Original Article

Combination treatment with an ET$_A$-receptor blocker and an ACE inhibitor is not superior to the respective monotherapies in attenuating chronic transplant nephropathy in a 'Fisher-to-Lewis' rat model

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Abstract

Background. Specific endothelin A (ET$_A$)-receptor blockade and ACE inhibition attenuate chronic transplant nephropathy (CTN) in the 'Fisher-to-Lewis' rat model. It is unknown (i) which of both pharmacological interventions attenuates CTN more effectively and (ii) whether combination therapy exerts additive nephroprotection.

Methods. We compared (i) the effects of specific ET$_A$-receptor blockade with LU 302146 (30 mg/kg bw/day) and ACE inhibition with trandolapril (0.3 mg/kg bw/day) and (ii) the effect of a combination therapy of both drugs on the development of CTN. Kidneys of Fisher rats were orthotopically grafted to Lewis rats. Untreated 'Fisher-to-Lewis' allografts served as controls (TX). All animals received low-dose cyclosporin A (1.5 mg/kg body weight) for 10 days post-transplant to inhibit early acute rejection episodes. The duration of the experiment was 36 weeks. Blood pressure (BP) was measured every other week by tail plethysmography. Indices of glomerulosclerosis (GS), tubulointerstitial and vascular damage, number of glomeruli, total glomerular volume and mean glomerular volume were measured using morphometric and stereological techniques, respectively. Albuminuria, blood chemistry and haematology were measured at the end of the experiment.

Results. LU 302146 did not affect systolic BP. In contrast, trandolapril and combination treatment significantly reduced systolic BP. Histological signs of CTN were almost completely prevented by LU 302146 and trandolapril as compared to TX, e.g. GS = 0.8 ± 0.08 and 0.9 ± 0.20 vs $1.8 \pm 0.21*$ (arbitrary unit; $*P < 0.001$ vs treated groups). Allograft weight was significantly lower in treated vs TX animals. Trandolapril and combination therapy, but not LU 302146 alone, abrogated glomerular hypertrophy, i.e. mean glomerular volume: TX 2.22 ± 0.43, trandolapril $1.61 \pm 0.38**$, LU 302146 2.22 ± 0.11, trandolapril + LU 302146 $1.78 \pm 0.28*$ (μm^3; $*P < 0.05$ vs control and LU 302146, $**P < 0.01$ vs control and LU 302146). Albuminuria was lower in treated compared to TX animals. Combination therapy did not confer additional benefit compared to the respective monotherapies.

Conclusions. We conclude that ET$_A$-receptor blockade abrogates GS, tubulointerstitial and vascular damage in the 'Fisher-to-Lewis' model of CTN to a similar extent as ACE inhibition. However, only ACE inhibition inhibits glomerular hypertrophy. In contrast to ACE inhibition, the effect of ET$_A$-receptor blockade is independent of BP. This finding is consistent with the notion that ET$_A$-receptor mediated events play a partly BP-independent role in the genesis of CTN. Combination therapy exerts no additive nephroprotection.

Keywords: ACE inhibitor; chronic rejection; chronic transplant nephropathy; endothelin; endothelin receptor antagonist; ET$_A$-receptor

Introduction

So-called 'chronic rejection' is the major cause of renal and cardiac allograft loss and no specific treatment of this entity is available. Although chronic rejection is triggered by immune mechanisms, damage is perpetuated and amplified by non-immune mechanisms, potentially including the endothelin (ET) and

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renin–angiotensin systems. We have recently performed a study to investigate the hypothesis that ET-1 plays a role in the genesis of chronic rejection: specific ET$_A$-receptor blockade in a standard rat model of chronic renal allograft nephropathy ('Fisher-to-Lewis' kidney transplantation) abrogated renal functional decline and largely prevented structural renal damage without lowering blood pressure (BP), as assessed by telemetry [1].

The issue arose whether the effect of ET$_A$-receptor blockade is superior to ACE inhibition. The latter has also been shown to be nephroprotective in a 'Fisher-to-Lewis' model [2]. To this end, in a head-on comparison the effect of specific ET$_A$-receptor blockade with LU 302146 on the development of chronic transplant nephropathy (CTN) was compared to that of the ACE inhibitor trandolapril in the above orthotopic kidney transplantation model. We chose a dose of the ET$_A$-receptor blocker LU 302146 which is known to completely block ET$_A$-receptors without affecting the ET$_B$-receptor [3]. LU 302146 is the follow-up molecule of LU 135252 with similar pharmacological properties (Dr M. Raschack, Knoll AG, personal communication). A BP-effective dose of the ACE inhibitor trandolapril (as assessed in a previous experiment [4]) was chosen to investigate whether the known BP-independent nephroprotection of ET$_A$-receptor blockade [1] can compete with that of a hypotensive dose of trandolapril. The latter would strengthen the hypothesis that the ET-system is of major importance in the pathogenesis of CTN, at least in rats. Furthermore, the question whether combination treatment confers additional nephroprotection was addressed by investigating a group of animals treated with both drugs. Combination therapy of these classes of drugs had been shown to exhibit additive nephroprotection in a different model of renal damage, i.e. 'Heyman nephritis' [5].

