Allogeneic kidney transplantation after active immunological enhancement: a model to study post-transplantation hypertension in rats

M. H. DE KEIJZER, A. P. PROVOOST, M. VAN AKEN, I. M. WEYMA, W. J. KORT, E. D. WOLFF AND J. C. MOLENAAR

1 Department of Paediatric Surgery, 2 Laboratory for Surgery, 3 Department of Paediatrics (Paediatric Nephrology), Erasmus University Medical School, Rotterdam, The Netherlands

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Summary
1. A model was developed to study post-transplant hypertension after allogeneic kidney transplantation between two inbred normotensive rat strains. Prolongation of graft survival was achieved by 'active immunological enhancement'.
2. Renal function, systolic blood pressure and plasma renin concentration were determined.
3. The systolic blood pressure started to rise in the second week after allogeneic transplantation. The glomerular filtration rate was impaired to a greater extent than the effective renal plasma flow.
4. Histopathological changes occurred indicating immunological reactions in the renal graft.
5. The plasma renin concentration was lower in transplant recipients than in controls.
6. We hypothesize that retention of sodium is immediately involved in the post-transplant hypertension observed in this model.

Key words: experimental surgery, hypertension, immunological enhancement, kidney transplantation.

Introduction
In renal transplant recipients hypertension is a frequent complication. Periods of elevated blood pressure have been reported to occur in large numbers of adult [1] as well as paediatric transplant recipients [2].

Various factors may be involved in post-transplant hypertension [3]. These include transplant artery stenosis, high doses of corticosteroids, acute and chronic allograft rejection, recurrence of the original disease and the presence of the recipient's own diseased kidneys. Disordered functioning of the renin-angiotensin system or persistent volume expansion may play a part in the post-transplant hypertension [4, 5].

Experimental renal transplantation in rats has been used to assess the role of the kidney in the pathogenesis of the blood pressure elevation in genetically hypertensive strains [6, 7]. Up till now, no model has been developed to study post-transplantation hypertension in rats with normotensive donors and recipients. In a previous study [8] it was shown that hypertension did not develop after isogeneic rat kidney transplantation. In the present experiment we determined the effect of allogeneic kidney transplantation on renal function and blood pressure in rats.

Methods

Laboratory animals

Adult male rats (body weight 250–300 g) of highly inbred Wistar (WAG/Ro) and Brown Norway (BN) strains were used for the transplantsations. These rat strains differ at the major histocompatibility locus. The WAG/Ro is RT-1a, the BN strain is RT-1b. The animals had unlimited access to food (Rat chow AMII, Hope Farms, containing 120 mmol of Na/kg) and tap water (containing 3 mmol of Na/l).
Surgery

A microsurgical technique, as described before [8], was used for rat renal transplantation. BN rats were used as donor, WAG/Ro rats as recipients. It is known that BN rats may develop spontaneous hydronephrosis [9]. Consequently, their renal function may be unequally divided over both kidneys. Since we wanted to know the pretransplant renal function of the donor kidney, intact kidneys had to be selected. When an intact kidney was present in the potential donor rat, the left kidney was removed 3 weeks before transplantation. In this way a compensatory hyperfunctioning donor kidney was transplanted. In the isogeneic rat model, developed previously, there were no major differences when comparing the transplantation of a normal or a hyperfunctioning kidney [8]. In allogeneic kidney transplantation the recipient's own kidneys were removed during the transplant operation.

Analytical procedures

The glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF) were measured as clearances of $^{51}$Cr-labelled EDTA and of $^{125}$I-iodohippurate ($^{125}$I-IOH) respectively. The method has been described in detail elsewhere [10]. In the BN rats it was necessary to determine the volume of distribution ($V$) of $^{51}$Cr-labelled EDTA and of $^{125}$I-IOH, to be able to calculate the GFR and the ERPF in this rat strain. In seven rats, with a body weight of 283 ± 29 g (mean ± sd), the $V$ of $^{51}$Cr-labelled EDTA was found to be 23.0 ± 0.7% of body weight and that of $^{125}$I-IOH 25.3 ± 1.6% of body weight.

The plasma renin concentration (PRC) was measured with a radioimmunoassay for angiotensin I (ANG I) after incubation of the plasma sample with excess rat renin substrate at pH 6.5 [11]. Plasma concentrations of creatinine ($P_Cr$) and urea ($P_Ur$) were determined with a Gilford auto-analyser.

