicylic acid (ASA) were not protected from the development of proteinuria and glomerulosclerosis, thus indicating that platelet TXA2 does not play an important role in renal disease.
Recent experiments documenting that blocking of TxA2 biological activity by the selective TxA2 receptor antagonist GR 32191 failed to influence progressive proteinuria, severity of glomerulonephritis, and hypertension have suggested that renal TxA2 does not contribute to the evolution of the disease of rats with remnant kidney (8). One of the theoretical possibilities to reconcile study of Purkerson and co-workers (6) with the above findings rests on the observation that the use of TxA2 synthase inhibitors at variance with the TxA2 receptor antagonists increases vasodilatory prostaglandins (PG). Evidence is indeed available to indicate that selective inhibition of the TxA2 synthase enzyme may lead to redirection of PG endoperoxides ultimately resulting in an enhanced prostacyclin (PGI2) and prostaglandin E2 (PGE2) formation (9,10). Of interest in the study of Purkerson et al. (6) is the observation that the amelioration of the disease after OKY 1581 treatment was associated with a decrease in blood pressure, which could have been related to an increase in vasodilatory PG. Another possibility is that TxA2 synthase inhibitors exert a higher degree of inhibition on renal and extrarenal TxA2 biological activity than do TxA2 receptor antagonists. The experiments described here were therefore designed to establish (1) whether a TxA2 synthase inhibitor different from the one employed previously (6) could retard the progression of glomerular disease in rats with remnant kidney and (2) whether this effect was associated with changes in the arachidonic acid metabolic pathway, including a redirection of PG endoperoxides toward an increase in the vasodilatory PGI2.

METHODS

Experimental Design

Studies were carried out in 38 Sprague-Dawley, CD-COBs male rats (Charles River Italia S.p.A., Calco, Italy) with initial body weights of 285 to 340 g. Animals were fed standard rat chow (Altromin, Rieper, Vandoies, Italy) and had free access to tap water. Twenty-eight rats were subjected to right nephrectomy and ligation of two or three branches of the left renal artery by the method of Olson et al. (11) under ether anesthesia. Ten days after renal ablation, 24 rats survived and were divided in two groups: Group 1, 12 rats which were daily given distilled water by gavage (vehicle) for 35 days; Group 2, 12 rats which were given the selective TxA2 synthase inhibitor FCE 22178 daily [5,6-dihydro-7-[(1H-imidazol-1-yl)-2-naphthalene-carboxylic acid] (Farmitalla Carlo Erba, Milan, Italy) (12) at the dose of 100 mg/kg (by gavage) and 200 mg/kg (in the drinking water) for 35 days. Ten additional rats were subjected to sham operation with manipulation of the renal pedicles. Ten days after the sham procedure, rats were subdivided in two groups: Group 3, five rats which served as controls and received distilled water by gavage (vehicle) for 35 days; Group 4, five rats which were given FCE 22178 with the same schedule and dosage as Group 2. By the end of the experimental period, three animals in Group 1 and one animal in Group 2 had died. In order to verify whether the chronic treatment with FCE 22178 did inhibit TxA2-dependent platelet aggregation, blood for platelet aggregation studies was collected from four rats from each group at the end of the study (i.e., 45 days after surgery). In all groups of rats, bleeding time, hematocrit, and serum creatinine were measured at the same period. Twenty-four-hour urine samples were collected from all animals in metabolic cages on days 0, 10, 20, 30, and 45 after the surgical procedure for urinary protein excretion measurement. Urinary samples for determination of TxB2 and 6-keto-PGF1α, the stable breakdown products of TxA2 and PGI2, respectively, were collected from all groups of rats before and at the end of the experimental period. It is generally accepted that urinary excretion of TxB2 and 6-keto-PGF1α largely reflects the renal synthesis of the vasoactive parent compounds TxA2 and PGI2 (13). Systolic blood pressure was determined by the tail-cuff method (14) in all animals on days 0, 10, 30, and 45. Renal clearance studies were carried out in four rats from each group on day 45 after surgery. Renal tissue specimens were obtained from all animals at the end of the study and were processed for light microscopy.

