The ET<sub>A</sub> Receptor Blocker LU 135252 Prevents Chronic Transplant Nephropathy in the “Fisher to Lewis” Model

STEPHAN R. ORTH,* GIULIO ODONI,* KERSTIN AMANN, ‡ PIOTR STRZELCZYK,* MANFRED RASCHACK, ‡ and EBERHARD RITZ*

Departments of *Internal Medicine and ‡Pathology, Ruperto Carola University, Heidelberg, Germany; and †Knoll AG, Ludwigshafen/Rhein, Germany.

Abstract. The effect of the orally highly bioavailable and specific endothelin A (ET<sub>A</sub>) receptor antagonist LU 135252 was assessed in a model of chronic renal allograft nephropathy. Kidneys of Fisher rats were orthotopically grafted to Lewis rats. Fisher autografts and kidneys after uninephrectomy served as controls. All animals received low-dose cyclosporin A (CsA; 1.5 mg/kg body wt) for 10 d after surgery. Allotransplanted animals were then randomized to receive standard diet or a diet designed to deliver 30 mg of LU 135252/kg body wt per d for 35 wk. BP was monitored telemetrically. Treatment with LU 135252 did not affect systolic or diastolic pressure.

Chronic transplant nephropathy has become the major cause of kidney graft failure (1). Although it is triggered by immune mechanisms, damage is perpetuated and amplified by nonimmune mechanisms (1), one of which is presumably the endothelin (ET) system.

This system is involved in several models of renal damage including progressive chronic renal failure (2). At least in some models of renal damage (2–4), ET<sub>A</sub> as well as mixed ET<sub>A/B</sub> receptor antagonists have been shown to be nephroprotective. The ET system seems to play a role in the genesis of chronic transplant nephropathy, since sixfold elevated ET-1 peptide levels have been reported in the vasculature of human renal transplant nephropathy, compared with normal kidneys (5). The highest expression of ET-1 was found in the neointima (5), a point of interest because ET-1 is a strong mitogen for vascular smooth muscle cells (VSMC) (6) acting via the ETA receptor (7). To investigate whether ET<sub>A</sub> receptor-mediated mechanisms play a role in a model of chronic transplant nephropathy, the highly orally bioavailable and highly specific nonpeptide ETA receptor antagonist LU 135252 (8) was studied in the “Fisher to Lewis” model of chronic transplant nephropathy (9).

Indices of glomerulosclerosis (GSI), and tubulointerstitial and vascular damage were measured. Chronic transplant nephropathy was almost completely prevented by LU 135252 compared with untreated allografts or kidneys of uninephrectomized controls, i.e., GSI 0.7 ± 0.12 versus 1.6 ± 0.25 (P < 0.001) versus 0.7 ± 0.06 (P < 0.001). Allograft weight and serum creatinine were significantly lower in treated versus untreated animals. The results are consistent with the notion that ETA receptor-mediated events play a role in the genesis of chronic transplant nephropathy.

Materials and Methods

Animals

Naive male inbred Lewis (LEW, RT1<sup>1</sup>) rats (n = 25) were used as recipients of Fisher (F344, RT1<sup>1v1</sup>) kidney allografts (n = 25; 200 to 240 g) (Charles River, Sulzfeld, Germany). A pilot experiment had shown that histology of Fisher isografts was not significantly different from uninephrectomized (UNX) Fisher kidneys. In the main experiment, UNX-Fisher rats were therefore used as controls. The rats were fed standard rat chow (0.25% NaCl and 19% of protein; ssnif GmbH, Soest, Germany).

Kidney Transplantation

Transplantation was performed under xylazine (5 mg/kg body wt) and ketamine (100 mg/kg body wt) anesthesia. The left kidney of the donor rat was isolated, perfused with ice-cold Collins solution, excised, and transplanted orthotopically into weight-matched Lewis recipients. The left renal vessels were mobilized, clamped, and the left kidney was excised. End-to-end anastomosis of donor and recipient renal artery, vein, and ureter (without ureteral stenting) was performed with 10-0 prolene sutures. The contralateral native kidney was excised 10 d later. All allograft recipients and isograft as well as UNX-control animals were treated with low-dose cyclosporine (1.5 mg/kg per d) for 10 d after surgery (10).