Systolic blood pressure was generally determined in conscious rats by means of tail plethysmography (Electro-sphygmonanometer PE 300 Narco Bio-System). In BN donor rats the assessment of normal systolic blood pressure values was not possible without anaesthesia. This strain could not be trained to sit still in the restraining cages. Consequently, the systolic pressure was measured under pentobarbital anaesthesia (60 mg/kg intraperitoneally) in a separate group of BN rats and compared with that of a group of pentobarbital anaesthetized WAG/Ro rats.

Experimental protocol

To prolong allograft survival, and to circumvent the use of corticosteroids, active immunological enhancement was used, which had been shown to be successful in this model [12]. To induce enhancement all WAG/Ro rats were given an intravenous injection of 1 ml of fresh citrated BN rat blood 2 weeks before transplantation. Three groups of rats were studied. The first group initially consisted of 15 WAG/Ro rats with a technically successful (i.e. survival for at least 7 days) BN kidney transplant. Of these 15 rats, nine survived for a period of over 21 days. In the analysis, these nine remaining rats of this group were compared with a second group of seven unilaterally nephrectomized (NX) WAG/Ro rats and a third group of six intact WAG/Ro rats.

The systolic blood pressure was measured three times a week, starting at least 1 week before transplantation. The GFR and the ERPF were determined before transplantation as well as 1 and 3 weeks after transplantation. Fifteen minutes after the induction of pentobarbital anaesthesia and before the injection of $^{51}$Cr-labelled EDTA and $^{125}$I-IOH, 400 µl of blood was sampled to determine the PRC, $P_Cr$ and $P_Ur$ values.

Histology

In a separate group of transplant recipients, animals were killed at various times after transplantation. The kidney was fixed in 4% buffered formaldehyde solution. The fixed tissue was dehydrated with ethanol and embedded in Paromat. Serial sections were cut to a thickness of 3–4 µm. Sections were stained with haematoxylin-eosin.

Statistics

Results are given as means ± sd. The divergence between the groups was determined by one-way analysis of variance (ANOVA).

Results

The GFR and the ERPF of WAG/Ro recipients of BN kidneys, NX and control rats, as well as those of BN donor rats, are given in Table 1. The GFR and the ERPF of the single compensatory hyperfunctioning BN kidney amounted to, respectively, 59% and 72% of the two intact kidneys of WAG/Ro recipient rats.
TABLE 1. Pre-transplant renal function of recipients and control groups of WAG/Ro rats, and of unilaterally nephrectomized BN donor rats

All results are means ± SD (n is the number of rats). TX, (Pre-operative) transplant recipients; NX, (pre-operative) unilaterally nephrectomized rats; C, intact control rats; BN, kidney donor rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt. (g)</th>
<th>GFR (ml/min)</th>
<th>ERPF (ml/min)</th>
<th>Ratio GFR/ERPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX (9)</td>
<td>245 ± 19</td>
<td>2.00 ± 0.10</td>
<td>5.09 ± 0.30</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>NX (7)</td>
<td>243 ± 16</td>
<td>2.09 ± 0.26</td>
<td>5.14 ± 0.61</td>
<td>0.41 ± 0.04</td>
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<tr>
<td>Control (6)</td>
<td>246 ± 21</td>
<td>1.97 ± 0.24</td>
<td>4.93 ± 0.35</td>
<td>0.40 ± 0.04</td>
</tr>
<tr>
<td>BN (9)</td>
<td>283 ± 22</td>
<td>1.18 ± 0.29</td>
<td>3.67 ± 0.82</td>
<td>0.31 ± 0.04</td>
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**Systolic blood pressure**

Fig. 1 shows the mean systolic blood pressure of the transplant recipients, the NX and the control WAG/Ro rats from 1 week before transplantation up to 4 weeks post-transplantation. The mean systolic blood pressure of the transplant recipients was not significantly different from that of NX and control rats before or during the first week after transplantation. However, from day 8 onwards, the pressure started to increase in the recipients. A significantly elevated mean systolic pressure in rats with a kidney transplant was present as from day 11. The mean systolic blood pressure of normal BN rats under pentobarbital anaesthesia was 128 ± 7 mmHg, not significantly different from that of WAG/Ro rats, which was 134 ± 18 mmHg under the same conditions.