Platelet Aggregation Experiments

Blood for platelet aggregation studies was drawn by cardiac venipuncture in 3.1% trisodium citrate (1:10). Platelet rich plasma (PRP) was obtained by centrifugation at 200 × g for 15 min at room temperature; after PRP was removed, the residual blood sample was centrifuged at 2,000 × g for 10 min to obtain platelet-poor plasma. The PRP platelet count was adjusted to 5 × 10^5 platelets/μL with autologous platelet-poor plasma. Platelet aggregation was induced by ADP (Sigma Chemical Co., St. Louis, MO) and arachidonic acid (AA) (Sigma) as previously described (15). Threshold aggregating concentration was defined as the lowest concentration of aggregating agent which induced irreversible platelet aggregation starting within 3 min after the addition of the aggregating agents to PRP.

Bleeding Time

Bleeding time was determined in nonanesthetized rats by using a standardized Simplate II device (General Diagnostic, Milan, Italy) as previously described (16). Bleeding time was measured and expressed in
seconds from the moment the tail was incised until bleeding stopped completely (no rebleeding within 30 s; normal range, 80 to 120 s).

**Serum Creatinine**

Serum was obtained after whole blood (1 mL) clotting at 37°C for 30 min. Blood was collected from the tail vein of anesthetized animals. The prepared sera were frozen and kept at −20°C until assayed. Creatinine was measured by the alkaline picrate method (17).

**Urinary Protein Excretion**

Proteinuria was determined by the modified Coomassie blue G dye-binding assay for proteins (18) with bovine serum albumin as standard.

**Urine Extraction**

Transferred to the stable breakdown product of TxAs, and 6-keto-PGF1α, the stable hydrolysis product of PGI2, were measured in urine by radioimmunoassay (RIA) after extraction and silicic acid column chromatography, as previously described (19). Briefly, after the addition of 6,000 dpm [3H]TxB2 or [3H]6-keto-PGF1α (Amersham International, Buckinghamshire, United Kingdom; 113 or 130 Ci/mmol, respectively) for the estimation of recovery (60 to 75%), urine (3 mL) was acidified to pH 3.5 and passed through a disposable (C18) column (Sep-pak; Millipore, Milford, MA) already activated with methanol and water. The column was activated with n-hexane, and the sample was eluted with ethylacetate. The elution fraction was dried on a rotary evaporator at 37°C for 30 mm. Blood was collected from the tail vein of anesthetized animals. The prepared sera were frozen and kept at −20°C until assayed. Creatinine was measured by the alkaline picrate method (17).

**Radioimmunoassay**

Extracted urinary samples were diluted 1:5 to 1:30 for TxB2 and 1:50 to 1:100 for 6-keto-PGF1α. RIA was performed as described previously (19). Results were expressed as nanograms per day. The smallest concentration that could be measured with 95% confidence was 2 pg/mL for both antisera. Cross-reactivity at 50% displacement of other arachidonate metabolites, interassay and intra-assay variability, and validation of RIA measurement were reported elsewhere (19).

**Renal Clearance Studies**

Rats were anesthetized with thiopental sodium (50 mg/kg body wt i.p.) and were placed on a temperature-regulated table. After tracheostomy, a polyethylene (PE-50) catheter was inserted in the left femoral artery for blood sampling and for monitoring the arterial blood pressure by a Statham pressure transducer and a carrier amplifier (Battaglia Rangoni, Bologna, Italy). Catheters were also placed in the left femoral vein and in the bladder. Inulin (5%) and p-aminohippuric acid (PAH) (0.25%) were infused into the femoral vein over 2 min as a priming dose (1.4 mL), followed by a constant infusion of a maintenance saline solution containing 1.5% inulin and 0.05% PAH at a rate of 2 mL/h. After 50-min equilibration, urine was collected from the bladder catheter during three 30-min periods and 200 μL was taken at the midpoint of each collection to quantify inulin and PAH concentrations. The concentration of inulin in plasma and urine was measured by the anthrone method (20). PAH concentration was determined by a colorimetric technique (21).