Materials and methods

Animals

Naive male inbred Lewis (LEW, RT1^1) rats ($n=58$) were used as recipients of Fisher (F344, RT$^{[v]}$) kidney allografts ($n=58$) (200–240 g; Charles River, Sulzfeld, Germany). The rats were fed standard rat chow (0.25% NaCl and 19% of protein; ssnif GmbH, Soest, Germany).

Kidney transplantation

Transplantation was performed under ether anaesthesia. 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 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 days later and the graft was checked macroscopically to exclude hydronephrosis. All allograft recipients were treated with low-dose cyclosporin A (CsA) (1.5 mg/kg/day) for 10 days post-transplant [1].

Experimental design

Untreated 'Fisher-to-Lewis' allografts (TX) were compared to allografts treated with LU 302146 (TX + LU 302146), trandolapril (TX + trandolapril), or a combination of both (TX + combination treatment). LU 302146 and trandolapril were added to the food calculated to deliver 30 mg/kg bw/day or 0.3 mg/kg bw/day, respectively. Treatment was started on the 11th post-transplant day. To exclude differences in body weight as a potential confounding factor, a pair-feeding protocol was used throughout the experiment. Blood pressure (BP) was measured every other week using tail plethysmography.

After 34 weeks animals were housed in metabolic cages for 24 h urine sampling. Urinary albumin and interleukin-6 (IL-6) excretion were measured using specific ELISA's following the guidelines of the manufacturers (ICN-Pharma, Eschwege, Germany; Pharmacia-Amersham Biotech, Freiburg, Germany). Thirty-six weeks after kidney transplantation blood samples were obtained (chemistry measured with Hitachi 9-17E) and retrograde perfusion fixation via the abdominal aorta at controlled pressure (110 mmHg) was performed, as described elsewhere [6].

Morphological 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 [6] for embedding in Epon-Araldite. The remaining tissue slices were embedded in paraffin, 4 µm sections were stained with haematoxylin/eosin (HE)/periocid-acid-Schiff (PAS) and analysed using morphometric and stereologic techniques (vide infra).

Morphological investigations of the kidney

Indices of renal damage (glomerular sclerosis, tubulointerstitial and vascular damage). The glomerular sclerosis index (GS) was determined in 100 glomeruli per animal on PAS-stained paraffin sections at a magnification of ×400, using a semiquantitative scoring system [6]. The glomerular damage was graded as follows: grade 0, no changes; grade 1, mild mesangial expansion and/or mild segmental hyalinosis/sclerosis (up to 25% of the glomerular tuft); grade 2, moderate segmental hyalinosis/sclerosis involving 25–50% of the glomerular tuft; grade 3, severe segmental hyalinosis/sclerosis involving 50–75% of the glomerular tuft; grade 4, diffuse sclerosis/obliteration involving >75% of the glomerular tuft. Tubulointerstitial (tubular atrophy, dilatation, 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. [7] applied to PAS-stained paraffin sections at a magnification of ×250. Ten fields per kidney were randomly sampled and graded as follows: tubulointerstitial damage (TID): grade 0, no changes; grade 1, lesions involving less than 25%; grade 2, lesions affecting 25–50%; grade 3, lesions involving more than 50% of the field. Vascular damage (VD): grade 0, normal vessels; grade 1, mild vascular thickening; grade 2, moderate thickening; grade 3, severe thickening

(onion skin pattern); grade 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_A) and volume density (V_V) of the renal cortex and medulla as well as the number of glomeruli per area (N_A) were measured using a Zeiss eyepiece (Integrationsplatte II, Zeiss Co., Oberkochen, Germany) and the point counting method ($P_P = A_A = V_V$) at a magnification of ×400 [6]. The number of glomeruli per area (N_A) was then corrected for tissue shrinkage (45%).

Total cortex volume (V_{cortex}) was derived from kidney mass divided by specific weight of the kidney (1.04 g/cm^3) times the volume density of the cortex.

Glomerular geometry was analysed as follows: volume density (V_V) of glomeruli and area density of glomerular tuft (A_{AT}) were measured by point counting according to $P_P = A_A = V_V$ [6] at a magnification of ×400 on HE sections.

Total area of glomerular tuft (A_T) was then determined as $A_T = A_{AT} × A_{cortex}$.

The number of glomeruli per volume (N_V) was derived from glomerular area density (N_A) and the volume density (V_V) of glomeruli using the formula: $N_V = k/\beta × N_A^{1.5}/V_V^{0.5}$ with $k = 1$ and $\beta = 1.382$.

The total number of glomeruli was derived from the total volume of the renal cortex and the number of glomeruli per cortex volume: $N_{glom} = N_V × V_{cortex}$.

