HYPERTENSION AFTER ALLOGENEIC KIDNEY TRANSPLANTATION IN THE RAT

HYPERTENSIE NA ALLOGENE NIERTRANSPLANTATIE BIJ DE RAT

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CHAPTER 1

GENERAL INTRODUCTION

1.1 PREFACE

With the use of cadaver donors, the number of kidney transplantations is growing rapidly. The total number for the whole world is estimated at over 100.000. Half of these transplantations are performed in Europe. For the Netherlands the total number of renal transplantations carried out until the end of 1985 amounts to 3.000. Out of these 3.000, nearly 200 involved children under 15 years of age.

In the unnatural situation of allogeneic kidney transplantation, where surgeons are attempting to contravene nature by inserting foreign proteins into an unwilling host, unusual compensatory host mechanisms may take place. One of these mechanisms is the development of post-renal transplantation hypertension.

Although a successful renal allograft may cure hypertension, it is now recognized that there is an alarmlingly high incidence of hypertension in transplanted patients. In this situation hypertension poses diagnostic and therapeutic problems in many patients. A variety of factors may be responsible for the genesis of blood pressure elevation in the post-transplantation period, but an analysis of the importance of the various factors is very problematic in humans.

The aim of the present experiments was to develop a model in rats in which post-transplantation hypertension could be studied. This model was used to reveal the mechanisms causing the hypertension after kidney transplantation in the rat and then to find ways to prevent this post-transplantation hypertension in rats.

GENERAL INTRODUCTION

1.2 HISTORICAL OVERVIEW

A number of problems had to be overcome to allow successful transplantation of organs. These problems can be divided roughly into two distinct sets. The first set comprises the surgical and technical problems of the transplantation procedure as such. After half a century of continuous research most of these have been solved. Conservation of the normal structure and function of the grafted organ is now secured by protocols for procurement, preparation, storage and surgical relocation of the graft into the living organism. The second set of problems in transplantation combines the process of rejection. An allograft, i.e. an organ exchange between genetically different individuals of one species, is not accepted in the recipient and rejected within one to two weeks. With xenografts, i.e. organs exchanged between individuals of different species, an even more rapid rejection may take place. On the other hand, there is the ideal situation of isografts where the organ is exchanged between genetically identical individuals and survival is indefinite. These two sets of problems involving transplantation procedure and rejection have been studied extensively in laboratory animals and no aspect of medical research has benefitted as much from experimental investigation as the field of transplantation research.

The first experimental success came in the early 1900's when Jaboulay (1906) anastomosed kidneys from a pig and a goat respectively to an artery and a vein in the arms of two patients. After one hour of diuresis the grafts ceased to function and were removed. Unger (1910) placed both kidneys from a monkey in the groin of a uremic patient. As the desiribality of genetic similarity between donor and recipient was already recognized at that time, it was no surprise to Unger that this transplant did not function. Carrel (1912), having perfected the surgical techniques for vascular anastomoses, showed that kidney autografts in dogs survived for a long time, whereas allografts transplanted by the same technique functioned for only a few days. This transplantation model was confirmed by the Boston group and on the basis of this proven laboratory model, the first successful renal transplantation in a human identical twin was carried out (Murray et al., 1955; Merril et al., 1956). The recipient obtained normal renal function and control of hypertension was achieved.

This first successful human transplantation led to new experiments to treat rejection in an effort to extend allogeneic transplantation to humans. Two laboratory methods of achieving skin allograft survival were adapted to potential recipients: acquired immunologic tolerance (Billingham et al., 1953) and x-irradiated marrow infused tolerance (Main and Prehn, 1955), unfortunately without success. In 1959 Schwartz and coworkers discovered that 6-mercaptopurine posessed specific immunodepressive properties and this new

drug was applied by Calne (1960) and by Zukovski and coworkers (1960) to canine renal transplants with good results. Two years later, the first 6-mercaptopurine-treated human recipient of a cadaver donor kidney survived for over a year (Murray et al., 1962). Corticosteroids were then introduced as the major immunosuppressive drugs (Goodwin et al., 1962) and combined with azathioprine, an analogue of 6-mercaptopurine. These drugs remained the basis of immunosuppression for the next 20 years.

Other points of progress in kidney transplantation were generated by the development of tissue typing and matching (Terasaki et al., 1966) and methods of kidneys preservation, which can now be maintained for up to three days. For practical reasons, kidneys are currently used within 24-36 hours after harvesting (Belzer et al., 1972). Other, ancillary methods of immunosuppression were studied. Localized x-ray therapy over the transplant seemed to prolong survival, while splenectomy, thymectomy and extracorporeal x-irradiation of circulating blood was also tried. The use of anti-lymphocyte serum and globulin as an immunosuppressive agent was developed by Waksman (1961) and Woodruff (1963) and their coworkers, while drainage of lymphocytes by thoracic duct cannulation was introduced by Franksson (1964).

Finally, in the late 1970's Cyclosporin A (CsA), a cyclic endecapeptide has gained interest as an addition to the existing immunosuppressive drugs. Its potent immunosuppressive activity has been shown in a number of models, e.g. the prolonged survival of kidney, pancreas, skin and heart

transplants in rats (Homan et al., 1980; Garvey et al., 1980; White et al., 1980; Kostakis et al., 1977). Clinical studies have proved that CsA is also immunosuppressive in humans (Calne et al., 1981; Starzl et al., 1981). Major side effects of CsA in man are its nephrotoxicity and its hepatotoxicity. Apart from being reversible, these effects can be contained with a reduction in dosage (Calne et al., 1978). Extensive clinical trials are carried out to study the properties of CsA compared to the conventional immunosuppressive drugs.

1.3 HYPERTENSION AFTER RENAL TRANSPLANTATION IN MAN

As mentioned above, human kidney transplantation is still in a developmental stage and although the techniques have improved considerably with time, optimal immunosuppression is not available yet. Consequently, a number of side effects of the immunosuppressive drugs have to be accepted. Examples are cancer transferred to recipients with allografts (Wilson et al.,1968) or the development of de novo cancer in immuno-suppressed patients (Penn et al., 1979; Calne et al.,1979). Infections with relatively non-pathogenic viruses, fungi and protozoa are a direct sequelae of immunosuppression too. Not a direct consequence, but strongly related to kidney transplantation and immunosuppression is the development of aseptic bone necrosis (Griffith et al., 1974), hyperlipidemia (Saldanha et al., 1976), cataracts (Wilson et al., 1979), growth retardation in children, (McEnergy, 1973) and hyper-

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tension.

The role of renal disease in the pathogenesis of hyper-
tension is well-known and has been studied extensively. For
example, hypertension is one of the most common complications
of end-stage renal failure (ESRF). It is present in 80% to 90%
of patients with irreversible uremia (DelGreco et al., 1975;
Lazarus et al., 1975), adding to the morbidity and mortality
of patients who receive maintenance haemodialysis. A
functioning renal allograft in a hypertensive ESRF patient may
result in blood pressure control by excreting excess salt and
water and by suppressing the release of renal pressor
substance(s) secreted by the recipient's own diseased kidneys.
Although the anti-hypertensive effects of renal
transplantation are well documented (Kolff et al., 1964;
Ducrot et al., 1965), the development or persistence of hyper-
tension have been noted to occur in an alarmingly high
proportion of patients who have undergone renal
transplantation.

1.4 PREVALENCE OF POST-TRANSPLANTATION HYPERTENSION

Adult recipients

In the literature there is a wide divergence concerning the
prevalence of post-transplantation hypertension in adult renal
recipients as can be seen in TABLE 1.

	% hypertensive	no of patients
Papadimitriou et al., 1969	29	24
Smellie et al., 1969	18	67
Coles et al., 1972	52	24
Cohen, 1973	51	81
Sampson et al., 1973	70	24
Grunfeld et al., 1975	73	41
Bachy et al., 1976	56	85
Curtis et al.,1976	24	74
Rao et al.,1977	49	164
Jacquot et al., 1978	20	50
Klarskov et al., 1979	50	79
Pollini et al., 1979	54	98
Whelton et al., 1979	47	93

TABLE 1. Prevalence of post-transplantation hypertension in adults. Criteria for hypertension differ in the various studies listed above.

The results of the various studies listed in the table can not be compared due to divergence in study populations, timing of hypertensive evaluation, immunosuppressive treatment, and peri-operative care. Apart from that, the definition of post-transplantation hypertension is used inconsistently by the various authors. Some include all cases that have once been hypertensive after transplantation, while others include only cases that become or remain hypertensive at a later stable phase after transplantation or include only those patients who have been given anti-hypertensive treatment.

The incidence of hypertension tends to vary with time following renal transplantation. In the 85 recipients reported by Bachy et al.(1976), the proportion of patients with hypertension rose during the first three months, stabilized around 50% to 60% for up to five years after transplantation and then tended to decrease slightly. In this study a third of the initially hypertensive patients became normotensive, whereas

one third of the initially normotensive patients became hypertensive. Studies carried out by Rao et al. (1977) also showed that a significant number of hypertensive patients became normotensive after transplantation. According to some authors, the incidence of post-transplantation hypertension may vary with the donor source of the transplant. Nerstrom et al. (1972) recorded an incidence of 36% in live-related donor kidney recipients, whereas Coles et al. (1972) observed an incidence of 83% in recipients of cadaver donor kidneys. Pollini and coworkers (1979), however, found no difference in the incidence of hypertension between cadaver kidney recipients and live, related kidney recipients. Whelton et al. (1979) found the incidence of hypertension to be significantly higher in cadaver graft recipients. This higher incidence might also be related to the renal function in cadaver recipients, which is generally considered to be much poorer than renal function in live, related donor kidney recipients.

Paediatric recipients

In juvenile kidney recipients, likewise, hypertension is a significant problem, but there are some differences in comparison with adult recipients. In the immediate post-operative period, acute hypertension is reported to occur in most paediatric transplant recipients, but in few adults. In one large series of 137 juvenile recipients the incidence of immediate post-transplantation hypertension was noted to be as

high as 98% (Ingelfinger, 1981). This high incidence has been confirmed by other investigators (Gonzalez et al., 1970; Najarian et al., 1971; Fine et al., 1970; Arbus et al., 1980). Almost all cases seem to be related to a disproportionate positive fluid balance. Once the volume expansion had been controlled, the blood pressure often returned to normal values (Potter et al., 1970). In the Sophia Children's Hospital 50% of the recipients required anti-hypertensive medication during the first half year after transplantation, but only 15 % of all patients needed anti-hypertensive treatment for more than two years (Nauta et al., 1986).

After the initial post-operative period the percentages of post-transplantation hypertension in juvenile patient series show as much variation as in the adult series (TABLE 2).

	% hypertensive	no of patients
Williams et al., 1969	50	14
La Plante et al., 1970	25	8
Gonzalez et al., 1970	10	19
Fine et al., 1970	4	45
Najarian et al., 1971	34	44
Lilly et al., 1971	38	42
Cerilli et al., 1972	19	31
Ingelfinger et al., 1975	86	49
Malekzadeh et al., 1975	9	77
Henriksson et al., 1975	25	16
Ingelfinger et al., 1981	93	134
Tejani, 1983	87	75
Broyer, 1985	59	197

TABLE 2. Prevalence of post-transplantation hypertension in juvenile recipients after the initial post-operative period.

A clear difference between adult and juvenile recipients is the relative mass of the kidney transplant itself, as most

children receive transplants from adult donors. Apart from other factors that will be discussed later, the lower cardiac output and arterial blood pressure might stimulate large kidneys to produce extra renin or, conversely, the large kidney might alter the cardiac output and/or the extracellular volume causing hypertension. It is also feasible that, due to the presence of the large grafted kidney, the perfusion of the native kidney(s) decreases, triggering the production of extra renin (Linas et al., 1978). To study this hypothesis, Caldicott and Ingelfinger (1981) use newborn puppies and measured the effects of increasing relative renal mass on cardiovascular and renal function. They found that blood pressure dropped after a large kidney had been implanted, remaining lower than before transplantation. Cardiac output did not change significantly in these animals, nor did the plasma volume. These experiments suggest that renal mass as such does not seem to explain the high frequency of post-transplantation hypertension in children.

1.5 POSSIBLE MECHANISMS OF HYPERTENSION IN TRANSPLANTED PATIENTS

The crucial role of the kidney in blood pressure regulation has been emphasized by the systems analysis approach used by Guyton and coworkers (1974). The kidney normally controls body fluid volumes while it also produces renin which, by generating angiotensin II, may increase peripheral vascular resistance. The kidney may also have an anti-hypertensive function through the production of prosta-

glandins from medullary interstitial cells, while it may well secrete other anti-hypertensive substances. Disturbed renal function can therefore contribute to a rise in blood pressure in a number of ways. The allografted kidney might be disturbed in its function by more than one factor. An analysis of the relative importance of the various factors involved in post-transplantation hypertension is complicated. Despite the large number of clinical kidney transplantations performed each year, every one of these patients presents a singular clinical picture. Some of the minor factors contributing to a rise in blood pressure deserve a mere mention only, while the major ones , falling within the context of this thesis, will be discussed extensively.

Possible explanations for the hypertension observed in transplanted patients include expansion of the extracellular fluid volume (Horvath et al., 1976), extrinsic compression of the graft (Cromie et al., 1976), polycythaemia (Swales and Evans, 1969), transplantation of a kidney from a hypertensive donor (Hume et al., 1966) and even transplantation of a kidney from a donor with a family history of hypertension (Bianchi et al.,1974; Guidi et al.,1982). Other mechanisms proposed are ischaemic damage to the graft during transplantation (Roguska et al., 1971), hypercalcaemia (Weidmann et al., 1977), peri-renal fibrosis (Faarup et al., 1973) or recurrent glomerulo-nephritis (Cameron and Turner, 1977).

The major factors involved in the genesis of post-transplantation hypertension are thought to include: the presence of the recipient's own kidneys, rejection, cortico-

steroids and renal artery stenosis. In one way or another, they are all linked to post-transplantation hypertension by the renin-angiotensin system.

1.6 PLASMA RENIN LEVELS IN POST-TRANSPLANTATION HYPERTENSION

The basal plasma renin levels in hypertensive transplant recipients with a good and stable graft function are reported to be either normal (Grunfeld et al., 1975; West et al., 1969; Sampson et al., 1972) or elevated (Beckerhoff et al., 1974; Verniory et al., 1972; McDonald et al., 1974). No correlation was found between the basal renin levels and the occurence of post-transplantation hypertension. Interpretation of these studies is questionable since several non-quantitative, variable factors are involved: the use of anti-hypertensive drugs and/or diuretics, the renin release from the patients' own kidneys and the salt intake by the recipient. In the absence of these variable factors, Kornerup (1977) found that the basal renin level is normal in hypertensive patients with stable graft function.

Transplanted patients probably differ in the way they react to postural changes, i.e. no significant rise in renin levels was found after orthostasis (Pedersen and Kornerup, 1976). The sympathic denervation of the kidney is a possible explanation, while this might also explain the cessation of the normal diurnal variation in plasma renin levels of renal transplant recipients (Armbruster et al., 1975). On the other

hand, it seems that the response of the plasma renin to changes in sodium intake is normal in patients with post-renal transplantation hypertension (Horvath et al., 1976; Keogh et al., 1976).

1.7 ROLE OF THE RECIPIENT'S OWN DISEASED KIDNEYS IN POST-TRANSPLANTATION HYPERTENSION

Several authors have found that the host kidneys are important in the development of hypertension following renal transplantation (Coles et al., 1972; Cohen, 1973; Ball et al., 1974). It seems likely that the diseased kidneys contribute to the development of hypertension by the hypersecretion of renin. This is in accordance with the observation of a significantly lower plasma concentration of renin in transplant recipients subjected to bilateral nephrectomy as compared with recipients retaining their own diseased kidneys (McHugh et al., 1980). The importance of renin secreted by the host kidneys in hypertension after transplantation concurs with the observations on the aetiology of hypertension at haemodialysis and the response to bilateral nephrectomy (Wilkinson et al., 1970).

There is probably a dual mechanism that causes hypertension in renal transplant recipients secondary to overproduction of renin by the own diseased kidneys. The hypotensive response to saralasin (a competitive inhibitor of angiotensin II) as observed in many hypertensive recipients, suggests a direct vasoconstrictor action of angiotensin II. In

addition, angiotensin II may contribute to the hypertension by causing sodium retention through stimulation of the aldosterone secretion (Brown et al., 1979). Urinary aldosterone excretion was noted to be high in hypertensive transplant recipients, while there was a significant increase in exchangeable sodium in these patients, not explained by their dietary sodium intake (McHugh et al., 1980).

1.8 REJECTION AND HYPERTENSION

Acute rejection

Acute rejection may occur within the first week of transplantation or, more usually, within the first month, while episodes of acute rejection crises may develop many months after transplantation. Prominent morphological features of the acute rejection process include interstitial cell infiltration, interstitial edema, changes in the small blood vessels and tubular changes. Glomerular lesions are less common. Although hypertension is said to be an inconstant marker of acute rejection (Coles et al., 1972) most researchers have found elevated plasma renin levels during the course of acute rejection (Beckerhoff et al., 1974; Blaufox et al., 1966; Gunnels et al., 1966; McDonald et al., 1974; Verniory et al., 1972). However, the relationship between plasma renin and hypertension in connection with acute allograft rejection is unclear. Some studies found a closely positive correlation between plasma renin and arterial blood

pressure, while others have found a weak correlation or none at all. Kornerup (1979) demonstrated that the plasma renin pattern was characterized by a moderately elevated plasma renin concentration at the start of the acute renal allograft reaction, after which the renin concentration normalized in the course of the reaction. This drop in plasma renin concentration was accompanied by an increase in body weight, which suggests that the increased renin release from the renal transplant may be involved in the initial phase of the development of hypertension in connection with acute renal allograft rejection, whereas salt and water retention may be responsible at a later stage.

Chronic rejection

Chronic rejection of kidney allotransplants becomes manifest months or even years after grafting. Morphologically, the chronic rejection process is characterized mainly by obliterative vascular changes and a wide variety of glomerular alterations. Chronic rejection is commonly associated with a rise in blood pressure (Ducrot et al., 1965; Bachy et al., 1976). Roguska and coworkers (1971) and Chrysant and coworkers (1974) found elevated plasma renin levels in chronic renal allograft rejection. In the latter study, blood pressure was closely related to the plasma renin activity, whereas this correlation was weak in the former study. Grunfeld and coworkers (1975) found normal renin secretion rates from the kidney grafts in post-renal transplantation hypertension in

connection with chronic renal allograft rejection.

The different outcomes of these studies are not surprising considering the many factors which may effect the renin system. Increased renin release from the recipients' own kidneys was not excluded as a possible reason for the elevated plasma renin levels. Furthermore, differences in the character of the allograft rejection reaction must be expected to depress renal function and to affect the transplant's juxtaglomerular apparatus in different ways, contributing to either stimulated or depressed renin release.

1.9 TRANSPLANT RENAL ARTERY STENOSIS AND HYPERTENSION

Renal artery stenosis is a well-known complication of renal transplantation (Vidne et al., 1976) which may contribute to post-transplantation hypertension. The quoted incidence of transplant renal artery stenosis varies from 1% to 10%. Bachy and coworkers (1976) studied 14 arteriograms in 11 out of 89 recipients and found a stenosis in 5 patients. Rao and coworkers (1978) noticed stenosis in 11 out of 15 patients who underwent angiography because of severe hypertension, an incidence of 4% of all allograft recipients. In children a similar incidence of 4% has been reported by the Boston group (Ingelfinger et al. 1981).

These figures, however, underestimate the actual incidence of renal artery stenosis in transplant recipients. Renal angiography is usually performed only in patients with severe hypertension or an unexplained deterioration of graft

function. Lacombe (1975) found that in 100 consecutive transplant patients routine arteriograms demonstrated stenosis in 25% of the cases. This 25% incidence in the transplant population was confirmed by Grunfeld and coworkers (1975). That group also reported that the frequency of graft artery stenosis rose to 33% in transplant recipients with hypertension. In another study, Bennett and coworkers (1974) found narrowing of the graft artery in 31% of the recipients with hypertension.

The success of reconstructive surgery in controlling hypertension varies from 33% to 100% in the different studies (Lacombe, 1975; Grunfeld et al., 1975; Goldman et al., 1975; Nerstrom et al., 1972). Procedures aimed at correcting the hypoperfusion may lead to severe complications such as loss of the graft and death of the patient, while recurrence of the stenosis and hypertension was very common in some studies (Lee et al., 1972; Dayle et al., 1975). An association between artery stenosis and hypertension does not always appear to be causal. In a large series of 367 patients who underwent angiography, 22 (6%) were found to have stenosis, but only 13 of them were hypertensive (Henriksson et al., 1975). Not even a correlation between plasma renin and hypertension in transplanted patients with graft artery stenosis could be established. Under standardized research conditions, Kornerup and coworkers (1977) demonstrated elevated basal values of the plasma renin concentration in only 3 of 9 bilaterally nephrectomized recipients with post-transplantation hypertension and stenosis of the renal graft artery. No

differences were found in plasma renin concentration, comparing hypertensive patients with transplant artery stenosis and normotensive recipients examined on either low or high sodium intake. These results are in agreement with studies carried out by other groups (Bennett et al., 1974; Grunfeld et al., 1975; Henriksson et al., 1976). In summary, the renin system's activity may be elevated but is generally normal in recipients with post-renal transplantation hypertension and stenosis of the renal graft artery.

1.10 CORTICOSTEROIDS AND HYPERTENSION

From studies of patients with systemic lupus erythematosus, rheumatoid arthritis and other chronic diseases, it is known that administration of glucocorticoids is implicated in the development of hypertension (Bulkley and Roberts, 1975). Several authors believe that corticosteroids are important in the genesis of post-transplantation hypertension (Lacombe, 1975; Starzl et al., 1964). Popovtzer and coworkers (1973) found a correlation between diastolic blood pressure and the dose of prednisone in a group of renal recipients. Other authors, however, were unable to demonstrate an association between the administration of corticosteroids and post-transplantation hypertension (Bachy et al., 1975; Coles et al., 1972; Cohen, 1973). In yet another report of a series of hypertensive renal transplant recipients, a normal blood pressure was noted to prevail as time elapsed and the dose of corticosteroids was reduced (Starzl et al., 1964).