**Renal function**

The GFR and the ERPF, measured 1 and 3 weeks after transplantation, are given in Table 2. The WAG/Ro rats with an allogeneic BN kidney
TABLE 2. Post-transplant measurements of renal function, plasma renin concentration and systolic blood pressure in allogeneic kidney transplant recipients and control groups

All results are means ± SD (n is the number of rats). TX, Transplant recipients; NX, unilaterally nephrectomized rats; C, control rats. Significance: * P < 0.05 of TX with NX and C; ** P < 0.05 of NX with C; † no significant differences between TX, NX and C.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Body wt. (g)</th>
<th>GFR (ml/min)</th>
<th>ERPF (ml/min)</th>
<th>GFR/ERPF</th>
<th>P_Cr (µmol/l)</th>
<th>P_Ur (mmol/l)</th>
<th>PRC (ng of ANG I h⁻¹ ml⁻¹)</th>
<th>Systolic BP (mmHg)</th>
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<td>Week 1</td>
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<tr>
<td>TX (9)</td>
<td>225 ± 12*</td>
<td>0.39 ± 0.08*</td>
<td>1.95 ± 0.45*</td>
<td>0.20 ± 0.02*</td>
<td>117 ± 25*</td>
<td>22 ± 4*</td>
<td>21.5 ± 5.6*</td>
<td>148 ± 24†</td>
</tr>
<tr>
<td>NX (7)</td>
<td>239 ± 9</td>
<td>1.16 ± 0.10**</td>
<td>3.41 ± 0.22**</td>
<td>0.33 ± 0.02**</td>
<td>53 ± 3**</td>
<td>9.4 ± 0.7</td>
<td>69.0 ± 11.4</td>
<td>129 ± 10</td>
</tr>
<tr>
<td>Control (6)</td>
<td>252 ± 15</td>
<td>1.88 ± 0.13</td>
<td>4.60 ± 0.32</td>
<td>0.41 ± 0.04</td>
<td>43 ± 4</td>
<td>8.6 ± 1.1</td>
<td>60.8 ± 18.7</td>
<td>131 ± 10</td>
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<tr>
<td>Week 3</td>
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<tr>
<td>TX (9)</td>
<td>224 ± 22*</td>
<td>0.46 ± 0.17*</td>
<td>2.75 ± 0.86*</td>
<td>0.17 ± 0.02*</td>
<td>118 ± 33*</td>
<td>29 ± 12*</td>
<td>18.3 ± 2.9*</td>
<td>175 ± 26*</td>
</tr>
<tr>
<td>NX (7)</td>
<td>259 ± 14</td>
<td>1.48 ± 0.13**</td>
<td>4.04 ± 0.48**</td>
<td>0.37 ± 0.02**</td>
<td>61 ± 7**</td>
<td>9.9 ± 0.4**</td>
<td>54.2 ± 7.2</td>
<td>124 ± 11</td>
</tr>
<tr>
<td>Control (6)</td>
<td>268 ± 20</td>
<td>2.34 ± 0.32</td>
<td>5.07 ± 0.14</td>
<td>0.44 ± 0.02</td>
<td>46 ± 2</td>
<td>8.1 ± 0.3</td>
<td>65.2 ± 13.9</td>
<td>128 ± 10</td>
</tr>
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</table>

graft had lower GFR and ERPF values than NX and control rats. However, the reduction in GFR was more pronounced than the reduction in the ERPF, leading to a reduction in the filtration fraction (GFR/ERPF ratio). The P_Cr and P_Ur of transplant recipients were significantly higher than those of NX and control rats (Table 2).

Although there was a significant increase in the ERPF of the transplanted kidneys between weeks 1 and 3, the improvement of the GFR was not very marked. At 3 weeks after transplantation the GFR amounted to 30% of NX, and to 20% of controls, whereas the ERPF amounted to 68% of NX and 54% of controls.

Histopathology

Allotransplanted kidneys taken from immunologically enhanced recipients showed marked alterations when compared with BN control kidneys. As examples, micrographs of kidney sections taken 4, 8 and 20 days after transplantation and those of a control BN kidney are given in Fig. 2 (a-d).

On day 4, the glomeruli showed some swelling of the visceral epithelium. Prominent perivascular infiltrates of mononuclear cells were noted. The vascular walls as such were not affected. The tubular cells showed vacuolization.

On day 8, the glomerular changes consisted of an increment in the cellularity and the matrix of the mesangium. Furthermore, the glomeruli showed lobulation. An increase in the interstitial infiltrate was also noted.

