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Hypertension accelerates the pace of chronic graft dysfunction in the rat*

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Abstract In this study we compared the effects of hypertension on chronic rejection in a rat model of renal transplantation utilizing genetically normotensive (BBOK) and spontaneously hypertensive rats (SHR). SHR received either a BBOK (BBOK → SHR) or an SHR (SHR → SHR) kidney; normotensive isografts served as controls. Before transplantation, SHR recipients were treated with hydralazine (50 mg/kg per day). To prevent acute rejection, an anti-CD4 antibody (3 mg/kg per day for 3 weeks) in combination with cyclosporin A (3 mg/kg per day for 1 week) was given to all groups. Six weeks after transplantation, blood pressure was measured, and the kidneys removed for histological and immunohistological analysis. SHR → SHR developed a significantly higher blood pressure than BBOK → SHR. Blood pressure in BBOK → BBOK was significantly lower than in the other two groups. The degree of glomerulosclerosis was similarly increased in allografted (BBOK → SHR) and SHR → SHR kidneys as compared with the BBOK → BBOK kidneys ($P < 0.05$). Infiltration of ED-1$^+$ monocyte/macrophages and OX19 pan-T-cells was most pronounced in allografts (BBOK → SHR) and was also increased in SHR → SHR as compared with BBOK → BBOK. Our results indicate that hypertension accelerates the morphological and immunohistological changes characteristic of grafts undergoing chronic rejection. However, our findings support the hypothesis that alloantigen-dependentfactors are of greater important.

Key words Kidney transplantation · Hypertension · Chronic rejection

Introduction

In recent years, chronic rejection has emerged as a major cause of late graft loss following transplantation. Although chronic rejection has been traditionally regarded as a repeated low-grade response to allogeneic tissue, recent evidence indicates that alloantigen-independent factors may also contribute to its pathogenesis [1–10]. Concerns over the inability of immunological advances to improve long-term allograft survival have focused attention on alloantigen-independent factors, such as hypertension, and its contribution to chronic renal allograft rejection [11, 12].

The role of hypertension in allograft failure is complex and has been difficult to define [11]. The prevalence of post-transplant hypertension varies from 6% to 80% [9, 13–16]. The presence of hypertension in the post-transplant period portends a poorer outcome for renal allografts, with a faster decline in creatinine clearance and a greater likelihood of return to dialysis or of death [11, 17, 18]. However, a beneficial impact of blood pressure control and consequent prevention or delay of renal allograft deterioration has been difficult to dem-

onstrate. Recent evidence suggests, in analogy to native kidney disease, that progressive allograft dysfunction can be prevented by a reduction in blood pressure [19, 20].

The present study was designed to investigate the effects of hypertension on chronic renal graft rejection in a rat model of renal transplantation.

Materials and methods

Animals

Experiments were performed with male normotensive (BBOK, $RT1^u$) and spontaneously hypertensive rats (SHR, $RT1^k$). BBOK rats have a genetic predisposition to develop diabetes mellitus type I [21]. In the colony from which the BBOK rats for the present study were derived, the incidence of diabetes is about 40% and the age at onset of diabetes is about 15 to 20 weeks [22]. In the present study, BBOK rats were transplanted at 8 weeks of age when they were in a nondiabetic metabolic state and the transplants were harvested 6 weeks thereafter, before any signs of diabetes-induced kidney disease became apparent.

Animals were obtained immediately after weaning from the rat breeding facilities at the Ernst-Moritz-Arndt-University, Greifswald, Germany. They were housed in standard plastic cages and maintained in a temperature- and humidity-controlled environment. The animals had access to standard chow (Ssniff, Soest, Germany) and tapwater *ad libitum*, except for the night before transplantation, when food was withheld for 12 h. All experiments were approved by a governmental committee on animal welfare. Secondary structural fixation of hypertension in SHR recipients and donors was prevented by antihypertensive treatment from weaning until transplantation.

Surgery

Surgery for nephrectomy, renal transplantation and implantation of femoral artery catheters was performed as previously described [23, 24]. Briefly, both donor and recipient were anaesthetized with ketamine (100 mg/kg body weight, i.p.; Ketamin 10%, cp-Pharma, Burgdorf, Germany), plus xylacine (10 mg/kg body weight, i.p.; Rompun, Bayer, Leverkusen, Germany), and simultaneously operated on by two investigators. During surgery, ketamine was supplemented as needed. The kidney, including the renal artery with a short piece of aorta, the renal vein with a patch of vena cava and the whole ureter were removed from the donor and immediately transferred to the recipient, which at that time was ready to receive the graft. An end-to-side anastomosis was performed between donor and recipient aortas. Similarly, the renal vein was anastomosed end-to-side to the recipient's vena cava. The ureter was placed into the bladder through a small incision in the apex of the bladder wall. During surgery, the graft was wrapped in gauze and repeatedly rinsed with ice-cold isotonic saline. Total ischaemic time was always less than 60 min.

