A Rat Model of Progressive Chronic Glomerular Sclerosis: The Role of Thromboxane Inhibition

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ABSTRACT
In order to evaluate a possible role of thromboxane A2 (TxA2) in the pathophysiology of chronic glomerular disease, we studied the effect of a 12-wk combined treatment with the thromboxane receptor blocker Daltroban (D) and the thromboxane synthesis inhibitor UK 38485 (UK) on glomerular function and morphology in a rat model of chronic progressive glomerular injury. The glomerular lesion was induced in unilaterally nephrectomized rats by the repeated i.v. injection of an antibody directed against mesangial cells. Control rats were uninephrectomized. Three months after the first antibody injection before D and UK treatment, albuminuria (35.8 ± 3.6 mg/24 h) and glomerular TxB2 formation (146 ± 20 pg/mg of protein/min) were significantly higher compared with control values (albuminuria, 14.3 ± 3.5 mg/24 h; TxB2, 59 ± 16 pg/mg/min). Six months after antibody, albuminuria in nephritic rats had increased to 135 ± 17 mg/24 h. In nephritic rats treated with D plus UK, albuminuria (44 ± 12 mg/24 h), however, was significantly (P < 0.001) inhibited. Quantitative morphological analysis (glomerular damage index) 6 months after antibody revealed significantly (P < 0.001) increased glomerular lesions in nephritic rats (0.353 ± 0.095) compared with that in uninephrectomized controls (0.045 ± 0.014). The treatment of rats with D and UK significantly (P < 0.001) reduced the glomerular damage index (0.101 ± 0.004) in nephritic rats. D plus UK treatment reduced glomerular TxB2 formation but increased prostaglandin E2 and 6-keto prostaglandin F1α release by isolated glomeruli. This study demonstrates that interventional treatment with D and UK ameliorates albuminuria and glomerular morphological lesions in a rat model of immunologically induced progressive glomerular injury.

Key Words: Mesangial cell injury, thromboxane B2, prostaglandins, glomerular sclerosis, albuminuria

A series of recent observations established thromboxane A2 (TxA2) as an important mediator of the initial reduction of glomerular hemodynamic function in several animal models of glomerular immune injury (1-8). This effect of TxA2 is probably due to vasoconstrictory influences on glomerular mesangial cells (9) and the renal vasculature (10).

There is also evidence that TxA2 exerts mitogenic effects on glomerular mesangial cells in culture (11) and influences extracellular matrix formation (12). Given its hemodynamic, mitogenic, and biosynthetic properties, TxA2 might be of pathophysiological importance in the development of chronic glomerular disease, because hemodynamic alterations (13), glomerular resident cell growth, and increased matrix formation (14) are characteristic features of progressive chronic glomerular disease. We therefore evaluated the effect of a combined Tx synthesis inhibition and Tx receptor blockade on the course of a rat model of chronic progressive glomerular disease.

Our results demonstrate that 12-wk interventional treatment with this drug combination significantly ameliorates albuminuria and prevents glomerular morphological damage. These beneficial effects appear in the presence of reduced glomerular Tx formation but stimulated prostaglandin (PG)E2 and PGI2 production. These findings suggest a role for eicosanoids in the mediation of this chronic model of glomerular disease.

METHODS

Experimental Procedures

Induction of Immune-Mediated Glomerular Injury. The experiments were carried out in male Wistar rats, which were unilaterally nephrectomized (80 to 100 g/body wt). Two weeks after surgery animals were injected i.v. with 5 mg/100 g body wt of an
immunoglobulin G (IgG) preparation of rabbit anti-rat thymocyte antiserum (ATS) in 1 mL of 0.9% NaCl. ATS was induced in New Zealand rabbits by repeated immunization with $2 \times 10^8$ thymocytes from Lewis rats, combined with Freund's complete or incomplete adjuvant. The IgG preparation of the rabbit serum was made by caprylic acid precipitation by the method described by Steinbuch and Andran (15). The IgG preparations were tested in vivo and in vitro for their specificity to react with mesangial cells. Rats were injected i.v. with the antibody, and after 1 to 2 h, kidneys were removed for immunofluorescence studies. Kidney sections were stained with a fluorescein isothiocyanate-labeled goat anti-rabbit IgG. Only IgG preparations that had a selective mesangial pattern, which was demonstrated earlier (16), were used for the experiments. In vitro testing of the antibody was performed on rat mesangial cells to test the titer of the reactivity with the epitope. Antibodies with titers between 1:800 and 1:2,400 were used. Antibodies of several positive rabbit bleedings were pooled and used throughout the experiments. Two weeks after nephrectomy, the antibody was given and a second injection was repeated after 6 wk.