**Histological Studies by Light Microscopy**

Sections including superficial and juxtamedullary glomeruli were evaluated. For each animal, at least 150 glomeruli were examined and the percentage of glomeruli presenting focal or global glomerulosclerosis was determined. Tubular (atrophy, casts, and dilatation) and interstitial changes (fibrosis and inflammation) were graded from 0 to 4+ (0, no changes; 1+, changes affecting less than 25% of the sample; 2+, changes affecting 25 to 50% of the sample; 3+, changes affecting 50 to 75% of the sample; 4+, changes affecting 75 to 100% of the sample). All renal biopsies were analyzed by the same pathologist, who was unaware of the nature of the experimental groups.

**Statistical Analysis**

Data were analyzed by using one-way or two-way analysis of variance as appropriate. Significance level of differences between individual group means, subjected to the analysis of variance, was established by using Tukey's test for multiple comparisons (22). Correlation among different variables, was performed by linear regression analysis. Estimates of renal damage by morphological studies were compared with the Mann-Whitney test. The statistical significance level was defined as P < 0.05.

**RESULTS**

Rats with RMR failed to gain weight during the first postoperative week (day 0, 323 ± 21 g; day 7, 21 g; day 7, 21 g; day 7, 21 g; day 7,
299 ± 12 g). During this week in sham-operated control rats, mean body weight rose from 296 ± 5 to 336 ± 9 g. Thereafter, rates of weight gain were fairly comparable in all groups (Table 1). As shown in Table 1, rats with RMR and sham-operated animals ingested similar amounts of food.

**Effect of FCE 22178 on Platelet Aggregation in Rats with Reduced Renal Mass**

In order to verify whether the dose of FCE 22178 employed was effective in inhibiting TxA2 platelet-dependent aggregation, we studied ADP and AA-induced platelet aggregation in four rats from each experimental group 45 days after surgery. Mean threshold aggregating concentrations of ADP in renal mass reduction or sham-operated rats given FCE 22178 were comparable with those of the two groups receiving vehicle (Table 2). In contrast, concentrations of AA up to 2 mM were unable to induce aggregation in PRP from either rats with reduced renal mass or sham-operated rats given FCE 22178.

**Effect of FCE 22178 on Bleeding Time in Rats with Reduced Renal Mass**

As previously reported (16), bleeding time in rats with reduced renal mass who were given vehicle was significantly (P < 0.01) longer than in control sham-operated rats (Table 1). FCE 22178 tended to further prolong the bleeding time of rats with renal mass reduction, although a statistical significance was not reached. Since reduction of renal mass per se leads to a marked prolongation of bleeding time (a likely result of the platelet function defect of uremia), it is possible that the pure effect of FCE 22178 in this condition cannot be established. That this is the case is supported by the finding of a significant (P < 0.01) prolongation of bleeding time values in sham-operated rats given FCE 22178 as compared with that in rats receiving vehicle alone.

**TABLE 1.**

General clinical and laboratory values measured in all experimental groups at the end of the study

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight (g)</th>
<th>Food Intake (g/day)</th>
<th>Bleeding Time (s)</th>
<th>Hematocrit (%)</th>
<th>Serum Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMR + vehicle</td>
<td>441 ± 33</td>
<td>22 ± 3</td>
<td>227 ± 49</td>
<td>45 ± 3</td>
<td>1.31 ± 0.29</td>
</tr>
<tr>
<td>RMR + FCE 22178</td>
<td>447 ± 42</td>
<td>21 ± 5</td>
<td>242 ± 51</td>
<td>46 ± 4</td>
<td>0.80 ± 0.18</td>
</tr>
<tr>
<td>Sham + vehicle</td>
<td>464 ± 29</td>
<td>24 ± 4</td>
<td>102 ± 16</td>
<td>51 ± 1</td>
<td>0.61 ± 0.06</td>
</tr>
<tr>
<td>Sham + FCE 22178</td>
<td>457 ± 23</td>
<td>25 ± 2</td>
<td>191 ± 55</td>
<td>52 ± 1</td>
<td>0.60 ± 0.08</td>
</tr>
</tbody>
</table>

* Values are mean ± SD.
* P < 0.01 versus sham + vehicle.
* P < 0.05 versus sham + vehicle.
* P < 0.01 versus RMR + vehicle.