Experimental Design

Fisher to Lewis (F to L) allografts with no treatment (TX) were compared with F to L treated with LU 135252 (TX + LU 135252). Uninephrectomized (UNX)-Fisher rats served as controls (UNX-control). LU 135252 was added to the food calculated to deliver 30 mg/kg body wt per d. Treatment was started on the 11th day after surgery. Food consumption was monitored. BP was measured with an aortic catheter by telemetry (Data Sciences International, St. Paul, MN) during the last 20 d of the experiment.

Thirty-five weeks after kidney transplantation, blood samples were...
obtained (chemistry measured with Hitachi 9–17E) and retrograde perfusion fixation via the abdominal aorta was carried out, as described elsewhere (11).

Morphologic Study

The kidneys were weighed and dissected in a plane perpendicular to the interpolar axis, yielding slices of 1-mm width. Ten small pieces of each kidney were selected by area weighted sampling (11) for embedding in Epon-Araldite. The remaining tissue slices were embedded in paraffin, and 4-μm sections were stained with hematoxylin and eosin (HE)/periodic acid-Schiff (PAS) and analyzed using morphometric and stereologic techniques.

Morphologic Investigations of the Kidney

Indices of Renal Damage: Glomerular Sclerosis and Tubulointerstitial and Vascular Damage. The glomerular sclerosis index was determined in 100 glomeruli per animal on PAS-stained paraffin sections at a magnification of ×400, using a semiquantitative scoring system according to El Nahas et al. (12). The glomerular damage was graded as follows: 0, no changes; 1, mild mesangial expansion and/or mild segmental hynalnosis/sclerosis (up to 25% of the glomerular tuft); 2, moderate segmental hynalnosis/sclerosis involving 25 to 50% of the glomerular tuft; 3, severe segmental hynalnosis/sclerosis involving 50 to 75% of the glomerular tuft; 4, diffuse sclerosis/obliteration involving >75% of the glomerular tuft. Tubulointerstitial (tubular atrophy, dilation, casts, interstitial inflammation, and fibrosis) and vascular changes as parameters of interstitial and vascular damage were determined using a semiquantitative scoring system according to Véniant et al. (13) applied to PAS-stained paraffin sections at a magnification of ×250. Ten fields per kidney were randomly sampled and graded as follows: Tubulointerstitial damage: 0, no changes; 1, lesions involving <25%; 2, lesions affecting 25 to 50%; and 3, lesions involving >50% of the field. Vascular damage: 0, normal vessels; 1, mild vascular thickening; 2, moderate thickening; 3, severe thickening (onion skin pattern); 4, fibrinoid necrosis. The respective indices in each animal were expressed as a mean of all scores obtained.

Glomerular Geometry: Paraffin Sections (PAS Stain, Light Microscopy using Various Magnifications). Area (A) and volume density (V) of the renal cortex and medulla as well as the number of glomeruli per area (N) were measured using a Zeiss eyepiece (Integrationsplatte II, Zeiss, Oberkochen, Germany) and the point-counting method (P = A / V) at a magnification of ×400 (14,15). The number of glomeruli per area (N) was then corrected for tissue shrinkage (45%). Total cortex volume (Vtotal) was derived from kidney mass divided by specific weight of the kidney (1.04 g/cm³) multiplied by the volume density of the cortex.

Glomerular geometry was analyzed as follows: Volume density (V) of glomeruli and area density of glomerular tuft (A50) were measured by point counting according to P = A / V at a magnification of ×400 on HE sections.

Total area of glomerular tuft (A50) was then determined as A50 = A / Vtotal.

The number of glomeruli per volume (N50) was derived from glomerular area density (N) and the volume density (V) of glomeruli using the formula:

\[ N50 = k / \beta \times N \times V^{0.5} \]  

The mean glomerular tuft volume was determined according to the equation:

\[ v = \beta / k \times A^{1.5} \]

Statistical Analyses

Data are given as mean ± SD. Statistical analyses were performed by one-way ANOVA, followed by Bonferroni–Dunn multiple range test.