**Isolation and Superfusion of Glomeruli.** Glomeruli were isolated by a sieving technique described earlier (17). Glomeruli were transferred onto a Millipore filter (Sartorius, Gottingen, Germany) and superfused for 30 min with Krebs-Ringer-HCO$_3$ buffer at 37°C in order to determine glomerular PGE$_2$ and TxB$_2$ production. The details of the superfusion technique were described earlier (17). Four milliliters of buffer was used as perfusate in a closed superfusion system at a perfusion rate of 7 mL/min. The perfusates were collected and frozen at −20°C until analysis for PGE$_2$, 6-keto PGF$_1\alpha$, and TxB$_2$. The glomeruli were solubilized in a 1 N NaOH, and protein analysis was performed by the method of Lowry et al. (18).

**Measurements of PGE$_2$, 6-keto PGF$_1\alpha$, and TxB$_2$.** PGE$_2$, 6-keto PGF$_1\alpha$, and TxB$_2$ levels were determined by direct RIA in the perfusates without prior extraction or chromatographic separation. The used assay procedures, and the sensitivity and specificity of the antisera were described earlier (17). Glomerular prostanoïd production is expressed in picograms per milligrams of glomerular protein per minutes of incubation time.

**Inulin Clearances.** The rats were anesthetized with Inactin (100 mg/kg/body wt i.p. Byk Gulden, Konstanz, Germany). A tracheostomy tube was inserted, and both jugular veins were cannulated with PE-50 polyethylene catheters. The right ureter was cannulated with a PE-10 polyethylene catheter for urine collection. Each animal received 10 mL/kg body wt of a 0.45% saline solution over 10 min to replace fluid losses during surgery. Then, a bolus (3.7 mL/kg body wt in 10 min) of a 1% inulin solution in 0.45% saline was given, followed by a 90-minute period during which 0.9 mL/kg/h of a 1% inulin/0.45% saline infusion was given to reach constant urine flow. A 30-min clearance period followed. Blood for the determination of inulin concentrations was collected in the middle of the clearance period. Inulin was determined by the method of Führ et al. (19).

**Albuminuria, Systolic Blood Pressure, Serum Cholesterol, and Serum Triglycerides.** Rats were kept for 24 h in metabolic cages with free access to tap water but no food. Twenty-four-hour urine samples were collected and centrifuged (2,000 × g), and urinary albumin was determined by nephelometry with a rabbit anti-rat albumin antibody. Systolic blood pressure was measured in awake rats by plethysmography (20). Serum cholesterol and serum triglycerides were determined by automated standard routine laboratory tests.

**Morphological Studies.** Kidney slices were obtained for morphological evaluation. For light microscopy, tissue was fixed in 4% buffered formaldehyde and embedded in paraffin. For electron microscopy, tissue was embedded in methacrylate. For immunohistological studies, kidney sections were frozen in liquid nitrogen and tissue sections were stained with fluorescein isothiocyanate-labeled goat anti-rabbit IgG and goat anti-rabbit IgG.