**Effect of FCE 22178 on Hematocrit in Rats with Reduced Renal Mass**

Rats with RMR given vehicle, exhibited mild anemia 45 days after the surgical procedure, with values for hematocrit of 45 ± 3%. These values were significantly (P < 0.01) lower than those of the sham-operated control rats (Table 1). Hematocrit values were not influenced by FCE 22178 treatment.

**Effect of FCE 22178 on Blood Pressure in Rats with Reduced Renal Mass**

Values of systolic blood pressure measured in all groups of animals at different time intervals are shown in Figure 1. In Group 1 rats, removal of renal mass resulted in the development of systemic hypertension within 10 days after ablation. Blood pressure values were significantly (P < 0.01) higher than basal ones during the entire course of the study (day 0, 125 ± 6 mm Hg; day 45, 189 ± 34 mm Hg). Systemic hypertension was observed over the first 10 days of the study in Group 2 rats. Institution of FCE 22178 therapy after this interval promptly lowered systolic blood pressure and maintained it at values averaging 142 ± 21 mm Hg at the end of the study. These values were significantly (P < 0.01) lower than those of rats with RMR given vehicle alone but were still numerically higher than those of sham-operated animals, although the difference did not reach statistical significance. During the study, the mean systolic blood pressure of sham-operated rats either receiving vehicle or FCE 22178 remained comparable to that measured on day 0 (with vehicle, values at day 0 and day 45 were 128 ± 1 and 127 ± 5 mm Hg, respectively; with FCE 22178, values at day 0 and day 45 were 127 ± 5 and 135 ± 10 mm Hg, respectively).

**Effect of FCE 22178 on Proteinuria in Rats with Reduced Renal Mass**

The time course of urinary protein excretion is shown in Figure 2. Animals with RMR given vehicle
Effect of FCE 22178 on Renal Function in Rats with Reduced Renal Mass

To assess whether chronic treatment with FCE 22178 retarded the progression of renal disease in rats with reduced renal mass, we measured serum creatinine and renal inulin and PAH clearances in all of the experimental groups. Animals with RMR given vehicle showed a significant (P < 0.01) increase in serum creatinine as compared with sham-operated control rats (Table 1). Serum creatinine was significantly (P < 0.01) lower in rats with renal mass ablation treated with FCE 22178 than in those with RMR given vehicle. In sham-operated animals, serum creatinine concentration was not modified by FCE 22178.

Rats with RMR given vehicle exhibited significantly (P < 0.01) lower GFR values than did sham-operated control animals (Table 3). FCE 22178 significantly (P < 0.01) ameliorated renal function of rats with RMR. However, in these animals, GFR values were still significantly (P < 0.01) lower than those of sham-operated rats. No difference in GFR was found in sham-operated rats given FCE 22178 or vehicle.

RPF as estimated by PAH clearance was significantly (P < 0.01) lower in rats with reduced renal mass given vehicle than in sham-operated controls (Table 3). Administration of FCE 22178 to rats with ablation of renal mass significantly (P < 0.01) increased RPF as compared with rats with reduced renal mass given vehicle without returning, however, to values of sham-operated rats (P < 0.01 versus sham-operated rats). In sham-operated rats treated with FCE 22178, RPF remained unchanged.
Renal Eicosanoids and Renal Disease Progression

Table 3. Effect of FCE 22178 on GFR and RPF in rats with RMR or sham-operated rats studied at the end of the experimental period.

<table>
<thead>
<tr>
<th></th>
<th>GFR (mL/min/100 g)</th>
<th>RPF (mL/min/100 g)</th>
</tr>
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<tbody>
<tr>
<td>RMR + vehicle</td>
<td>0.25 ± 0.07²</td>
<td>0.66 ± 0.09²</td>
</tr>
<tr>
<td>RMR + FCE 22178</td>
<td>0.51 ± 0.07³</td>
<td>1.20 ± 0.17³</td>
</tr>
<tr>
<td>Sham + vehicle</td>
<td>0.75 ± 0.07</td>
<td>2.18 ± 0.25</td>
</tr>
<tr>
<td>Sham + FCE 22178</td>
<td>0.72 ± 0.05</td>
<td>2.25 ± 0.17</td>
</tr>
</tbody>
</table>

² Values are mean ± SD.
³ P < 0.01 versus sham-operated rat groups.
⁴ P < 0.01 versus RMR + vehicle.

