Inhibition of thromboxane synthesis ameliorates the progressive kidney disease of rats with subtotal renal ablation

(renal hyperperfusion and hyperfiltration/intraglomerular coagulation/glomerulosclerosis/uremia/hypertension)

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ABSTRACT Ablation of >70% of renal mass in the rat results in hypertension, proteinuria, and glomerular sclerosis of the remnant kidney. Rats with a remnant kidney have increased excretion of thromboxane in the urine when compared with normal rats. Chronic oral administration of OKY 1581, an inhibitor of thromboxane synthesis, in rats with a remnant kidney increases renal blood flow and glomerular filtration rate (GFR), decreases protein and thromboxane excretion in the urine, lowers blood pressure and cardiac index, and improves renal histology. The degree of hyper trophy of the remnant kidney was unaffected by administration of OKY 1581. Calculated values for single nephron plasma flow and GFR were significantly greater in rats with remnant kidneys given OKY 1581 than in rats given saline. Acute i.v. administration of OKY 1581 increased renal plasma flow and GFR in rats with a remnant kidney but not in normal rats or rats with a remnant kidney previously treated with acetylsalicylic acid. OKY 1581 markedly inhibited platelet aggregation. We suggest that in this model of renal disease platelet aggregation and intraglomerular thrombosis play a key role in the development of glomerulosclerosis. Inhibition of platelet aggregation prevents development of glomerulosclerosis, hypertension, and cardiac hypertrophy. We suggest that hyperperfusion and hyperfiltration per se occurring in remnant glomeruli are not directly responsible for the development of glomerulosclerosis.

Subtotal renal ablation in the rat results in proteinuria, hypertension, and progressive kidney disease (1, 2). The factors responsible for progressive renal failure in this model are incompletely understood (3, 4). Previous studies have shown that heparin administration retards the development and progression of the renal damage, the hypertension, and the uremia and prolonged survival of these rats with experimentally induced renal infarction (5). Warfarin administration has a similar effect (6). These findings suggest that inhibition of blood coagulation prevents the development of hypertension and progressive renal failure in rats with severe hyperfiltration and support the concept that glomerular thrombosis plays a role in their pathogenesis.

A role for increased glomerular perfusion and hyperfiltration (7) in causing the glomerular sclerosis that occurs in rats with decreased renal mass has been proposed (8, 9). A linkage between glomerular hyperfiltration and structural changes was suggested in studies involving dietary protein restriction. Feeding diets low in protein to rats with remnant kidneys prevented the increases in glomerular plasma flow and capillary pressures that lead to hyperfiltration. Also, the accompanying proteinuria and structural alterations were less severe (10). It has been proposed that the progression of chronic renal insufficiency after a critical reduction in renal mass depends on a final common pathway, glomerular hyperfiltration. As the functional contribution of the sclerosing glomeruli is lost, less severely affected glomeruli undergo further compensatory hyperfiltration with subsequent injury, favoring progression and eventual total loss of glomeruli (10, 11).

The present studies examine the potential role of endogenous thromboxane on the progression of renal disease in rats with reduced kidney mass. We reasoned that in this model selective inhibition of thromboxane synthesis, a powerful vasoconstrictor of the renal circulation, may increase perfusion pressure and hyperfiltration and accelerate the progression of renal disease. However, if other events occurring concomitantly with or subsequent to the development of hyperfiltration and hyperperfusion participate in the progression of the renal disease, inhibition of thromboxane synthesis may have no effect or indeed may retard the onset and progression of renal damage. It has been found that oral administration of a selective inhibitor of thromboxane synthesis (12, 13) ameliorates the progressive renal disease that occurs in rats with a reduced renal mass despite a concomitant increase in renal plasma flow and single nephron glomerular filtration rate (GFR).

MATERIALS AND METHODS

All studies were carried out with female Sprague-Dawley rats weighing 240–300 g and fed a standard rat chow containing 22.8% protein (Ralston-Purina). Renal mass was reduced by removing one kidney and infarcting three-fourths of the contralateral kidney ("1/4 nephrectomy"). Several groups of rats were studied. In group 1 with subtotal renal ablation, 5 animals were given vehicle (1.5 ml of saline) and 4 were given OKY 1581 (provided by Ono Pharmaceuticals, Osaka, Japan; lot 17) at 20 mg/kg body weight in 1.5 ml of saline per gavage daily for 26 days beginning the day after 1/4 nephrectomy. At the end of this period, blood was obtained for bound urea nitrogen (BUN) and creatinine determination and blood pressure was measured. The rats were killed, the heart was excised and weighed, and the kidneys were removed for histological examination. Group 2 was composed of 11 rats with 1/4 nephrectomy. Of these rats, 6 received 1.5 ml of saline per gavage twice daily and 5 were given OKY 1581 (lot 205A) at 20 mg/kg body weight in 1.5 ml of saline twice a day for 35 days. At the end of this period, clearance studies were carried out, blood pressure was measured, and the animals were killed and organs were obtained as described above. Group 3 was composed of 5 normal rats and 10 rats with 1/4 nephrectomy, 5 untreated and 5 that received acetylsalicylic acid at 5 mg/kg body weight by gastric gavage daily until the time of the clearance experiments; these rats were studied 26–49 days after nephrectomy. In this case.