**Glomerular Damage Index.** Histological studies were performed in a blind fashion. Glomerular damage was assessed by a semiquantitative score (Grades 0 to 4), by the methods of Raij et al. (21) and Olson et al. (22). Grade 1 represents involvement of up to 25% of the glomerulus (including a range of abnormalities from mild increase in mesangial matrix [equal to increased amounts of periodic acid-Schiff positive material in the mesangial region] to segmental mesangial sclerosis and/or hyalinosis with focal adhesions), whereas Grade 4 represents damage of 75 to 100% of the glomerulus. The glomerular damage index was calculated in the following way. The number of glomeruli with a score of 1 was multiplied by 1, the ones with a score of 2 were multiplied by 2, with a score of 3 by 3, and with a score 4 by 4. These numbers were added and divided by the number of glomeruli assessed, including those with a score of 0. The incidence of glomerular damage was calculated by dividing the total number of damaged glomeruli by the number of all glomeruli. A minimum of 140 glomeruli (range, 140 to 190) was examined in each specimen. We defined glomerular sclerosis as the disappearance of cellular elements from the tuft, collapse of capillary lumens, and folding of the basement membrane with the entrapment of amorphous material.

The morphometry of the glomerular cross-sectional area was assessed as follows. Planimetric examinations of glomerular sizes were performed with a Zeiss
drawing tube in combination with a semiautomatic interactive image analysis system (Zeiss-Morphomat 30; Zeiss, Oberkochen, Germany). By using a serpentine movement from cortex to medulla and vice versa, the circumference of 50 cortical and 50 juxtamedullary consecutively encountered capillary tufts were manually traced and the mean glomerular random cross-sectional area was determined.

Experimental Protocols

Induction of Immune-Mediated Glomerular Injury. Male Wistar rats were uninephrectomized. Two weeks after surgery, animals were divided into two groups. One group of animals received ATS i.v.; the corresponding control animals were injected i.v. with 0.9% saline. Both groups had free access to tap water and standard rat chow. Six weeks after the first antibody injection, rats received ATS again and were kept on standard chow. Twelve weeks after the first ATS injection, body weight and systolic blood pressure were evaluated in all animals. Control and nephritic rats were placed in metabolic cages for 24-hour urine collections. Inulin clearances were evaluated in subgroups of the controls and the nephritic animals. Glomeruli were isolated for TxB2 and PGE2 evaluation.

Effect of Daltroban and UK 38485 on Renal Function and Morphology. UK 38485 was kindly supplied by Pfizer (Karlsruhe, Germany). It is a Tx synthesis inhibitor that significantly inhibits glomerular TxB2 in rats (23). Daltroban was kindly supplied by Boehringer Mannheim (GmbH, Germany). Daltroban is a competitive, specific TxA2 receptor blocker that acts on several TxA2-induced effects in rats (24,25) in the doses given to our animals. The combined treatment was applied to prevent unspecific endoperoxide binding to the Tx receptor blocker, which could be induced by an exclusive treatment with a synthesis inhibitor (26).

Table 1 summarizes some characteristics of control and nephritic animals 12 wk after the first antibody injection. Albuminuria in rats with antibody was significantly higher compared with that in the controls. There was also increased formation of TxB2.

**RESULTS**

Characteristics of Rats 12 wk After the First Antibody Injection

Table 1 summarizes some characteristics of control and nephritic animals 12 wk after the first antibody injection. Albuminuria in rats with antibody was significantly higher compared with that in the controls. There was also increased formation of TxB2.

### TABLE 1. Characteristics of the animals 12 wk after the first antibody injection before treatment with Daltroban and UK 38485

