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Influence of acute selective endothelin-receptor-A blockade on renal hemodynamics in a rat model of chronic allograft rejection

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Abstract We have recently demonstrated up-regulation of renal endothelin (ET) synthesis in a rat model of chronic renal allograft rejection. Treatment with a selective ET-A receptor antagonist improved survival and reduced functional and morphological kidney damage. However, the underlying mechanisms have not yet been elucidated, as ET exhibits both hemodynamic and inflammatory properties. Therefore, in the present study we investigated acute hemodynamic effects of the selective ET-A receptor antagonist LU 302146 (LU) on chronic renal allograft rejection in rats. Experiments were performed in the Fisher-to-Lewis model of chronic renal allograft rejection. Lewis-to-Lewis isografts served as controls. After 2, 12, and 24 weeks, hemodynamic measurements were performed on anesthetized animals. Measurement of mean arterial pressure (MAP) was performed via a catheter in the femoral artery. Renal blood flow (RBF) was measured by an ultrasonic flow probe placed around the renal transplant artery. Medulla blood flow (MBF) and cortex blood flow (CBF) were determined with laser Doppler probes. Hemodynamic response upon intravenous bolus injection of LU (50 mg/kg) was investigated. The application of LU was followed by a decline in MAP that reached statistical significance only in isografts (ISOs) after 12 weeks and allografts (ALLOs) after 24 weeks. RBF slightly decreased in all groups; however, without reaching statistical significance. MBF showed a small increase in ALLO12 and ALLO24 whereas CBF slightly decreased in all groups. Acute ET-A receptor blockade does not induce important hemodynamic effects in kidneys undergoing chronic rejection. The lack of response to ET-A receptor blockade suggests that the beneficial effect of ET receptor antagonists in this model is likely to be due to improvement of renal morphology.

Keywords LU 135252 · Kidney transplantation · Microcirculation

Introduction

Chronic transplant nephropathy is one of the most common causes of graft loss in renal transplantation. The clinical course is characterized by progressive deterioration of renal function, arterial hypertension, and increasing proteinuria. Histomorphological findings include gradual vascular obliteration, glomerulopathy, interstitial fibrosis with variable mononuclear cell infiltration, and tubular atrophy. In addition to immunological factors such as HLA-mismatch, non-immunological factors are also considered to be

responsible for chronic graft dysfunction. Among these, ischemia appears to be an important determinant of delayed graft function [21, 31]. Despite a number of therapeutic approaches successfully tested in animal models, there is presently no specific treatment available for chronic rejection in human transplantation [1, 2, 3, 12]. Endothelin (ET) was first described by Yanagisawa et al. in 1988 and has been identified as the most potent endogenous vasoconstrictor on a molecular basis known to date [35]. The ET system plays a substantial role in both acute and chronic renal failure. Beside its well-characterized hemodynamic effects, its mitogenic and profibrotic properties have generated increased interest in the pathophysiology of chronic renal failure [25, 30]. In an experimental rat model, Herrero et al. were able to show that the combined ET-A/B receptor antagonist bosentan, given 1 day before and 7 days after transplantation, was able to attenuate ischemia–reperfusion injury [22]. Our group could demonstrate that kidney isografts from rats treated with a selective ET-A receptor antagonist after transplantation recovered faster from ischemic injury [13]. The course of chronic renal allograft failure was also dramatically improved by this treatment [12, 26]. In contrast to these observations, treatment with a combined ET-A/B receptor antagonist did not prevent progression of chronic renal rejection nor did it prolong survival [13]. Therefore, the ET-A receptor seems to play an important role in the development of chronic organ failure, and selective ET-A receptor antagonists should provide nephro-protection in this setting. Whether these effects are mediated via hemodynamic or anti-inflammatory effects remains unclear. Therefore, in the present study we investigated acute hemodynamic effects of the selective ET-A receptor antagonist LU 302146 (LU) in chronic renal allograft rejection in rats.

Materials and methods

Animals

Inbred male Fisher rats (F344, $RT1^{lvl}$) and male Lewis rats (LEW, $RT1^{l}$), all weighing between 200 and 220 g, were used as graft donors and recipients, respectively. The animals were purchased from Harlan Winkelmann, Borchen, Germany. The F344-to-LEW strain combination has been used extensively in experimental studies of chronic renal allograft rejection [16]. All experimental protocols were approved by the local institutional animal care committee (Regierungspräsidium Karlsruhe; AZ 37-9185.81/99/98) and were in compliance with EEC Directive 86/609 and with the *Principles of Laboratory Animal Care* (NIH publication No. 86-23, revised 1985) as well as with national animal welfare regulations [Tierschutzgesetz (TSG) and Gesellschaft für Versuchstierkunde/Society of Laboratory Animal Science (GV-SOLAS)].