Abbreviations: GFR, glomerular filtration rate; PAH, p-aminophenylurea; 6-keto-PGF1α, 6-ketoprostaglandin F1α.
group of normal and 1/4 nephrectomized rats, data for three baseline clearance periods for inulin and p-aminophthippurate (PAH) were obtained and then an i.v. infusion of OKY 1581 was given and data for three additional clearance periods were obtained.

Group 4, composed of 10 rats with 1/4 nephrectomy, was studied using micropuncture techniques. Five of these rats received vehicle and 5 received OKY 1581 for 5 weeks prior to study.

Group 5, composed of 18 rats, had been 1/4 nephrectomized 3–7 days prior to study. These rats were given a single oral dose of OKY 1581 (20 mg/kg of body weight) in 1.5 ml of saline or 1.5 ml of saline only and killed at specified time intervals to determine effects on platelet aggregation.

Clearance Studies. Renal function in groups 2 and 3 was determined in awake rats using standard clearance techniques (14). A priming dose of inulin (Fish) to produce plasma levels of 75–100 mg/dl and of PAH (Merck Sharp & Dohme) to give plasma levels of 1–2 mg/dl was infused in 0.6 ml of saline over a 3-min period and then a solution containing sufficient inulin and PAH to maintain their plasma levels constant was infused. After an equilibration period of 60 min, data for three clearance periods were collected and, for group 3, data for additional clearance periods were also obtained. After baseline clearance data were obtained, a priming dose of OKY 1581 (lot 205A) at 5 μg/kg of body weight in 0.3 ml of 0.9% saline was given over a 5-min period. This was followed by sustaining i.v. infusion of OKY 1581 at 12 μg/hr. Urine collections for these additional periods were begun after an equilibration period of 10–15 min. Urine for all clearance periods was collected under oil in previously weighed tubes immersed in ice water. Urine volume was determined by weighing. Arterial blood samples were collected at the beginning, midpoint, and termination of each clearance period. Plasma was separated for each clearance period for determination of inulin, PAH, sodium, potassium, and BUN. Urine aliquots were obtained for inulin, PAH, Na+, and K+ and a 100-μl sample was frozen and stored at −70°C for determination of thromboxane and 6-ketoprostaglandin F1α (6-keto-PGF1α).

Micropuncture Studies. Rats that had been fasted for 12 hr were anesthetized with Inactin (Promonta, Hamburg, F.R.G.). Immediately before the abdomen was open and the kidney was prepared for micropuncture all rats received a priming dose of inulin and a sustaining infusion to maintain plasma levels of 50 and 100 mg/100 ml. An equilibration period of 45–60 min was allowed before timed fluid collections were obtained from the end of the accessible portion of the proximal tubules of surface nephrons as described (15). Blood samples were obtained at 30-min intervals or immediately after collection of tubular fluid. Volume and inulin content of tubular fluid were measured as described (15).

Determination of Urine Protein, Blood Pressure, and Platelet Aggregation. Urine protein and blood pressure were measured in rats of groups 1 and 2. Prior to clearance studies, animals were placed in metabolic cages for 48-hr urine collection for determination of protein. After clearance studies, rats were anesthetized with Na pentobarbital at 30 mg/kg of body weight i.p. and mean arterial blood pressure was measured via the right femoral artery with PE 50 tubing connected to a mercury manometer.

Blood for platelet aggregation studies was collected via the abdominal aorta. Platelet aggregation was examined by a turbidimetric technique in platelet-rich plasma after addition of 3 μM ADP, acid-soluble collagen (5 μg/ml), or 1.25 and 2.5 mM arachidonic acid (16). The maximal change in light transmission occurring within 5 min after stimulation was recorded.