The mean glomerular tuft volume was determined according to $v = \beta/k × A_T^{1.5}$ with $\beta = 1.382$ and $k = 1.1$ [6].

Statistics

Data are given as mean ± SD. Statistical analysis was performed using one-way ANOVA, followed by Bonferroni–Dunn multiple range test. A probability of error of $P < 0.05$ was defined as statistical significant.

Results

The body weight of the animals did not differ between the groups during the whole duration of the experiment (weight at the end of the experiment see Table 1).

Twenty animals died before the end of the experiment: 10 animals of the TX control group, 2 animals in the TX + trandolapril group, 5 in the TX + LU 302146 group and 3 in the TX + combination treatment group. None of the treated animals died after the 10th week of the experiment, whereas 8 animals of the TX control group died after the 10th week.

Haemoglobin, haematocrit, white blood cell and platelets counts, serum potassium, sodium and calcium did not differ between the groups. In contrast, serum cholesterol was significantly lower in LU 302146-treated animals (both monotherapy and combination therapy). Triglycerides were lower in all treatment groups as compared to untreated animals. Serum creatinine and urea tended to be lower in treated animals, but this was statistically significant only for serum creatinine in the trandolapril-treated group (Table 1).

Albuminuria was lower in treated as compared to untreated animals. The effect of ACE inhibition and ET_A-receptor blockade was comparable; combination therapy did not exert additional benefit (Table 1).

Urinary IL-6 excretion was significantly lower in ET_A-receptor blocker treated animals (both monotherapy and combination therapy with ACE inhibition). In contrast, ACE inhibition alone had no significant effect on this parameter (Table 1).

Trandolapril monotherapy and combination treatment of trandolapril and LU 302146 significantly reduced systolic BP. Combination treatment did not further decrease systolic BP compared to trandolapril alone (Table 2).

Morphological studies

Morphology of allograft controls showed signs of severe CTN, i.e. glomerulosclerosis, tubulointerstitial fibrosis and vascular damage. Monotherapy with LU 302146 and trandolapril abrogated transplant nephropathy to a similar extent. Combination therapy did not confer additional nephroprotection (Figures 1–4).

The number of glomeruli did not differ significantly. The same was true for total glomerular volume. In contrast, mean glomerular volume was significantly lower in trandolapril-treated animals (both monotherapy and combination therapy) (Table 3).

Table 1. Body and organ weights, haematology, blood chemistry, urinary albumin and IL-6 excretion (week 36, urinary measurements at week 34)

Group	Body weight (g)	Heart weight (g)	Kidney weight (g)	S_{Cr} (µmol/l)	S_{urea} (mmol/l)	Cholesterol (mmol/l)	Triglycerides (µmol/l)	Urinary albumin excretion (g/day)	Urinary IL-6 excretion (pg/day)°
TX control (*n* = 9)	406 ± 32	1.975 ± 0.146	1.95 ± 0.49	132 ± 62	65 ± 40	4.80 ± 1.48	1.41 ± 0.40	0.914 ± 0.122	290 ± 111
TX + trandolapril (*n* = 8)	382 ± 40	1.579 ± 0.161†	1.78 + 0.21‡	80 ± 16*	37 ± 11	3.76 ± 0.49	1.03 ± 0.07*	0.332 ± 0.104‡	116 ± 110
TX + LU 302146 (*n* = 9)	377 ± 71	1.657 ± 0.237*	1.70 ± 0.39‡	97 ± 27	36 ± 18	3.10 ± 0.44*	1.06 ± 0.23*	0.322 ± 0.102‡	32 ± 39†
TX + combination treatment (*n* = 12)	368 ± 35	1.761 ± 0.237	1.82 ± 0.50‡	92 ± 26	38 ± 8	3.00 ± 0.46*	1.08 ± 0.21*	0.341 ± 0.089‡	66 ± 90*

Data are mean ± SD. TX, allograft; S_{Cr}, serum creatinine. *$P < 0.05$ *vs* TX control; †$P < 0.01$ *vs* TX control; ‡$P < 0.001$ *vs* TX control. °Only five animals per group were chosen randomly for measurement of urinary IL-6 excretion.

Table 2. Systolic blood pressure (mmHg, measured by tail plethysmography) during the experiment

Group	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36
TX control ($n=9$)	118 ± 8	117 ± 9	126 ± 12	134 ± 8	143 ± 11	152 ± 17	159 ± 17
TX + trandolapril ($n=8$)	$79 \pm 3^*$	$85 \pm 6^*$	$104 \pm 13\dagger\P$	$89 \pm 13^*\P$	$104 \pm 12^*\P$	$109 \pm 12^*\P$	$107 \pm 14^*\P$
TX + LU 302146 ($n=9$)	115 ± 5	117 ± 7	125 ± 13	130 ± 16	139 ± 19	152 ± 17	156 ± 15
TX + combination treatment ($n=12$)	$84 \pm 8^*$	$85 \pm 13^*$	$98 \pm 15\ddagger\P$	$101 \pm 14^*\P$	$108 \pm 11^*\P$	$109 \pm 11^*\P$	$110 \pm 13^*\P$

Data are mean $\pm$ SD. TX, allograft. $^*P<0.001$ *vs* TX control and TX + LU 302146; $\dagger P<0.05$ *vs* TX control; $\ddagger P<0.001$ *vs* TX control; $\P P<0.001$ *vs* TX + LU 302146.