On day 20, most of the glomeruli showed severe pathological changes with lobulation, capillary loop necrosis and extra-capillary proliferation. Furthermore a dense interstitial infiltrate was noted, as well as vasculitis and necrosis of vascular walls.

Plasma renin concentration

The plasma obtained from the WAG/Ro rats with a kidney transplant, after 15 min of pentobarbital anaesthesia, had significantly lower PRC values than that of NX and control rats. Despite a significant increase in the systolic blood pressure, there was no difference in the PRC when this was measured at 1 and 3 weeks post-transplantation. In order to determine whether the low PRC values were intrinsic to BN kidneys, the PRC was also measured in unilaterally nephrectomized BN rats. After 15 min of pentobarbital anaesthesia, the PRC of these rats amounted to 73 ± 29 ng of ANG I h⁻¹ ml⁻¹. The PRC of the same rats, determined 1 week later without anaesthesia, from blood obtained after decapitation, was 32 ± 7 ng of ANG I h⁻¹ ml⁻¹.

Discussion

Hypertension was noted to occur after allogenic kidney transplantation between two inbred normotensive rat strains. The systolic blood pressure started to rise during the second week after transplantation and remained high for the duration of the study, which was completed 28 days after transplantation. In a previous study we have established that hypertension does not occur after isogeneic transplantation within the same strain of rats as those we used as recipients in the present study [8]. That finding indicated that the mere presence of a transplanted kidney, or the trans-
FIG. 2. Micrographs from sections of a normal BN kidney (a) and of kidneys allo-
grafted into immunologically enhanced WAG/Ro recipients, removed on days 4 (b),
8 (c) and 20 (d) after transplantation. (Haematoxylin–eosin, x500).
planted as such, did not cause a rise in blood pressure.

Untreated WAG/Ro recipients of BN kidneys usually die within 2 weeks after transplantation [13]. In the present study, active immunological enhancement achieved with a single intravenous injection of donor strain whole blood [14] was successful in prolonging allograft survival in nine out of 15 rats. In this way the use of corticosteroids or other immunosuppressive agents could be avoided. Consequently, the hypertension we observed could not be due to the effect of drugs.

Despite the prolonged survival, immunological reactions to the transplanted kidney were not entirely suppressed. Marked histopathological changes were seen in the allografted kidneys during the first 3 weeks after transplantation. Measurements of renal function also indicate extensive damage. At 3 weeks after isogeneic kidney transplantation (WAG/Ro to WAG/Ro), the GFR amounted to 80–85% [8], and the ERPF to 90–95% (A. P. Provoost & M. H. De Keijzer, unpublished work) of that of single, hyperfunctioning kidneys after unilateral NX. In the isogeneic BN to WAG/Ro combination, these values were 35–40% and 80–85% respectively for the GFR and the ERPF. This would indicate that the process of glomerular filtration is markedly impaired after isogeneic transplantation in this model. The functional impairment, together with the histological changes, resembles the picture of experimental glomerulonephritis [15, 16]. Consequently, the increase in systolic blood pressure observed in the transplant recipients may have a nephritic nature, associated with sodium retention [16, 17].

Determination of the PRC in the transplant recipients revealed no increase, whereas there was a considerable rise in blood pressure. The PRC of transplant recipients was even significantly lower than that of control rats. The PRC was measured in plasma obtained after 15 min of pentobarbital anaesthesia, which is known to stimulate renin release [18]. Unilaterally nephrectomized BN rats showed a normal increase in the PRC during pentobarbital anaesthesia. One might argue that in denervated transplanted BN kidneys renin release was not stimulated by pentobarbital. However, the fact that the transplanted kidneys are denervated cannot play an important part, since we have found that the pentobarbital-stimulated renin release was independent of an intact sympathetic nervous system [18]. Furthermore, the PRC of transplant recipients was even lower than that of unstimulated values, obtained after decapitation in unilaterally nephrectomized BN rats.

It is a well-known fact that the PRC is reduced during high sodium intake or after sodium reten-
tion. The release of renin from the kidney was found to be inversely related to the sodium load sensed by the macula densa cells in the distal tubule [19]. Consequently, we think that the reduced PRC observed in the transplant recipients might well be the result of the retention of sodium and an expansion of the extracellular fluid volume.

In conclusion, the hypertension observed in this particular model, after renal transplantation in rats, might be mediated by sodium retention as a result of immunological alterations in the kidney. Further studies are being carried out to test this hypothesis.

Acknowledgments

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