Gross and Microscopic Morphology. After exsanguination, the heart was removed and its weight was determined to calculate cardiac index [weight of heart (in mg)/body weight (in g)]. Tissue was obtained from the viable portion of the remaining kidney for weight determination and examination by light and transmission electron microscopy. Light microscopy was carried out on p-aminosalicylic acid-stained midline sagittal plane sections of the remnant kidney of each rat. To assess the number of abnormal glomeruli, 50 consecutive glomeruli were examined at 100× magnification in each rat.

Chemical and RIA Determinations. Inulin in urine and plasma was determined using the microanthone method (17), and PAH was determined by a modification of the method of Smith et al. (18). Na+ and K+ were determined by flame photometry (Instrumentation Laboratories model 43). Urine protein was quantitated using the Lowry method (19). Thromboxane B2 (the stable metabolite of thromboxane A2) and 6-keto-PGF1α in the urine were determined by RIA.

Calculations and Statistical Analysis. Clearances were calculated using standard formulae. An unpaired t test was used when comparing data from control vs. experimental rats. A paired t test was used when comparing clearance values in the same animals before and after administration of OKY 1581.

RESULTS

Values for mean arterial blood pressure and cardiac index in normal and 1/4 nephrectomized rats given vehicle or OKY 1581 are shown in Fig. 1. Arterial blood pressure and cardiac index values were significantly less in 1/4 nephrectomized rats receiving OKY 1581 than in those given vehicle. Data for body weight, blood pressure, urine flow, inulin and PAH clearances, BUN, 24-hr protein excretion, and weights of the whole remnant kidney and its viable portion in 1/4 nephrectomized rats given vehicle or OKY 1581 orally for 5 weeks are given in Table 1. There were no differences in initial body weight or in weight gain in the two groups of rats. Rats receiving OKY 1581 had higher inulin and PAH clearance values than rats receiving vehicle. Levels of BUN and 24-hr excretion of protein in the urine were significantly less in treated rats than in those receiving vehicle. Weights of the
Table 1. Body weight, mean arterial blood pressure, and renal function in 13/4 nephrectomized rats given vehicle or OKY 1581

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BW, g</th>
<th>35-day gain, g</th>
<th>MABP, mm Hg</th>
<th>V, ml/min</th>
<th>C_{in}, ml/min per kg</th>
<th>C_{PAH}, ml/min per kg</th>
<th>BUN, mg/dl</th>
<th>24-hr protein, mg/ml</th>
<th>Kidney weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>269.0</td>
<td>21.80</td>
<td>190.0</td>
<td>0.070</td>
<td>2.34</td>
<td>6.25</td>
<td>48.0</td>
<td>610.7</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>± 10.06</td>
<td>± 4.26</td>
<td>± 3.83</td>
<td>± 0.01</td>
<td>± 0.15</td>
<td>± 0.15</td>
<td>± 3.08</td>
<td>± 149.6</td>
<td>± 0.05</td>
</tr>
<tr>
<td>OKY 1581</td>
<td>291.0</td>
<td>29.20</td>
<td>137.0</td>
<td>0.086</td>
<td>5.34</td>
<td>10.92</td>
<td>31.6</td>
<td>111.4</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>± 9.77</td>
<td>± 9.25</td>
<td>± 5.43</td>
<td>± 0.004</td>
<td>± 0.66</td>
<td>± 0.91</td>
<td>± 2.34</td>
<td>± 21.57</td>
<td>± 0.11</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>&lt;0.02</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Studies were carried out on groups of five rats after 35 days of treatment. Values are mean ± SEM. BW, body weight; MABP, mean arterial blood pressure; V, urine flow; C_{in}, inulin clearance; C_{PAH}, PAH clearance. NS, no significant difference (2P > 0.1).

The whole remnant kidney and its viable portion were comparable in the two groups, indicating comparable degrees of renal hypertrophy over the period of observation.

As shown in Table 2, urinary excretion of thromboxane B_2 was greater in 13/4 nephrectomized rats than in normal animals. Administration of OKY 1581 decreased thromboxane excretion in rats with 13/4 nephrectomy to values that did not differ from those in normal rats. Excretion of 6-keto-PGF_{ia} was not significantly different between normal and 13/4 nephrectomized rats.

As shown in Table 3, acute i.v. administration of OKY 1581 increased PAH and inulin clearance values untreated in 13/4 nephrectomized rats but not in normal rats or 13/4 nephrectomized rats that had received acetylsalicylic acid for 4–5 weeks prior to administration of OKY. These data suggest that increased thromboxane production, either by the kidney per se or by cells infiltrating the kidney, has physiological effects on renal blood flow and GFR in rats with reduced renal mass.