Furthermore, Curtis and coworkers (1976) noted an incidence of hypertension in only 24% of their transplant population treated with alternate-day steroid therapy, contrasting with a much higher frequency reported by other centers where patients received steroids daily. Sampson and Albert (1973) found that after conversion to alternate-day therapy, all renal transplant recipients had a fall in blood pressure and those previously requiring anti-hypertensive drugs no longer needed them. All these studies implied a possible cause and effect relationship between the level of blood pressure and the dose of steroids.

Several mechanisms have been proposed to explain the hypertensive effects of steroids. In a group of renal allograft recipients an elevated aldosterone secretion was found in spite of normal plasma activity (Sampson et al., 1972). Resolution of hypertension was observed with conversion of daily prednisone therapy to alternate-day methyl-prednisolone therapy and this was associated with a significant reduction in the aldosterone secretion rate (Sampson and Albert, 1973). Sampson and coworkers (1973) also reported a prompt decrease in blood pressure when the hyper-tensive recipients were treated with the aldosterone antagonist spironolactone. The conclusion of this study was that prednisone was metabolized to aldosterone, causing a syndrome of mineralocorticoid excess and subsequent development of hypertension. Experimental animal studies subsequently suggested that the redistribution of body fluid volumes (Haack et al., 1977) and increased activity of the

renin-angiotensin system is due to an increase of renin substrate production (Krakoff et al., 1975). The latter study also indicated that glucocorticoids are able to potentiate the vasoconstrictor action of angiotensin II. Finally, mineralocorticoids are known to cause sodium retention (Gomez-Sanchez et al., 1970; Marcus et al., 1952).

1.11 THE PRESENT STUDY

In the present study rats were chosen as experimental animals for a number of reasons. Nowadays, the rat is the most generally used animal in hypertension and transplantation research. The use of inbred rat strains enables large series of allogeneic transplantations in which rejection is a reproducible phenomenon, while isogeneic kidney transplantation may serve as control since rejection will not occur. Moreover, rats are relatively cheap and easy to maintain. A disadvantage of renal transplantation in the rat is the difficulty of the operation, causing a number of technical failures.

Many reports have demonstrated the usefulness of rats for renal transplantation studies. The chronic and acute rejection patterns after transplantation between two inbred rat strains were studied extensively (Guttmann et al., 1967; White et al., 1969; Ippolito et al., 1972). Immunosuppressive properties of different agents were studied in allogeneic kidney transplantation (Tinbergen, 1968; Guttmann et al., 1969). The role of

the kidney in the pathogenesis of hypertension was studied with the aid of isogeneic kidney cross-transplantation between spontaneously hypertensive rats and their normotensive counterparts (Bianchi et al., 1974; Dahl and Heine, 1975; Kawabe et al., 1969). The effects of aging upon functional and cellular aspects of kidney transplants have also been studied in the rat (van Bezooijen et al., 1974). Finally, a method has been developed to determine the glomerular filtration rate in rats, based on a report by Layzell and Miller (1975). This method allows for repeated measurements in the same animal (Provoost et al., 1983). Assessment of the function of the grafted kidney in experimental renal transplantation has received little attention to date, this method provided an ideal opportunity to study the functional changes after transplantation in rats.

As mentioned before, the pathogenesis of post-transplantation hypertension can be divided into those related to the donor, those associated with the recipient in combination with the transplantation procedure itself and those uniquely related to the post-transplantation course. The complex interrelationships of these factors make it very difficult to analyze precisely the etiology of hypertension after renal transplantation. We chose to start with an isogeneic transplantation model in which rejection and the use of immunosuppressive drugs is not present. In this rat model we would be able to investigate the effects of the transplantation procedure itself. After that investigation a new feature would be included in the transplantation model,

namely the effect of rejection by transplanting a renal allograft from an antigenically disparate source. The rat donor rat recipient combination used by us allows for prolonged allograft survival with the aid of donor strain blood transfusions (Marquet, 1978). Consequently, at this stage the use of other immunomodulating drugs could still be avoided. To mimic the clinical situation, the next step was the introduction of different immunosuppressive drugs and the evaluation of the effects of these drugs on the development of post-transplantation hypertension. Finally, we tried to define the role of sodium in the genesis of post-transplantation hypertension in the original model. This involved the use of a low-sodium diet, while in a separate metabolic experiment the renal allograft handling of electrolytes and water would be examined in more detail.

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CHAPTER 2

THE GLOMERULAR FILTRATION RATE OF ISOGENEICALLY
TRANSPLANTED RAT KIDNEYS

The glomerular filtration rate of isogeneically transplanted rat kidneys

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The glomerular filtration rate of isogeneically transplanted rat kidneys. The glomerular filtration rate (GFR) was determined in rats with an isogeneic kidney transplant and compared with that of unilaterally nephrectomized rats. Experiments were carried out in adult rats, 3 months of age, weighing approximately 300 g, as well as in juvenile rats, 6 to 8 weeks of age, weighing approximately 170 g. All donor kidneys were taken from adult rats. The GFR was measured regularly, using a chromium 51-EDTA clearance technique which permitted repeated measurements to be taken in the same animals, during a 15-week followup period. After unilateral nephrectomy the GFR per 100 g body weight (BW) increased compared with that of a single normal kidney. Adult transplant recipients had a GFR per 100 g BW of about 80% of that of unilaterally nephrectomized rats. There was no statistical difference in the GFR when comparing adult recipients of either a normal or a hyperfunctional kidney. When isografts were transplanted to juvenile recipients, there was an initial decrease in the absolute GFR compared with the donor value in the case of a normal adult kidney. This decrease was even more pronounced when a hyperfunctioning kidney was transplanted to a juvenile recipient. However, when related to BW the GFR was, as in the adult recipients, about 80% of that of unilaterally nephrectomized juvenile rats. During the followup period the systolic blood pressure was measured regularly by tail plethysmography, in order to detect any blood pressure elevations, which are a frequent complication in adult and pediatric human renal transplantation. However, no hypertension was observed after isogeneic kidney transplantation in the various groups. These results show that the GFR of isogeneically transplanted rat kidneys amounts to about 80% of the maximally attainable level. Isogeneic transplantation of an adult kidney to a juvenile recipient results in a rapid adaptation of the GFR to the smaller size of the body and does not cause an increase in blood pressure.

Débit de filtration glomérulaire des reins de rat en situation de transplantation isogénique. Le débit de filtration glomérulaire a été déterminé chez des rats ayant un transplant rénal isogénique et comparé à celui de rats ayant eu une néphrectomie unilatérale. Les expériences ont été conduites chez des rats adultes, agés de trois mois, dont le poids corporel était de 300 g et chez des rats jeunes, agés de six à huit semaines, dont le poids corporel était de 170 g. Tous les transplants provenaient de rats adultes. Le débit de filtration glomérulaire a été mesuré à intervalles réguliers pendant une période de 15 semaines au moyen de la clearance de ^{51}Cr-EDTA, cette technique permet des mesures répétées chez le même animal. Après néphrectomie unilatérale, le débit de filtration glomérulaire par 100 g de poids corporel augmente par comparaison avec celui d'un rein unique. Les receveurs adultes ont un débit de filtration glomérulaire par 100 g d'environ 80% celui des rats ayant subi une néphrectomie unilatérale. Il n'y a pas de différence statistiquement significative entre les débits de filtration glomérulaire des adultes ayant reçu un rein normal ou un rein hyperfonctionnant. Quand les isogreffes ont été transplantées à des receveurs jeunes, il a été observé une diminution initiale du débit de filtration glomérulaire absolue par comparaison avec la valeur obtenue chez le donneur dans le cas d'un donneur adulte normal. Cette diminution est encore plus importante quand un rein hyperfonctionnant est transplanté à un receveur jeune. Cependant, le débit de filtration glomérulaire rapporté au poids corporel est, comme chez les rats jeunes ayant subi une néphrectomie unilatérale. Durant la période d'observation la pression sanguine systolique a été mesurée régulièrement par plethysmographie de la queue, de façon à détecter une élévation de la pression artérielle, ce qui est une complication fréquente de la transplantation humaine aussi bien chez l'adulte que chez l'enfant. Cependant, il n'a pas été observé d'hypertension artérielle après la transplantation rénale isogénique dans les différents groupes. Ces résultats montrent que le débit de filtration glomérulaire des reins de rat transplantés de façon isogénique est d'environ 80% du niveau maximum qui peut être atteint. La transplantation isogénique d'un rein adulte à un receveur jeune a pour conséquence une adaptation rapide du débit de filtration glomérulaire à la plus petite taille corporelle. La transplantation rénale isogénique ne détermine pas d'augmentation de la pression sanguine.

Kidney transplantation in rats is one of the most frequently used models in organ transplantation research. Basic information concerning rejection and immunosuppression has been gathered. However, the function of the transplanted kidney has received much less attention in renal research. Apart from a number of recent studies in the early post-transplantation period [1–3], no measurements of the glomerular filtration rate (GFR) of transplanted kidneys have been reported. Followup studies of rat kidney transplant recipients, exceeding one week, mainly used plasma levels of urea and/or creatinine as indicators of renal function [4–7]. These parameters are not very well suited to determine a relatively small impairment of the GFR.

Recently, a method was developed to measure the GFR in rats, which permitted repeated measurements in the same animals [8]. This method was used to follow the changes in the GFR after isogeneic kidney transplantation of adult donor kidneys to adult and juvenile recipient rats. Isogeneic kidney transplantation circumvents kidney damage caused by rejection. Consequently, this model provides information about the degree of damage caused by the transplantation procedure.

Juvenile recipients were used, because in pediatric renal transplantation most donor kidneys stem from adults. In juvenile recipients it is important to know what level of renal function is to be expected. It remains to be seen whether or not the adult kidney continues to function as it does in the donor body or adapts to the immature size of the juvenile recipient.

The GFR of transplanted kidneys was compared with that of kidneys remaining after unilateral nephrectomy, that is, the maximally attainable level of a single intact kidney. Because it may be possible that the compensatory response is reduced in a transplanted kidney, compensatory hyperfunctioning kidneys were also used as donor kidneys.

Hypertension has been noted to be a frequent complication of human renal transplantation in adults [9, 10] as well as in children [11]. The occurrence of a rise in blood pressure has been studied after kidney transplantation between rat strains with various types of genetically mediated hypertension [12, 13]. However, no studies have been reported on the effects of kidney transplantation in normotensive rats, especially from adult donors to juvenile recipients, upon the post-transplant blood pressure. We therefore measured the systolic blood pressure regularly up to the end of the followup period.

Methods

The experiments were performed with male rats of a highly inbred (continuous brother-sister mating) Wistar strain (WAG/Ro) bred at the Central Animal Breeding Center of the Erasmus University, Rotterdam. This strain was obtained originally from TNO (Rijswijk, The Netherlands) as the WAG/Rij strain. Genetic homogeneity of this particular strain has been confirmed by the permanent survival of intrastrain skin-, heart-, and kidney grafts [14, 15].

The animals had food (rat chow AM II, Hope Farms, containing 90 mEq Na/kg) and water (containing 3 mEq Na/l) available ad libitum. The adult rats used were about 13 to 15 weeks old with a body weight (BW) of 275 to 325 g. The juvenile rats were 6 to 8 weeks old with a BW of 135 to 200 g at the time of transplantation.

Surgery

The microsurgical technique as described by Fisher and Lee [16] was used for heterotopic renal transplantation, with a slight modification of the ureter-bladder anastomosis. Only right donor kidneys were used for transplantation.

Donor kidney preparation. Under ether anesthesia the abdomen of the donor rat was opened; the right kidney together with its renal artery and vein was mobilized. The ureter was freed and transected near the bladder. After i.v. injection of heparin (50 I.U.), the animal was bled to death, and the kidney was removed from the body in such a way that elliptical cuffs of the aorta and the vena cava were left at the end of the renal vessels. The kidney was then kept at 4° C in a 0.9% sodium chloride solution.

Transplantation. Under ether anesthesia the abdomen of the recipient rat was opened and the aorta and the vena cava were clamped below the renal vessels with a Satinsky-like curved clamp. Small openings were made in the clamped part of the vessels to which the cuffs of the transplant vessels were anastomosed. End-to-side anastomoses were made by continuous suturing using 7-0 silk in the adult and 8-0 nylon in juvenile

recipients. After the completion of the suturing, recirculation was effected by removing the clamp. Bleeding was controlled by the gentle pressure of a cotton wool roll.

The total ischemic time varied between 30 and 40 min and did not differ in regard to transplantation of either adult or juvenile recipients.

After restoring the blood supply to the transplanted kidney, the ureter-bladder anastomosis was made. A small opening was made in the bladder fundus. The ureter tip was then drawn into the bladder through the opening. After securing the ureter to the low dorsal part of the bladder wall with one suture, the bladder opening was closed with a short continuous silk suture.

Bilateral nephrectomy of the recipient's own kidneys was performed under ether anesthesia with an abdominal approach, one week after the transplantation.

Unilateral nephrectomy. Because we transplanted only right kidneys, we removed the left kidney of the control rats. This nephrectomy was carried out under ether anesthesia through a midline incision in adult and juvenile rats. Thus the GFR of the transplanted right kidneys was then compared with the maximally attainable GFR, that is, that of hyperfunctioning single right kidneys in the control group.

Glomerular filtration rate

The GFR was measured under pentobarbital anesthesia (60 mg/kg i.p.), using an i.v. injection of chromium 51-EDTA and a single timed (60 min) blood sample [8, 17]. The GFR (clearance of chromium 51-EDTA) is then calculated from the formula:

$$GFR = \frac{V}{t} \cdot \ln P_o/P_t$$

where GFR = clearance (ml/min), V = distribution volume of chromium 51-EDTA (ml). P_o, P_t = plasma concentration of chromium 51-EDTA at time zero and t min (cpm/ml). P_t is derived from the plasma sample at t (60) min, P_o is calculated from $P_o = I/V$, where I is the injected amount of chromium 51-EDTA (cpm) and V the distribution volume.

From the clearance formula it is obvious that V plays an important role in the calculation of the GFR. The V is related to the BW of the rat, $V = v' \cdot BW$. In adult rats v' was reported to be 0.198 ± 0.012 (mean ± SD, $N = 5$) [17]. In juvenile rats, with a mean BW of 172 g, V was 0.225 ± 0.011 (mean ± SD, $N = 8$).

It may well be that v' changes in the different experimental situations. The effect of a change in the actual v' upon the calculated GFR is variable. When we substitute $V = v' \cdot BW$ and $t = 60$, and relate the GFR to 100 g BW, the clearance formula becomes:

GFR per 100 g BW = $1.667 \, v' \cdot \ln (I \div BW \cdot P_t) \cdot (1 \div v')$

Thus, v' is present as an augmenting as well as a depressing factor in the formula. The overall effect of a change in the actual v' from the one used to calculate the GFR (0.20 in the case of adult rats) is dependent upon the value of $I \div (BW \cdot P_t)$, that is, the actual GFR per 100 g BW.

Effects of changes in v' from 0.20 upon the calculated GFR per 100 g BW are depicted in Figure 1. In the normal GFR range (0.6 to 0.8 ml/min per 100 g BW) changes of up to 10% in the GFR will be the result of an increase in the actual v' with about 20% (0.20 to 0.24) or a decrease of about 15% (0.20 to 0.17). In
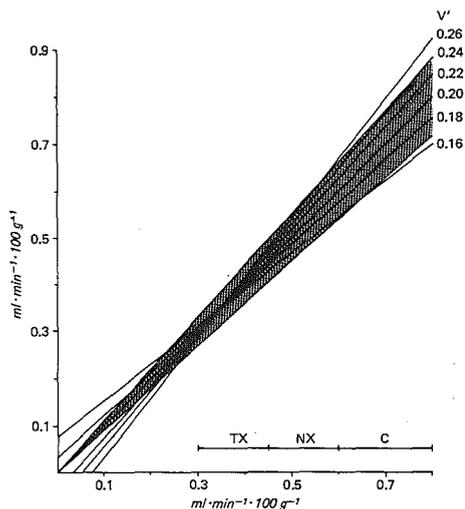
Fig. 1. *The effect of a change in the actual value of v' upon the calculated GFR per 100 g BW in rats.* The abscissa shows the GFR per 100 g BW calculated with v' = 0.20. The ordinate gives the GFR per 100 g BW calculated for various values of v', as indicated on the righthand side of the figure and represented by the various lines. The shaded area is the GFR per 100 g BW calculated for v' = 0.20 plus or minus 10%. C is the GFR range in two kidney control rats. NX is the GFR range in rats with a hyperfunctioning single kidney. TX is the GFR range in rats with isogeneically transplanted kidneys.

the GFR range found in rats with a single compensatory hyperfunctioning kidney (0.45 to 0.6 ml/min per 100 g BW) the effect of a change in the actual v' is much less, while it is almost absent in the GFR range found in rats with an isogeneically transplanted kidney (0.3 to 0.5 ml/min per 100 g BW). Large relative deflections will be found in the very low GFR range, although absolute differences from the calculated GFR will remain small. For example, a value of 0.10 ml/min per 100 g BW, calculated with v' is 0.20, would be 0.05 ml/min per 100 g BW if the actual value of v' were 0.24.

From these considerations we concluded, that although v' may be different in the various experimental models, the effects upon the calculated GFR will remain relatively small and do not outweigh the advantages of this method provided by the facility and the possibility of repeated use in the same animals.

Systolic blood pressure

The systolic blood pressure was obtained from trained, conscious but restrained animals by the tail cuff method, using a sphygmomanometer (Narco Bio-systems, Houston, Texas). Blood pressure recordings were taken in a quiet surrounding, twice a week throughout the followup period.

Experimental protocol

Six groups of rats undergoing different treatments were studied. Group 1 consisted of adult rats that underwent a unilateral nephrectomy. Group 2 consisted of adult rats that received a normal kidney transplant from a two-kidney adult donor rat. In group 3 adult rats received a hyperfunctioning kidney transplant from an adult donor rat that had been unilaterally nephrectomized three weeks earlier. Group 4 contained juvenile rats that underwent a unilateral nephrectomy. Group 5 contained juvenile rats that received a normal kidney from a two-kidney adult donor rat and group 6 contained juvenile rats that received a hyperfunctioning kidney from an adult donor rat.

The initial number of successful operations as well as the number of animals completing the study are given in Table 1. Most of the losses were due to complications during the repeated GFR measurements. In donor rats the GFR was measured about one week before transplantation of the kidney. The GFR of the control groups 1 and 4 was first measured one week before unilateral nephrectomy. In the followup period all rats had their GFR determined 1, 3, 9, and 15 weeks after either bilateral removal of the recipient's own kidneys or after unilateral nephrectomy in control rats.

Statistics

Longitudinal differences within each group of rats between the GFR values before and after unilateral nephrectomy or between the donor GFR and the GFR after kidney transplantation were compared using paired Student's *t* tests.

Differences in means among the groups in either the adult rat experiments (groups 1, 2, 3) or the juvenile rat experiments (groups 4, 5, 6) were compared by a one-way analysis of the variance. In case of significant differences among these three groups ($P < 0.05$), the Newman-Keuls test was used to find which pairs of means were different [18].

Results

Body weight

Changes in the BW of the rats after the various treatments are given in Table 2. In adult rats kidney transplantation causes a decrease in BW. A 5 to 12% drop in BW was noted one week after the removal of the recipients own kidneys. From then on BW increased again. At the end of the 15-week followup period the BW of rats with a kidney transplant was about 5% less than that of unilaterally nephrectomized adult control rats.

In juvenile rats there was an arrest in growth until two weeks after kidney transplantation, while subsequently there was a period of a rapid increase in BW. At the end of the followup period the BW of rats with a kidney transplant was about 5 to 10% less than that of unilaterally nephrectomized juvenile control rats.

Glomerular filtration rate

The changes in the GFR and in the GFR per 100 g BW noted in adult rats after unilateral nephrectomy or after the transplantation of either a normal or a hyperfunctioning kidney are depicted in Figure 2 (a and b).

In unilaterally nephrectomized rats (A NX) there was a rapid increase in the GFR, compared with 50% of the prenephrectomy level, during the first week after nephrectomy. The GFR per 100 g BW at the end of the first week amounted to 131% of the single kidney level. Subsequently, there was a further slight

Table 1. Experimental treatments and numbers of rats starting the study (N) and completing (n) the 15-week followup period

Group	Treatment	Code	n/N
1	Unilateral nephrectomy in adult rats	A NX	10/12
2	Transplantation of a normal adult kidney to an adult recipient	A TX_N	8/12
3	Transplantation of a hyperfunctioning kidney to an adult recipient	A TX_H	6/10
4	Unilateral nephrectomy in juvenile rats	Y NX	12/12
5	Transplantation of a normal adult kidney to a juvenile recipient	Y TX_N	13/16
6	Transplantation of a hyperfunctioning kidney to a juvenile recipient	Y TX_H	7/10

Table 2. Changes in body wt of adult or juvenile rats following unilateral nephrectomy or isogeneic kidney transplantation[a]

Code[b]	PRE	TX	NX	Weeks post NX 1	3	9	15	N
A NX	316 ± 3		316 ± 4	306 ± 3	313 ± 3	344 ± 3	356 ± 3	(10)
A TX_N	307 ± 8[c]	315 ± 6		277 ± 5	293 ± 6	332 ± 6	344 ± 8	(8)
A TX_H	306 ± 7[c]	305 ± 6		288 ± 7	304 ± 9	322 ± 8	338 ± 10	(6)
Y NX	162 ± 4		171 ± 5	188 ± 6	217 ± 6	280 ± 5	313 ± 4	(12)
Y TX_N	306 ± 6[c]	181 ± 6		178 ± 5	208 ± 6	261 ± 8	301 ± 7	(13)
Y TX_H	310 ± 10[c]	161 ± 6		167 ± 6	209 ± 8	257 ± 12	284 ± 12	(7)

[a] Data given are mean values ± SEM; the number of rats (N) is given in parentheses.
[b] For explanation of abbreviations see Table 1.
[c] Body wt of donor rat is given.
Abbreviations used are defined: PRE, data obtained one week before transplantation or unilateral nephrectomy; TX, data obtained at the day of transplantation; NX, data obtained at the day of unilateral nephrectomy in A NX and Y NX, and at the day of bilateral nephrectomy of the recipients own kidneys in the transplant groups, being one week after transplantation.

increase. The GFR per 100 g BW was at a stable level of about 145% of the prenephrectomy value from 3 to 15 weeks.