Glomerulosclerosis Index

Tubulointerstitial Damage Index
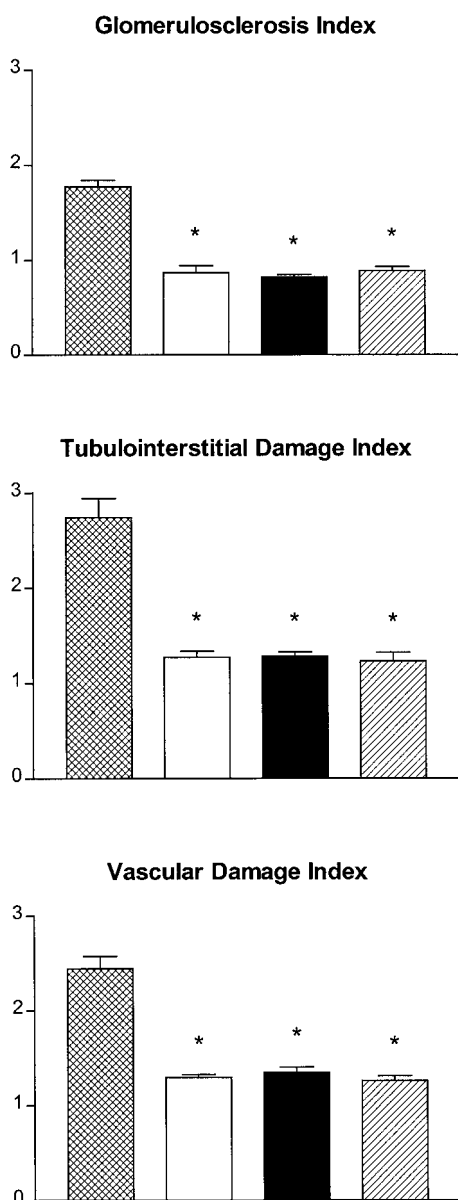
Vascular Damage Index

Fig. 1. Glomerulosclerosis, tubulointerstitial damage, and vascular damage indices in untreated 'Fisher-to-Lewis' renal allograft controls ($n=9$), allografts treated with trandolapril ($n=8$), allografts treated with LU 302146 ($n=9$), and allografts treated with combination treatment (trandolapril + LU 302146; $n=12$). Indices: arbitrary units (see methods), $^*P<0.001$ *vs* untreated allografts.

Discussion

The present study shows that development of CTN is abrogated to a similar extent by treatment with the ETₐ-receptor blocker LU 302146 and the ACE inhibitor trandolapril. The salient features of the present study are that (i) LU 302146 exerts a similar nephroprotective effect as trandolapril without lowering blood pressure and (ii) that combination therapy of LU 302146 and trandolapril does not confer additional nephroprotection. The efficacy of LU 302146 indicates that (i) ET plays a crucial role in progression of CTN, and that (ii) the effects of ETs are mainly mediated via the ETₐ-receptor. The fact that combination therapy of ACE inhibitor and ETₐ-receptor blocker did not confer additive benefit on the parameters investigated is compatible with, but not proof for, the idea that ACE inhibition and ETₐ-receptor blockade share, at least in part, similar pathogenetic pathways. One possibility may be the reduction of ET-1 synthesis by ACE inhibition [8]. The fact that BP was lowered by ACE inhibition, but not by ETₐ-receptor blockade, may raise the issue of not entirely comparable experimental settings. We had, however, chosen a BP-effective dose of trandolapril with the aim to compare LU 302146 with an adequate positive treatment control. BP was not lowered to a greater extent by combination therapy than by ACE inhibition alone. This observation is of note, because some studies had reported that combination of angiotensin II receptor blockers with ETₐ-receptor blockers had an additive hypotensive effect. The lack of such an effect in our study may be due to the different animal models used and the use of ACE inhibitors instead of angiotensin II receptor blockers.

We acknowledge the lack of an untreated isotransplanted group to evaluate the magnitude of nephroprotection confered by treatment. We had such a control group in our previous study [1] and could document that ETₐ-receptor blockade largely prevented CTN. Thus, it can cautiously be concluded that the same is true in the present study.