**Light and Transmission Electron Microscopy.** Alterations observable by light microscopy in rats with partial renal infarction have been described (5). The glomerular involvement is foci and consists of segmental thickening of capillaries, cellular proliferation, accumulation of proteinaceous material, occlusion of capillaries, and structureless glomeruli (5). Focal interstitial inflammatory cell infiltration and dilated tubules containing hyaline casts are usually present. Morphologically, rats given OKY 1581 had qualitatively similar changes although quantitatively they were markedly fewer.

Thus, using the criteria described above, untreated rats had 45.6 ± 11% abnormal glomeruli whereas the treated group had only 12.2 ± 5.1%.

The ultrastructural changes seen with renal infarction have been described (20). Differences between the treated and untreated groups in this study were more quantitative than qualitative. Many glomeruli were abnormal in the untreated group, in keeping with the light microscopic findings. Altered glomeruli showed swollen epithelial cells frequently containing electron-dense bodies; segmental fusion of foot processes was occasionally noted. Electron-dense proteinaceous material was present in the lumen. Hyaline-like material was seen in the subendothelium and in the mesangium. The mesangial regions were variably expanded and often contained lipid. Severely altered glomeruli were collapsed and structureless. These alterations were markedly reduced in rats given OKY 1581.

**Effects of OKY 1581 on Platelet Aggregation.** The effects on platelet aggregation in rats with 13/4 nephrectomy of a single oral dose of OKY 1581 are shown in Fig. 2. Blood was obtained 1, 2, 4, 6, 7, 9, and 13 hr after administration of OKY 1581. Inhibition of platelet aggregation in response to addition of collagen or arachidonic acid was observed 1 hr after administration of OKY 1581. The inhibitory effect was maximal at 4 hr and disappeared ~7 hr after administration of the drug. In contrast, OKY 1581 given in vivo did not affect ADP-induced platelet aggregation.

**DISCUSSION**

Oral administration of OKY 1581, an inhibitor of thromboxane synthesis, to rats with subtotal renal ablation ameliorates the progressive renal disease that occurs in this model. Renal histology improved, as judged by fewer abnormal glomeruli on light microscopy and more moderate pathologic changes on electron microscopy in treated rats as compared with controls. In addition, OKY 1581 increased PAH and inulin clearances, measures of renal plasma flow and GFR, and decreased blood pressure and urinary protein excretion in these rats.

The mechanisms responsible for these effects are not clear. However, the amelioration of renal disease with OKY 1581 occurred despite an increase in renal plasma flow and GFR. Based on determinations of PAH and inulin clearances and of PAH extraction in normal rats and in rats with 13/4 nephrectomy (21), it is possible to calculate values for single-nephron plasma flow and single-nephron GFR, making some assumptions as to the number of nephrons present in normal rats and in rats with renal ablation (Table 4). Using a figure of 60,000 as the total number of nephrons in both kidneys in the rat and the values for inulin and PAH clearances reported in Table 4, we obtained mean values of 56.3 nl/min for single-nephron GFR and 174.3 nl/min for single-nephron plasma flow in normal rats. In rats with 13/4 nephrectomy (total number of nephrons ~ 7500), calculated values for single-nephron GFR were 84 nl/min in rats given saline and 205.3 nl/min in rats receiving OKY 1581. Values for single-nephron plasma flow averaged 317.3 nl/min in rats with renal ablation receiving saline and 592 nl/min in rats treated with OKY 1581. These calculations indicate that both single-nephron plasma flow and single-nephron GFR are increased in rats with a remnant kidney as compared with normal rats, in agreement with previous findings using micropuncture techniques (22–24). Administration of OKY 1581 increased single-nephron plasma flow and GFR in rats with a remnant kidney. Micropuncture studies showed that single-nephron
Thus, receiving vehicle my hyperfiltration, weeks after renal 13/4 after occurring concomitant tology ment of el. a or parable enlargement (B), tained before and after renal ablation with subsequent aggregation of platelets. This, in turn, may lead to release of platelet products

GFR averaged 76.2 ± 12.7 ml/min in rats with 1/4 nephrectomy receiving vehicle and 115.4 ± 4.2 ml/min in those given OKY. Thus, inhibition of endogenous thromboxane synthesis increased hyperperfusion and hyperfiltration further in this animal model. Despite this increase in hyperperfusion and hyperfiltration, there were fewer abnormal glomeruli 5 weeks after renal ablation in rats treated with OKY 1581 than in animals receiving saline, indicating that other events occurring concomitant with or subsequent to the development of hyperperfusion and hyperfiltration may be responsible for the progressive renal disease that occurs in this model.