After the transplantation of a normal kidney to adult recipient rats, the GFR as well as the GFR per 100 g BW was not significantly different from 50% of the donor value one week after the removal of the recipients' own kidneys. However, from three weeks onward both values were significantly higher than the donor values. The average GFR per 100 g BW of adult recipient rats (A TX_N) who received the transplantation of normal adult kidneys, between 3 and 15 weeks, amounted to 78% of that of A NX control rats.

After the transplantation of a hyperfunctioning kidney to adult rats, a significant drop in the GFR and in the GFR per 100 g BW, compared with the donor level, was observed at one and three weeks after the removal of the recipient's own kidneys. Although the GFR remained lower than the donor value, this difference was no longer significant after 9 and 15 weeks. Between 3 and 15 weeks the average GFR per 100 g BW of adult recipient rats (A TX_H) transplanted with hyperfunctioning kidneys was 80% of that of A NX control rats.

As a consequence of the divergent changes after the transplantation of either a normal or a hyperfunctioning kidney, analysis of variance showed no significant differences between the GFR and the GFR per 100 g BW of A TX_N and that of A TX_H rats. Both transplant groups, however, had a GFR and a GFR per 100 g BW that was significantly lower than that of A NX rats (GFR: $P < 0.01$ for both A TX_N and A TX_H at weeks 1, 3, 9, and 15; GFR per 100 g BW: $P < 0.01$ for A TX_N at weeks 3, 9, and 15 and for A TX_H at weeks 1, 3, and 15, $P < 0.05$ for A TX_N at week 1, and for A TX_H at week 9).

From the combined data of both adult transplant groups, it can be deduced that the GFR of isogeneically transplanted kidneys was about 80% of the maximally attainable level.

The changes in the GFR and the GFR per 100 g BW noted in juvenile rats after unilateral nephrectomy or after the transplantation of either a normal or a hyperfunctioning adult kidney are depicted in Figure 3 (a and b).

After the transplantation of a normal adult kidney to juvenile recipient rats, there was an initial decrease in the GFR to about 70% of the pretransplant donor GFR. The level of the GFR increased again as BW of the recipient increased. At the end of the 15-week followup period, when the recipient's BW was about the same as that of the adult donor rats, the GFR was significantly increased to about 125% of the GFR of the donor kidney. Despite the initial decrease in the GFR after the transplantation, the GFR per 100 g BW of the juvenile recipient rats (Y TX_N) transplanted with normal adult kidneys was significantly higher than 50% of that of the donor rats and remained higher throughout the followup period.

After the transplantation of a hyperfunctioning adult kidney to a juvenile recipient, the decrease in the GFR was much more pronounced than after the transplantation of a normal adult kidney. The GFR was, one week after the removal of the recipient's own kidneys, about 50% of the donor value. The GFR increased again as the BW of the recipient increased but remained significantly lower than the donor value throughout the followup period.

The GFR per 100 g BW of the juvenile recipients was not significantly different from 50% of that of the donor rats during the followup period. As in adult recipients analysis of the variance did not show any significant differences in either the GFR or the GFR per 100 g BW when comparing the recipients of a normal or a hyperfunctioning adult kidney. Both transplant groups had a GFR and a GFR per 100 g BW that were, in general, significantly lower than those of juvenile rats unilaterally nephrectomized (Y NX) (GFR: $P < 0.01$ for Y TX_N at
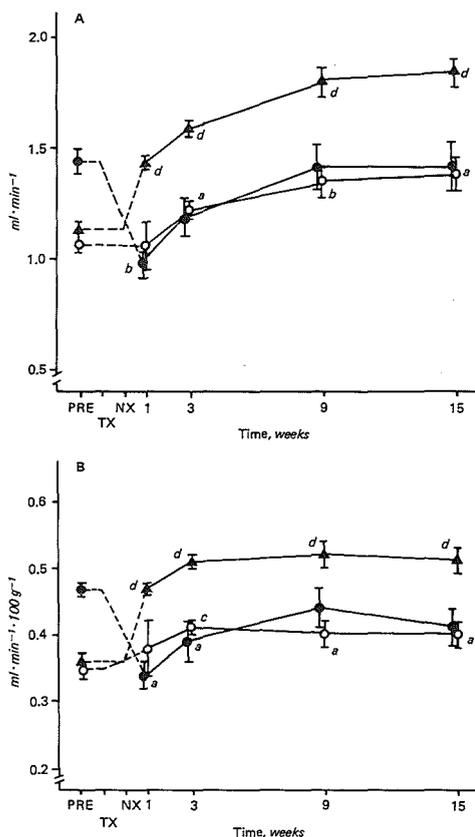
Fig. 2. A *The glomerular filtration rate (GFR) and* **B** *the GFR per 100 g body wt of normal and hyperfunctioning adult kidneys transplanted isogeneically to adult rat recipients compared with that of unilaterally nephrectomized rats.* Symbols used are: ▲——▲ A NX (10); ○——○ A TX_N (8); ●——● A TX_H (6). The number in parentheses is the number of rats in that particular group. For group abbreviations see Table 1; PRE control and N donor values represent 50% of the actual measured GFR in order to obtain the GFR of a single kidney; TX, the week of transplantation; NX, the week of either unilateral nephrectomy of the controls or bilateral nephrectomy of the transplant recipient's own kidneys. Letter symbols are defined: *a*, $P < 0.05$; *b*, $P < 0.01$; *c*, $P < 0.005$; *d*, $P < 0.001$ compared with the PRE value (paired *t* test). (Data given are means ± SEM.)

weeks 1, 3, 9, and 15 and for juvenile recipients transplanted with hyperfunctioning kidneys (Y TX_H) at weeks 1, and 15, $P < 0.05$ for Y TX_H at weeks 3, and 9; GFR per 100 g BW: $P < 0.01$ for Y TX_N at weeks 1, 3, and 9, and for Y TX_H at weeks 1, and 15, $P < 0.05$ for Y TX_N at week 15 and for Y TX_H at week 3).

By combining Y TX_N and Y TX_H, it can be stated that the GFR per 100 g BW of isogeneically transplanted adult kidneys

to juvenile recipients amounted to about 75 to 80% of the GFR of juvenile unilaterally nephrectomized rats.

Systolic blood pressure

No major changes were observed in the normotensive systolic blood pressure level after the rats received either a unilateral nephrectomy or an isogeneic kidney transplantation. For comparison the averages are given for 10 to 12 successive blood pressure recordings obtained between 6 and 12 weeks after nephrectomy (Table 3).

Discussion

The GFR of isogeneically transplanted adult rat kidneys to adult recipients amounted to 80% of the maximally attainable level, that is, the GFR of a single kidney remaining after contralateral nephrectomy. Studies concerning the long-term changes in the GFR of transplanted kidneys have not been reported. GFR measurements taken shortly after renal transplantation showed a reduced GFR up to 6 hours [2] or 15 hours [3] after the operation. At that time the GFR amounted to 60 to 70% of the control value. Studies covering a longer period did not indicate that damage had occurred to the kidney during transplantation [6], even after two hours of cold ischemia [7], according to the postoperative serum creatinine levels up to 16 days after transplantation. Serum creatinine and urea, however, are not very sensitive measures to identify minor changes in renal function. Normal or near normal creatinine and urea levels may cover a wide range of GFR values [17]. During long-term studies near normal [4] or elevated [5] levels of blood urea after isogeneic kidney transplantation have been reported, suggesting that some degree of renal damage had occurred.

Several factors may be involved in the observed reduction in the GFR when compared with the maximally attainable level. It is not probable that an incomplete compensatory response of the transplanted kidneys causes the reduction in the GFR. After the transplantation of already hyperfunctioning kidneys, the level of the GFR was almost indistinguishable from that of rats with a normal kidney transplant. This observation suggests that the transplanted kidney is partially damaged. Damage due to rejection can be excluded, since the kidneys were transplanted between male rats of a highly inbred strain, in which permanent survival of intra-strain skin-, heart-, and kidney grafts had been obtained [14, 15].

Therefore, it is most likely that the approximate 20% impairment in the GFR of isogeneically transplanted rat kidneys is caused by the transplantation procedure. We speculate that, apart from the damaging effect of the temporary ischemia, ureteral obstruction occurring after the transplantation is of importance. This opinion is based on several observations. The WAG/Ro recipients of WAG/Ro donor kidneys generally did not survive kidney transplantation for more than a week when their own kidneys were removed at the same time. During this period no urine was excreted. Clearance studies with chromium 51-EDTA a few hours after transplantation showed that the kidneys did filtrate, but no radioactive material appeared in the bladder. Furthermore, those transplanted kidneys ($N = 20$) subjected to an IVP at the end of the followup period all revealed a moderate to severe degree of hydronephrosis. All these findings point, in our opinion, to urinary outflow problems as a possible cause for renal damage observed in this
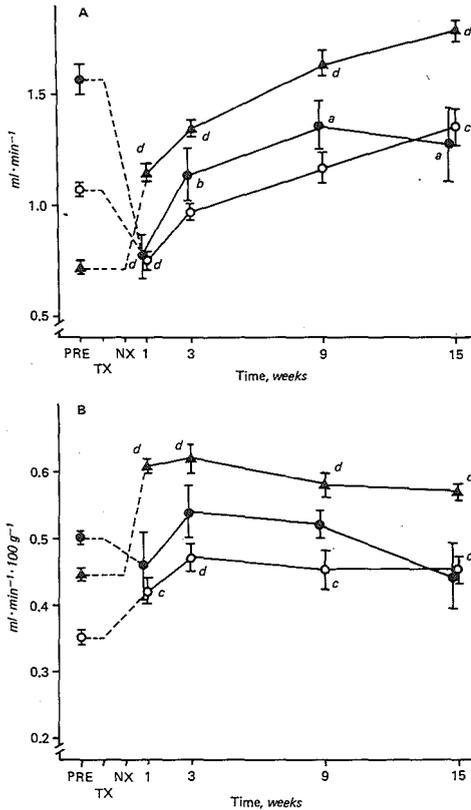
Fig. 3. A *The glomerular filtration rate (GFR) and* **B** *the GFR per 100 g body wt of normal and hyperfunctioning adult kidneys transplanted isogeneically to juvenile rat recipients compared with that of unilaterally nephrectomized rats.* Symbols used are: ▲——▲ Y NX (12); ○——○ Y TX$_N$ (13); ●——● Y TX$_H$ (7). For further explanation of abbreviations, see the legend for Figures 1 and 2.

Table 3. Systolic blood pressure after isogeneic kidney transplantation in rats[a]

Group code[b]	N[c]	mm Hg
A NX	(10)	121 ± 2
A TX$_N$	(8)	113 ± 4
A TX$_H$	(6)	120 ± 4
Y NX	(12)	129 ± 4
Y TX$_N$	(13)	118 ± 3
A TX$_H$	(7)	127 ± 4

[a] Mean ± SEM of 10 to 12 blood pressure determinations taken between 6 and 12 weeks after transplantation.
[b] For abbreviations see Table 1.
[c] N represents the number of rats in each group.

amounted to over 300 g. At that time the GFR was of the same order as that of adult recipients, indicating that the degree of renal damage was also of the same order. Thus, we conclude that the initial GFR decrease in transplanted adult kidneys is the result of a rapid adaptation to the size of the immature recipient rat.

In rats, the GFR per 100 g BW of single kidneys remaining after contralateral nephrectomy increases to about 70 to 80% of the two-kidney value in a relatively short period [17]. The GFR of transplanted kidneys amounted to a level of about 80% of that of single hyperfunctioning kidneys in adult as well as in juvenile recipients. Assuming that the 20% impairment of the GFR is due to damage caused during and after the transplantation, it appears that the GFR of transplanted adult kidneys is in one way or another influenced by the recipient. Thus, the present findings provide evidence that, in the case of a successful transplantation, the GFR is not determined by the previous function of the undamaged donor kidney. The transplanted kidney, however, behaves like a single kidney of the recipient in establishing the ultimate GFR level.

No clinical data substantiating these findings in human transplantation are known to us. However, conversely juvenile kidneys should adapt rapidly when transplanted to adult recipients. In rats a rapid increase in size has been noted after the transplantation of "baby" rat kidneys to adult recipients [20]. In human transplantation a rapid increase in renal function has been reported to occur when kidneys were transplanted from (very) young children to adult recipients [21, 22].

Hypertension, which is a common finding after clinical renal transplantation [9–11], was not observed in rats with isogeneically transplanted kidneys. This finding indicates that the presence of a kidney transplant as such, in this model, does not cause a rise in blood pressure. Thus other factors, such as rejection and immunosuppressive therapy, may be of importance in raising the blood pressure level.

particular model of rat kidney transplantation. The degree of GFR reduction was in the same order as that of kidneys which had recovered from a complete ureteral obstruction of a one-week duration [19].

After the transplantation of normal adult kidneys to juvenile recipients, we observed a significant decrease in the GFR, which was even more marked when hyperfunctioning kidneys were transplanted. This decrease in the GFR could be due to a greater damage brought about during and after the transplantation. The transplantation of adult kidneys to juvenile rat recipients, however, did not cause additional microsurgical problems, nor did the ischemic time differ from that of kidneys transplanted to adult recipients. Furthermore, during the followup period the juvenile rats reached adulthood, and the BW

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CHAPTER 3

ALLOGENEIC KIDNEY TRANSPLANTATION AFTER ACTIVE IMMUNOLOGICAL
ENHANCEMENT: A MODEL TO STUDY POST-TRANSPLANTATION
HYPERTENSION IN RAT RENAL ALLOGRAFT RECIPIENTS

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

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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 mediately 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 num-

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bers 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 transplantations. These rat strains differ at the major histocompatibility locus. The WAG/Ro is RT-1^y, the BN strain is RT-1^n. The animals had unlimited access to food (Rat chow AM II, 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 $\pm$ SD), the V of ^{51}Cr-labelled EDTA was found to be $23.0 \pm 0.7\%$ of body weight and that of ^{125}I-IOH $25.3 \pm 1.6\%$ 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-sphygmomanometer 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 Paramat. Serial sections were cut to a thickness of 3–4 μm. Sections were stained with haematoxylin-eosin.

Statistics

Results are given as means $\pm$ 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.

Group (n)	Body wt. (g)	GFR (ml/min)	ERPF (ml/min)	Ratio GFR/ERPF
TX (9)	245 ± 19	2.00 ± 0.10	5.09 ± 0.30	0.35 ± 0.02
NX (7)	243 ± 16	2.09 ± 0.26	5.14 ± 0.61	0.41 ± 0.04
Control (6)	246 ± 21	1.97 ± 0.24	4.93 ± 0.35	0.40 ± 0.04
BN (9)	283 ± 22	1.18 ± 0.29	3.67 ± 0.82	0.31 ± 0.04
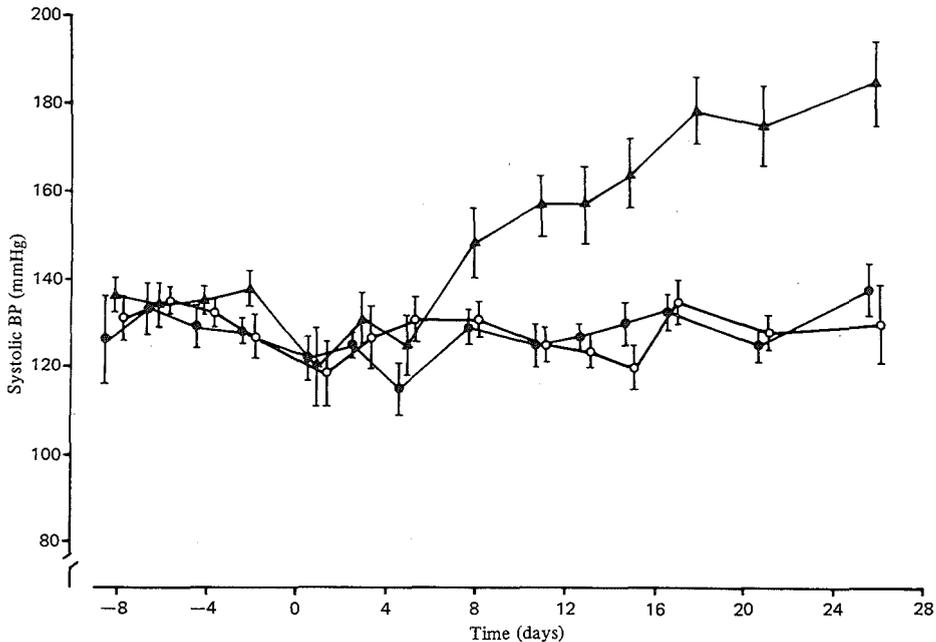
FIG. 1. Systolic blood pressure of allogeneic kidney recipients (▲), of unilaterally nephrectomized (NX: o) and of two-kidney control (•) rats.

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.

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

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-
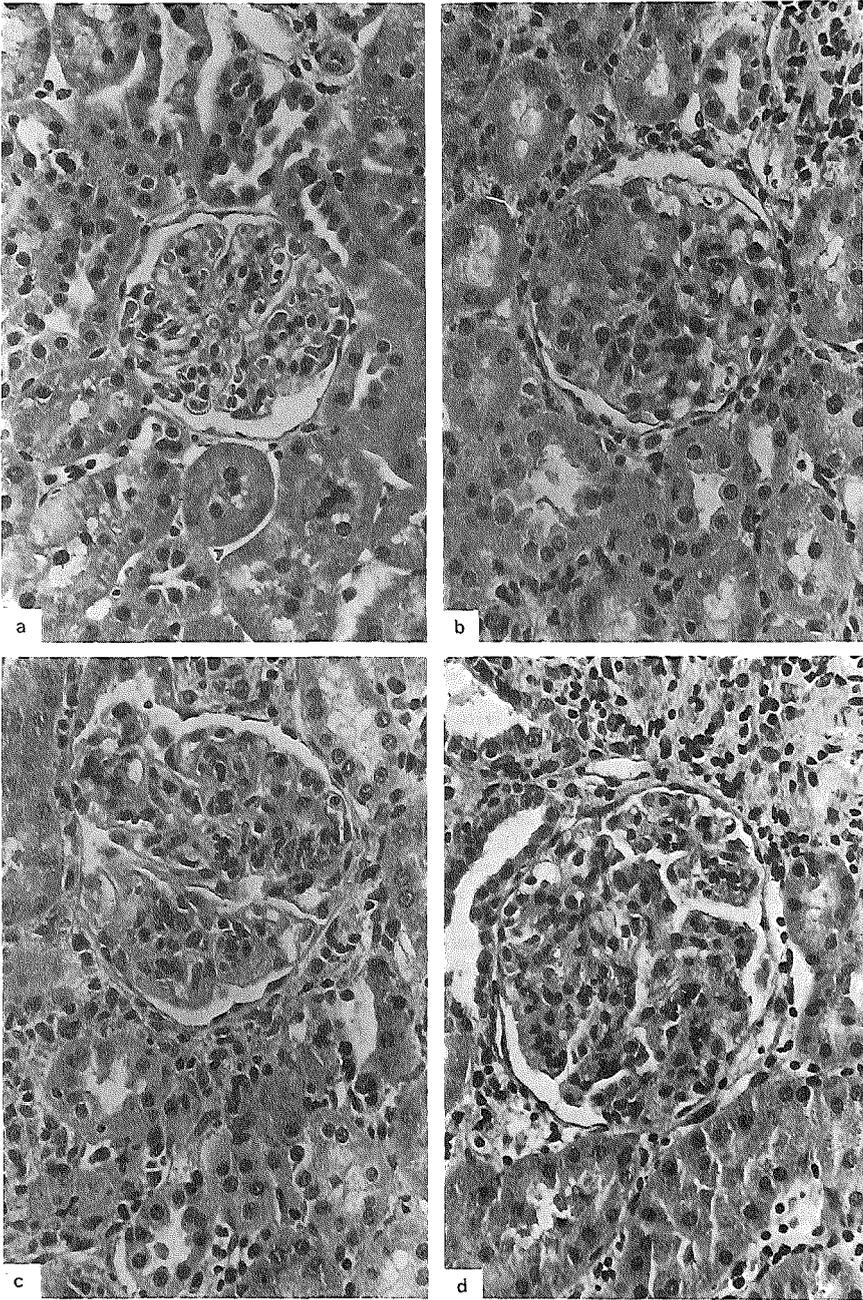
FIG. 2. Micrographs from sections of a normal BN kidney (*a*) and of kidneys allografted into immunologically enhanced WAG/Ro recipients, removed on days 4 (*b*), 8 (*c*) and 20 (*d*) after transplantation. (Haematoxylin–eosin, ×500).

plantation procedure 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 allogeneic 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 allogeneic 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.

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CHAPTER 4

EFFECT OF CYCLOSPORIN A ON THE DEVELOPMENT OF POST-
TRANSPLANTATION HYPERTENSION IN RAT RENAL ALLOGRAFT RECIPIENTS

Effect of Cyclosporin A on the Development of Posttransplantation Hypertension in Rat Renal Allograft Recipients

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Key Words. Experimental hypertension · Kidney transplantation · Renal function · Cyclosporin A · Rats

Abstract. Hypertension secondary to renal transplantation was studied in our experimental model in the rat. In this model an intravenous injection of donor strain blood into recipients of allogeneic donor kidneys prior to transplantation was used to prolong the allograft survival. A reduced renal function associated with the hypertension was suggestive of incomplete prevention of the rejection process. We studied the effect of cyclosporin A, either alone or as adjuvant immunosuppressive therapy, on renal function and systolic blood pressure. Either way, cyclosporin A resulted in normotensive allograft recipients and in a better function of the graft when compared with recipients pretreated with donor strain blood only.