An interesting finding is reduction of mortality in treated as compared to untreated animals. Most of the untreated animals died after the 10th week of the experiment. It can be speculated that the absence of mortality after the 10th week of the experiment in treated animals is a direct result of ETₐ-receptor
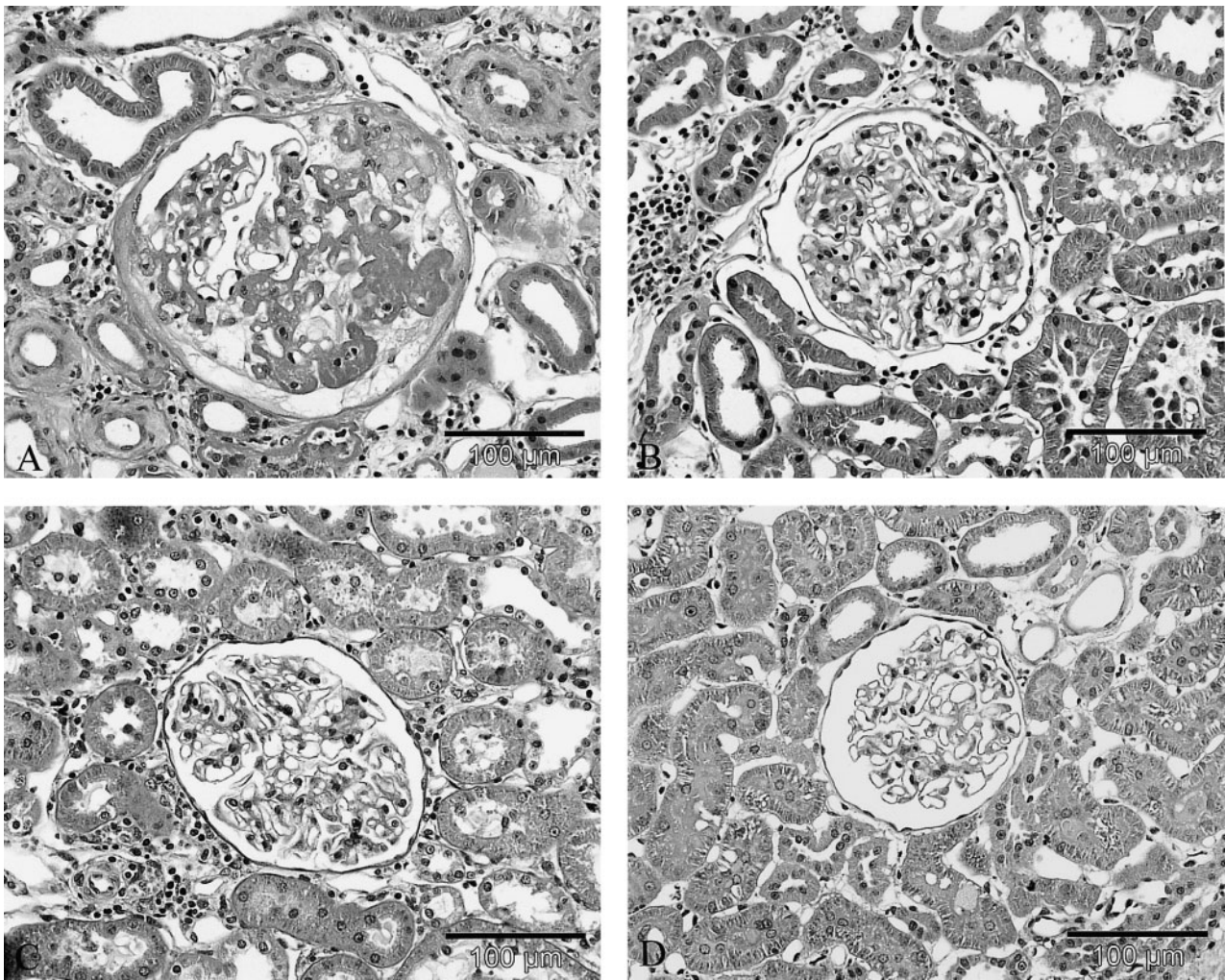
Fig. 2. Representative figures of glomerular morphology in untreated allografts (**A**), trandolapril-treated allografts (**B**), LU 302146-treated allografts (**C**), and allografts + combination treatment (**D**). Paraffin sections, PAS-stain. (A) Glomerulus with marked glomerulosclerosis, mesangial matrix expansion, thickening and crinkling of capillary basement membranes with slight collapse of the capillary tuft and segmental adhesion to Bowman's capsule. (B) Signs of very mild glomerulosclerosis, i.e. only mild diffuse mesangial matrix expansion. (C) As in panel B glomerulosclerosis is only very mild with segmental mesangial matrix expansion. (D) Normal appearance of the glomerulus, no signs of mesangial matrix expansion.

and/or ACE inhibitor treatment, respectively, since reduced mortality with these treatments has also been documented in other renal damage models. We have excluded hydronephrosis of the graft macroscopically at the time when nephrectomy of the remaining native kidney was performed (10th day post-transplantation). Since hydronephrosis is a potential confounder for structural alterations of the graft, we used a more sophisticated method to exclude hydronephrosis at the end of the experiment, i.e. histological evaluation of renal papilla. None of the animals showed signs of papillar necrosis (data not shown).