The weight of the viable portion of the kidney 5 weeks after 1/4 nephrectomy was ~1 g and comparable in rats given saline or OKY 1581 (Table 1). Thus, the improved renal histology seen in OKY 1581-treated rats cannot be attributed to a lesser degree of renal hypertrophy in this group. The comparable enlargement of the remnant kidney in both groups of rats also suggests that the greater values for single-nephron plasma flow and GFR in the OKY 1581-treated group are related to hemodynamic changes.

The fall in blood pressure observed in rats with subtotal renal ablation receiving OKY 1581 may play a role in improving renal histology in these animals. However, previous studies from our laboratory have shown that administration of antihypertensive drugs (hydralazine plus thiazides) in this model of renal disease ameliorates histological damage to the kidney but not to the extent seen in rats receiving heparin (5) or OKY 1581.

The finding that OKY 1581, at the doses administered, inhibited platelet aggregation suggests the possibility that platelet aggregation and intraglomerular thrombosis play an important role in the development of glomerular sclerosis and progressive renal disease that occurs in this model. Based on the present findings and previous observations (5, 6), we suggest the following pathogenetic events in the glomerulopathy that occurs in rats with severe renal ablation (Fig. 3). Decreased renal mass causes vasodilatation of the afferent arteriole and increased hyperperfusion. Vasodilatation of the afferent arteriole may increase intraglomerular hydrostatic pressure, leading to damage of the glomerular capillary endothelium with subsequent aggregation of platelets. This, in turn, may lead to release of platelet products

Table 4. Inulin and PAH clearances, calculated single-nephron plasma flow, and GFR in normal and 1/4 nephrectomized rats given vehicle or OKY 1581

\[
\begin{array}{ccc}
\text{C}_{\text{in}}, \text{ ml/min per kg of body weight} & \text{Normal rats} & \text{Vehicle treated} & \text{OKY 1581 treated} \\
\hline
\text{Nephrons, n} & 60,000 & 7,500 & 7,500 \\
\text{SNGFR, ml/min} & 56.3 & 84.0 & 205.3 \\
\text{C}_{\text{PAH}}, \text{ ml/min} & 9.84 & 1.69 & 3.15 \\
\text{A-V extraction of PAH, } \% & 0.94 & 0.71 & 0.71 \\
\text{Renal plasma flow, ml/min} & 10.46 & 2.38 & 4.44 \\
\text{Plasma flow per nephron, ml/min} & 174.3 & 317.3 & 592 \\
\end{array}
\]

Groups of five rats were examined. C_{\text{in}}, inulin clearance; SNGFR, single-nephron GFR; C_{\text{PAH}}, PAH clearance; A-V extraction, arteriovenous extraction.

Fig. 2. Platelet aggregation in response to ADP (A), collagen (B), and 1.25 mM arachidonic acid (C) in platelet-rich plasma obtained before and 1, 2, 4, 6, 7, 9, and 13 hr after oral administration of OKY 1581 at 20 mg/kg of body weight to rats with 1/4 nephrectomy (n = 18). The inhibition of platelet aggregation in response to collagen and arachidonic acid observed after OKY 1581 treatment disappeared 7–8 hr after the treatment.
Fig. 3. Proposed scheme for pathogenesis of the progressive glomerulosclerosis that occurs in rats with subtotal renal ablation.

such as serotonin and platelet-derived growth factor, which may affect capillary permeability and produce proliferation of smooth muscle. Increased capillary permeability together with hyperperfusion and hyperfiltration may cause proteinuria. Platelet aggregation, in turn, may cause intraglomerular coagulation with subsequent fibrosis and sclerosis, resulting in permanent loss of nephrons and a decrease in the number of functional units in the viable portion of the remnant kidney. OKY 1581, heparin, or warfarin would interrupt this chain of events by preventing intraglomerular coagulation, platelet aggregation, or both. Whether all these agents act at one or more steps in the proposed scheme is not clear. OKY 1581, by inhibiting thromboxane synthesis in platelets, may affect platelet aggregation and hence prevent liberation of platelet products, which may, in turn, influence permeability of glomerular capillaries. The decrease in thromboxane excretion in the urine with OKY 1581 treatment, coupled with the vasodilatory effects of the drugs, may be related to inhibition of renal thromboxane synthesis and not to inhibition of thromboxane synthesis by platelets (25). Of interest is the finding that despite the increase in hyperfiltration and hyperfusion seen with administration of OKY 1581 there was amelioration of the renal disease in this experimental model.

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