Introduction

In man, hypertension is a common complication of a surgically successful kidney transplantation. The hypertension is usually attributed to various factors [1], the relative contribution of which is not easy to establish in man. Recently we developed an experimental model to study hypertension following allogeneic kidney transplantation in rats. Treatment with donor strain blood prior to transplantation was used to prolong graft survival. This posttransplantation hypertension was associated with a reduced renal function and a low plasma renin concentration [2]. The latter was considered to result from sodium retention, and in a subsequent experiment it was shown that a reduction in the daily sodium intake prevented the development of hypertension [3].

The reduced renal function and the observed histopathological changes [2] indicated an incomplete prevention of the rejection process by the pretreatment with donor strain blood. Consequently, we wanted to study the effect of additional immunosuppression upon the renal function and the systolic blood pressure (SBP) in our model. Clinically, a combination of azathioprine and corticosteroids is most frequently used to prevent rejection. Steroids, however, are hypertensiogenic themselves [4, 5]. Recently, cyclosporin A (CsA) proved to be a powerful immunosuppressive [6], effective in experimental kidney transplantation [7–9] as well as in human transplantation [10]. To avoid the use of corticosteroids, CsA was used to improve immunosuppression in our allogeneic kidney transplantation model in rats.

Materials and Methods

Laboratory animals consisted of adult male rats with a body weight (BW) of 250–300 g of highly inbred Wistar (WAG/Ro) and Brown Norway (BN/Ro) strains. These strains differ at the major histocompatibility locus. The rats had free access to tap water and food (rat chow AM II, Hope Farms, containing 120 mmol sodium/kg).

The BN rats were used as kidney donors, WAG rats as recipients and as unilaterally nephrectomized controls. Potential kidney donors, i.e., BN rats without an apparent hydronephrotic right kidney, had their left kidney removed 3 weeks before transplantation. In this way a compensatory hyperfunctioning kidney was transplanted. A number of WAG recipients and controls received an intravenous injection of 1 ml of fresh citrated BN donor strain blood 2 weeks prior to transplantation or unilateral nephrectomy. All recipients were subjected to bilateral nephrectomy of the native kidneys at the time of transplantation. Transplantation was performed under ether anaesthesia, utilizing a microsurgical technique as previously described [11].

The CsA was administered intramuscularly at a dose of 15 mg/kg on days 0, 4, and 7 after transplantation. Before administration CsA was dissolved in olive oil by heating it to 60 °C.
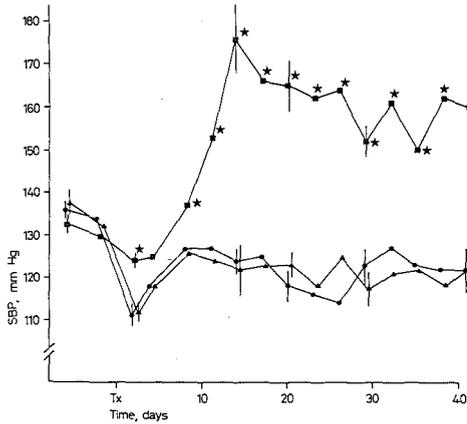
Fig. 1. SBP of renal allograft recipient rats on different immuno-suppressive regimens. Tx = Transplantation; ■ = group 1, donor strain blood pretreatment; ● = group 2, donor strain blood pretreatment plus CsA; ▲ = group 3, CsA alone. Asterisks denote significant difference from groups 2 and 4 (p < 0.05).

Experimental Protocol

Recipient rats were divided into 4 groups: group 1 was pretreated with donor strain blood, but received only olive oil after transplantation; group 2 animals were pretreated with donor strain blood and with CsA in olive oil after transplantation; group 3 had no pretreatment and received only olive oil, and group 4 animals had no pretreatment and received CsA in olive oil after transplantation. A

renal transplantation was considered technically successful if the recipient rats survived for at least 7 days.

Group 1 initially consisted of 14 rats, group 2 of 12, group 3 of 6, and group 4 of 12 rats. Only data from rats surviving for 6 weeks, except those from group 3, were included in the statistical analysis of the differences in SBP or renal function. The different groups of allograft recipients were also compared with groups of 6 unilaterally nephrectomized (NX) control rats receiving identical treatments.

The SBP was measured 6 days per week, starting 1 week before operation and continuing until 6 weeks after operation. The glomer-ular filtration rate (GFR) and the effective renal plasma flow (ERPF) were determined before transplantation in the BN donor rats and 1, 3, and 6 weeks after operation in transplant recipients and in unilaterally nephrectomized controls. At the end of the follow-up period, the kidneys were weighed and the number of glomeruli of transplanted and control kidneys counted.

Measurements

Systolic Blood Pressure. The SBP of non-anaesthetized animals was determined by tail plethysmography (electro sphygmoma-nometer; Narco Bio System, Houston, Tex., USA). At least three SBP readings were taken to obtain the average daily SBP. The SBP data are given as an average of three consecutive daily values.

Renal Function. GFR and ERPF were measured as clearances of ^{51}Cr-EDTA and ^{125}I-iodohippurate, respectively. This method has been described in detail elsewhere [11, 12]. At the time of the renal function measurement, 200 μl blood was sampled to determine the plasma concentrations of creatinine (P_{cr}) and urea (P_{ur}). P_{cr} and P_{ur} were determined by kinetic techniques with the use of an auto-ana-lyzer.

Glomeruli were counted after staining with India ink according to a modification [13] of the method of *Damanian* et al. [14].

Statistics

Differences in mean values between groups were compared by one-way analysis of variance. In case of significant differences (i.e.,

Table I. Pre-operative and posttransplant renal functional parameters in allogeneic transplant recipients on different immunosuppressive regimens

	Pre-operative								Week 1	
	group 1		group 2		group 3		group 4		group 1	group 2
	don (n=9)	rec (n=9)	don (n=9)	rec (n=9)	don (n=6)	rec (n=6)	don (n=8)	rec (n=8)	(n=9)	(n=9)
BW, g	250 ± 19	289 ± 28	268 ± 19	293 ± 21	264 ± 21	290 ± 17	252 ± 27	297 ± 33	264 ± 31	264 ± 22
SBP, mm Hg		130 ± 9		132 ± 13		132 ± 3		134 ± 4	137 ± 12	126 ± 11
GFR, ml/min	1.04 ± 0.22		1.13 ± 0.14		1.04 ± 0.25		1.04 ± 0.23		0.74 ± 0.25	0.69 ± 0.20
GFR, % NX									63	57
ERPF, ml/min	3.33 ± 0.65		3.91 ± 0.44		3.65 ± 0.80		3.35 ± 0.56		3.25 ± 0.99	3.00 ± 0.64
ERPF, % NX									97	87
P_{cr}, μmol/l	64 ± 3		65 ± 3		67 ± 5		65 ± 3		82 ± 15	95 ± 41
P_{ur}, mmol/l	13.1 ± 1.1		13.5 ± 1.6		14.9 ± 2.8		14.2 ± 1.8		17.4 ± 5.5	16.8 ± 3.7

[a] Non-survivors.

Data are mean values ± SD; Don = donor; rec = recipient; % NX = percentage of unilaterally nephrectomized controls; *p < 0.05 when compared with group 1.

an F value indicating a probability less than 0.05), the Newman-Keuls test was used to find which pair(s) of means were different [15]. To test statistical differences in survival rates between the groups, the χ^2 test was used.

Results

Systolic Blood Pressure

The SBP of allograft recipients of groups 1, 2, and 4 is depicted in figure 1. Recipients who were pretreated with donor strain blood only (group 1) showed an increase in SBP after transplantation. The SBP started to rise 1 week after transplantation and remained elevated till the end of the 6-week follow-up period. Kidney transplant recipients receiving either CsA in additional to donor blood pretreatment (group 2) or CsA alone (group 4) remained normotensive throughout the observation period. Compared with these two groups, recipients in group 1 showed a significantly higher SBP during the follow-up period. There were no statistical differences in SBP between recipients in groups 2 and 4 and in comparison with unilaterally nephrectomized rats regardless the treatment. The recipients that had neither pretreatment with blood nor CsA (group 3) all died within 10 days. Although they remained normotensive for that short period of survival, their SBP started to rise at the end of the 1st week after transplantation.

Renal Function

Pre-operative BW and SBP of the four groups of recipients and renal functional parameters and BW of the corresponding donor rats are given in table I. This table also lists renal function parameters, BW and SBP of each group of recipient rats determined 1, 3, and 6 weeks after transplantation.

1 week after transplantation no statistical differences were found in the GFR or ERPF of recipient rats which received either donor strain blood alone or CsA alone or a combination of both. After the 1st week, the recipients of groups 2 and 4 showed an improvement of their GFR, while there was a decrease in GFR of recipients in group 1. After week 3 the GFR of the rats in this group improved again, but remained significantly lower than the GFR of recipients in groups 2 and 4. No differences in the ERPF between recipients in groups 1, 2 and 4 were noted throughout the follow-up period. Recipients in group 3 had a very low GFR and ERPF at the end of the 1st week after transplantation. When expressed as a percentage of the donor GFR, the GFR of the recipients in group 1 was 70% at week 1, 43 at week 3, and 69% at week 6. The GFR of recipients in groups 2 and 4, respectively, was 59 and 62% of the donor GFR at week 1 and steadily increased to 86 and 116% at week 6. Expressed as a percentage of the donor ERPF, the ERPF of the recipients in group 1 was 98% at week 1, 85 at week 3, and 99% at week 6. The percentages of the recipients in groups 2 and 4 were, respectively, 77 and 86% at week 1, 88 and 97% at week 3, and 98 and 132% at week 6.

Number of Glomeruli

The number of glomeruli present in transplanted kidneys of recipients in group 1 was less in comparison with that of recipients in groups 2 and 4. The number amounted to 27,920 ± 9,297 in group 1 against

		Week 3				Week 6			
group 3	group 4	group 1	group 2	group 3	group 4	group 1	group 2	group 3	group 4
(n=6)	(n=8)	(n=9)	(n=9)	(n=6)[a]	(n=8)	(n=9)	(n=9)	(n=6)[a]	(n=8)
264 ± 13	269 ± 24	264 ± 23	271 ± 14	—	283 ± 19	276 ± 25	283 ± 18	—	300 ± 23
138 ± 13	127 ± 8	165 ± 19	123 ± 8*	—	118 ± 10*	160 ± 34	123 ± 13*	—	122 ± 14*
0.19 ± 0.08	0.64 ± 0.15	0.44 ± 0.23	0.79 ± 0.28*	—	0.82 ± 0.20*	0.71 ± 0.40	0.98 ± 0.13*	—	1.20 ± 0.31*
17	50	37	65	—	66	57	81	—	95
0.36 ± 0.23	2.87 ± 0.72	2.82 ± 0.99	3.45 ± 0.84	—	3.24 ± 1.02	3.30 ± 1.34	3.81 ± 0.55	—	4.42 ± 0.46
11	88	71	94	—	85	88	106	—	113
536 ± 273	83 ± 13	87 ± 34	70 ± 8*	—	71 ± 6*	98 ± 71	71 ± 8	—	68 ± 8
94.0 ± 35.9	16.8 ± 3.0	27.0 ± 16.6	13.9 ± 2.5*	—	12.9 ± 0.9*	27.1 ± 25.9	15.7 ± 4.2	—	13.0 ± 1.2

33,523 ± 5,083 in group 2 and 31,984 ± 3,838 in group 4. There was no statistical difference between the recipients of groups 2 and 4 and the unilaterally nephrectomized control rats. We also counted the glomeruli of 2 moribund allograft recipients in group 3. These kidneys had 10,290 and 11,520 glomeruli which was about 30% of the glomeruli of controls.

Survival Rate

All transplant recipients of group 3 died before day 11 after transplantation. During the 6-week observation period several transplanted rats from the other three groups were also lost due to rejection. 5 (36%) of the 14 technically successful transplanted rats died in group 1; 3 (25%) of the 12 rats in group 2, and 4 (33%) of the 12 rats in group 4 did not complete the follow-up period. There were no statistically significant differences in mortality rates between those latter groups (χ^2 test).

Discussion

In experimental kidney transplantation in rats, isograft recipients remained normotensive [11], while hypertension developed in allograft recipients pretreated with donor strain blood to prolong graft survival [2]. This may have been due to incomplete suppression of rejection. In the present study the use of the potent immunosuppressive CsA [6], as adjuvant therapy as well as alone, prevented the development of hypertension after allogeneic kidney transplantation. The recipients treated with CsA also had a better renal function, and, at the end of the 6-week follow-up period, more glomeruli were present in the allografts when compared with recipients pretreated with donor strain blood only. These findings demonstrate that a better immunosuppression was achieved by the use of CsA. With improved immunosuppression the recipients remained normotensive as previously reported for recipients with isogeneic kidney transplants [11].

In human recipients, hypertension following renal transplantation has been attributed to various factors [1]. Among the most frequently mentioned are renal transplant artery stenosis, presence of the diseased native kidneys, rejection, and the use of corticosteroids. Since no diseased native kidneys were present, and corticosteroids were not used in the present experiment, these factors can be excluded as a cause for hypertension in this model, nor is transplant renal artery stenosis likely to be involved here. The transplantation technique, using end-to-side anastomosis, is not prone to the development of artery stenosis. Furthermore, the blood pressure response to a reduction in salt intake and the presence of an intact native kidney convinced us that the posttransplantation hypertension model was at variance with the 1-kidney-1-clip Goldblatt hypertension in rats [3]. Consequently, rejection remains as the most likely cause for the rise in SBP.

After stimulation of the host's immune system, rejection is the result of a combined attack on the allograft by several arms of the immune response. Rejection consists of a number of processes, tackling the graft in a different way, and each probably capable of graft destruction by itself. After transplantation, rejection has been classified as hyperacute, acute, or chronic. These terms are not well defined and refer not only to the relationship between rejection and the time elapsed after transplantation, but also to the rate of functional deterioration of the allograft. Another classification is based on the effector mechanism of the immune response. Following cellular infiltration of the graft by a mixture of T and B lymphocytes, macrophages, and polymorphonuclear cells, graft rejection may occur by direct cell-mediated killing or through the production of antibodies. The cellular type of rejection involves mainly the T lymphocytes, whereas the humoral response is mediated by B lymphocytes, albeit T cell dependent: The chronic rejection process is usually attributed to damage caused by antibodies.

The cellular and humoral types of rejection may differ in their response to various immunomodulating agents. Pretreatment of the recipient rats with donor strain blood partially prevents the rejection reactions. The greatest effect is on the cellular response, although the humoral response is also diminished [16]. After allogeneic kidney transplantation in recipients pretreated with donor strain blood, the histopathological findings in the allografts are consistent with chronic rejection [2]. Although the precise action of CsA is not completely known, the main effect of CsA is directed to the cellular mediated immune response, probably due to a selective action on helper T lymphocytes [6, 8, 17, 18]. Through these cells inhibition of B cell antibody production may occur as well. Consequently, while the action of CsA involves the same target as pretreatment with donor strain blood, it is much more effective. This may explain the apparent lack of synergism between CsA and donor strain blood pretreatment. The use of CsA alone was as effective as the combination of CsA and donor strain blood pretreatment.

From the present and previously published results [2, 3, 11] we postulate that in our rat model of postrenal transplantation hypertension the increase in SBP is the result of

the following chain of events. Pretreatment with donor strain blood partially suppresses the rejection process, leading to a chronic type of rejection. Structural damage within the allograft due to immunological reactions will cause functional alterations. As a result of these changes, the transplanted kidney has an intrinsic deficiency in the excretion of the daily sodium intake at a normal blood pressure level. Accumulation of sodium will cause a volume-dependent hypertension. Either sodium restriction or the prevention of chronic rejection, i.e., a more successful immunosuppression, should prevent the development of hypertension. As shown in the present and a previous study [3] we found both to be effective.

Some of the general concepts of rejection may be applicable to man. The human situation, however, appears far more complex than that observed in rats. In the rat short courses ·of immunosuppression are generally sufficient for prolonged allograft survival. Three injections with CsA up to 1 week after transplantation resulted in good functioning grafts. In man, most immunosuppressives are administered as long as a functioning graft is present. Cessation of the treatment usually results in rejection, indicating that the antigenic stimulation and/or the immune response in man is much stronger than in the rat.

In clinical studies, renal transplant recipients treated with CsA showed neither a lower incidence nor a less severe form of hypertension when compared with recipients treated with a combination of azathioprine and prednisolone [19–21]. The reason for this divergence from our findings in rats may be twofold. Firstly, in these studies renal function was generally reduced in the CsA-treated recipients. This is most likely due to CsA nephrotoxicity [22, 23]. However, it is difficult to differentiate between nephrotoxicity and rejection [23, 24]. Therefore, a less effective prevention of chronic rejection is also possible. Secondly, recent findings point to a direct effect of CsA on blood pressure. In rats an acute, albeit transient, increase in blood pressure has been noted during CsA infusion [25]. Chronic administration of CsA at a dose of 20 mg/kg was found to attenuate hypertension in spontaneously hypertensive rats [26]. This increase in SBP was associated with an increased plasma renin activity. Furthermore, in cardiac transplant recipients receiving CsA, there is an almost universal development of systemic arterial hypertension [27, 28], while this was less than 20% in patients receiving azathioprine plus prednisolone. The absence of hypertension in CsA-treated rats in the present experiment may be due to the very short duration of the treatment.

These findings indicate that extrapolation of the experimental results to the clinical situation has to be done with great care. Due to the stronger immunogenicity of man as compared with rats, chronic rejection phenomena may be present despite continuous immunosuppressive treatment. In our model adequate immunosuppressive treatment resulted in well-functionning grafts and normotensive allograft recipients. In man, despite stable graft function with continuous immunosuppression, hypertension frequently occurs. This may indicate that the immunosuppression is still insufficient, or the drugs used have hypertensive properties.

In conclusion, our experimental research demonstrated that postrenal transplantation hypertension is secondary to insufficient immunosuppression. In view of post-transplantation salt retention, a peri-operative low salt intake is recommended. Experimentally, the use of CsA successfully suppressed rejection, and hypertension did not occur. Since clinical results differ from these findings, there is still room for improvement of the antirejection treatment in human renal transplantation.

Acknowledgements

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CHAPTER 5

PREDNISOLONE AND POST-TRANSPLANTATION HYPERTENSION IN RAT
RENAL ALLOGRAFT RECIPIENTS

PREDNISOLONE AND POST-TRANSPLANTATION HYPERTENSION

IN RAT RENAL ALLOGRAFT RECIPIENTS

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SUMMARY

Rat recipients of renal allografts and unilaterally nephrectomized control rats were studied to evaluate the response in blood pressure to prednisolone in diverse doses and to determine the dosage required to achieve adequate immunosuppression without undue complication of hypertension. While a continuous infusion of 2 mg/kg/day or more of prednisolone proved effective in prolonging allograft survival time, this dosage increased the blood pressure of recipients as well as unilaterally nephrectomized control rats. In contrast to control rats, the recipients remained hypertensive after the cessation of prednisolone administration. This suggests that the high blood pressure observed during prednisolone administration was due to its hypertensive action. On the other hand, the high blood pressure remaining after cessation of the prednisolone administration is likely to be caused by an incomplete prevention of rejection. If recipients had received a transfusion of donor strain blood prior to transplantation in combination with the infusion of 2 mg/kg/day or more of prednisolone, they became normotensive when the prednisolone infusion was ceased. By reducing the prednisolone dosage to 1 mg/kg/day in combination with donor strain blood pretreatment, hypertension could also be eliminated during the first two weeks. In conclusion, effective immunosuppression can be achieved with prednisolone in rats, without inducing hypertension, provided prednisolone is administered in a low dose in combination with adjuvant immunosuppression, i.e. donor strain blood pretreatment.

INTRODUCTION

In previous studies we have found that pretreatment with donor strain blood (DSB) prolongs the survival time of rats after renal allotransplantation. A relatively low renal function indicated that there was only partial suppression of rejection (1,2). Incomplete prevention of rejection resulted in the development of hypertension. With additional immunosuppression, e.g. by administering Cyclosporin A, the recipient rats remained normotensive throughout the observation period with a good renal function (3). We also found that a low sodium diet would prevent a rise in blood pressure after transplantation, suggesting that the retention of sodium might be a precursor of the observed hypertension (2).

In humans, hypertension is a common complication of renal transplantation in the immediate and subsequent post-operative period. Prednisolone is a substantial element in almost every human immunosuppressive regimen. As this drug raises the blood pressure, it is generally assumed that post-transplantation hypertension is a side-effect of the steroid medication (4,5).

The effects on blood pressure and immunosuppression of prednisolone either with or without adjuvant immunosuppressive treatment (DSB) were studied in our rat model of post-transplantation hypertension. We wanted to determine to what extent the immunosuppressive effect of DSB would enable lowering the prednisolone dosage, resulting in a normotensive recipient. In previous experiments we found that a continuous

infusion of prednisolone would enhance the immunosuppressive effect as compared with single daily injections (6). We now studied the effects of a continuous infusion of prednisolone either with or without DSB pretreatment upon blood pressure after allogeneic kidney transplantation in rats.

MATERIAL AND METHODS

Rats

Adult male rats of the inbred WAG/Ro ($RT-1^u$) and BN/Ro ($RT-1^n$) histo-incompatible strains, weighing 250-300 g, were used. The rats had free access to tap water and food (Rat chow AM II, Hope Farms, Woerden, The Netherlands) containing 120 mmol sodium per kg.