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Nephritic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>450 ± 20 (&lt;i&gt;N&lt;/i&gt; = 12)</td>
<td>NS</td>
<td>448 ± 15 (&lt;i&gt;N&lt;/i&gt; = 19)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>126 ± 1.5 (&lt;i&gt;N&lt;/i&gt; = 12)</td>
<td>NS</td>
<td>130 ± 3.1 (&lt;i&gt;N&lt;/i&gt; = 19)</td>
</tr>
<tr>
<td>Albuminuria (mg/24 h)</td>
<td>14.3 ± 3.5 (&lt;i&gt;N&lt;/i&gt; = 12)</td>
<td>&lt;0.001</td>
<td>35.8 ± 3.6 (&lt;i&gt;N&lt;/i&gt; = 19)</td>
</tr>
<tr>
<td>Inulin Clearance (µl/min/100 g body wt)</td>
<td>571 ± 73 (&lt;i&gt;N&lt;/i&gt; = 5)</td>
<td>NS</td>
<td>570 ± 57 (&lt;i&gt;N&lt;/i&gt; = 5)</td>
</tr>
<tr>
<td>Glomerular PGE2 (pg/mg/min)</td>
<td>272 ± 47 (&lt;i&gt;N&lt;/i&gt; = 5)</td>
<td>&lt;0.001</td>
<td>581 ± 66 (&lt;i&gt;N&lt;/i&gt; = 5)</td>
</tr>
<tr>
<td>Glomerular TxB2 (pg/mg/min)</td>
<td>59 ± 16 (&lt;i&gt;N&lt;/i&gt; = 7)</td>
<td>&lt;0.05</td>
<td>146 ± 20 (&lt;i&gt;N&lt;/i&gt; = 7)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Twelve weeks after the first antibody injection, nephritic rats had a significantly higher albumin excretion compared with controls. There were no differences in body weights, systolic arterial blood pressures, or inulin clearances. Glomerular TxB2 and PGE2 formation was also higher in nephritic rats compared with in controls.

<sup>b</sup>NS, not significant.
and PGE₂ in isolated glomeruli from nephritic rats compared with that in the uninephrectomized controls. There were no significant differences in insulin clearances, systolic blood pressures, and body weights between both groups.

**Effect of Daltroban and UK 38485 on Control and Nephritic Rats**

Body weights and systolic blood pressures in controls and nephritic rats were not different between both animal groups before treatment (Table 1). There was a slight increase in the body weight of nephritic animals over the 12-wk treatment period (Table 2). Rats treated with Daltroban and UK 38485 had slightly higher body weights at 8 and 12 wk (Table 2). Control rats, which were evaluated only at 12 wk, had body weights comparable with nephritic rats. Systolic blood pressure in the nephritic rats did not change during the 12-wk treatment period and remained normotensive (Figure 1). Daltroban and UK treatment, however, significantly reduced systolic blood pressure. This difference became significant at 8 and 12 wk. Systolic blood pressure was not different between controls (134 and 6 mm Hg) and nephritic rats at 12 wk.

**Albuminuria**

Albuminuria (in milligrams per 24 h) in nephritic animals increased significantly over the 12-wk treatment interval (4 wk, 40.8 ± 13; 8 wk, 152 ± 41; 12 wk, 135 ± 72) (Figure 2). This progressive increase, however, was significantly ameliorated when the rats received Daltroban and UK 38485 (4 wk, 29 ± 10; 8 wk, 43 ± 12; 12 wk, 30 ± 10) (Figure 2). Albuminuria in uninephrectomized control rats also increased (4 wk, 14.6 ± 4; 8 wk, 33.7 ± 1; 12 wk, 23 ± 14) but was significantly (P < 0.001 at all time points) lower than that in nephritic controls.

**Morphological Analysis**

The qualitative morphological analysis of the glomeruli revealed that rats that received the antibody had glomerular morphological lesions 6 months after uninephrectomy (Figure 3A and B) that were not present in controls (Figure 3C and D). These lesions were characterized by an increase in the extracellular matrix and an expansion of the mesangium. In immunohistological studies, rat glomeruli stained positive for rat IgG in the mesangium 6 months after the first antibody (Figure 4). The stain for rabbit IgG was negative (not demonstrated). The semiquantitative assessment of glomerular morphology revealed a significantly higher degree of glomerular damage in rats with antibody compared with that in controls. Ne-

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**TABLE 2. Body weights (in grams) of nephritic rats and control rats during treatment**

<table>
<thead>
<tr>
<th>Prior Treatment</th>
<th>4 wk</th>
<th>8 wk</th>
<th>12 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephritic (N = 8)</td>
<td>448 ± 15</td>
<td>477 ± 16</td>
<td>482 ± 5</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Nephritic Plus</td>
<td>495 ± 11</td>
<td>518 ± 9</td>
<td>494 ± 17</td>
</tr>
<tr>
<td>Daltroban Plus</td>
<td>UK 38485</td>
<td>(N = 9)</td>
<td></td>
</tr>
<tr>
<td>Uninephrectomized Controls</td>
<td>452 ± 17</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Body weights in nephritic and uninephrectomized animals increased over the 12-wk treatment period. The treatment of nephritic rats with Daltroban and UK 38485 had no effect on body weights.
* NS, not significant; ND, not determined.
Role of Thromboxane Inhibition