Results

Animal Data

Survival. Two animals of the TX + LU 135252 died during the experiment (the first at week 1 due to thrombosis of the transplant artery and the second for unknown reasons at week 30). Furthermore, one animal of the TX group and two animals of the TX + LU 135252 group died 1 d after implantation of the BP telemetry catheter. No animal died in the UNX group.

Blood Pressure. Systolic and diastolic pressures were not different in TX and TX + LU 135252, respectively: Systolic BP: 133.6 ± 23.3 mmHg versus 135.4 ± 25.0 mmHg; diastolic BP: 102.3 ± 18.4 mmHg versus 99.9 ± 20.1 mmHg.

Body and heart weights did not differ between the groups (Table 1). Serum creatinine was significantly higher in untreated TX compared with TX + LU 135252 and UNX-control. No significant differences were noted concerning Na, K, Ca, Cl, total protein, white blood cell count, and platelets. Hemoglobin was significantly higher in treated compared with untreated TX animals. Serum cholesterol and triglycerides were significantly higher in untreated TX animals (Table 1).

Structural Changes of the Kidney

Hydronephrosis was excluded by histologic examination of the papilla in all animals. Glomerulosclerosis and tubulointerstitial and vascular damage indices were significantly higher in untreated compared with treated TX animals. LU 135252 completely prevented glomerular changes as illustrated in Figures 1 and 2.

Renal Weight. The weight of the perfusion-fixed kidney allografts was significantly lower in TX + LU 135252 animals (1.87 ± 0.09 g versus 2.06 ± 0.18 g in TX [P < 0.01] and 1.79 ± 0.13 g in UNX-controls [P < 0.001]).

Number and Volume of Glomeruli. The number of glomeruli did not differ significantly (UNX-control = 28,260 ± 3,276; TX untreated = 31,590 ± 5,454; TX + LU 135252 = 28,960 ± 3,578). The same was true for total glomerular volume per kidney: UNX-control 0.063 ± 0.01 cm³, TX untreated 0.072 ± 0.01 cm³, TX + LU 135252 0.062 ± 0.01 cm³.

Discussion

This stereologic investigation documents that development of chronic transplant nephropathy, as assessed by histology, can almost completely be prevented by ETA receptor blockade in the standard “Fisher to Lewis” model. This suggests: (1) that ET plays a crucial role in progression of chronic transplant...
Table 1. Body and organ weights, hematology, and blood chemistry.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight (g)</th>
<th>Heart Weight (g)</th>
<th>Kidney Weight (b) (g)</th>
<th>Hemoglobin (g/L)</th>
<th>S Cr (m mol/L)</th>
<th>S urea (mmol/L)</th>
<th>Cholesterol (mmol/L)</th>
<th>Triglycerides (m mol/L)</th>
<th>Total Protein (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNX-control</td>
<td>408 ± 35</td>
<td>1.664 ± 0.198</td>
<td>0.094 d</td>
<td>152 ± 17</td>
<td>269 ± 93</td>
<td>3.74 ± 1.56</td>
<td>2.22 ± 0.46</td>
<td>654 ± 21</td>
<td>6.4 ± 14</td>
</tr>
<tr>
<td>TX (n = 9)</td>
<td>420 ± 35</td>
<td>1.741 ± 0.139</td>
<td>0.094 d</td>
<td>152 ± 17</td>
<td>269 ± 93</td>
<td>3.74 ± 1.56</td>
<td>2.22 ± 0.46</td>
<td>654 ± 21</td>
<td>6.4 ± 14</td>
</tr>
<tr>
<td>TX + LU 135252</td>
<td>408 ± 35</td>
<td>1.741 ± 0.139</td>
<td>0.094 d</td>
<td>152 ± 17</td>
<td>269 ± 93</td>
<td>3.74 ± 1.56</td>
<td>2.22 ± 0.46</td>
<td>654 ± 21</td>
<td>6.4 ± 14</td>
</tr>
</tbody>
</table>

\(a\) S Cr, serum creatinine; S urea, serum urea; UNX, uninephrectomized rats; TX, transplanted rats.