Surgery

The microsurgical technique of Fisher and Lee (7) with a modification of the bladder-ureter anastomosis was used for heterotopic kidney transplantation. The recipients' native kidneys were removed at the time of transplantation. Control WAG/Ro rats had a unilateral nephrectomy through an abdominal approach.

Immunosuppression

Blood for donor strain blood (DSB) pretreatment was obtained from adult BN/Ro rats by cardiac puncture. Two weeks before transplantation, WAG/Ro recipients received 1 ml of fresh citrated BN blood intravenously. Prednisolone (Di-

Adreson-F aquosom, Organon, Oss, The Netherlands) was administered in diverse doses as a constant, 24-hour, subcutaneous infusion during two weeks, by means of an osmotic mini-pump (Alzet 2001). These minipumps were designed to deliver fluid at a constant rate with a nominal value of 0.9 μl/hr for seven days. At the end of the first week the minipumps were replaced by new ones.

Blood pressure

Tail systolic blood pressure (SBP) was measured plethysmographically in conscious, acclimatized rats after prewarming for 20 minutes. The average of three consecutive SBP readings was taken as the daily blood pressure.

Renal function

Renal function was estimated by means of the glomerular filtration rate (GFR) and determined as the clearance of 51 Cr-EDTA. This method allows for repeated measurements in the same animal and has been described in detail elsewhere (8). The GFR was measured one, three, and six weeks after transplantation or unilateral nephrectomy.

Experimental protocol

After determining control values of SBP, the rats receiving a kidney transplant (Tx) were divided into 10 groups with divergent immunosuppressive regimens (see Table 1) . Three groups (Tx1, Tx3, Tx5) received prednisolone alone in doses of 8, 4, or 2 mg/kg/day; five groups (Tx2, Tx4, Tx6,

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------------------------------------------------------------------
        TABLE 1   IMMUNOSUPPRESSIVE REGIMENS
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RECIPIENTS	n/N*	PREDNISOLONE	DSB	CONTROLS	n
group Tx1	6/10	8 mg/kg/day	no		
group Tx2	5/10	8 mg/kg/day	yes	group Nx1	12
group Tx3	6/11	4 mg/kg/day	no		
group Tx4	5/9	4 mg/kg/day	yes	group Nx2	11
group Tx5	3/8	2 mg/kg/day	no		
group Tx6	5/6	2 mg/kg/day	yes	group Nx3	10
---------	-	1 mg/kg/day	no		
group Tx7	7/10	1 mg/kg/day	yes	group Nx4	6
---------	-	0.5 mg/kg/day	no		
group Tx8	6/7	0.5 mg/kg/day	yes	group Nx5	5
group Tx9	7/9	no	yes		
group Tx10	0/5	no	no	group Nx6	10

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*   number  of  rats  starting  (N)  and  completing  (n)  the
    observation period.
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Tx7, Tx8) received DSB pretreatment in combination with prednisolone in doses of 8, 4, 2, 1, or 0.5 mg/kg/day; group Tx9 received only DSB pretreatment; and finally, group Tx10 received no immunosuppressive treatment at all.

These 10 groups of recipient rats were compared with six groups of unilaterally nephrectomized control rats (Nx), also listed on Table 1. The original plan called for the inclusion of control groups with or without DSB pretreatment. Half of all control rats received pretreatment with DSB. As DSB had no significant effect on the blood pressure of the Nx rats,

the pretreatment was subsequently disregarded. After unilateral nephrectomy, the first five groups of control rats received prednisolone in doses of 8, 4, 2, 1 or 0.5 mg/kg/day. The last group (Nx6) had no prednisolone.

Statistics

Only transplant recipients with a survival of 6 weeks were included in the statistical analysis. Differences in means within the Tx or Nx groups were compared by a one-way analysis of the variance. In case of significant differences ($p < 0.05$), the Newman-Keuls test was used to find which pairs of means were different (9).

RESULTS

Prednisolone in combination with DSB

The results for the recipient rats who received DSB pretreatment are given in figure 1. Recipient rats who received DSB pretreatment alone (Tx9) showed the expected rise in blood pressure in the second week after allogeneic kidney transplantation. These rats remained hypertensive till the end of the observation period. The allografted rats who received DSB in combination with the higher doses of prednisolone (8, 4, or even 2 mg/kg/day for 2 weeks) showed a similar rise in blood pressure. However, upon cessation of the prednisolone infusion, the rats in these groups (Tx2, Tx4, Tx6) became normotensive with blood pressure levels significantly below those of the Tx9 group who received DSB alone ($p < 0.05$).
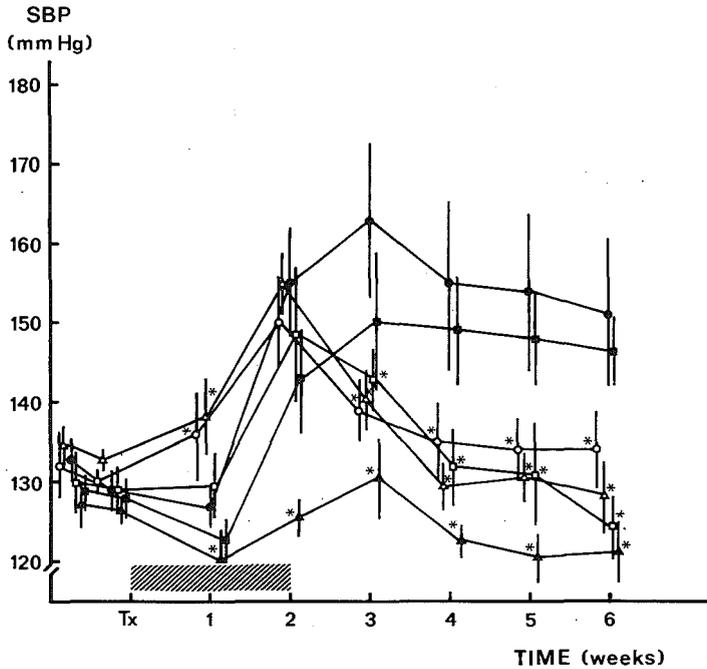
Figure 1: SBP of allogeneic kidney transplant recipients pretreated with DSB and infused continuously with divergent doses of prednisolone. Bar indicates duration of infusion in weeks. Means ± SEM are given. *p<0.05 compared with recipient rats receiving DSB only.

o: Tx2, n=5 ▲: Tx7, n=7
△: Tx4, n=5 ■: Tx8, n=6
□: Tx6, n=5 ●: Tx9, n=7

For specification of groups see Table 1.
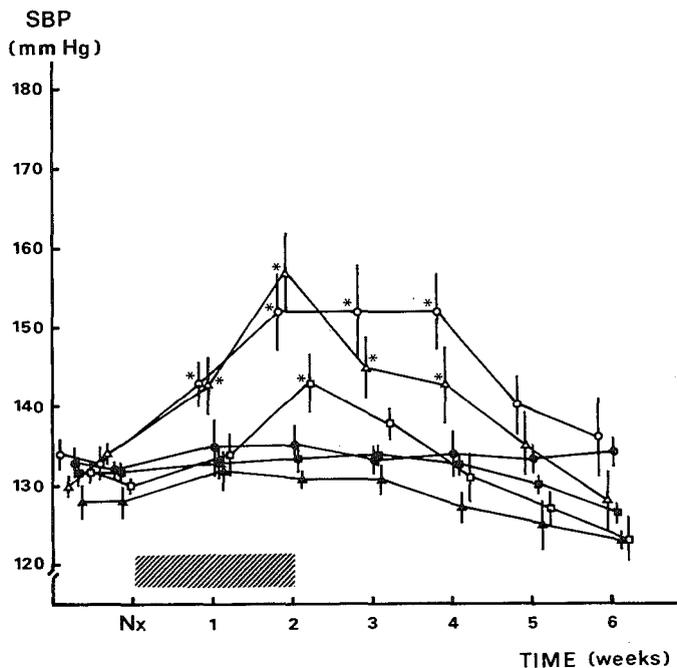
66

Figure 2: SBP of unilaterally nephrectomized control rats continuously infused with divergent doses of prednisolone. Bar indicates duration of infusion. *p<0.05 compared with Nx rats receiving no prednisolone.

o:	Nx1, n=12	▲:	Nx4, n=6
△:	NX2, n=11	■:	Nx5, n=5
□:	Nx3, n=10	●:	Nx6, n=10

For specification of groups see Table 1.

No hypertension was found at all in group Tx7 (1 mg/kg/day prednisolone + DSB). Throughout the study, the blood pressure of these rats remained significantly lower than that of the Tx9 rats who received DSB alone (p<0.05). Group Tx8 (0.5 mg/kg/day prednisolone + DSB) had a slightly but not significantly lower SBP than group Tx9.

Figure 2 gives the results for the six groups of Nx rats. The three groups that received prednsiolone in doses of 2mg/kg/day or more showed a rise in blood pressure. However, 6 weeks after the unilateral nephrectomy, all differences between the groups had disappeared regardless of dosage. At that stage all control rats had become normotensive.

The results for the GFR measurements are given in Table 2. When compared with Tx rats pretreated with DSB only, infusion with 4 or 8 mg/kg/day of prednisolone resulted in an increase of the GFR with about 50%. Infusion of 2 or 1 mg/kg/day had little effect on the GFR, but Tx rats infused with 0.5 mg/kg/day showed a decrease of the GFR. After discontinuation of the prednisolone infusion, the differences in GFR between the various Tx groups disappeared. A similar effect was noted in the prednisolone infused Nx rats.

Prednisolone without DSB pretreatment

Figure 3 gives the SBP of allograft recipients that did not receive DSB pretreatment (Tx1, Tx3, Tx5, Tx10) and were either infused with diverse doses of prednisolone for two weeks or received no immunosuppressive treatment at all.
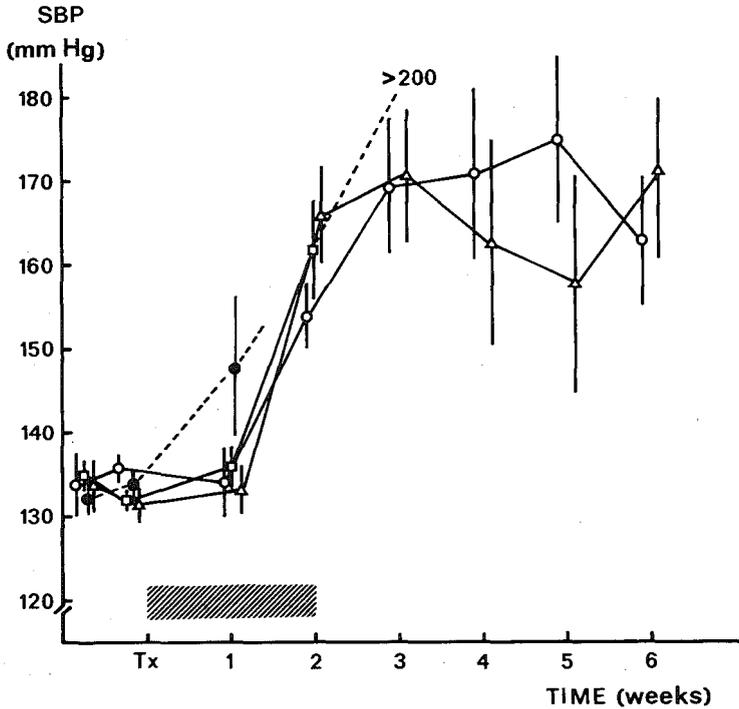
Figure 3: SBP of allogeneic kidney transplant recipients continuously infused with divergent doses of prednisolone. Bar indicates duration of infusion in weeks. Means ± SEM are given.
   o:Tx1, n=6
   Δ:Tx3, n=6
   □:Tx5, n=8
   ●:Tx10, n=5
For specification of groups see Table 1.

Table   2.   POSTOPERATIVE   GLOMERULAR   FILTRATION   RATE   OF
              ALLOGRAFTED   AND UNILATERALLY NEPHRECTOMIZED RATS

GROUP	GLOMERULAR FILTRATION RATE ml/min/100 g body weight		
	WEEK 1	WEEK 3	WEEK 6
Tx1	0.50 ± 0.17	0.30 ± 0.11	0.28 ± 0.11
Tx2	0.51 ± 0.10**	0.34 ± 0.13	0.30 ± 0.14
Tx3	0.36 ± 0.19	0.30 ± 0.10	0.23 ± 0.12
Tx4	0.48 ± 0.10	0.35 ± 0.09	0.33 ± 0.06
Tx5	0.13 ± 0.10**	(0.15 ± 0.01)#	(0.09 ± 0.02)#
Tx6	0.29 ± 0.11	0.25 ± 0.04	0.25 ± 0.06
Tx7	0.28 ± 0.11	0.23 ± 0.06	0.28 ± 0.08
Tx8	0.18 ± 0.07**	0.20 ± 0.06	0.20 ± 0.08
Tx9	0.31 ± 0.11	0.29 ± 0.10	0.26 ± 0.11
Tx10	0.08 ± 0.02**	no survivors	no survivors
Nx1	0.69 ± 0.08*	0.47 ± 0.16	0.49 ± 0.05
Nx2	0.64 ± 0.07*	0.50 ± 0.06	0.48 ± 0.06
Nx3	0.59 ± 0.04*	0.47 ± 0.04	0.45 ± 0.03
Nx4	0.49 ± 0.05	0.47 ± 0.05	0.42 ± 0.05
Nx5	0.44 ± 0.06	0.46 ± 0.05	0.46 ± 0.06
Nx6	0.50 ± 0.07	0.50 ± 0.07	0.50 ± 0.04

Data are mean $\pm$s.d.
* $p<0.05$ compared to unilaterally nephrectomized rats
    receiving no prednisolone (Nx6).
** $p<0.05$ compared to recipient rats receiving DSB but no
    prednisolone (Tx9).
    Three out of eight rats.For specification of groups see
    table 1.

Doses of 8 and 4 mg/kg/day caused a rise in blood pressure

during the infusion period, while in these groups (Tx1, Tx3)

the hypertension persisted after cessation of prednisolone

infusion. The rats in these groups remained hypertensive

throughout the follow-up period. The GFR of these two groups

was almost identical to the clearance of allografted rats

receiving the same dose of prednisolone in combination with

DSB (see Table 2). Five of the eight rats in group Tx5

receiving prednisolone alone in a dose of 2 mg/kg/day, died

within 7 days after cessation of the prednisolone infusion. The remaining three recipient rats treated with this low dose of prednisolone alone, survived for the remaining 4 weeks of the follow-up, albeit with a very high blood pressure. The GFR of these three rats was reduced and in the same low order as allografted rats receiving no immunosuppressive treatment at all. These rats (Tx10) all died within 11 days after transplantation. During this period the rats were normotensive until day 8, from then on their SBP started to rise (see figure 3).

DISCUSSION

The use of prednisolone effectively prolongs the survival of kidney and heart allografts in rats (10,11). We found that the administration of prednisolone by continuous infusion with subcutaneously implanted osmotic minipumps resulted in a prolongation of rat heart allograft survival enabling lower dosage than with daily injections (6). The present study revealed that continuous administration of prednisolone had a dosage-related effect on rat renal allograft survival. In the absence of adjuvant immunosuppressive therapy, the minimal dose of prednisolone required for an immunosuppressive effect was shown to be 2 mg/kg/day in continuous infusion. However, at the end of the two-week follow-up period, the function of these grafts was comparatively lower than that of recipient rats treated with Cyclosporin A for one week after allogeneic renal transplantation (3). In combination with DSB

pretreatment, a dose of only 1 mg/kg/day prednisolone for two weeks had a similar effect as Cyclosporin A.

In agreement with other experimental studies in rats using methylprednisolone (12,13), our present study also revealed that prednisolone has a distinct dosage-related effect upon blood pressure. Several mechanisms have been suggested to explain the rise in blood pressure caused by glucocorticoids, such as a) metabolization of prednisone to aldosterone (14); b) increased activity of the renin-angiotensin system, although elevated blood pressure levels were noted even during continuous captopril adminstration suggesting participation of other mechanisms (12); c) salt retention (15); or d) activation of the sympathetic nervous system (16).

Various authors have reported that corticosteroids contributed to the development of hypertension after renal transplantation in man. One study (5) described a correlation between diastolic blood pressure and the dose of prednisone in a group of allograft recipients. These findings were not confirmed in other studies (17,18). It was reported, however, that a reduction in dosage of the corticosteroids or conversion to alternate-day therapy resulted in a fall in blood pressure (19,20).

The results of our present study show that the hypertension observed in rat renal allograft recipients treated with prednisolone may be due to two different mechanisms. Recipient rats treated with 2 mg/kg/day prednisolone or more may develop high blood pressure due to a

direct action of steroids. This type of hypertension is reversible, as shown in the allograft recipients pretreated with DSB and receiving 2 mg/kg/day or more, as well as in the unilaterally nephrectomized rats. All these rats became normotensive after cessation of the prednisolone infusion. In some groups of recipient rats hypertension persisted after cessation of prednisolone infusion. These rats had either not been pretreated with DSB or if they had, prednisolone was administered in a very low dose (0.5 mg/kg/day). Such treatment obviously amounts to insufficient immunosuppression and consequently, to an incomplete prevention of rejection of the allograft. In comparison, we have previously found that in this rat model the use of Cyclosporin A prevented the development of post-transplantation hypertension (3). In humans, the chronic types of rejection processes are also associated with a rise in blood pressure (17,21).

The administration of prednisolone in combination with DSB pretreatment as adjuvant immunosuppressive therapy led to allografted rats either becoming normotensive after cessation of the corticosteroid infusion or to them not developing hypertension at all. These findings illustrate the important role of the rejection processes in the development of hypertension as surmised in our previous experiment using DSB pretreatment as the only immunosuppressive procedure (1).

In conclusion, the present experiment using prednisolone in rat renal allograft recipients, clearly shows the effectiveness of this drug. However, unless extreme care is taken to find the optimal dosage of prednisolone in

combination with adjuvant immunosuppression, there is always the inherent risk of hypertension.

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CHAPTER 6

THE EFFECT OF A REDUCED SODIUM INTAKE ON POST-RENAL
TRANSPLANTATION HYPERTENSION IN RATS

The effect of a reduced sodium intake on post-renal transplantation hypertension in rats

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Summary

1. In an experimental model of post-renal transplantation hypertension in rats, we studied the effect of a reduction of sodium intake on the development of this type of hypertension.

2. Systolic blood pressure, plasma renin concentration and renal function were measured regularly in recipients of an allogeneic kidney transplant that had previously undergone active immunological enhancement.

3. Transplant recipients on a normal diet showed a rise in systolic blood pressure during the second week after transplantation. The systolic blood pressure of recipients on a low sodium diet remained normotensive throughout the 15 weeks follow-up period.

4. The plasma renin concentration was low in the hypertensive recipients on a normal diet, as compared with unilaterally nephrectomized controls. Although the plasma renin concentration of recipients on a low sodium diet fell below that of unilaterally nephrectomized controls on a low sodium diet, it was higher than that of recipients on a normal diet.

5. The renal function of transplant recipients was greatly reduced compared with that of control rats. The glomerular filtration rate was reduced to a greater extent than the effective renal plasma flow.

6. In a separate experiment it was revealed that a similar reduction in the glomerular filtration rate of kidneys permanently damaged by temporary ischaemia did not result in an increase in the systolic blood pressure.

7. Survival up to 6 weeks after transplantation was the same for both groups of recipients. Recipients on a low sodium diet, however, showed a better 15 weeks survival, probably owing to the absence of hypertension in this group.

8. The prevention of the development of hypertension by means of a reduction of sodium intake, points to an involvement of sodium retention in this post-transplantation hypertension model.

Key words: hypertension, kidney transplantation, plasma renin concentration, sodium intake.

Introduction

Hypertension after clinical renal transplantation is a relatively common medical complication [1]. It has been reported in a large percentage of adult [2] as well as paediatric transplant recipients [3]. This hypertension after renal transplantation is usually attributed to various factors [1]. Among these, the corticosteroid therapy, renal artery stenosis, the presence of the recipient's own diseased kidney, and acute or chronic rejection are most frequently mentioned. Investigation of the relevance of the individual hypertensive mechanisms in human renal transplantation is difficult, owing to the great variation of factors normally present.

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We have recently succeeded in developing an experimental model of post-renal transplantation hypertension [4]. This model consisted of an allogeneic kidney transplantation between normotensive donor and recipient rats. The survival time of the recipients was prolonged by means of active immunological enhancement, avoiding the use of corticosteroids. It was noted that the hypertension was associated with a low plasma renin concentration. We surmized that the hypertension and the low plasma renin concentration might be the result of sodium retention by the transplanted kidney. Consequently, we studied the effect of a reduction of sodium intake upon the blood pressure after allogeneic renal transplantation in rats.

Methods

Rats

Adult male rats, circa 3 months of age with a body weight of 250–300 g, of highly inbred Wistar (WAG/Ro) and Brown Norway (BN) strains were used. These strains differ at the major histocompatibility locus. The WAG/Ro rats are RT-1^y, the BN rats are RT-1^n [5].

Diets

The rats had free access to food and water. Both normal food and sodium-poor diet were obtained from Hope Farms, Woerden, The Netherlands. The normal diet contained 120 mmol of Na/kg, the sodium-poor diet contained 5.6 mmol of Na/kg, and the drinking fluid (tap water) contained 2.4 mmol of Na/l. Besides a difference in sodium content there was also a small difference in protein content: the normal diet contained 24% of protein, the sodium-poor diet contained 17–18% of protein.

From separate balance studies we know the food and water intake of transplant recipients on a normal diet. The intake is variable just after transplantation but stabilizes after a few weeks at about 6 g of food and 20 ml of water per day per 100 g body weight. The calculated sodium intake then amounts to about 0.77 mmol day^{-1} 100 g^{-1} body weight. No data are at present available on the food intake of recipients on a sodium-poor diet. When the same amount is consumed, however, the daily sodium intake amounts to about 0.085 mmol/100 g body weight. In these rats the major part of the sodium intake stems from the drinking fluid.