Kidney transplantation

The microsurgical technique used in this study is a modification of the technique described by Lee [26]. Surgical procedures were performed under isoflurane anesthesia. The donor kidney was flushed with 1 ml ice-cooled University of Wisconsin solution and transplanted into the recipient rat, from which the left kidney had been removed. Perfusion of the grafted organ was restored by release of the vessel clamps after 35 min. At the end of surgery, the rats were given 100,000 U penicillin i.p. The right kidney was removed 10 days later.

Experimental protocols

Forty-eight transplanted rats were used (24 allogeneic, 24 isogeneic). All animals were treated with cyclosporin A (5 mg/kg per day i.m.; Sandimmun, Novartis, Nuremberg, Germany) for 10 days after transplantation. Hemodynamic studies were performed in eight allografted and eight isografted recipient rats at weeks 2, 12, and 24 after transplantation. The animals received an i.v. bolus injection of either LU (50 mg/kg b.w.) or vehicle solution.

Hemodynamic studies

The rats were anesthetized with an i.p. injection of 100 mg/kg thiobutabarbital and were placed on a heating table. After tracheotomy had been performed, arterial and venous catheters (PE 50) were inserted into the femoral vessels. An intravenous infusion of isotonic saline with albumin at a concentration of 5 g/dl was given at a rate of 3 ml/h throughout the experiments. Measurement of mean arterial blood pressure (MAP) was continuously monitored via the arterial line with a Statham pressure transducer p23Db and a Gould pressure processor. The allograft was exposed through midline incision. An ultrasonic transit-time flow probe (1RB, Transonic Systems, Ithaca, N.Y., USA) was placed around the left renal artery for measurement of renal blood flow (RBF). The signals were transmitted to a transit-time flow meter (T106, Transonic Systems). This device measures absolute RBF in milliliters per minute with a precision of ± 5%. For determination of cortex blood flow (CBF) a blunt superficial laser Doppler probe (p10B Prisma, Moor Instruments, Lawrenz, Sulzbach, Germany) was placed on the kidney surface and covered with body-warm mineral oil. Outer medulla blood flow (MBF) was measured by insertion of a fine-needle laser Doppler probe (p10B, Moor Instruments) into the outer medulla. Both probes were mounted on micromanipulators so that movement artifacts were avoided. The signals of the two probes were transmitted to a laser flow meter (MBF 3D, Moor Instruments). Validation of transit time and laser Doppler methods are given elsewhere [17, 20, 32]. The experimental setting was established and has been used in several projects in our laboratory. A high level of experience in the use of these methods has been achieved [9, 10, 11]. The correct positioning of the medulla probe was assured with an operation microscope at the end of each experiment by kidney dissection. Only experiments with correct placement of the probe were used for further assessment. After instrumentation had been carried out, a 60-min equilibration time was allowed for baseline values. Then, LU 302146 (50 mg/kg b.w.) was given i.v. as a single bolus injection, while the controls received vehicle (VEH) injections. All above-mentioned parameters were continuously recorded by means of a multichannel Gould Brush 2600 recorder for 60 min after the bolus was given. Values are given as changes from baseline in percent. After the measurements had been taken the animals were killed.

Endothelin receptor antagonist

LU 302146 was supplied by Abbott, Ludwigshafen, Germany. The chemical name is (+)-(*S*)-2-(4.6-dimethoxy-pyrimidine-2-yloxy)-3-methoxy-3.3-diphenyl-propionic acid. The molecular mass is 410.43. Control animals received a VEH (saline buffer). LU 302146 is a derivate of LU 135252 with a higher ET-A receptor specificity [34]. The pharmacology and kinetics of LU have been described extensively [29]. The LU dose chosen in this study is comparable to that in other studies. LU has been shown to exert positive effects in congestive heart failure, hypertension, and transplant rejection [8, 12, 24, 26, 27]. Higher doses of LU did not further promote the specific results.

Data calculation and statistical evaluation

Data are shown as means ± SEM. Differences between groups were assessed by the unpaired Student's *t*-test. Statistical significance was defined as $P<0.05$. StatsDirect statistical software (version 1.9.1) was used for statistical analysis. Differences between means were considered to be significant at a level of $P<0.05$.