Effect of FCE 22178 on Urinary Excretion of TxB₂ and 6-keto-PGF₁α in Rats with Reduced Renal Mass

Results of urinary TxB₂ and 6-keto-PGF₁α excretion measurements are depicted in Figure 3. Urinary TxB₂ excretion in rats with renal mass reduction given vehicle significantly (P < 0.01) increased during the course of the experiment as compared with basal values (22.05 ± 6.76 versus 9.10 ± 2.55 ng/day). FCE 22178 completely prevented the enhanced urinary excretion of TxB₂ in rats with renal mass ablation with values at day 45 averaging 5.69 ± 0.95 ng/day. These values were significantly (P < 0.01) lower than those obtained at the same time in rats with RMR receiving vehicle alone. In sham-operated animals, the urinary excretion of TxB₂ did not change through the experimental period either in animals given vehicle or FCE 22178 (values with vehicle were 9.32 ± 1.95 ng/day at day 0 and 9.58 ± 2.07 ng/day at day 45; values with FCE 22178 were 9.51 ± 1.84 ng/day at day 0 and 9.22 ± 3.09 ng/day at day 45).

Urinary excretion of 6-keto-PGF₁α in rats with remnant kidney given vehicle remained unchanged during the whole study (values at day 0 and day 45 were 19.94 ± 2.49 and 19.17 ± 7.24 ng/day, respectively). In contrast, after FCE 22178 treatment, urinary excretion of 6-keto-PGF₁α of rats with RMR significantly (P < 0.01) increased over the baseline values (36.61 ± 12.35 versus 22.45 ± 2.59 ng/day). Thus, at the end of the study, urinary excretion of 6-keto-PGF₁α of rats given FCE 22178 was significantly (P < 0.01) higher than that of rats with RMR given vehicle. In sham-operated animals receiving vehicle, the values of urinary 6-keto-PGF₁α excretion obtained at the end of the study were comparable to basal ones (day 0, 17.42 ± 4.22 ng/day; day 45, 18.43 ± 3.72 ng/day). In sham-operated rats given FCE 22178 urinary excretion of 6-keto-PGF₁α by the end of the study was numerically higher than basal values but the difference did not reach statistical significance (day 0, 20.03 ± 4.16 ng/day; day 45, 28.46 ± 8.33 ng/day). In groups 1 and 2 of rats with RMR, a negative correlation coefficient was found between urinary excretion of 6-keto-PGF₁α and systolic blood pressure (r = 0.66; P < 0.01) and between urinary excretion of 6-keto-PGF₁α and proteinuria (r = 0.60; P < 0.02).

Pathological Studies

Glomerular and tubulointerstitial changes are quantified in Table 4. At the end of the 45-day observation period, rats with reduced renal mass given vehicle showed irregularly distributed glomerular alterations, characterized by focal and segmental collapse of capillaries, hyaline deposition, and adhesion of the glomerular tuft to Bowman’s capsule. In the glomeruli spared by sclerotic changes, the mesangial matrix was moderately expanded and the cytoplasm of podocytes showed pronounced swelling. Hypertrophy and a moderate number of proteinaceous casts were seen in the distal and collecting tubules. Inter-
Table 4. Pathological changes in rats with RMR chronically given vehicle or FCE 22178

<table>
<thead>
<tr>
<th></th>
<th>Glomerular with Sclerotic Changes (%)</th>
<th>Tubulo-interstitial Damage</th>
</tr>
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<tbody>
<tr>
<td>RMR + vehicle (N = 9)</td>
<td>20.9 (2–54)</td>
<td>1.08 (0.5–1.5)</td>
</tr>
<tr>
<td>RMR + FCE 22178 (N = 11)</td>
<td>5.8 (0–14)</td>
<td>0.21 (0–0.5)</td>
</tr>
</tbody>
</table>

* Values are mean percentages and mean scores; values in parentheses are ranges.

P < 0.05 versus RMR + vehicle.