Experimental procedure

Comparison of a normal and a low sodium diet upon post-renal transplantation hypertension. Two groups of WAG/Ro transplant recipients and two groups of WAG/Ro unilaterally nephrectomized control rats were compared. The remaining kidneys of the previously unilaterally nephrectomized BN rats were used as donor kidneys. The renal transplantation was performed with microsurgical techniques as described previously [4, 6]. The recipients underwent bilateral nephrectomy at the time of transplantation. Active immunological enhancement by means of a single intravenous injection of 1 ml of BN rat blood to the recipient, 2 weeks before transplantation, was carried out to prolong graft survival [4, 7]. Transplantations were considered technically successful when recipients survived for at least 1 week.

One group, initially consisting of 17 successful allograft recipients, had free access to normal food and so did a group of four unilaterally nephrectomized controls. A third group, initially consisting of 16 successful allograft recipients, had free access to a low sodium diet from the day of transplantation. A group of six unilaterally nephrectomized control rats also had a low sodium diet from the day of nephrectomy.

In all four groups, systolic blood pressure, renal function and plasma renin concentration were measured regularly. The systolic blood pressure was measured six times a week from 1 week before, to 3 weeks after transplantation or nephrectomy. After 3 weeks the systolic blood pressure was measured every other day. The glomerular filtration rate and the effective renal plasma flow were measured 1, 3, 6, 9 and 15 weeks after transplantation or nephrectomy. At these times, 400 μl of blood was sampled, 15 min after initiation of pentobarbital anaesthesia (60 mg/kg intraperitoneally), to determine the plasma renin concentration and the plasma levels of creatinine and urea. At the end of the 15 week follow-up period, the glomeruli in the kidneys were counted.

Effect of the presence of an intact native kidney. A group of five allograft recipients, surviving for at least 9 weeks, was studied. These recipients had one of their own kidneys removed at the time of transplantation, the second one being removed 3 weeks after transplantation. The rats received a normal diet. Systolic blood pressure and renal function were measured as in the previous experiment. At week 3 renal function was measured 2–3 h after nephrectomy.

Effect of a reduction in function of non-transplanted kidneys. In a group of 10 WAG/Ro rats renal function was reduced by means of an

induced temporary ischaemia. A clamp (Schwarz aneurysm clamp) was applied to the right renal artery for 105 min. After a recovery period of 1 week, the contralateral kidney was removed. Systolic blood pressure was measured regularly, starting 1 week before the induced ischaemia. Renal function was measured 1, 3, 6 and 9 weeks after ischaemia. The measurement at week 1 was taken 2–3 h after nephrectomy.

Measurements

Systolic blood pressure. This was measured in conscious rats by tail plethysmography (Electro Sphygmo Manometer PE 300, Narco Bio-System). The animals were prewarmed at an environmental temperature of 32°C and to take their blood pressure were placed in small restraining cages. The daily systolic blood pressure was the average of at least three consecutive measurements. The systolic blood pressure data given in the Results section consist of the mean systolic blood pressure taken on 3 consecutive days.

Glomerular filtration rate and effective renal plasma flow. These were measured, under pentobarbital anaesthesia, as the plasma clearances of ^{51}Cr-labelled EDTA and [^{125}I]iodohippurate respectively. This method, which allows for repeated measurements in the same rat, has been described in detail elsewhere [6, 8].

Plasma creatinine, urea and renin concentrations. These were determined in samples obtained during the measurements of renal function, before

the injection of the radioactive compounds. The samples were taken about 15 min after the intraperitoneal injection of pentobarbital (60 mg/kg). Creatinine and urea were measured by kinetic methods on a computer directed analyser in 50 μl and 10 μl of plasma respectively [9].

The plasma renin concentration was determined through the angiotensin I (ANG I) generated after incubation of 100 μl of plasma with an excess of rat renin substrate at pH 6.5 [10].

Number of glomeruli. At the end of the 15 week follow-up period, the remaining animals were killed just after the intra-arterial injection of 1 ml of India ink close to the renal artery. The kidney was removed, weighed and the glomeruli were counted by a modification [11] of the method of Damanian *et al.* [12].

Statistics

All results are given as means ± SD. Comparison of the differences in mean values of the transplant recipients on a normal and a low sodium diet was done by Student's *t*-test. A difference was considered statistically significant when the probability level reached $P < 0.05$.

Results

Comparison of recipients on either a normal or a low sodium diet after allogeneic transplantation

Systolic blood pressure. Systolic blood pressures of both groups of allograft recipients are depicted
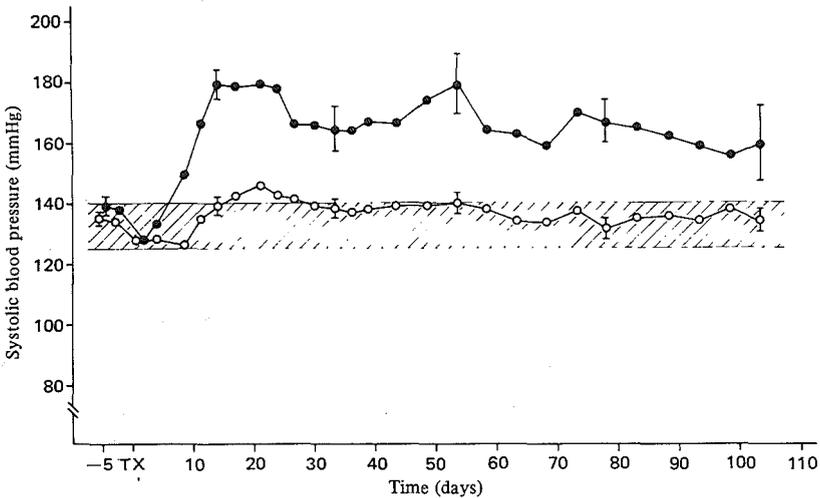

FIG. 1. Systolic blood pressures of renal allograft recipients on a normal (●) and a sodium-poor (○) diet. The cross-hatched area represents the normotensive systolic blood pressure of unilateral nephrectomized control rats. TX, Day of transplantation.

in Fig. 1. In recipients on a normal diet the systolic blood pressure started to increase from day 8 after transplantation. From then on the systolic blood pressure remained elevated until the end of the 15 weeks follow-up period.

In contrast, the systolic blood pressures of transplant recipients on a low sodium diet remained within the range of normotensive unilaterally nephrectomized control rats, except for a slight elevation around week 3 after transplantation. The systolic blood pressures of recipients on a low sodium diet were significantly lower than those of recipients on a normal diet from day 8 onwards.

Renal function. Renal function measurements made at various times after transplantation or unilateral nephrectomy are given in Tables 1 and 2, together with data on body weight, systolic blood pressure and plasma renin concentration. Renal function results and the body weights of donor rats, and pre-operative data regarding body weight and systolic blood pressure of recipients, are also included.

The glomerular filtration rate of transplanted kidneys in rats on a normal diet amounted to 30–40% of that of nephrectomized rats on a normal diet. In general, the glomerular filtration rate of recipients on a low sodium diet was somewhat higher than that of recipients on a normal diet. The difference was statistically significant at weeks 3, 6 and 9.

There was less divergence in the effective renal plasma flow of transplanted kidneys when rats on a normal diet were compared with those on a low sodium diet. In comparison with that of unilaterally nephrectomized control rats, the effective renal plasma flow of transplanted kidneys appeared less impaired than the glomerular filtration rate, leading to a fall in the glomerular filtration rate/effective renal plasma flow ratio.

Plasma renin concentration. The renin concentration of plasma obtained under pentobarbital anaesthesia from transplant recipients on both diets was less than that of their respective controls. The plasma renin concentration in recipients on a normal diet remained at the same low level throughout the follow-up. In the recipients on a low sodium diet, the mean plasma renin concentration values were higher than those of recipients on a normal diet, but there was a tendency to decrease. Statistically, however, the difference was only significant at weeks 1 and 3.

Wet kidney weight and number of glomeruli. The glomeruli of non-transplanted BN kidneys were counted in a separate group of unilaterally nephrectomized BN rats. In nine rats with a body weight of 399±25 g, the wet kidney weight 15

TABLE 1. *Pre- and post-transplant renal function in allogeneic kidney transplant recipients on a normal or a sodium-poor diet*

All data are means ± SD. *P<0.05 compared with recipients on a normal diet. BN donor, BN donor rat before kidney transplantation; TX recip., WAG recipient rat before kidney transplantation; SBP, systolic blood pressure; PRC, plasma renin concentration; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; P_{cr}, plasma creatinine concentration; P_{ur}, plasma urea concentration. N.D., Not determined.

| | Pre-transplant | | | | Post-transplant | | | | | | | | | |
| | Normal diet | | Na-poor diet | | Week 1 | | Week 3 | | Week 6 | | Week 9 | | Week 15 | |
	BN donor	TX recip.	BN donor	TX recip.	Normal diet	Na-poor diet	Normal diet	Na-poor diet	Normal diet	Na-poor diet	Normal diet	Na-poor diet	Normal diet	Na-poor diet
No. of rats	17	17	16	16	17	16	12	14	11	11	9	11	5	11
Body wt. (g)	280±25	299±22	281±21	299±25	279±19	255±28*	274±18	275±26	291±17	300±21	300±22	319±19	321±18	338±22
SBP (mmHg)	N.D.	138±8	N.D.	134±8	149±16	125±9*	179±31	146±17*	166±36	139±14*	163±14	134±13*	159±29	134±13*
PRC (ng of ANG I h⁻¹ ml⁻¹)	N.D.	N.D.	N.D.	N.D.	23±12	70±25*	19±2	43±18*	23±10	34±16	15±5	28±9	20±9	32±16
GFR (ml/min)	1.02±0.21	N.D.	1.08±0.13	N.D.	0.50±0.22	0.41±0.20	0.41±0.15	0.60±0.24*	0.51±0.30	0.74±0.23*	0.63±0.23	0.96±0.32*	0.69±0.44	0.88±0.32
ERPF (ml/min)	3.51±0.61	N.D.	3.78±0.34	N.D.	2.21±1.01	1.75±0.80	2.77±0.80	3.21±1.10	2.86±1.16	3.99±0.63*	3.68±0.80	4.44±0.68	3.64±1.35	4.03±0.89
GFR/ERPF	0.29±0.03	N.D.	0.29±0.03	N.D.	0.24±0.05	0.23±0.07	0.15±0.02	0.19±0.06*	0.18±0.08	0.18±0.04	0.17±0.03	0.21±0.05*	0.17±0.06	0.22±0.03
P_{cr} (μmol/l)	78±8	N.D.	77±9	N.D.	134±61	143±0.74	126±26	87±31*	112±30	64±9*	93±24	59±7*	94±37	62±7*
P_{ur} (mmol/l)	13.4±2.2	N.D.	13.4±2.2	N.D.	24.9±11.3	16.2±12.3	27.2±8.0	23.9±15.8	27.4±8.2	10.2±3.8*	24.0±7.4	15.9±2.6*	23.3±9.6	15.8±3.1*

TABLE 2. *Renal function in unilaterally nephrectomized control rats on a normal or a sodium-poor diet*

Abbreviations are defined in Table 1.

| | Pre-nephrectomy | | Post-nephrectomy | | | | | | | | | |
| | | | Week 1 | | Week 3 | | Week 6 | | Week 9 | | Week 15 | |
	Normal diet	Na-poor diet	Normal diet	Na-poor diet	Normal diet	Na-poor diet	Normal diet	Na-poor diet	Normal diet	Na-poor diet	Normal diet	Na-poor diet
No. of rats	4	6	4	6	4	6	4	6	4	6	4	6
Body wt. (g)	299±31	285±35	286±29	269±29	298±26	285±31	318±27	308±40	332±29	331±37	344±32	344±32
SBP (mmHg)	137±6	133±6	135±10	128±11	134±10	139±5	137±10	137±9	129±9	139±8	129±8	137±6
PRC (ng of ANG I h^{-1} ml^{-1})	N.D.	N.D.	69±25	89±22	58±14	109±34	68±16	131±95	66±14	60±3	91±26	83±1
GFR (ml/min)	N.D.	N.D.	1.33±0.05	1.28±0.13	1.36±0.17	1.44±0.36	1.50±0.12	1.66±0.15	1.72±0.12	1.62±0.30	1.73±0.14	1.44±0.15
ERPF (ml/min)	N.D.	N.D.	3.73±0.10	3.09±0.31	4.13±0.22	4.00±0.42	4.14±0.17	4.36±0.42	4.37±0.24	4.20±0.48	4.45±0.39	4.20±0.19
GFR/ERPF	N.D.	N.D.	0.36±0.01	0.35±0.02	0.33±0.02	0.36±0.03	0.36±0.02	0.38±0.03	0.39±0.01	0.38±0.03	0.39±0.05	0.34±0.04
P_{cr} (μmol/l)	N.D.	N.D.	59±5	56±4	60±6	55±5	54±3	51±4	56±4	46±3	57±2	54±3
P_{ur} (mmol/l)	N.D.	N.D.	10.5±1.4	8.6±0.9	10.6±1.4	8.4±1.1	9.8±0.3	9.1±1.2	11.3±0.6	9.4±1.6	10.3±0.5	8.6±0.7

weeks after unilateral nephrectomy amounted to 436 ± 59 mg/100 g body weight and the number of glomeruli was $31\,240 \pm 4765$.

The glomerular counting of transplanted BN rat kidneys at the end of the follow-up period showed a reduction in the number of glomeruli. Transplanted kidneys from recipients on a normal diet ($n = 5$) weighed 654 ± 123 mg/100 g body weight and contained $21\,089 \pm 4297$ glomeruli. The wet kidney weight of transplanted kidneys from recipients on a sodium-poor diet ($n = 11$) was 515 ± 45 mg/100 g body weight and the kidneys contained $26\,911 \pm 3164$ glomeruli. The difference between the wet kidney weights as well as the numbers of glomeruli were significant when recipients on a normal diet were compared with those on a sodium-poor diet ($P < 0.05$).

Kidneys from unilaterally nephrectomized WAG rats on either a normal or a low sodium diet contained $35\,350 \pm 4174$ ($n = 4$) and $34\,512 \pm 5322$ ($n = 5$) glomeruli respectively.

Survival. The sodium-poor diet after transplantation had a marked effect on the survival of the recipients. As depicted in Fig. 2, both groups had a mortality of 30% between week 1 and week 3. After the week 3 only recipients on a normal diet died. This resulted in a 15 week survival of 30% of the recipients on a normal diet and 70% of the recipients on a sodium-poor diet.

Effect of the presence of an intact kidney

The systolic blood pressure of transplanted recipients on a normal diet with one native kidney still *in situ* remained at a normal level till the end of week 3. After the removal of the native kidney the systolic blood pressure increased. Just before removal of the native kidney, the mean systolic blood pressure was 114 ± 11 mmHg, and was 159 ± 23 mmHg 3 weeks later.

Directly upon the removal of the native kidney of the transplanted rats, the function of the transplanted kidney was very low. The glomerular filtration rate and effective renal plasma flow gradually improved and 6 weeks after nephrectomy they both reached values comparable with those of kidneys of transplanted rats on a normal diet, The glomerular filtration rate was 0.77 ± 0.33 ml/min and the effective renal plasma flow was 3.90 ± 0.70 ml/min.

Effect of a reduction in renal function

Renal function results and systolic blood pressures of rats that underwent temporary clamping of the renal artery are given in Table 3. The glomerular filtration rate and effective renal
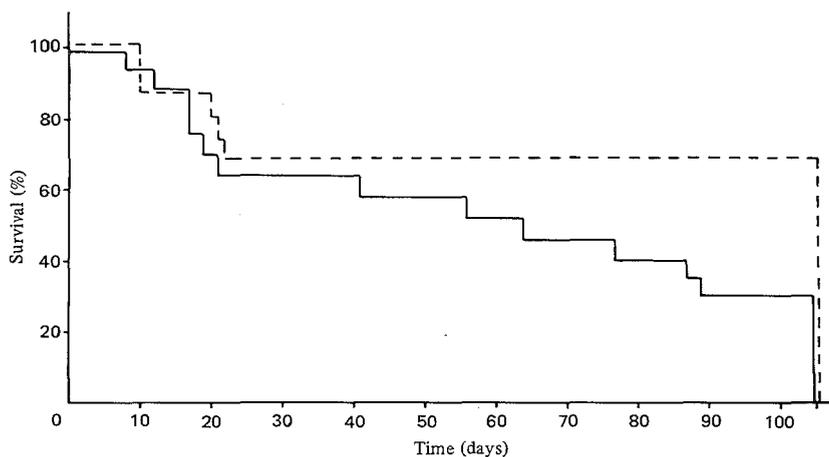
FIG. 2. Mortality of renal allograft recipients on a normal (———) and a sodium-poor diet (— — —). Surviving rats were killed 15 weeks after transplantation. For rats on a normal diet 100% = 17; for rats on a sodium-poor diet 100% = 16.

TABLE 3. *Renal function parameters and systolic blood pressure of rats with a renal artery temporarily clamped, and contralateral nephrectomy after 1 week*

All data are means ± SD. Abbreviations are defined in Table 1.

	Post-nephrectomy			
	Week 1	Week 3	Week 6	Week 9
No. of rats	10	8	7	6
Body wt. (g)	250 ± 9	271 ± 10	277 ± 11	285 ± 9
SBP (mmHg)	112 ± 8	120 ± 5	130 ± 8	128 ± 9
GFR (ml/min)	0.32 ± 0.09	0.42 ± 0.14	0.38 ± 0.22	0.40 ± 0.21
ERPF (ml/min)	1.36 ± 0.39	1.82 ± 0.48	1.88 ± 0.69	1.96 ± 0.80
GFR/ERPF	0.24 ± 0.04	0.23 ± 0.02	0.19 ± 0.06	0.20 ± 0.03
P_{cr} (μmol/l)	207 ± 40	128 ± 21	112 ± 23	127 ± 24
P_{ur} (mmol/l)	31.9 ± 5.8	23.6 ± 4.1	23.6 ± 4.5	27.5 ± 5.0

plasma flow of the damaged kidneys were similar to or lower than those of transplanted rats on a normal diet. In spite of their diminished renal function these rats remained normotensive throughout the 9 weeks follow-up period.

Discussion

The present findings confirm the previously reported elevation in blood pressure after allogenic kidney transplantation in actively enhanced recipients [4]. The hypertension persisted during a follow-up period of 15 weeks. The experiments demonstrated that hypertension after renal transplantation in rats can be prevented by a reduction of the sodium intake. The normal blood pressure

range in these rats significantly increased survival at the end of a 15 weeks follow-up period.

There are other models of experimental hypertension in rats in which only one kidney is present. Of these models renovascular, one-kidney, one-clip (1K1C) hypertension is the one most investigated. 1K1C hypertension is associated with an increase in extracellular volume and exchangeable sodium and a low or normal plasma renin activity [13-16]. According to the criteria of the vasoconstrictor-volume concept, the hypertension in the 1K1C model is considered to be volume-dependent [17, 18].

Our model of post-renal transplantation hypertension, however, is different from the renovascular 1K1C type. A reduction of the dietary intake

of sodium alone, which in our model prevented the post-transplant hypertension, does not depress the systolic blood pressure in the 1K1C rats. In those rats the hypertension becomes renin-dependent [14, 19–22], and only extreme sodium and volume depletion reduce the systolic blood pressure [23].

If renovascular alterations at the transplanted kidney were of importance, renal transplantation with one native kidney still *in situ* should resemble the two-kidney, one-clip hypertension model. However, in our experiment no hypertension was observed. Our findings indicate that the observed hypertension does not have a renovascular origin. Consequently, the transplanted kidney itself should be considered of primary importance in the development of this type of hypertension.

Despite the divergence from the 1K1C model, we consider that sodium retention is involved in the hypertension after allogeneic kidney transplantation. However, in contrast to the Goldblatt models, where the cause of sodium retention lies outside the kidney, i.e. the stenotic artery, we think that in post-transplant hypertension sodium retention is primarily a renal process.

Although active immunological enhancement does prolong the survival time of rats after renal transplantation, it does not completely suppress rejection. Consequently, damage of the transplanted kidney, due to immunological reactions, is present. This was not only shown by histopathology [4], but is also indicated by the impairment of the glomerular filtration rate and by the number of deaths occurring between days 7 and 21 after transplantation. The renal functional impairment of the allogeneic recipients resembles the picture of acute experimental glomerulonephritis, secondary to immunological reactions [24, 25]. In acute experimental glomerulonephritis in rats, changes in renal function similar to those in allografted kidneys, i.e. a greater impairment of the glomerular filtration rate than of the effective renal plasma flow, together with sodium retention and suppression of the renin secretion, was noted.

Rats with an allogeneic transplanted kidney together with one intact native kidney *in situ* did not develop hypertension. In this case the decreased sodium excretion by the allografted kidney is probably adequately compensated for by the native kidney at a normal blood pressure. Once the native kidney is removed, the kidney graft apparently cannot handle the sodium excretion and a volume-dependent hypertension will develop owing to sodium accumulation.

The plasma renin concentration was measured in plasma obtained after 15 min of pentobarbital anaesthesia, which is known to stimulate renin release [26]. The low plasma renin concentration values, found in both hypertensive and normotensive recipients, must therefore indicate that the renin–angiotensin system is not involved in the development of post-transplant hypertension. Instead, the low plasma renin concentration values indicate sodium and water retention, as postulated before, whereas the higher plasma renin concentration values in recipients on a low sodium diet suggest that the extent of sodium retention is less than in recipients on a normal diet.

It can be argued that the observed hypertension might be the result of the impaired renal function alone. However, rats with a diminished renal function, due to temporary clamping of the renal artery, did not develop hypertension at all, although their kidney function was the same or worse than that of transplanted rats on a normal diet. This finding is in agreement with those reported by other investigators [27]. Consequently, an impaired renal function as such did not cause hypertension in our model.