We were concerned about additional possible confounders. Food as well as sodium intake were similar in all groups. CsA and some of its metabolites are known to (i) interact with endothelial cell function, (ii) increase ET secretion from endothelial cells and vascular smooth muscle cells (VSMC), (iii) elevate

ET plasma level, (iv) modulate ET receptor expression, and (v) cause renal vasoconstriction. A potential confounding effect of CsA on the development of CTN was ruled out by treating all animal groups with CsA for the first 10 days post-transplant. In view of recent findings that kidney/body mismatch may contribute to histological lesions through 'nephron underdosing' [9] we emphasize that kidneys from donors were transplanted to weight-matched recipients and the number of glomeruli was quantitated. The body weights between the groups did not differ throughout the experiment.

Our experiment does not specifically exclude effects of ET_A-receptor blockade on immune recognition or effector steps in the allograft. In this context it is of interest that both ET_A-receptor blocker monotherapy and combination treatment with the ACE inhibitor reduced urinary IL-6 excretion, but ACE inhibition
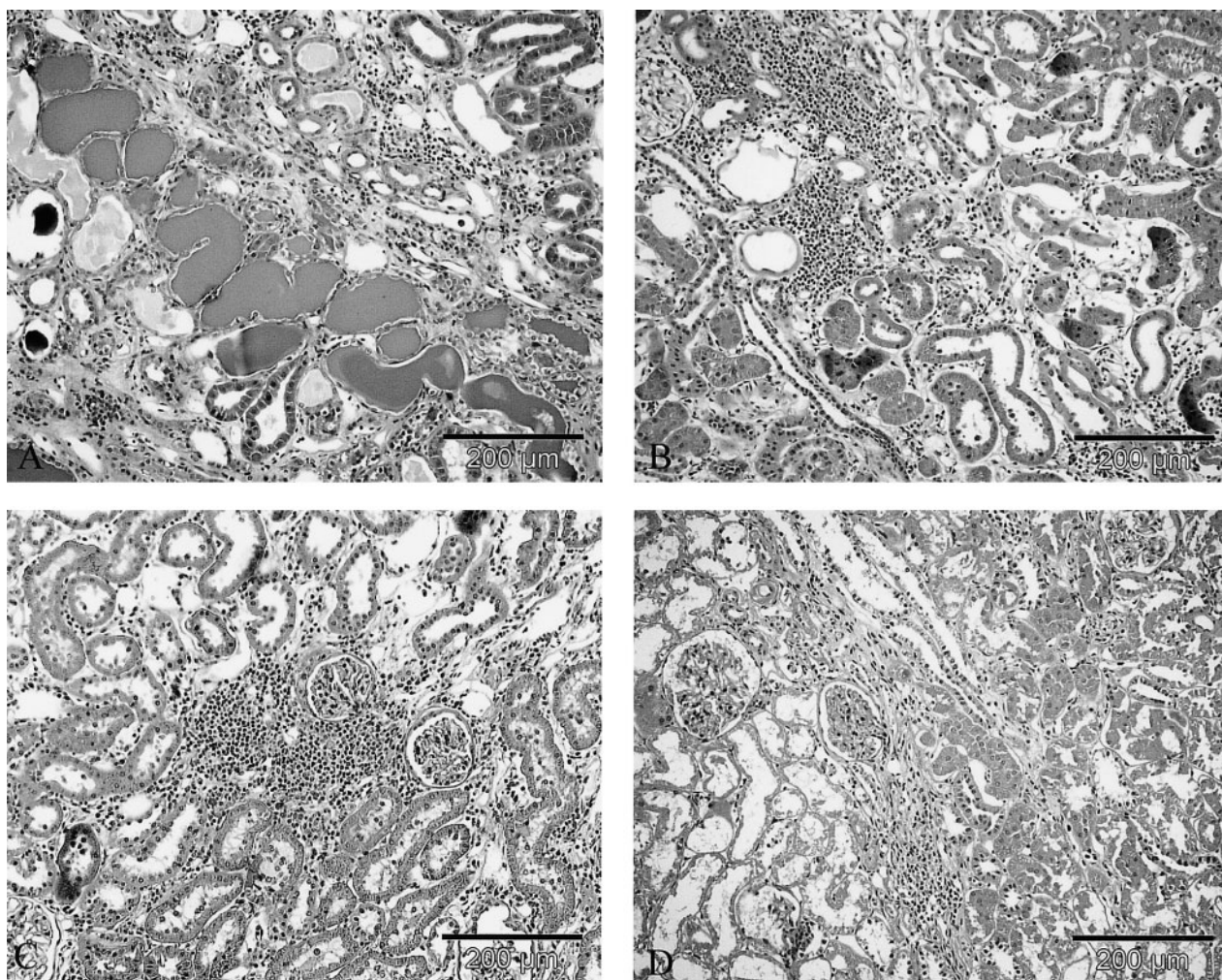
Fig. 3. Representative figures of tubulointerstitial morphology in untreated allografts (**A**), trandolapril-treated allografts (**B**), LU 302146-treated allografts (**C**), and allografts+combination treatment (**D**). Paraffin sections, PAS-stain. (A) Tubulointerstitium with marked tubulointerstitial damage (dilatation of tubuli with intraluminal casts, tubular atrophy with thickened basement membrane, diffuse interstitial inflammation and fibrosis). (B) Tubulointerstitial damage is less pronounced with only focal dilatation of tubuli without intraluminal casts and diffuse interstitial inflammation. No tubular atrophy, no interstitial fibrosis. (C) Tubulointerstitial damage is very mild with only focal dilatation of tubuli without intraluminal casts and diffuse interstitial inflammation. No tubular atrophy, no interstitial fibrosis. (D) There is only focal interstitial inflammation. No tubular atrophy, no interstitial fibrosis.