Results

Bolus injection of LU was followed almost immediately by a decline in MAP (Fig. 1a). However, this observation only reached statistical significance 12 weeks after transplantation in isografts (ISOs) and 24 weeks after transplantation in allografts (ALLOs) [relative changes to baseline (%): ISO2: −20 ± 1; ISO12: −24 ± 1 ($P<0.01$); ISO24: −18 ± 4; ALLO2: −14 ± 1; ALLO12: −17 ± 1; ALLO24: −23 ± 2 ($P<0.05$)]. Measurement of RBF revealed a small, though non-significant decrease in all groups (Fig. 1b) (ISO2: −20 ± 3; ISO12: −21 ± 4; ISO24: −22 ± 2; ALLO2: −18 ± 1; ALLO12: −16 ± 1; ALLO24: −23 ± 1). No relevant changes occurred in renal microcirculation. MBF showed a non-significant slight increase in ALLO12 and ALLO24 (ALLO12: 17 ± 5; ALLO24: 14 ± 4), whereas CBF decreased slightly in all groups (Fig. 1c). The variability in flow measurements was within expected ranges, as described previously [9, 20, 32]. The calculated vascular resistances did not indicate a decreasing vascular tone in macro-vasculature or micro-vasculature (Table 1).

Discussion

Activation of the renal ET system is involved in the pathogenesis of chronic renal allograft rejection. We have previously demonstrated that selective ET-A receptor blockade [12], as opposed to non-selective combined ET-A/B receptor antagonism [13], improves survival and markedly reduces histological damage in allogeneic kidney transplantation. Most importantly, this effect was paralleled by a significant increase in glomerular filtration rate. However, on the basis of these results, it was not possible to attribute the nephro-pro-

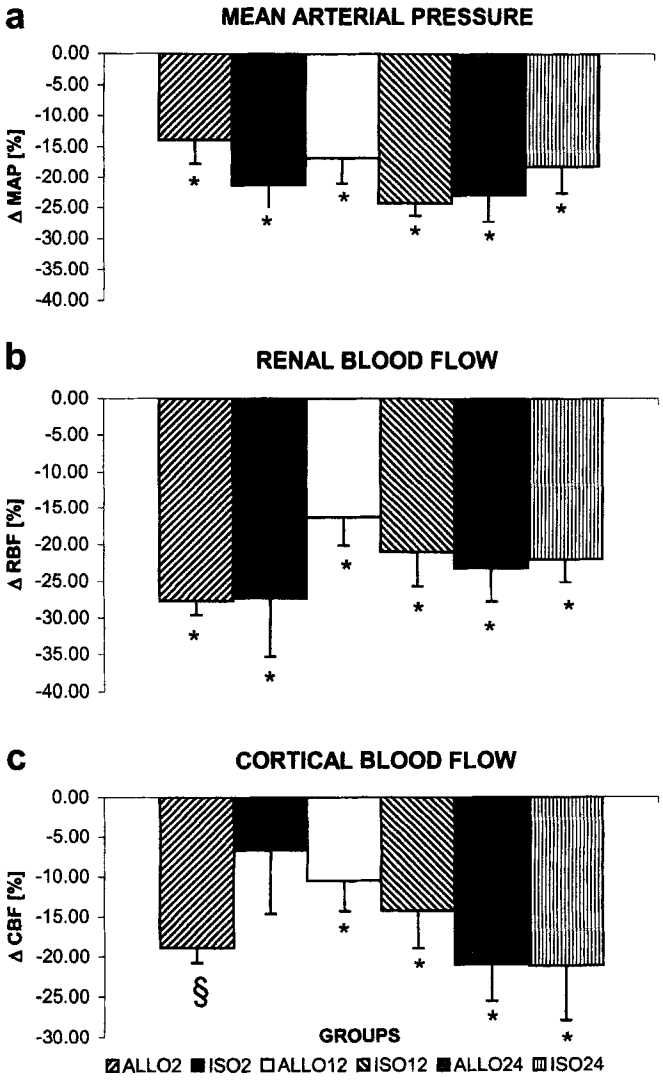

Fig. 1a–c Relative changes to baseline of (**a**) MAP, (**b**) RBF, and (**c**) CBF after application of LU 302146. *$P<0.05$, §$P<0.01$ compared to baseline. *ALLO/ISO2*, *ALLO/ISO12*, and *ALLO/ISO24* indicate allografts/isografts 2 weeks, 12 weeks, or 24 weeks after transplantation