Stititual inflammation and fibrosis were patchy and not particularly severe. In rats with reduction of renal mass given FCE 22178, sclerosis was strikingly limited, with the mean values being significantly (P < 0.05) lower than in rats with reduced renal mass receiving vehicle. Tubulointerstitial changes were mild. All sham-operated animal groups did not show glomerular or tubulointerstitial changes.

**Discussion**

Rats with subtotal nephrectomy received chronic treatment with a TxA<sub>2</sub> synthase inhibitor, FCE 22178, or vehicle for 35 days. Our results showed that the TxA<sub>2</sub> synthase inhibition reduced proteinuria, protected animals against renal function deterioration, and ameliorated the histological evidence of renal injury in respect to vehicle-treated animals. In animals receiving FCE 22178 in particular, urinary protein excretion was lower than in the corresponding controls with a significant difference at day 30 that remained until day 45 when the animals were killed. GFR and RPF were higher than in vehicle-treated animals. Analysis of histopathological changes indicated that the degrees of glomerulosclerosis and tubulointerstitial damage were significantly lower in animals given FCE 22178 than in vehicle-treated animals. The present findings are in keeping with previous data of Purkerson et al. (6) who found that chronic administration of a different molecule, OKY 1581, with the same pharmacological activity of blocking TxA<sub>2</sub> synthase enzyme to rats with subtotal nephrectomy, decreased proteinuria and ameliorated renal disease progression. Since hyperperfusion increased in animals given the TxA<sub>2</sub> synthase inhibitor, those authors suggested that OKY 1581 was beneficial because of an effect on platelet aggregation and intraglomerular thrombosis. In subsequent studies, the possibility that inhibiting TxA<sub>2</sub>-mediated platelet aggregation would be of any value in protecting rats with remnant kidney from the development of progressive disease has been formally tested. Selective inhibition of platelet cyclooxygenase has been achieved in rats with reduced renal mass by low-dose ASA, with an almost complete prevention of platelet Tx generation (7). Glomerular and urinary TxB<sub>2</sub> and 6-keto-PGF<sub>1α</sub>, the hydrolysis products of TxA<sub>2</sub> and PGI<sub>2</sub>, respectively, were not inhibited by low-dose ASA nor was blood pressure lowered. This study (7) indicated that both platelet-derived TxA<sub>2</sub> and TxA<sub>2</sub>-dependent platelet aggregation were not relevant factors for renal disease progression in the remnant kidney model. However, the experiments with low-dose ASA could not rule out the possibility that an excessive generation of TxA<sub>2</sub> by resident renal cells was involved in renal disease progression. A more recent study investigated the latter possibility and explored whether renal disease progression in the remnant kidney could have been influenced by a selective TxA<sub>2</sub> receptor blocking, which, in addition to platelet TxA<sub>2</sub>, also blocks the biological activity of TxA<sub>2</sub> formed at the renal level by resident cells (8). Rats with renal mass ablation given the selective TxA<sub>2</sub> receptor antagonist GR 32191, at a dose which completely inhibited TxA<sub>2</sub> mimetic-induced decrease in GFR and RPF, were not protected from the progressive renal injury. This experiment (8) again reinforced the concept that inhibiting TxA<sub>2</sub> biological activity per se of either platelet or renal cell origin does not protect animals from progressive renal injury. To reconcile the data of the beneficial effect of OKY 1581 with those of low-dose ASA or GR 32191, one can consider the difference in the pharmacological profile of these classes of platelet-inhibiting drugs. Low-dose ASA irreversibly acetylates platelet cyclooxygenase in the "presystemic" circulation (23), thus inhibiting platelet TxA<sub>2</sub>. Since inactivation of cyclooxygenase activity by ASA is permanent, de novo synthesis of the enzyme is required to restore normal eicosanoid formation (24). Such a process can easily occur within hours in nucleated cells but cannot take place in platelets which are anucleated. Therefore, the kinetics of ASA, together with the different renewal rates of cyclooxygenase in platelets and vascular or renal cells, should allow to "selectively" inhibit platelet but to spare vascular and renal cyclooxygenase (25–27). Tx receptor antagonists block the activity of TxA<sub>2</sub> at platelet, vessel wall, and renal levels and also antag-
onize the effects of PG endoperoxides that bind to a common TxA2/PG endoperoxide receptor (8, 28, 29). At variance, Tx synthase inhibition is associated with a concomitant accumulation within the cell of PG endoperoxides which are redirected toward PGI2 and PGE2 synthesis (9, 10, 30–32). At the renal level, such a mechanism might increase synthesis and urinary excretion of PGI2 and its metabolites and PGE2.