Figure 3. Light microscopy of glomeruli from uninephrectomized rats that received ATS had structural changes that are characterized by an increase in mesangial area (A and B) and occasional segmental sclerosis. These changes were not found in uninephrectomized control rats (C and D). The glomeruli of these animals appeared normal. When rats that received ATS were treated with Daltroban and UK 38485, morphological changes were less severe (E and F) compared with those in the untreated rats (A and B). The mesangial area that occupied the glomerulus was reduced, and the sclerotic lesions were less prominent. (Sections were stained with periodic acid-Schiff; magnification, ×495).

Phritic animals also had an increased incidence of glomerular structural lesions. When rats were treated with Daltroban and UK 38485, qualitative (Figure 3E and F) and quantitative (Table 3) glomerular damage was significantly reduced in nephritic animals. There was also a significant increase in glomerular cross-sectional areas in the superficial glomeruli of nephritic rats compared with that in...
controls—alterations that, however, remained unef-
fected by Daltroban and UK 38485. Glomerular cross-sectional areas (in square millimeters × 10⁻³) of juxtamedullary glomeruli were not different between the animal groups (controls, 20.7 ± 0.48; nephritic, 20.53 ± 0.45; nephritic plus Daltroban plus UK 38485, 20.86 ± 0.49). Ultrastructural analysis demonstrated fusion of foot processes of podocytes in some glomerular capillaries of nephritic kidneys and detachment of podocytes from the basement membrane in other areas (Figure 5). The basement membrane and the subepithelial space were free of immune deposits.

Glomerular PGE₂, 6-keto PGF₁α, and TxB₂ Formation

Glomerular PGE₂, 6-keto PGF₁α, and TxB₂ formation from nephritic rats was significantly higher compared with that in non-nephritic controls (Table 4). Treatment with Daltroban and UK 38485 significantly reduced glomerular TxB₂ formation in nephritic animals but stimulated glomerular PGE₂ and 6-keto PGF₁α formation (Table 4).

Serum Cholesterol and Serum Triglycerides

Levels of serum triglycerides (175 ± 28 mg/dL) and total serum cholesterol (122 ± 18 mg/dL) were significantly (P < 0.05) higher in nephritic rats 6 months after the first antibody injection when compared with those in controls (triglycerides, 97 ± 21; cholesterol, 70 ± 6 mg/dL). Daltroban and UK 38485 reduced serum triglyceride levels (67 ± 12 mg/dL) and serum cholesterol levels (82 ± 6 mg/dL) significantly (P < 0.05) in nephritic rats.

DISCUSSION

In this article, we describe an animal model of chronic glomerular disease, which was induced in unilaterally nephrectomized rats by repeated i.v. injection of an antibody directed to epitopes on mesangial cells (16). Morphologically, this model is characterized by the expansion of the glomerular mesangial area and occasional segmental sclerosis in glomeruli. This morphological feature of the glomerular lesion is associated with a progressive increase in albuminuria (Figure 2). The morphological and functional characteristics appear independent of changes in body weight and arterial blood pressure.

TABLE 3. Semiquantitative morphological analysis 6 months after antibody

<table>
<thead>
<tr>
<th></th>
<th>Non-Nephritic Controls (N = 7)</th>
<th>Nephritic Controls (N = 8)</th>
<th>Nephritic Plus Daltroban Plus UK 38485 (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular Damage Index</td>
<td>0.045 ± 0.014</td>
<td>0.343 ± 0.095b</td>
<td>0.101 ± 0.023c</td>
</tr>
<tr>
<td>Incidence of Glomerular Damage (%)</td>
<td>3.2 ± 0.9</td>
<td>21.2 ± 5.2b</td>
<td>6.7 ± 1.2c</td>
</tr>
<tr>
<td>Glomerular Cross-Sectional Area (Superficial Glomeruli) (mm² × 10⁻³)</td>
<td>14.9 ± 0.44</td>
<td>16.9 ± 0.6b</td>
<td>16.02 ± 0.58</td>
</tr>
</tbody>
</table>