The low sodium diet may have had better immunosuppressive properties than the normal diet. However, the mortality between day 7 and day 21 is the same for the transplanted rats on either diet. In both groups about 30–35% of the rats died during this period. This may indicate a similarity of the immunological rejection process in this period. In transplanted rats on a sodium-poor diet no rats succumbed after day 21, probably due to the prevention of hypertension.

The sodium-poor diet contained less protein than the normal diet. It has been shown, in rats with a moderate renal insufficiency, that daily protein intake influences the deterioration rate of renal function as well as survival [28]. A difference in daily protein intake between transplant recipients on either a normal or a sodium-poor diet could also have had a different effect on renal function and survival rate. However, the difference in the protein content of both diets is relatively small, especially when compared with other studies relating protein intake and the progression of renal disease [27, 28]. Consequently, we assume that the changes induced during the first 3 weeks after transplantation are of major importance in explaining the present findings.

In conclusion, the increase in systolic blood pressure after allogeneic renal transplantation in immunologically enhanced recipients was prevented by reducing the daily sodium intake. The hypertension was not caused by the reduced renal function as such. These findings indicate that sodium is involved in this model of hypertension after renal transplantation in rats.

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CHAPTER 7

RENAL FUNCTION, SODIUM METABOLISM AND PLASMA VOLUME DURING THE
DEVELOPMENT   OF   HYPERTENSION   AFTER   ALLOGENEIC   KIDNEY
            TRANSPLANTATION IN THE RAT.

            This chapter has been submitted

RENAL FUNCTION, SODIUM METABOLISM AND PLASMA VOLUME DURING THE DEVELOPMENT OF HYPERTENSION AFTER ALLOGENEIC KIDNEY TRANSPLANTATION IN THE RAT

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SUMMARY

This study was undertaken to establish the relative significance of alterations in renal function and sodium and water intake and output during the development of hypertension after allogeneic kidney transplantation in our rat model. In this model the graft survival of the recipient rats was prolonged by pretreatment with donor strain blood. An inverse correlation between blood pressure and renal function of the recipient rats was noted 3 and 6 weeks after transplantation. Sodium was retained during the rise in blood pressure and excreted once the hypertension was established. Furthermore, polydipsia and polyuria developed after transplantation and more water was retained. Urinary osmolality was markedly decreased after transplantation and calciuria and proteinuria were present. The plasma volume of the recipient rats was significantly elevated when compared with unilaterally nephrectomized control rats.

These results substantiate our hypothesis that in this rat model post-transplantation hypertension is mediated by a different water and sodium handling as a result of functional alterations in the allografted kidney.

INTRODUCTION

Post-transplantation hypertension is a frequent complication of human kidney transplantation occuring in about 50 % of the recipients (1,2). Several hypotheses have been reported to explain this phenomenon. These involve the use of

corticosteroids (3), the presence of the recipients' own diseased kidneys (4) and rejection (5).

We have developed a model to study post-transplantation hypertension in the rat using two normotensive rat strains. The survival of the allograft was prolonged by pretreatment of the recipient rats with donor strain blood. In this model the blood pressure of the recipient rats started to rise at the end of the first week after transplantation. The function of the transplanted kidney was markedly impaired and although the rats were hypertensive, the plasma renin concentration was suppressed (6). A later study confirmed these results and showed that the hypertension could be prevented by a reduction in the sodium intake (7). Further experiments showed that the development of hypertension could also be prevented by improving the immunosuppressive therapy by the use of cyclosporin A (8). From our experimental data we surmized that the development of hypertension was the result of structural and functional changes in the allografted kidney causing sodium retention and volume expansion, which are both known to be the mediators in the development of hypertension. In the present study we evaluated the relation between changes in renal function and blood pressure. We also carried out metabolic studies to determine the water and sodium balance. Finally, plasma volumes were measured at several intervals after allogeneic kidney transplantation.

MATERIALS AND METHODS

General: adult male rats of the inbred Wag/Ro and BN/Ro strains, weighing 250 - 300 gram, were used. The rats had free access to food (Rat chow AM II, Hope Farms, the Netherlands) containing 120 meq sodium per kilogram and to tap water (3 meq sodium per litre). Transplantation of the BN donor kidney was performed using a method described previously (6). To prolong graft survival the Wag/Ro recipient rats were pretreated with 1 ml of donor strain blood intravenously 2 weeks before transplantation.

First experiment: this consisted of a retrospective analysis of data concerning 27 allograft recipient rats who survived for at least 6 weeks and who were included in several other studies. In this case we studied the correlation between the systolic blood pressure (SBP) and the glomerular filtration rate (GFR). The SBP was obtained from trained and conscious rats by means of the tail cuff method, using a sphygmomanometer (Narco Bio Systems, Houston, USA). The GFR was determined 1, 3, and 6 weeks after transplantation as the clearance of 51 Cr-EDTA. This method has been published in detail elsewhere (9).

Second experiment: 21 Wag/Ro rats were placed in metabolic cages (Techniplast). Body weight (BW), water and food intake and urine volume were measured daily between 10 and 11 a.m. After 2 - 8 days of adaptation, control values were determined. Then 12 rats received a kidney allograft and 9 control rats were unilaterally nephrectomized. These two

groups were followed for 6 weeks. Urinary sodium was measured by flame photometry (Klina Flame, Beckmann) using lithium as internal standard. Calcium was measured colorimetrically (Rapid Stat Kit, Pierce Chemical Company) and the osmolality of the urine was determined by its freezing point (Vogel micro Osmometer, Roebling). Urinary protein was measured colorimetrically (Bio Rad Laboratories).

Third experiment: the plasma volumes of a group of 7 allografted Wag/Ro rats were compared with the plasma volumes of 7 unilaterally nephrectomized control rats. Plasma volumes were determined 1, 2, 3, and 6 weeks post-operative in ether anaesthetized animals using Evans Blue solution. Via the penis vein 0.2 ml Evans Blue solution (2 mg/ml) per 100 gram BW was injected slowly. After 5 minutes equilibration time a blood sample was drawn from the eye plexus. The extinction of the plasma was measured at a wavelength of 600 nm. The haematocrit (Ht) was determined after centrifugation of a blood sample taken in a capillary tube. The plasma volume (PV) and blood volume (BV) were calculated from standard formulas.

Statistics: in the first experiment the relationship between the GFR and the SBP was calculated by linear regression. To evaluate the differences between the transplanted and the control group, the unpaired t-test was used in the other two experiments.
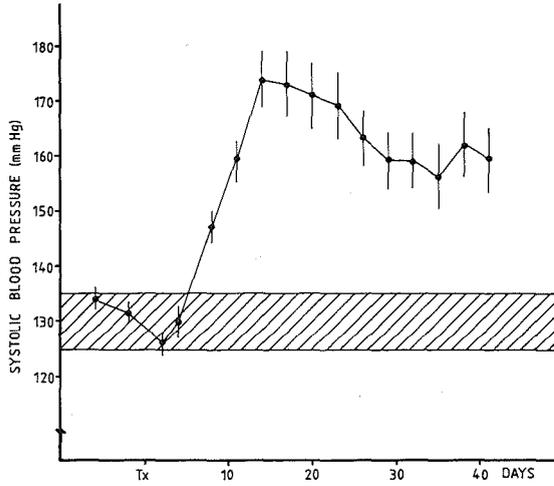
Fig 1. Systolic blood pressure of allograft recipient rats
pretreated with donor strain blood. Shaded area
represents the normotensive blood pressure range of
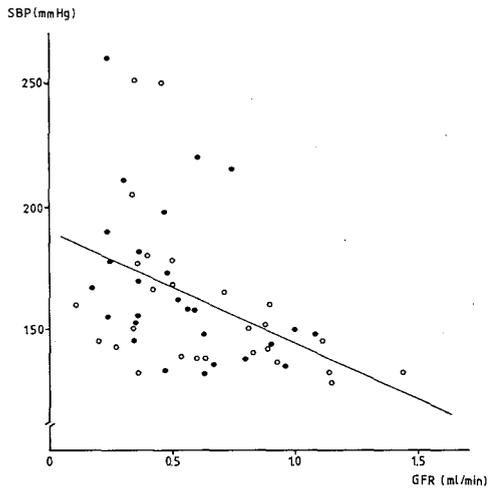the recipient rat strain. Data are shown as means±SEM

Fig 2. The systolic blood pressure as a function of the GFR at
week 3 (o) and week 6 (●).
SBP=190-45xGFR, r=-o.44; p<0.001.

RESULTS

First experiment: the mean SBP of the 27 recipient rats is shown in fig. 1 with the shaded area representing the normotensive SBP range. The SBP started to increase by the end of the first week after transplantation and was significantly elevated from day 8 till the end of the 6 week follow-up period. Fig. 2 shows the correlation between the GFR and the SBP at week 3 and 6. There was a weak relationship between both parameters ($r = -0.44$), which was significant ($p < 0.001$). No correlation was found between the GFR and the SBP one week after transplantation.

Second experiment: seven of the 12 recipient rats survived for more than 6 weeks and were included in this study. Fig. 3 and 4 depict the urinary calcium and protein excretion. The excretion of protein and calcium started to rise in the first week after transplantation and remained elevated throughout the study ($p < 0.05$ versus control group). In the unilaterally nephrectomized group no differences in calcium or protein excretion were noted after nephrectomy compared to pre-operative values. In fig. 5 urinary osmolality is given. Unilateral nephrectomy resulted in a significant decrease in urinary osmolality ($p < 0.05$, paired data), while the osmolality of the allografted recipients was further reduced ($p < 0.05$ versus control group). Water turnover in the recipient rats was markedly elevated (fig. 6) as water intake and urine volume were both increased after the insertion of an allograft ($p < 0.05$ versus control group).

Fig 3. Urinary calcium excretion before and after allogeneic
        kidney   transplantation   (o--o)   or   unilateral
        nephrectomy (●--●). Data are shown as means±SEM.
        *p <0.05 compared to unilaterally nephrectomized rats.

Fig 4. Urinary protein excretion before and after allogeneic
        kidney   transplantation   (o--o)   or   unilateral
        nephrectomy (●--●). Data are shown as means±SEM.
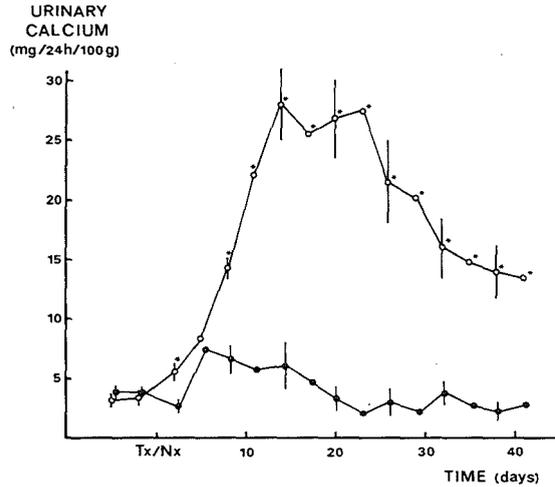   *p <0.05 compared to unilaterally nephrectomized rats.

Fig 5. Urinary osmolality before and after allogeneic kidney
transplantation (o--o) or unilateral nephrectomy (●--●)
Data are shown as means±SEM.
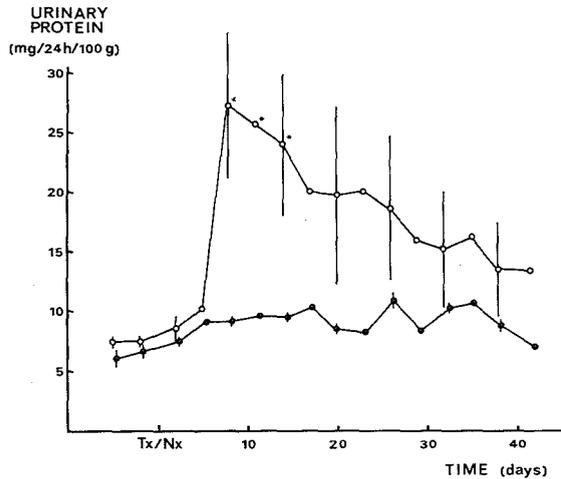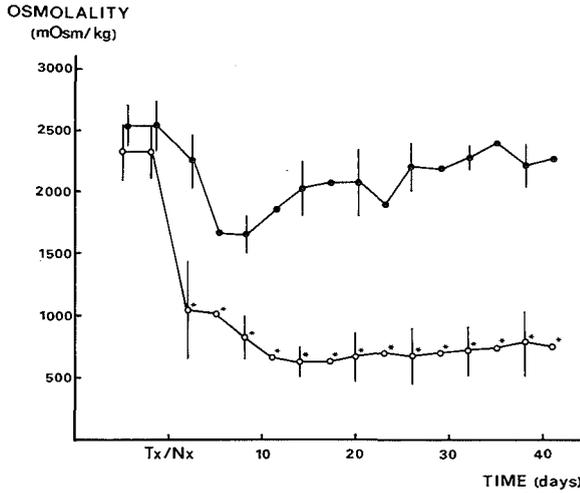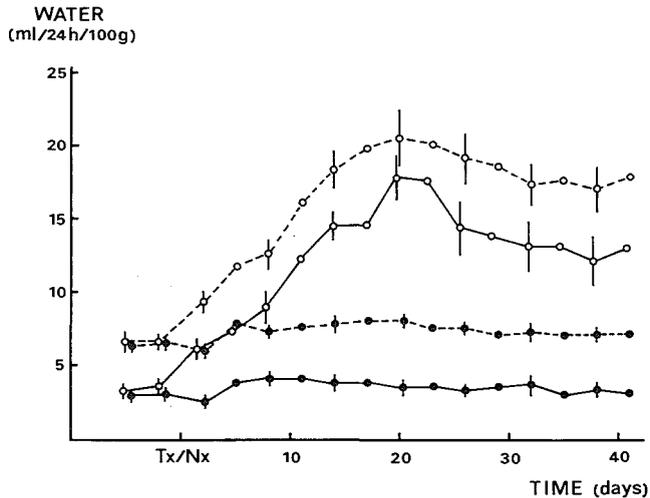*p <0.05 compared to unilaterally nephrectomized rats.

Fig 6. Water intake (broken lines) and urinary volume (solid
lines) before and after allogeneic kidney
transplantation (o) or unilateral nephrectomy (●).
Data are means ±SEM.

Fig. 7 gives the sodium intake and output of the allografted recipient rats and the unilaterally nephrectomized control rats. The transplantation or nephrectomy resulted in a fall in sodium intake and output in both groups. In the control group the balance between intake and output is restored within 3 days. The sodium intake and output in the recipient rats show a disbalance in the first 10 days. After this period intake and output are at a higher level compared to pre-operative values.

Third experiment: the plasma volume of the recipient rats of an allogeneic kidney was significantly higher ($p<0.05$) than that of unilaterally nephrectomized rats throughout the follow-up period of 6 weeks (table 1). During this time, however, the haematocrit was markedly decreased ($p<0.05$ versus control group). Consequently, the two groups did not differ in blood volume per 100 g BW except at week 6, when the blood volume of the unilaterally nephrectomized rats was somewhat less than that of the allograft recipients.

DISCUSSION

The results of the present study indicate that in our model of post-transplantation hypertension the rise in blood pressure was associated with marked alterations in renal function. We found a weak, but significant, inverse correlation between the SBP and the GFR at 3 and 6 weeks after transplantation. We previously showed that a low GFR in itself did not produce hypertension (7). However, in the present

analysis a more severe reduction in GFR points to a more severe rejection reaction. Consequently the present analysis points to an association of the degree of rejection with the level of SBP after transplantation.

SODIUM
(µmol/24h/100g)

TIME (days)
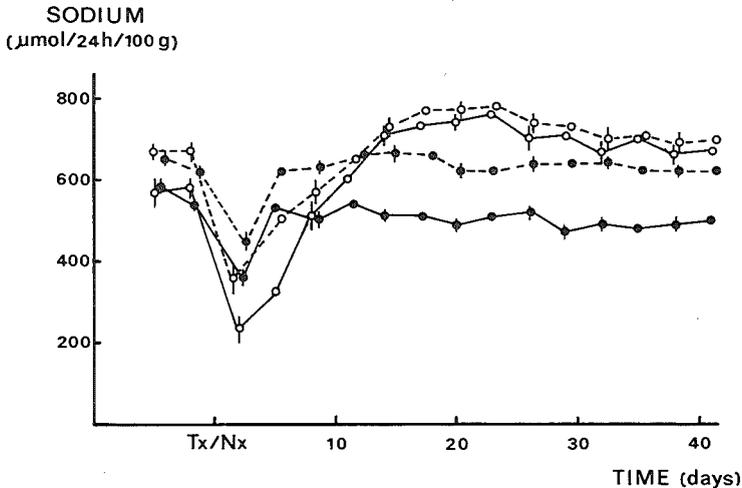
Fig 7. Sodium intake (broken lines) and urinary sodium excretion (solid lines) before and after allogeneic kidney transplantation (o) or unilateral nephrectomy (●). Data are means±SEM.

The metabolic experiments showed that in rats allogeneic transplantation led to polyuria, proteinuria and a different handling of sodium and water by the allograft. Analysis of the latter revealed that transplanting a renal allograft initially resulted in a diminished excretion of sodium for 8 to 10 days. The retention of sodium was accompanied by an increased difference between water intake and urinary output. However, the exact measurement of water retention is very difficult because some factors involved are hard to measure: extra renal water loss, metabolic water and water retention due to growth.

Table 1. Body weight (BW), haematocrit (Ht), plasma volume
          (PV) and blood volume (BV) of allografted recipient
          rats (Tx) and unilaterally nephrectomized rats (Nx).

		PRE-OPERATIVE	
		Nx(7)	Tx(7)
BW (gram)		279$\pm$10	269$\pm$13
Ht (%)		48$\pm$1	47$\pm$2
PV (ml/100g)		4.7$\pm$0.4	4.8$\pm$0.3
BV (ml/100g)		9.0$\pm$0.9	9.0$\pm$0.8

WEEK 1		WEEK 2	
Nx	Tx	Nx	Tx
270$\pm$11	254$\pm$13 *	273$\pm$13	247$\pm$23 *
45$\pm$2	35$\pm$3 *	43$\pm$3	38$\pm$3 *
5.4$\pm$0.4	5.9$\pm$0.6	5.3$\pm$0.3	6.2$\pm$1.0 *
9.8$\pm$0.9	9.1$\pm$1.1	9.4$\pm$0.6	9.9$\pm$1.4

WEEK 3		WEEK 6	
Nx	Tx	Nx	Tx
282$\pm$19	256$\pm$19 *	296$\pm$9	278$\pm$16 *
44$\pm$3	35$\pm$5 *	46$\pm$3	42$\pm$2 *
5.1$\pm$0.6	6.4$\pm$0.7 *	4.5$\pm$0.4	5.6$\pm$0.6 *
9.1$\pm$1.2	9.9$\pm$0.8	8.4$\pm$0.8	9.7$\pm$0.5 *

Values are means$\pm$SD; number of rats in brackets.
*$p < 0.05$ compared with Nx.

As a result of the diminished excretion of water and sodium
the plasma volume of the recipient rats was increased. After
the initial period in which the blood pressure started to
rise, the blood pressure remained more or less stable. In this
phase the excretion of sodium increased to a level higher than
the values observed before transplantation, while in the
recipient rats the increase in plasma volume persisted. The
reason for the heightened rate of urinary calcium excretion is
unknown, although it is reported that in response to expansion
of the extracellular space the urinary calcium excretion is
increased (10).

Appearance of protein in the urine preceeded the
establishment of hypertension, suggesting that nephritis-like

changes led to hypertension in our model. As we have demonstrated in previous studies (6,8) this malfunction of the allograft is due to an incomplete suppression of the rejection. Donor strain blood administration prior to transplantation prolonged the graft survival, but marked functional and structural changes were noted in the allograft. An increase in renin secretion is suggested as a common factor in hypertension associated with certain forms of renal malfunction, but in our model increased secretion of renin seems less likely to have caused the high blood pressure as plasma renin concentration levels were low. This study has supplied an explanation for the suppressed renin activity. It is well-known that renin concentration is reduced during high sodium intake or after sodium retention. Our metabolic studies suggested that sodium retention occurs after allogeneic kidney transplantation in the rat.

Sodium retention in post transplantation hypertension in man has been described in some papers but most authors found no changes in exchangeable sodium or postulated that the retention was caused by a mineralocorticoid excess due to the conversion of prednisone to aldosterone (11). In one study (12) the amount of exchangeable sodium was measured in patients before and after renal transplantation. In the hypertensive recipients exchangeable sodium was increased significantly in contrast to an insignificant decrease in the normotensive recipients, while corticosteroid dosage was similar in both groups. The change in exchangeable sodium was also positively correlated to the mean blood pressure after

renal transplantation. These results were confirmed by another study carried out during the first weeks after kidney transplantation (13).

The reason for sodium retention after renal transplantation is most probably intrinsic to the allografted kidney. As shown before (6) the GFR of the allograft was impaired to a greater extent than the effective renal plasma flow (ERPF), leading to a fall in the filtration fraction (ratio GFR : ERPF). This impaired renal function reflects the immunological processes present in the allografted kidney after donor strain blood pretreatment of the recipient. The rejection induces glomerular injury with relative preservation of tubular function, similar to that found in acute glomerulonephritis (14), where the total surface available for filtration is reduced. This is due to loss of functional renal mass as whole glomeruli are destroyed and also to functional nephrons being served by glomeruli with occluded capillaries. Another possible explanation for the sodium retention in allograft recipients is an altered renal tubular handling of sodium in the kidney transplant.

The mechanism whereby sodium retention may lead to hypertension in kidney transplant recipients remains unknown. However, a close relationship between sodium and water balance and blood pressure regulation has been described in end-stage renal disease (15). In this context the view of Guyton and coworkers (16) is of importance. According to their hypothesis, the regulation of body fluid volume and sodium content is not related to the total renal mass, but to the balance between

glomerular filtration and tubular reabsorption. Very minute disorders in glomerular filtration capacity or tubular reabsorption capacity can lead to extreme changes in body water and sodium content. When the body water and sodium content becomes enhanced, the blood pressure rises to a level where the balance is restored. At that level sufficient amounts of water and sodium can be excreted. This is probably the reason for the correlation between renal function and blood pressure. Other possibilities involve sodium and water accumulation in the vessel walls, thereby compressing the lumen (17) or an increase of the vascular sensitivity for pressor substances, due to the positive sodium balance (18).