Histology

Formalin-fixed sections were stained with haematoxylin/eosin for routine histology and with periodic acid-Schiff to evaluate the extent of glomerulosclerosis, as defined by a collapse of capillaries, adhesion of obsolescent segments of Bowman's capsules, and the entrapment of hyaline [25]. At least 100 glomeruli were counted in each kidney, and the proportion of sclerosed to total glomeruli expressed as a percentage.

Antibodies

Monoclonal antibodies against macrophages (ED1), $CD5^+$ T lymphocytes (OX19), fibronectin and laminin were purchased from Serotec Camon Labor Service (Germany) and for secondary (rabbit antimouse) and tertiary (mouse alkaline phosphatase anti-alkaline phosphatase, APAAP) staining from Dako, Denmark).

Immunohistology

Representative portions of kidney grafts were snap-frozen in liquid nitrogen, cut (4 μm), fixed in acetone, air dried, and stained with appropriate antibodies (mouse antirat antibodies). After staining, the sections were interacted with rabbit antimouse IgG by the APAAP or peroxidase antiperoxidase (PAP) methods. Fibronectin and laminin were quantified on a 0–4+ scale (0 = one, 4+ = dense). Positive cell counts are expressed as the mean (± SEM) number of cells per field of view (cells/FV). More than 20 FV per section were evaluated per specimen at 400 ×.

Blood pressure measurements

For direct measurements of arterial pressures, a femoral artery catheter was connected to an Isotec pressure transducer and a linear recorder WR 3300 (both from Hugo Sachs Elektronik, March-Hugstetten, Germany). Blood pressure signals were recorded continuously for 60 min or longer if stable values had not been achieved during this time. During measurements, rats were conscious, undisturbed and unrestrained in their standard cages.

Plasma urea and creatinine concentrations

Plasma urea and creatinine concentrations were determined photometrically with commercially available test kits (Harnstoff Liquicolor and Kreatinin Liquicolor, respectively, both from Human, Taunusstein, Germany).

Experimental protocol

At 4 weeks of age, animals were randomly assigned to three experimental groups. Group I consisted of 11 SHR donors and 11 SHR recipients (SHR → SHR), group II consisted of 15 BBOK donors and 15 SHR recipients (BBOK → SHR), and group III consisted of 8 BBOK donors and 8 BBOK recipients (BBOK → BBOK). Between 4 weeks of age and the time of renal transplantation, SHR recipients and donors were treated with hydralazine (50 mg/kg per day in the drinking fluid) to keep their blood pressure within the normal range. This treatment was stopped on the day of renal transplantation.

Renal transplantations were performed at 8 weeks of age. The left native kidney of the recipient was removed immediately before implantation of the renal graft. One week after transplantation, the recipient was anaesthetized again and the right native kidney was removed. After transplantation, all recipients received immunosuppressive treatment with anti-CD4 antibody and cyclosporine.

Table 1 Blood pressure, glomerulosclerosis, lymphocyte and macrophage infiltration, and quantification of extracellular matrix deposition of fibronectin and laminin in transplanted kidneys (*FV* field of view)

	Blood pressure (mm Hg)		Glomerulosclerosis (%)	Lymphocytes (cells/FV)	Macrophages (cells/FV)	Fibronectin (scale 0–4)	Laminin (scale 0–4)
	Systolic	Diastolic					
SHR → SHR	214 ± 11*	155 ± 6*	24 ± 3	33 ± 5+	50 ± 5+	2.7 ± 0.2	2.2 ± 0.3
BBOK → SHR	184 ± 8+	129 ± 5+	27 ± 3+	54 ± 4*	101 ± 14*	3.5 ± 0.2*	2.3 ± 0.2
BBOK → BBOK	123 ± 8	80 ± 7	18 ± 4	11 ± 6	24 ± 8	2.7 ± 0.4	2.2 ± 0.5

* $P < 0.05$ vs SHR → SHR and BBOK → BBOK
+ $P < 0.05$ vs BBOK → BBOK

Both agents were administrated intraperitoneally at a dose of 3 mg/kg per day according to the following schedule: anti-CD4 antibody was injected on days − 1, 0, 1, 2, 3, 6, 11, 16 and 20, and cyclosporine on days 0–7 with day 0 being the day of renal transplantation [26].