* The semiquantitative morphological analysis of glomeruli revealed a significant increase in the structural lesions in the nephritic control animals compared with those in uninephrectomized non-nephritic controls. The treatment of nephritic rats with Daltroban and UK 38485 significantly reduced the glomerular damage index and the incidence of the glomerular lesions. The glomerular cross-sectional area of the superficial glomeruli of nephritic controls was also significantly greater compared with that of the non-nephritic controls. This morphological alteration, however, was unaffected by the pharmacological treatment.

* P < 0.05 versus non-nephritic controls.

* P < 0.025 versus nephritic controls.
The current experimental design of repeated antibody injection to uninephrectomized rats was chosen because a single antibody application leads to a proliferative glomerular lesion (28), which reverses almost completely after several weeks. We have therefore proposed that the reinduction of the disease might induce a chronic lesion. The repeated antibody injection was combined with unilateral nephrectomy, because earlier experiments have demonstrated that a reduction in renal mass might accelerate disease progression (29–31).

The ultimate mechanisms underlying the described chronic inflammatory and sclerotic lesion are, however, unclear. Arterial blood pressure, which is a factor that influences the progression of glomerular disease (13), is probably not operative in this model, because there were no differences between uninephrectomized controls and nephritic rats. Theoretically, the lesion could be mediated by changes in glomerular hemodynamics (13) or by the effects of autacoids or growth factors (14) that are locally released by inflammatory or glomerular resident cells. One local mediator that has been demonstrated to play a role in the regulation of glomerular function in the early phase of this model is TxA2 (16). Because glomerular TxB2 formation was increased in this chronic disease (Table 1), we tested the hypothesis that TxB2 might be a candidate to play a role in the development of the lesion. A chronic interventional protocol with Daltroban and UK 38485 was therefore started. This combined treatment with Tx synthesis inhibitor and receptor blocker resulted in a reduction in TxB2 formation and significant amelioration of albuminuria and glomerular morphological lesions, when the animals were studied after the 12-wk treatment. Daltroban and UK 38485 significantly reduced systolic arterial blood pressure. Arterial hypertension has been demonstrated to be an important factor in the progression of structural glomerular lesions (13). The mechanisms of how Daltroban and UK induced the reduction in blood pressure are unclear; however, the increase in vasodilatory PG12 found in the glomeruli might also appear in the arterial walls and could reduce peripheral vascular resistance. Furthermore, there might exist the possibility that Tx blockade ameliorates angiotensin II effects (32,33). These

| Glomerular PG and Tx formation in nephritic controls was significantly higher 26 wk after uninephrectomy and antibody compared with that in non-nephritic controls. Treatment with Daltroban and UK 38485 significantly reduced glomerular TxB2 formation but increased PG12 and PGE2 formation. The increase in PG12 formation was significant. |}

TABLE 4. Effect of Daltroban and UK 38485 on glomerular prostanoid and Tx formation (pg/mg/min) *  

<table>
<thead>
<tr>
<th></th>
<th>Non-Nephritic Controls (N = 7)</th>
<th>P value</th>
<th>Nephritic Controls (N = 8)</th>
<th>P value</th>
<th>Nephritic Plus Daltroban Plus UK 38485 (N = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TxB2</td>
<td>125 ± 17</td>
<td>&lt;0.05</td>
<td>214 ± 40</td>
<td>&lt;0.001</td>
<td>71 ± 5</td>
</tr>
<tr>
<td>PGE2</td>
<td>370 ± 61</td>
<td>&lt;0.001</td>
<td>756 ± 63</td>
<td>&lt;0.05</td>
<td>928 ± 96</td>
</tr>
<tr>
<td>α-keto PGF1α</td>
<td>95 ± 10</td>
<td>&lt;0.001</td>
<td>162 ± 11</td>
<td>&lt;0.05</td>
<td>213 ± 20</td>
</tr>
</tbody>
</table>