In conclusion, our model of hypertension after kidney transplantation resembles the models of experimental glomerulonephritis, also characterized by proteinuria, reduction in GFR and suppression of the renin secretion. Due to an imbalance between glomerular filtration and tubular reabsorption, caused by the moderate rejection, water and sodium are retained, leading to a rise in extra cellular volume. Therefore, the observed hypertension is volume dependent and can be managed with an improvement of the immunosuppression or by a lower sodium intake.

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CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS

8.1 RATIONALE OF THE STUDY

Patients with kidney transplants are often hypertensive and investigators have described the characteristics of this hypertension in man. Moreover, the hypertension of kidney transplant recipients is more likely to result in surgical intervention than hypertension in the general population. Evenso the mechanisms of the many varieties of post-transplantation hypertension are not completely known and studies, at best carried out in animal models, are rare. However, several animal studies have been performed that were designed to look at the pathophysiology of post-transplantation hypertension. The renal cross-transplantation experiments of Dahl and Heine (1975) are the best known experimental studies of hypertension after transplantation, yet these were not designed to look at post-transplantation hypertension as such. Mc Dougall et al. (1981) have used sheep to develop a model of renal transplantation hypertension in which the vascular supply to the transplanted kidney in the animal can easily be contricted to mimic transplant artery stenosis.

Our aim was to develop a model of post-transplantation hypertension in rats in which the clinical problems of renal transplantation, such as rejection and immunsuppression, could be investigated.

8.2 MEASUREMENT OF RENAL FUNCTION

As a parameter of renal function the glomerular filtration rate (GFR) was measured in the recipient rats and the control rats. Conventional methods to measure the GFR in rats require extensive surgery, a constant infusion and a increased urine production for accurate urine collection. All these procedures may influence the GFR measurement apart from being time consuming. In our series of experiments, we measured the GFR without urine collection using 51 Cr-EDTA and a single timed blood sample (Layzell and Miller, 1975) together with a formula to calculate the clearance rate (Bryan et al., 1972). This method has the advantage of enabling measurement in large number of rats at one and the same time, while it allows for repeat measurements in the same rats at different intervals.

Although inulin remains the reference substance to measure the GFR, studies have ascertained the suitibility of 51 Cr-EDTA (Chantler et al., 1969). In clinical practice 51 Cr-EDTA has been accepted and is widely used as a compound to measure the GFR (Ditzel et al., 1972; Brochner-Mortensen and Rodbro, 1976). In contrast with inulin infusion for the determination of the GFR, the single injection technique can be used in adult as well as young rats. In adult rats, a good correlation was found between the filtration rates calculated by the present method and by other methods to establish the clearance (Bryan et al., 1972; Layzell and Miller, 1975; Provoost and Molenaar, 1980). In young rats, validation of the method we used with conventional measurements is technically almost impossible. Comparing our findings with data reported in the

literature showed the usefullness of the single injection technique (Aperia and Herin, 1975; Bengele and Solomon, 1974; Horster and Lewy, 1970).

In our experiments a single injection of 51 Cr-EDTA with a single plasma sample obtained after one hour, proved to be a very suitable method to determine the GFR in adult as well as young rats.

8.3   ISOGENEIC KIDNEY TRANSPLANTATION (chapter 2)

For the isogeneic kidney transplantation model, we used rats of a highly inbred strain. The genetic homogeneity of this strain has been confirmed by the permanent survival of intrastrain skin, heart and kidney grafts. In this setting, kidney damage caused by rejection is circumvented and, with donor and recipient rats of equal size, renal function of the transplanted kidney will only be influenced by damage caused by the transplantation procedure including storage as well as vascular and ureteral complications. Renal function may improve as the result of compensatory hyperfunction of the single grafted kidney. With donor and recipient of unequal size, post-transplantation renal function may likewise be influenced by the difference in size. From our studies it appears that the size of the recipient plays an important role in the functional outcome after renal transplantation (Provoost et al., 1984). The transplanted kidney behaves like a single organ pertaining to the recipient.

Hypertension was not found in rats with isogeneically

transplanted kidneys, even when donor and recipient rat were different in size. This indicates that neither the presence of a kidney transplant as such nor differences in the ratio bodyweight/kidneyweight between donor and recipient will lead to a rise in blood pressure after transplantation. Therefore, other factors such as rejection and/or immunosuppressive therapy may cause the elevation of blood after renal transplantation in rats.

8.4 ALLOGENEIC KIDNEY TRANSPLANTATION WITH DONOR STRAIN BLOOD PRETREATMENT OF THE RECIPIENT RATS (chap. 3)

In the allogeneic kidney transplantation model, the recipient rats were from the same strain as those used in the isogeneic model. The donor kidneys were from Brown Norway rats and in this setting kidney grafts would be rejected within two weeks. To prolong graft survival, preimmunization with donor strain blood was used. Preimmunization has been commonly defined as the delay or inhibition of an immune response to specific antigens by antibodies directed against those antigens (Marquet, 1978). The antibodies may be produced by active preimmunization and are generated by the injection of antigens. The antigens may be presented to the host by injection of viable cells, lyophilized tissue, tissue extracts or by injection of serum proteins. Passive preimmunization by the injection of donor-specific antisera may also lead to prolonged graft survival.

In the allogeneic kidney transplantation model the systolic blood pressure of the recipients started to rise in the second

week after transplantation. With active immunological
enhancement to prolong graft survival, corticosteroids or
other immunosuppressive drugs could be avoided. Consequently,
the observed hypertension could not be due to the effect of
drugs but must have originated from the allograft. Impairment
of the GFR itself did not cause a rise in blood pressure as
was seen in rats with one ischemic kidney and contralateral
nephrectomy. The presence of one intact native kidney in
combination with an allograft also resulted in normotensive
recipients. Removal of the native kidney six weeks after
transplantation again led to hypertensive allografted
recipient rats. Marked histopathological changes in the
allograft together with impairment of the GFR and low plasma
renin concentrations of the recipient rats led us conclude
that the observed hypertension in this model is the result of
immunological alterations in the kidney mediated by sodium
retention. A better immunosuppressive regimen or a low sodium
intake appear indicated to circumvent this kind of
hypertension.

8.5 ALLOGENEIC KIDNEY TRANSPLANTATION WITH IMMUNOSUPPRESSIVE DRUGS (chapters 4 and 5)

In our opinion, donor strain pretreatment will prolong
graft survival in the allogeneic transplantation model,
although it will not entirely prevent rejection. Therefore,
two different immunosuppressive agents were tested alone or in
combination with preimmunization with donor strain blood. We
wanted to know if additional or more effective immuno-

suppression could circumvent post-transplantation hyper-tension. The immunosuppressive agents we used were Cyclo-sporin A and prednisolone. The first drug was chosen because no direct effects of Cyclosporin A on the blood pressure had been reported at that time. The second drug was chosen because corticosteroids are still frequently used in transplanatation centers and because these drugs have a potential for raising blood pressure. In contrast to the combination of prednisolone and donor strain blood pretreatment, Cyclosporin A together with blood pretreatment did not show any synergistic effects on the survival or the graft function of the recipient rats. We believe that this is due to the high dosage of Cyclosporin we have used. Such dosage probably outweighs the effects of donor strain blood pretreatment on the blood pressure and the renal allograft function. In case of prednisolone, synergism was noted, albeit only found when prednisolone was given in low dose.

With either Cyclosporin A or low dose prednisolone, no elevation of the blood pressure was observed on our allogeneic transplantation model. Adequately immunosuppressed recipient rats (with either Cyclosporin A as only treatment or with prednisolone as adjuvant treatment) do not show any elevation in blood pressure after transplantation. This points to an important role for rejection in the development of hypertension in this model.

8.6 ALLOGENEIC KIDNEY TRANSPLANTATION AND SODIUM
(chapters 6 and 7)

As mentioned above, sodium retention might be the precursor of the observed hypertension in the original allogeneic transplanation model. We therefore speculated that a decrease in the sodium intake might result in normotensive recipient rats. We tested a semi-synthetic sodium-poor diet containing less than 15% sodium compared with normal food. On this diet recipient rats did remain within the blood pressure range of normotensive control rats. The sodium-poor diet had a positive effect both on the GFR and the survival of the recipient rats. The results from the metabolic studies suggest a different handling of water and sodium by the allograft in the first ten days after transplantation. An increase in the plasma volume of recipient rats after transplantation was also noted. These findings all point to the conclusion that sodium is involved in the rise in blood pressure in this model.

Several studies in humans also suggest that sodium accumulation in the body is involved in the patho-genesis of essential hypertension. Studies of the importance of body sodium content in post-renal transplantation hyper-tension have only rarely been carried out. Ducrot and coworkers (1961) found no significant changes in exchangeable sodium measured before and after renal transplantation in recipients with post-transplantation hypertension, whereas Swales (1967) found that changes in exchangeable sodium were correlated to changes in blood pressure in two recipients examined during the first weeks after renal transplantation.

In another study (Horvath et al., 1976), a correlation was found between changes in extracellular volume during oral sodium loading and changes in blood pressure in hypertensive and normotensive renal transplant recipients. Finally, in a controlled study Kornerup (1977) found a significant rise in exchangeable sodium in hypertensive recipients, in contrast with an insignificant drop in normotensive recipients before and after transplantation. These findings may suggest that sodium retention could be involved in the pathogenesis of post-renal transplantation hypertension in humans.

8.7 CONCLUSIONS

The following conclusions can be drawn from the experiments described in the preceding chapters.

- Isogeneic and allogeneic kidney transplantation models in the rat are suitable to study hypertension after transplantation.

- The presence of a grafted kidney in the isogeneic transplantation model does not cause a rise in blood pressure in the recipient rats.

- In the allogeneic transplantation model, where graft survival is prolonged with donor strain blood pretreatment, blood pressure starts to rise in the second week after transplantation.

- Chronic rejection of the allograft results in functional alterations in the kidney, causing retention of sodium and water and leading to a volume-dependant hypertension.

- The rise in blood pressure can be prevented by either better

  immunosuppression or a reduction in the daily sodium intake.

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SUMMARY

Kidney transplantation has become one of the most important therapeutic modalities for terminal renal insufficiency. During the past thirty years a great deal of research has been carried out concerning, in particular, more effective means of protecting the graft against rejection. An optimal method has not yet been found and the postoperative course of kidney recipients is complicated by incompletely treated graft rejection, coupled with the side effects of the immunosuppressive drugs administered to counteract that rejection. One of these postoperative complications consists of a rise in blood pressure. The development of hypertension causes diagnostic and therapeutic problems in many cases. This dissertation describes methods of inducing and combatting hypertension after allogeneic kidney transplantation between two inbred rat strains. A hypothesis is developed on the cause of this hypertension in the kidney recipient. Finally, this hypothesis is experimentally substantiated.

Chapter 1 gives an overview of the new developments in the field of renal transplantation in general and of the effects of immunosuppressive drugs in particular. Percentages are supplied and discussed regarding the occurrence of hypertension after kidney transplantation in children and adults. Finally, the various causative factors for hypertension after kidney transplantation as mentioned in the literature, are discussed.

Chapter 2 deals with the isogeneic renal transplantation model. It appears that in this model the recipients remain normotensive. It is affect concluded that the transplantation procedure as such does not influence the blood pressure of the recipient, not even when kidneys are transplanted from adult donors into juvenile rats.

Chapter 3 deals with the allogeneic renal transplantation model, whereby the allograft survival is prolonged by means of pretreatment with donor strain blood. It appears that the allogeneic kidney recipient becomes hypertensive in the second week after transplantation. This hypertension is associated with a reduction in the glomerular filtration rate (GFR) and a suppression of the plasma renin release. It is assumed that a partial rejection does occur in spite of the prolonged graft survival, causing damage in the allografted kidney, which leads to sodium retention and eventually hypertension.

Chapter 4 describes the effects on blood pressure and renal function of an improved method of immunosuppression using Cyclosporin A. With Cyclosporine A administration, the recipients appear to remain normotensive, while renal function is markedly improved. It is assumed that the use of Cyclosporine A prevents the partial rejection that does occur with donor strain blood pretreatment. The combined use of Cyclosporine A and pretreatment with donorstrain blood does not lead to an extra improvement of renal function in the recipients and/or survival of these rats in comparison with recipients that were treated with Cyclosporine A alone.

Chapter 5 describes the immunosuppressive effect and

potential for raising blood pressure of continuous prednisolone infusion. This method of immunosuppression appears to raise the systolic blood pressure (SBP) and the GFR, while it results in a significant loss of body weight. These effects appear to be dose-related; prednisolone was administered in diverse doses during the first two weeks after transplantation. The same effects were found in unilaterally nephrectomized control rats. After cessation of the prednisolone administration, the recipients remain hypertensive, while the GFR decreases. Pretreatment of the recipients with donor strain blood results in a drop in blood pressure to normotensive values, after cessation of the prednisolone administration. A reduction in the dose of prednisolone for the recipients pretreated with donor strain blood, results in recipients whose blood pressure remains normotensive throughout the study period. A further reduction in the dose of prednisolone, once again leads to hypertension.

In chapter 6, the premise of sodium retention as causative factor for post-renal transplantation hypertension is investigated by putting the rat kidney recipients on a low-sodium diet. It appears that these recipients do not become hypertensive, while their GFR is significantly higher than the GFR of recipients on a standard diet. The survival time for rats on a sodium-poor diet is also significantly higher. In this chapter, it is also demonstrated that a poor renal function as such does not lead to a rise in blood pressure in the rat. It is also shown that the presence of a native, intact kidney in the recipient will prevent a rise in blood

pressure.

Chapter 7 described the feasibility of a connection between the GFR and the SBP of recipients pretreated with donor strain blood, amounting to a weak, albeit significant, inverse correlation. In addition, a metabolic experiment is described, revealing a positive sodium and water balance during the first week after transplantation. The recipients show proteinuria and calciuria, while the osmolality of the urine is markedly reduced. Finally, it appears that the recipient rats have a higher plasma volume than unilaterally nephrectomized rats, suggesting an enlarged extracellular volume of the recipient rats.

The general discussion highlights the methods applied. It appears that the data obtained from the metabolic experiments are not conducive to simple explanations regarding the sodium and water balance. Finally, a feasible hypothesis is drawn up that would explain the post-transplantation hypertension. A partial graft rejection causes changes in the kidney, resulting in retention of sodium and water, which leads to a rise in blood pressure.

SAMENVATTING

Om terminale nierinsufficiëntie te behandelen is nier-transplantatie een van de meest belangrijke behandelingen geworden. In de afgelopen dertig jaar is veel onderzoek verricht naar met name effectievere manieren om het transplantaat tegen afstoting te beschermen. De meest optimale methode is echter nog niet gevonden en ontvangers van nieren ondervinden problemen door enerzijds een niet optimaal behandelde afstotingsreactie en anderzijds door bijwerkingen van de toegediende immunosuppressiva. Een van de complicaties in de post-operatieve periode is het ontstaan van een verhoogde bloeddruk. In dit proefschrift wordt een methode beschreven die na niertransplantatie tussen ratten van twee ingeteelde stammen leidt tot een verhoogde bloeddruk bij de nierontvangers. Een hypothese is opgesteld om de hypertensie te verklaren en wordt daarna getoetst op zijn juistheid.

In hoofdstuk 1 wordt een overzicht gegeven van de ontwikkelingen op het niertransplantatiegebied in het algemeen en van de gebruikte immunosuppressieve middelen. Tevens worden van kinderen en volwassenen percentages van hypertensieve recipienten vermeld en toegelicht. Tenslotte wordt ingegaan op de verschillende factoren die in de literatuur genoemd worden als mogelijke oorzaak voor hypertensie na niertransplantatie.

Hoofdstuk 2 beschrijft het isogene transplantatie model. Het blijkt dat in dit model de ontvangers normotensief blijven. Geconcludeerd kan worden dat de transplantatie procedure op zich geen invloed op de bloeddruk heeft, zelfs

niet indien er nieren getransplanteerd worden van volwassen naar jonge ratten.

Hoofdstuk 3 behandelt het allogene transplantatie model, waarbij de transplantaat overleving verlengd wordt door voorbehandeling van de ontvanger met donor bloed. De allogene nierrecipienten blijken in de tweede week na transplantatie hypertensief te worden. Dit gaat gepaard met een verlaging van de glomerulaire filtratie snelheid en een onderdrukking van de plasma renine afgifte. Verondersteld wordt dat er ondanks de verlengde transplantaat overleving toch een gedeeltelijke afstoting plaatsvindt, waardoor veranderingen in de nier optreden die aanleiding geven tot natrium retentie en daardoor tot hypertensie.

Hoofdstuk 4 beschrijft daarom de effecten van een verbeterde immunosuppressie met behulp van Cyclosporine A op de bloeddruk en de nierfunctie. Bij gebruik van Cyclosporine A blijken de ontvangers normotensief te blijven, terwijl de nierfunctie aanzienlijk verbeterd. Het idee is dat door het gebruik van Cyclosporine A de gedeeltelijke afstoting, die wel plaats vindt na voorbehandeling met donorbloed, achterwege blijft. Het gebruik van Cyclosporine A plus voorbehandeling met donorbloed geeft geen extra verbetering in nierfunctie en/of overleving van de aldus behandelde nierontvangers in vergelijking met nierontvangers die met Cyclosporine A alleen behandeld werden.

In hoofdstuk 5 worden de immunosuppressieve en hyper-tensiogene effecten beschreven van een continu prednisolone infuus. Prednisolone wordt in verschillende doses gedurende de

eerste twee weken na transplantatie toegediend. Dit blijkt de systolische bloeddruk en de glomerulaire filtratie snelheid te verhogen. Tevens vindt er een aanzienlijke afname van het lichaamsgewicht plaats. Deze effecten zijn dosis afhankelijk en komen ook voor bij de eenzijdig genefrectomeerde controle ratten. Na stopzetting van de prednisolone toediening blijven de nierontvangers hypertensief, terwijl de glomerulaire filtratie snelheid daalt. Voorbehandeling van de ontvangers met donorbloed resulteert na het stoppen van de prednisolone toediening in een daling van de bloeddruk tot normotensieve waarden. Verlaging van de prednisolone dosis in de met donorbloed voorbehandelde ratten leidt tot ontvangers die gedurende het onderzoek een normotensieve bloeddruk houden. Een verdere verlaging van de prednisolone dosis geeft weer aanleiding tot hypertensie.

In hoofdstuk 6 wordt de eerder veronderstelde natrium retentie nader onderzocht door de met donorbloed voorbehandelde ontvangers een laag zout dieet aan te bieden. Het blijkt dat deze ratten niet hypertensief worden, terwijl de glomerulaire filtratie snelheid aanmerkelijk hoger is dan die van ontvangers op standaard dieet. Ook de overleving van de ratten op het laag zout dieet is beter. In dit hoofdstuk wordt ook aangetoond dat bij ratten een slechte nierfunctie op zich geen aanleiding geeft tot een verhoogde bloeddruk. Tevens wordt aangetoond dat de aanwezigheid van een intacte eigen nier naast de getransplanteerde nier de stijging van de bloeddruk voorkomt.

In hoofdstuk 7 wordt een mogelijk verband beschreven tussen

de glomerulaire filtratie snelheid en de systolische bloeddruk van ontvangers die voorbehandeld waren met donorbloed. Er wordt een zwak significante, lineaire en omgekeerde correlatie aangetoond. Tevens wordt in een metaboolexperiment aangetoond dat er gedurende de eerste week na transplantatie sprake is van een positieve natrium- en waterbalans. De ontvangers vertonen proteinurie en calciurie, terwijl de osmolaliteit van de urine na transplantatie aanzienlijk verlaagd is. Tenslotte blijkt dat het plasma volume van de recipient ratten hoger is dan dat van eenzijdig genefrectomeerde ratten, reden om te veronderstellen dat het extracellulair volume van de recipient ratten vergroot is.

In de discussie wordt ingegaan op de gebruikte methodieken en wordt onder meer geconcludeerd dat uit de gegevens verkregen uit de metaboolexperimenten een natrium- en water balans moeilijk op te maken is. Als conclusie van dit onderzoek wordt een hypothese opgesteld om de hypertensie te verklaren. Door een gedeeltelijke afstoting van het transplantaat vinden in de nier veranderingen plaats die aanleiding geven tot retentie van natrium en water, waardoor een verhoging van de bloeddruk plaatsvindt.

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Iedereen, die op welke wijze dan ook, bijgedragen heeft tot de totstandkoming van dit boekje, wil ik van harte bedanken.

CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 23 augustus 1954 te Rotterdam. De middelbare schoolopleiding aan het Sint Franciscuscollege te Rotterdam sloot hij af in 1971 met het behalen van het einddiploma H.B.S. B. In datzelfde jaar werd hij als chemisch student ingeschreven aan de Rijks Universiteit te Utrecht. Het kandidaatsexamen S1 werd afgelegd in 1974 en het doctoraalexamen met hoofdrichting biochemie (Prof. Dr. L.L.M. van Deenen) en bijvak klinische chemie (Dr. G.J. van Stekelburg) in 1978. Van november 1978 tot januari 1980 vervulde hij zijn militaire dienstplicht bij de Koninklijke Landmacht. Sinds februari 1980 is hij werkzaam bij het instituut Kinderheelkunde (Prof. Dr. J.C. Molenaar) van de Erasmus Universiteit te Rotterdam, eerst in dienst van de Nierstichting Nederland en later van de Sophia Stichting voor Wetenschappelijk Onderzoek. Op het Laboratorium voor Chirurgie werd onder leiding van Dr. A.P. Provoost het onderzoek verricht wat tot dit proefschrift heeft geleid.