To address this possibility, we have investigated TxB2 (the stable hydrolysis product of TxA2) and 6-keto-PGF1α (the hydrolysis product of PGF2α) urinary excretion rates in animals given FCE 22178 or vehicle. As documented by previous studies (7, 8), the RMR was associated with a significant increase in TxB2 but not in 6-keto-PGF1α urinary excretion, which was comparable to that of vehicle-treated animals. FCE 22178 significantly lowered urinary TxB2 to values comparable with those of sham-operated animals. Concomitantly, with the reduction in urinary TxB2, the urinary excretion of 6-keto-PGF1α significantly increased in animals given FCE 22178 but not in vehicle-treated animals. Therefore, the effect of FCE 22178 on these animals could at least in part be mediated by the simultaneous inhibition of TxA2 together with an increase in renal PGI2 synthesis. Apparently, this explanation may not apply to previous results obtained by Purkerson et al. (6) with OKY 1581 since, in their study, urinary excretion of 6-keto-PGF1α was not increased. However, given the similarity in the pharmacological profile of the two compounds, one cannot exclude that a partial redirection of endoperoxides toward PGI2 and PGE2 also occurs with OKY 1581. The fact that FCE 22178 is more effective than OKY 1581 in increasing urinary excretion of 6-keto-PGF1α could reflect a higher rate of endoperoxide generation as a consequence of the inhibition of renal TxA2 synthase. This possibility is consistent with recent findings showing a higher susceptibility of glomerular versus platelet TxA2 synthase to FCE 22178, a peculiarity which is not shared by other imidazole-like molecules (12).

The significant decrease in blood pressure observed with FCE 22178, but not with low-dose ASA or GR 32191, is consistent with a possible decrease in vascular peripheral resistance which can be mediated by PGI2. As far as PGE2 is concerned, previous studies have documented that its increased synthesis has a protective effect on progressive renal disease in rats with renal mass ablation (33).

It is possible that the simultaneous increase in renal PGI2 and PGE2 synthesis acts synergistically to the effect of TxA2 inhibition (34) and controls glomerular hypertension, thus limiting glomerular injury. This possibility is based on micropuncture studies showing that in normal rats, PGI2 infusion in the presence of the angiotensin II receptor antagonist saralasin markedly decreased afferent and efferent arteriolar resistance with a concomitant decline in glomerular capillary hydraulic pressure (35). If this interpretation is correct, the beneficial effect of Tx synthase inhibitors in remnant kidney further supports the view that glomerular hemodynamic changes mediate progressive renal injury when a critical number of nephrons is reduced (1–3, 36).

One could argue whether simply reducing systemic blood pressure, as obtained with both FCE 22178 and OKY 1581, would be enough to protect animals from renal injury. Against this possibility is the experimental evidence that a triple antihypertensive therapy does not offer significant protection unless a parallel reduction of glomerular capillary hydraulic pressure is achieved (36). Similarly, the β-blocking agent tertatolol does not protect rats with remnant kidney from progressive disease, despite normalizing systolic blood pressure (8).

An alternative explanation for the superior effect of TxA2 synthase inhibitors over TxA2 receptor antagonists on renal disease progression in remnant kidney is a higher degree of inhibition in TxA2 biological activity, possibly achieved by the former versus the latter class of compounds.

In conclusion, our results indicate that a TxA2 synthase inhibitor different from OKY 1581 (6) also retards renal disease progression in rats with remnant kidney. The beneficial effects of FCE 22178 are associated with a substantial inhibition of urinary TxA2 together with an increase in urinary 6-keto-PGF1α. Whether a possible redirection of endoperoxides is responsible for the observed effect of FCE 22178 must remain speculative.

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REFERENCES