Table 1 Relative changes of vascular resistances compared with baseline after application of LU. *ALLO/ISO2*, *ALLO/ISO12*, and *ALLO/ISO24* indicate allografts/isografts 2 weeks, 12 weeks, or24 weeks after transplantation. *RVR* renal vascular resistance, *CVR* cortical vascular resistance, *MVR* medullary vascular resistance

Graft	RVR	CVR	MVR
ALLO2	0.50 ± 2.05	0.73 ± 2.17	0.90 ● 0.27
ISO2	0.78 ± 0.56	3.18 ± 0.45	2.88 ± 0.75*
ALLO12	1.03 ± 1.11	1.60 ± 1.91	−0.99 ± 0.54
ISO12	1.16 ± 0.43	1.70 ± 0.69	1.17 ± 0.17
ALLO24	0.99 ± 0.80	1.09 ± 1.09	0.99 ± 0.37
ISO24	1.42 ± 1.42	0.86 ± 0.66	−1.29 ± 0.32

*$P<0.05$

tective effect of ET-A receptor blockade to its known anti-proliferative properties or to a concomitant improvement of renal hemodynamics.

ET constitutes the most potent endogenous vasoconstrictor known so far [35]. This property has been shown to play an important role in the pathogenesis of a series of cardiovascular diseases, i.e., ischemic acute renal failure [9, 19], cerebrovascular spasm after subarachnoidal hemorrhage [18], and renal dysfunction in ischemic chronic heart disease [5]. These examples represent the hemodynamic role of ET in chronic disease states. In the present study, we were able to show that the acute bolus injection of a selective ET-A receptor antagonist produced no different hemodynamic effects in allografted kidneys than in isografted kidneys. This finding was observed at weeks 2, 12, and 24 after renal transplantation, indicating that the renal vasculature is not vasoconstricted via stimulated ET-A receptors to a larger degree in rats with chronic rejection than in those without. This is in fundamental opposition to results observed after acute renal failure [9, 33], where ET-A receptor blockade restored impaired renal perfusion parameters. Our results, therefore, indicate that the beneficial effect of ET receptor antagonism is exclusively mediated via anti-mitogenic and/or immunomodulatory properties. This is further supported by previous findings that the nephro-protective properties of selective ET-A blockade are independent of the lowering of proteinuria or blood pressure [12]. ET-1 transgenic mice develop severe glomerulosclerosis and chronic renal failure in the absence of arterial hypertension [23], which has led to the concept that ET is an important growth factor involved in the pathogenesis of chronic nephropathies. This hypothesis has been confirmed in a number of experimental investigations [6, 7].

In previous studies, treatment with a selective ET-A receptor antagonist reduced the expression of cellular markers of immunological activation after renal transplantation [12, 14]. In addition, it markedly reduced allograft infiltration of monocytes/macrophages, cells known to play a seminal role in the development of chronic allograft nephropathy [4]. The beneficial effect of long-term treatment with LU 135252 on chronic allograft nephropathy has been confirmed by Orth et al. [28]. In contrast to the nephro-protection provided by selective ET-A antagonism, combined ET-A/B receptor blockade had no effect on the course of chronic renal allograft rejection [13]. It is, therefore, conceivable that the ET-A receptor plays a pivotal role in chronic renal allograft failure. However, only incomplete data exist for ET receptor distribution in transplanted kidneys. Deng et al. investigated expression of ET receptor mRNA in the Fisher-to-Lewis kidney transplantation model [15]. They were able to demonstrate significant down-regulation of ET-A mRNA and unchanged ET-B mRNA levels in allografted versus isografted kidneys. However, these data have not been confirmed by other groups, and no information was provided on the receptor status on a protein level. In addition, no information is available on the distribution of the ET receptors, i.e., expression in vascular and glomerular structures as opposed to tubulointerstitial compartment. The observation of ET-A receptor mRNA down-regulation may, therefore, reflect a self-protecting mechanism of the kidney and not the actual receptor distribution. Further studies are needed to clarify this issue.

In summary, renal hemodynamic effects of acute ET-A receptor blockade do not differ between allo-geneically and isogeneically transplanted rats. These results demonstrate that the protective effect of long-term ET-A receptor antagonism is likely to be mediated through blockade of proliferative and/or immunomodulatory effects of the activated allograft ET system.

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