alone did not. IL-6 has been shown to correlate with macrophage infiltration of grafts in the 'Fisher-to-Lewis' model [10] and urine IL-6 excretion is a sensitive indicator for rejection in humans [11]. ET-1 exerts chemotactic properties on monocytes/macrophages [12]. It was not the aim of this study to investigate these immune mechanisms, but it may be rewarding to investigate this issue in the future, because ET$_A$-receptor blockade may be superior to ACE inhibition in this regard.

There is a large body of evidence that ET-1 amplifies non-immune effector mechanisms in chronic allograft rejection. The latter possibility appears plausible in view of the known actions of ET-1 on VSMC. Transplant vasculopathy, the hallmark of chronic rejection, is characterized by endothelial cell damage and VSMC proliferation and migration. ET-1 is a potent mitogen for VSMC and mesangial cells (for review see [13]) and the mitogenic effect of ET-1 is mediated via the

ET$_A$-receptor [14]. The nephroprotective effect of LU 302146 may be, at least partly, explained by inhibition of cell proliferation. Similarly, as far as ACE inhibition is concerned, it can be speculated that besides the known haemodynamic mechanisms of these drugs attenuation of CTN may also be related to inhibition of angiotensin II-mediated cell proliferation [15].

Simonson et al. [16] recently reported increased immunoreactive ET-1 levels in the vasculature of chronic rejecting renal allografts in humans. This 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. [18] reported that endothelin-converting enzyme (ECE) is increased in arteries of human renal allografts with CTN suggesting that ET-1 is generated from big ET-1 by ECE. Inhibition of ECE with phosphoramidon in a rat model of chronic cardiac
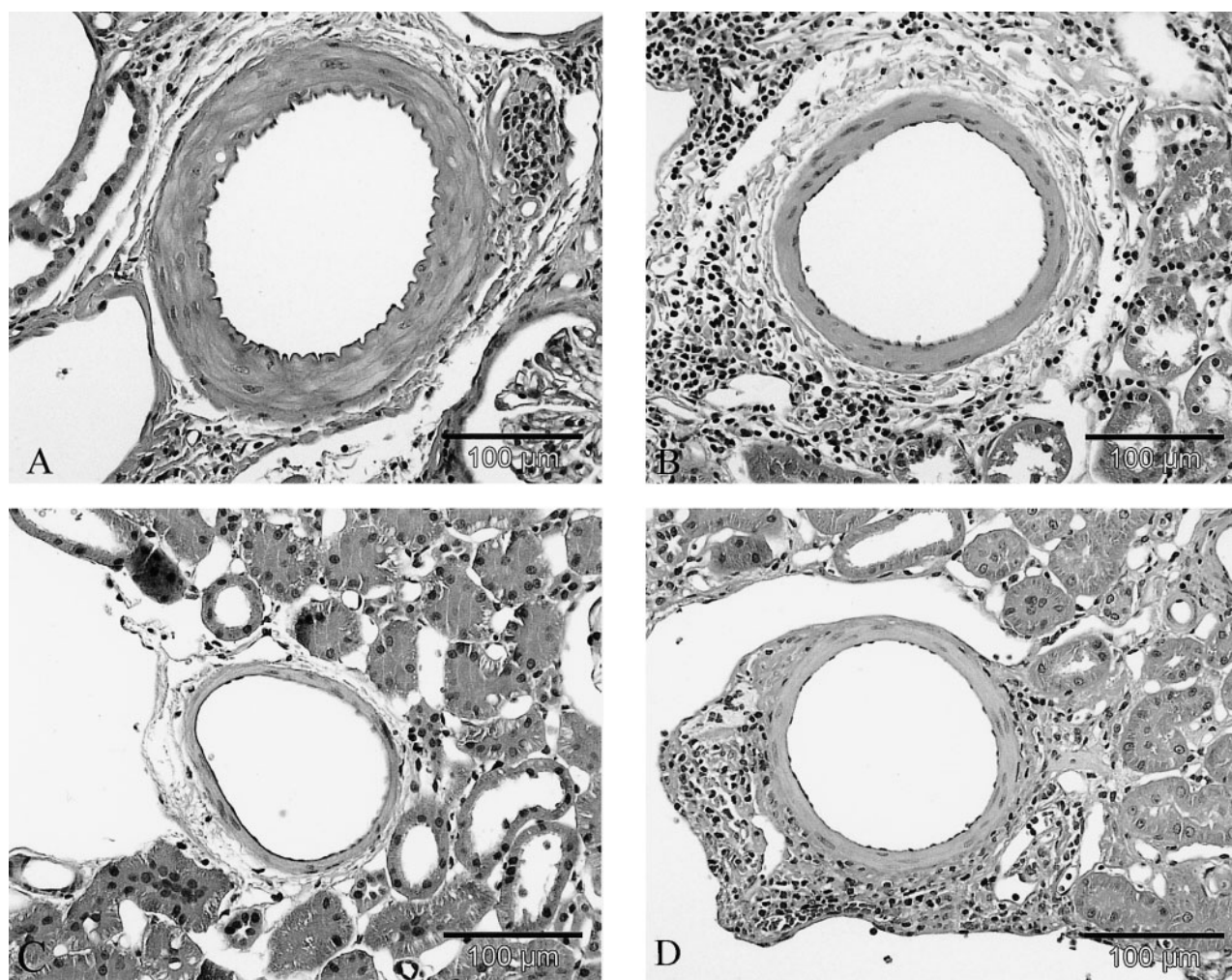
Fig. 4. Representative figures of vascular morphology in untreated allografts (**A**), trandolapril-treated allografts (**B**), LU 302146-treated allografts (**C**), and allografts + combination treatment (**D**). Paraffin sections, PAS-stain. (A) Intrarenal artery with marked thickening of the media. No intimal fibrosis. (B) Intrarenal artery with an only slight increase in media thickness and a mild perivascular inflammation. (C) Intrarenal artery showing no increase in media thickness. (D) As in panel B a mild increase in media thickness and a mild perivascular inflammation is visible.