*Glomerular PG and Tx formation in nephritic controls was significantly higher 26 wk after uninephrectomy and antibody compared with that in non-nephritic controls. Treatment with Daltroban and UK 38485 significantly reduced glomerular TxB2 formation but increased PG12 and PGE2 formation. The increase in PG12 formation was significant.
hormonal interactions might also be of relevance in the regulation ofglomerular intracapillary pressure in the sense that Tx inhibitors might reduce filtration pressure because of a reduction of effects on mesangial cells (9) and efferent arterioles (34). This might result in a consequent improvement of hemodynamically mediated glomerular structural lesions which have been attributed to increased glomerular hypertension (35).

Another possibility of how Daltroban and UK 38485 could have improved glomerular morphology might be independent of hemodynamic mechanisms. TxA2 has been demonstrated to exert mitogenic effects on glomerular mesangial cells (11) and to increase the formation of the extracellular matrix (12). Increased matrix is a characteristic feature present in the glomeruli of rats that received ATS. Daltroban and UK 38485 might thus have affected morphology by the reduction of the effect of Tx on matrix formation. This suggestion is supported by recent findings in diabetic mice where treatment with a Tx synthesis inhibitor significantly reduced the expression of mRNA for collagen type IV in glomeruli (36).

Another important beneficial role of the combined D and UK 38485 treatment might be the increased formation of PGE2 and PGI2 in the treated animals. This change in glomerular prostanooid formation by the combined treatment might depend on the effect of UK 38485 to inhibit Tx synthetase and to build up PG endoperoxides that could be metabolized to PGE2 and PGI2 (26). PGE2 and PGI2 have antimitogenic effects on glomerular mesangial cells (11,37) and can inhibit collagen formation (38,39). It might therefore be possible that a reduction of TxA2 and the stimulation of PGE2 and PGI2 might affect glomerular cells and might be important for the Daltroban and UK 38485 treatment.

A role of Tx on glomerular structural damage has been demonstrated earlier in the remnant kidney model (40). The beneficial effects in this model were attributed to an inhibition of platelet aggregation and Tx formation in the glomerulus. Platelets, in addition to their effects due to Tx, might be significant in this model by other mechanisms. Using a similar animal model, Johnson et al. (41) have recently demonstrated that platelets, probably by the production of platelet-derived growth factor (42,43), may mediate the proliferative phase of this disease. Furthermore, transforming growth factors beta (44,45) might also have a role in the accumulation of matrix components.

The glomerular lesion was also associated with a significant progressive increase in albuminuria and enhanced serum lipids, showing characteristics of a nephrotic syndrome. There is evidence from several animal models (46,47) that lipids might play a role in the progression of glomerular injury. Therefore, the possibility exists that increased serum lipids could participate in the structural lesions present in nephritic animals. The beneficial effects of Daltroban and UK 38485, which reduce albuminuria and lower serum cholesterol and triglycerides levels, could thus partially be attributed to an indirect lipid-lowering effect.

The underlying pathomechanisms of the development of albuminuria in this chronic model are unclear. There is, however, evidence from other animal models that glomerular epithelial cell damage due to structural glomerular hypertrophy might influence albuminuria (48,49). The ultrastructural analysis of glomeruli demonstrated fusions of foot processes of podocytes in some capillaries of nephritic rats and occasional signs of epithelial cell detachment from the basement membrane. Together with the semi-quantitative assessment of the glomerular cross-sectional areas of the glomeruli, which demonstrated larger glomeruli at least in the superficial glomerular subpopulation, the possibility of structural hypertrophy as a factor of epithelial cell damage has to be considered (48,49). On the other hand, however, treatment with Daltroban and UK 38485 did not reduce glomerular cross-sectional surface area, which makes the therapeutic effect of the pharmacological changes unlikely. Further studies need to be performed to obtain more detailed information on these pathomechanisms.

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