A catheter was implanted into the right femoral artery 6 weeks after renal transplantation, tunnelled under the skin and exteriorized at the scruff of the neck. The catheter was filled with isotonic saline containing 20 IU/ml heparin. After placement of the catheter, animals were allowed to rest for 2 days before direct blood pressure measurements were performed. After the blood pressure measurement, recipients were sacrificed and the kidney grafts were removed and processed for histology and immunohistology.

Statistics

Results are expressed as means ± SEM. Data on blood pressure, body weight, plasma urea, creatinine and immunohistology were analysed by one-way analysis of variance followed by the Newman-Keuls' post-hoc test where appropriate. Data on glomerulosclerosis were analyzed by Kruskal-Wallis one-way analysis on ranks. Statistical significance was accepted at $P < 0.05$.

Results

Blood pressure and body weight

By 6 weeks after transplantation and the ending of antihypertensive treatment, SHR recipients of an SHR kidney (SHR → SHR) and a BBOK kidney (BBOK → SHR), respectively, had developed hypertension (Table 1). However, SHR recipients of a BBOK kidney (BBOK → SHR) had about a 30 mm Hg lower systolic ($P < 0.05$) and about a 25 mm Hg lower diastolic blood pressure ($P < 0.05$) than SHR with an SHR kidney (SHR → SHR). BBOK recipients of a BBOK kidney (BBOK → BBOK) remained normotensive.

Body weight was lower in SHR recipients of a BBOK kidney (BBOK → SHR) than in the other two groups ($P < 0.05$).

Histology

To determine the long-term effects of alloantigen-mediated graft rejection, we evaluated the histology of harvested kidneys. Whether or not acute rejection occurred, the damage observed was compatible with the picture of chronic rejection. The most prominent sign of chronic rejection in rats is glomerulosclerosis. Therefore, we analysed the kidneys for glomerulosclerosis. Glomerulosclerosis was most apparent in BBOK kidneys that had been transplanted into SHR recipients (BBOK → SHR) (27 ± 3%) and least frequent in BBOK kidneys transplanted into BBOK recipients (BBOK → BBOK) (18 ± 4%); ($P < 0.05$). However, the difference between SHR → SHR (24 ± 3%) and BBOK → SHR was not significant.

Immunohistology

In SHR rats with a BBOK kidney (BBOK → SHR), cellular infiltration was significantly elevated ($P < 0.05$) as compared with the other two groups (Table 1). Cellular infiltration was most intense in interstitial areas, particularly around vessels and glomeruli. Fibronectin, as an indicator of extracellular matrix production, was markedly upregulated in BBOK → SHR, but there was no significant difference between the other two groups (Table 1). In contrast, there were no major differences in the expression of laminin between the groups (Table 1).

Plasma urea and creatinine

Plasma urea and creatinine concentrations did not differ significantly between the three groups.

Discussion

The increased glomerulosclerosis and cell infiltration in BBOK kidneys that had been transplanted into SHR recipients (BBOK → SHR) as compared to syngeneically transplanted kidneys (SHR → SHR, BBOK → BBOK) suggest that alloantigen-dependent factors dominate over hypertension in the development of chronic rejection. On the other hand, glomerulosclerosis and cell infiltration in hypertensive syngeneic grafts was elevated

as compared to the normotensive syngeneic kidneys, which taken together indicates that hypertension may play a major role in chronic renal graft injury.

It has been suggested that the damaging effect of hypertension on the renal graft arises from higher glomerular capillary wall tension and pressure. An increased traffic of macromolecules through the mesangium and mesangial expansion culminates in glomerulosclerosis [27]. A number of experimental studies have demonstrated that sustained transmission of the systemic blood pressure to the glomerular capillary wall is the major mechanism leading to hypertensive renal disease [10]. This increased wall stress (shear stress) is one of the most important activators of endothelial cells [28, 29]. Endothelial activation may facilitate the interaction of host leucocytes with foreign antigens inducing acute rejection [30]. In parallel to this interaction, several vasoconstricting substances such as angiotensin-II, endothelin-I and thromboxane-A2 and growth factors (TGF-β, PDGF-AA, b-FGF) are released which induce a migration of smooth muscle cells into the intima where they proliferate [31–33]. Once intimal proliferation has occurred, the thickening of the intima results in an enhanced shear stress on the endothelial cells [34]. This vicious cycle may cause more and more intimal thickening and other signs of chronic rejection, and finally the destruction of the graft.