Table 3. Number of glomeruli and glomerular geometry

Group	Number of glomeruli (n)	Total glomerular volume (cm^3)	Mean glomerular volume (μm^3)
TX control ($n=9$)	$30\,910 \pm 4\,927$	0.070 ± 0.02	2.220 ± 0.431
TX + trandolapril ($n=8$)	$30\,890 \pm 5\,550$	0.050 ± 0.02	1.611 ± 0.379*
TX + LU 302146 ($n=9$)	$29\,590 \pm 3\,251$	0.066 ± 0.01	2.225 ± 0.112
TX + combination treatment ($n=12$)	$29\,750 \pm 3\,904$	0.053 ± 0.013	1.784 ± 0.284†

Data are mean $\pm$ SD. TX, allograft. *$P < 0.01$ *vs* TX control and TX + LU 302146; †$P < 0.05$ *vs* TX control and TX + LU 302146.

allograft rejection attenuates transplant vasculopathy and rejection [19]. These findings together with the present study document that ET-1 plays a major role in the genesis of chronic rejection of different organs both in animals and humans. Thus, besides ACE inhibitor administration, prevention of chronic rejection with specific ET$_A$-receptor blockers may open a new perspective in human transplantation. The present study, however, documents that no additive effects of both treatments are observed in the 'Fisher-to-Lewis' rat model. This is in contrast to what was observed in another model of renal damage, i.e. the Heyman nephritis model of membranous nephropathy [5].

It is of note that the features of chronic transplant nephropathy observed in the 'Fisher-to-Lewis' rat lack some characteristic findings of human chronic transplant nephropathy. We and others do not observe the formation of a neointima in intrarenal arterioles.

Furthermore, we did not find splitting of the glomerular basement membrane in an unpublished electron microscopical study. The relevance of our findings is, however, strongly supported by another study using the same treatment regimens in rat models of chronic transplant vasculopathy [20], i.e. models which differ completely from the 'Fisher-to-Lewis' model. In these latter studies, we could document that under normo- and hypertensive conditions ET$_A$-receptor blockade and ACE inhibition were equally effective in abrogating chronic transplant vasculopathy of aorta allografts. In contrast to the 'Fisher-to-Lewis' model, these aorta allotransplantation models exhibit massive neointima formation. As in the present study, combination treatment was not superior to the respective monotherapies [20].

In view of species differences of the renal ET-system and the above limitations of the 'Fisher-to-Lewis' model it is unknown whether the strikingly beneficial effects of selective ET$_A$-receptor blockade and ACE inhibition in the present study can be extrapolated to chronic transplant nephropathy in humans [13]. This question can only be addressed by sorely needed prospective clinical trials.

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