\(\star\) * \(P < 0.001\) versus TX; \(\star\) * \(P < 0.001\) versus TX + LU 135252.

\(\star\) * \(P < 0.01\) versus TX; \(\star\) * \(P < 0.01\) versus TX + LU 135252.

\(\star\) * \(P < 0.05\) versus UNX-control.

Figure 1. Glomerulosclerosis (A), tubulointerstitial damage (B), and vascular damage (C) indices in uninephrectomized Fisher rats (UNX-control; \(n = 13\)), treated (TX; \(n = 13\)) and untreated (TX + LU 135252; \(n = 13\)) transplant-receiving rats, as indicated by asterisks (\(\star\)). Indices: arbitrary units (see Materials and Methods), \(\star\) * \(P < 0.001\) versus TX; \(\star\) * \(P < 0.001\) versus TX + LU 135252.
nephropathy, at least in this model, and (2) that the adverse effects of ET on progression are mainly mediated via the ET\textsubscript{A} receptor. Inhibition of transplant glomerulopathy and interstitial or vascular lesions appears plausible in view of the known renal actions of ET-1.

Transplant vasculopathy, the hallmark of chronic transplant nephropathy, is characterized by endothelial cell damage, VSMC proliferation, and migration. ET-1 is a potent mitogen for VSMC and mesangial cells (6), and the mitogenic effect of ET-1 is mediated via the ET\textsubscript{A} receptor (7). Several reports suggested a role of ET during posttransplant acute tubular necrosis and acute vascular rejection (16), but whether ET also plays a role in chronic transplant nephropathy has remained less clear. Simonson et al. (5), however, recently reported

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Figure 2. Paraffin sections, periodic acid-Schiff stain. Representative examples of: (A) Severe transplant glomerulosclerosis (grade 2) in an untreated allotransplant (×240). (B) Absence of glomerulosclerosis (grade 0) in an allotransplant treated with LU 135252 (×240). (C) Marked tubulointerstitial lesions (tubular casts and lymphocellular infiltrates; grade 2) in an untreated allotransplant (×60). (D) Minimal tubulointerstitial lesions (tubulointerstitial damage grade 1) in an allotransplant treated with LU 135252 (×60). (E) Intrarenal artery with transplant vasculopathy, i.e., with marked thickening of the media (vascular damage grade 2; ×100). (F) Minimal signs of transplant vasculopathy (vascular damage grade 1; ×100).
increased expression of immunoreactive ET-1 in the vasculature of chronic rejecting renal allografts in humans. This observation parallels earlier findings in coronary artery disease after heart transplantation. Ravalli et al. (17) documented increased ET-1 immunoreactivity in myointimal cells, macrophages, and endothelial cells. Tanabe et al. reported (18) that endothelin-converting enzyme (ECE) is increased in arteries of human renal allografts with chronic transplant nephropathy, suggesting that ET-1 is generated from big ET-1 by ECE. In most kidney graft biopsies, immunostaining for ET was found in glomeruli, tubules, vasculature, and inflammatory infiltrates, and staining was correlated with glomerular and interstitial lesions (19). ET also increases renal vascular resistance and reduces renal blood flow. These hemodynamic alterations are seen in chronic transplant nephropathy and possibly are reversed by LU 135252.

We were concerned about possible confounders. Food as well as sodium intake were similar in all groups. BP by telemetry and heart weight also were not different. CsA and some of its metabolites are known to (1) interact with endothelial cell function, (2) increase ET secretion from endothelial cells and VSMC, (3) elevate ET plasma level, (4) modulate ET receptor expression, and (5) cause renal vasoconstriction. A potential confounding effect of CsA on the development of chronic transplant nephropathy was ruled out by transiently treating all animal groups with CsA.

In view of recent findings that kidney/body mismatch may contribute to histologic lesions through “nephron underdosing” (1,10), we emphasize that kidneys from donors were transplanted to weight-matched recipients and the number of glomeruli was quantified. In view of species differences of the renal ET system, it is unknown whether the strikingly beneficial effects of selective ET<sub>A</sub> receptor blockade in the “Fisher to Lewis” model can be extrapolated to humans. The results are sufficiently encouraging, however, to warrant further studies.

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**References**


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