The role of hypertension in the development of chronic renal dysfunction in native kidney disease has been well described [35]. Hypertension is common among renal transplant recipients, but very few data are available regarding its impact on long-term graft structure, function or prognosis [10]. A significant negative correlation has been found between renal allograft survival and the presence of hypertension [36]. In a widely used animal model of chronic renal allograft rejection (F344 → LEW), treatment of hypertension results in decreased glomerular hypertension in association with reduced proteinuria and glomerular damage [37].

It is difficult to ascertain whether hypertension is the cause or a consequence of renal graft dysfunction. In this experiment, we investigated the impact of hypertension on chronic rejection. Transmission of hypertension by the donor kidney to the recipient has been demonstrated in various animal models in the setting of stable allograft function [38–40]. Many other factors have been implicated in the pathogenesis of hypertension after renal transplantation, particularly cyclosporine [41]. In rats, the increase in blood pressure and nephropathy under cyclosporine treatment depends on the dose, the duration of treatment, the route of administration and the strain of rat. SHR rats appear to be particularly sensitive to the nephrotoxic and blood pressure-increasing effects of cyclosporine. To our knowledge, the effects of cyclosporine on kidney function and blood pressure in BBOK rats have not yet been investigated. In the present study all BBOK recipients remained normotensive while the SHR recipients developed hypertension and glomerulosclerosis, albeit to a different degree depending on the source of the transplanted kidney. While we cannot exclude the possibility that differences in blood pressure and glomerulosclerosis between BBOK and SHR recipients may have been influenced by different reactions of the two strains, low-dose cyclosporine was given only during the first 7 day after transplantation.

In conclusion, these results indicate that hypertension accelerates the morphological and immunohistological changes characteristic of grafts undergoing chronic rejection. However, our findings support the hypothesis that alloantigen-dependent factors are more important.

References

1. Mackenzie HS, Azuma H, Rennke HG, Tilney NL, Brenner BM (1995) Renal mass as a determinant of late allograft outcome: insights from experimental studies in rats. Kidney Int 48 [Suppl 52]: 38–42
2. Tilney NL, Whitley WD, Diamond JR, Kupiec-Weglinski JW, Adams DH (1991) Chronic rejection – an undefined conundrum. Transplantation 52: 389–398
3. Paul LC (1995) Chronic renal transplant loss. Kidney Int 47: 1491–1499
4. Hostetter TH (1994) Chronic transplant rejection. Kidney Int 46: 266–279
5. Heemann UW, Azuma H, Tullius SG, Mackenzie HS, Brenner BM, Tilney NL (1994) The contribution of reduced functioning mass to chronic kidney allograft dysfunction in rats. Transplantation 58: 1317–1322
6. Heemann UW, Azuma H, Tullius SG, Scmid C, Phillipp T, Tilney NL (1996) Infections and reduced functioning kidney mass induce chronic rejection in rat kidney allografts. Clin Nephrol 46: 34–38
7. Conolly JK, Dyer PA, Martin S (1996) Importance of minimizing HLA-DR mismatch and cold preservation time in cadaveric renal transplantation. Transplantation 61: 709–713
8. Brenner BM, Cohen RA, Milford EL (1992) In renal transplantation, one size may not fit all. J Am Soc Nephrol 3: 162
9. Burke JF, Pirsch JD, Ramos EL (1994) Long-term efficacy and safety of cyclosporine in renal transplant patients. N Engl J Med 331: 358
10. Paul LC, Benediktsson H (1995) Post-transplant hypertension and chronic renal allograft failure. Kidney Int 48 [Suppl 52]: 34–37
11. Sanders CE Jr, Curtis JJ (1995) Role of hypertension in chronic renal allograft dysfunction. Kidney Int 48 [Suppl 52]: 43–47
12. Bia MJ (1995) Nonimmunologic causes of late renal graft loss. Kidney Int 47: 1470–1480

13. Legendre C, Saltiel C, Kreis H, Grunfeld J-P (1989) Hypertension in kidney transplantation. Klin Wochenschr 67: 919–922
14. Lake RG (1987) Hypertension in renal transplant recipients. Kidney Int 31: 1024–1037
15. Kasiske BL (1987) Possible causes and consequences of hypertension in stable renal transplant patients. Transplantation 44: 639–643
16. Ponticelli C, Montagnino G, Angelini C, Braga M, Tarantino A (1993) Hypertension after renal transplantation. Am J Kidney Dis 21 [Suppl 2]: 73–78
17. Cheigh JS, Stenzel KHS, Wang J, Fine P, Riggio RR, Rubin AL, Stentel KH (1995) Hypertension is associated with decreased graft survival in long-term kidney transplant recipients. Transplant Proc 17: 174–175
18. Brazy PC, Pirsch JD, Belzer FO (1992) Factors affecting renal allograft function in long-term recipients. Am J Kidney Dis 19: 558–566
19. Vianello A, Mastrosimone S, Calconi G, Gatti PL, Calzavara P, Maresca MC (1993) The role of hypertension as a damaging factor for kidney grafts under cyclosporine therapy. Am J Kidney Dis 21 [Suppl 2]: 79–83
20. Mourad G, Ribstein J, Mimran A (1993) Converting-enzyme inhibitor versus calcium antagonist in cyclosporine-treated renal transplants. Kidney Int 43: 419–425
21. Klöting I, Vogt L (1991) BB/O(ttawa)K(arlsburg) rats: features of a subline of diabetes-prone BB rats. Diabetes Res 18: 79–87
22. Klöting I, Vogt L (1992) On the influence of metabolic state of parents at conception and pregnancy on incidence and age at onset of diabetes in the progeny of BB/OK rats. Diabetes Res 19: 37–40
23. Kopf D, Waldherr R, Rettig R (1993) Source of kidney determines blood pressure in young renal transplanted rats. Am J Physiol 265: F104–F111
24. Sander S, Ehrig B, Rettig R (1996) Role of the native kidney in experimental post-transplantation hypertension. Pfluegers Arch 431: 971–976
25. Rennke HG (1989) Pathogenesis and significance of nonprimary focal and segmental glomerulosclerosis. Am J Kidney Dis 13: 443
26. Lehmann M, Kuttler B, Riedel H, Müller A, Hahn HJ, Brock J, Volk HD (1995) Anti-CD4 induced tolerance after rat kidney transplantation. In: Zanazana N (ed) T-cell, tolerance, transplantation, tumor. Pabst Science Publishers, Lengerich, pp 100–107
27. Bochicchio T, Ron O, Sandoval G, Perez-Grovas H, Bordes J, Herrera-Acosta J (1991) Hemodynamic alterations associated with progression of renal allograft injury. Transplant Proc 23: 1773–1774
28. Pohl U, Holtz J, Busse R, Bassenge E (1986) Crucial role of endothelium in the vasodilatator response to increased flow in vivo. Hypertension 8: 37–47
29. Langille BL, O'Donell F (1986) Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium dependent. Science 231: 405–407
30. Heemann UW, Tullius SG, Azuma H, Kupiec-Weglinsky J, Tilney NL (1994) Adhesion molecules and transplantation. Ann Surg 219: 4–12
31. Dzau VJ, Gibbons GH (1991) Endothelium and growth factors in vascular remodeling of hypertension. Hypertension 18: 111–115
32. Akyürek LM, Paul LC, Funa K, Larsson E, Fellström BC (1996) Smooth muscle cell migration into intima and adventitia during development of transplant vasculopathy. Transplantation 62: 1526–1529
33. Fellström BC, Larsson E (1993) Pathogenesis and treatment perspectives of chronic graft rejection (CVR). Immunol Rev 134: 83–98
34. Geraghty JG, Stoltenberg RL, Sollinger HW, Hullet DA (1996) Vascular smooth muscle cells and neointimal hyperplasia in chronic transplant rejection. Transplantation 62: 502–509
35. Walker WG (1993) Hypertension-related injury: a major contributor to end stage renal disease. Am J Kidney Dis 22: 164–173
36. Cheigh JS, Haschemeyer RH, Wang JCL, Riggio RR, Tapia L, Stenzel KH, Rubin AL (1989) Hypertension in kidney transplant recipients – effect on long-term renal allograft survival. Am J Hypertens 2: 341–348
37. Paul LC (1994) Functional and histologic characteristics of chronic renal allograft rejection. Clin Transplant 8: 319–323
38. Dahl LK, Heine M, Thompson K (1974) Genetic influence of the kidney on blood pressure. Evidence from chronic renal homografts in rats with opposite predisposition to hypertension. Circ Res 40: 94–101
39. Rettig R, Strauss H, Folberth C, Ganten D, Waldherr B, Unger T (1989) Hypertension transmitted from stroke-prone spontaneously hypertensive rats. Am J Physiol 257: F197–F203
40. Patschan O, Kuttler B, Heemann UW, Rettig R (1997) Kidneys from genetically normotensive donors lower blood pressure in young transplanted spontaneously hypertensive rats. Am J Physiol (in press)
41. Remuzzi G, Perico N (1995) Cyclosporine-induced renal dysfunction in experimental animals and humans. Kidney Int 48 [Suppl 52]: 70–74