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A Technique for In Vivo and In Vitro Studies on the Preserved and Transplanted Rat Kidney

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Summary. A modified technique for preservation and transplantation of rat kidneys, allowing precise control of the periods of warm and cold ischaemia, is presented. The donor kidney is placed in a continuously cooled micropuncture cup during the insertion. End-to-end anastomosis of arteries and veins is performed. The technique causes negligible circulatory changes on restoration of the blood flow. Eighty-nine consecutive transplantations are analysed. Complications from the vascular anastomoses occurred in 6% (5/89). In 53 survival experiments complications from the ureteric anastomoses occurred in 9% (5/53). The methodological scatter was small, with a distinct difference in the serum creatinine course and mortality of recipients obtaining kidneys subjected to cold ischaemia for different lengths of time.

Key words. Kidney transplantation - Rat - Renal ischaemia - Renal preservation - Microsurgical technique.

Treatment of chronic uraemia with renal transplantation is a well established clinical method. Preservation methods have improved to such an extent, mainly through experiments in dogs (1, 9, 16), that human kidneys can now be stored safely for up to 48 hours and still retain good function. The pathophysiology underlying the differences between functioning and non-functioning kidney grafts is, however, still unclear and a better knowledge of these differences is necessary to improve the preservation methods. The present paper will report a modified method for strictly controlled renal preservation and transplantation in the rat, giving standardized and reproducible organ quality. Studies on regional blood flow (13) and results of micropuncture experiments (15) are published elsewhere.

MATERIAL

Male rats of two different strains were used, 11 Lewis x Brown Norway F_1 hybrids (Microbiological Associates Corp., Walkersville, Maryland, USA) and 78 Sprague-Dawley (AB Anticimex, Stockholm, Sweden). They weighed 220 - 310 g. The Sprague-Dawley rats were taken from the same population and from the same animal house. The rats were fed with a standardized laboratory diet (Astra-Ewos, Södertälje, Sweden) and kept under conditions as uniform as possible with respect to such factors as lighting, temperature, and the number of rats per cage. They were kept in the animal house of the laboratory for at least four days before the experiment, and were separated from other experimental animals to minimize the risk of infection. The rats were starved for at least 12 h before the experiment but had free access to water.

The investigation comprised a total number of 89 transplantations. In all these experiments the complications from the vascular anastomoses were analysed. Only in 53 of these 89 experiments was an ureteric anastomosis performed, the rest being acute experiments in which the ureter was catheterised. The course of the serum creatinine levels after transplantation was studied in 19 Sprague-Dawley and 11 Lewis-Brown Norway rats comprised in this series.

METHOD

Donor Kidney

The donor animals were anaesthetized with Inactin^R (Promonta, GmbH, Hamburg, West Germany) given intraperitoneally in a dose of 120 mg/kg body weight. They were placed on an operating table with a servo-controlled heating pad which

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kept the body temperature constant at 37°C. Phenoxybenzamine, an alpha-adrenergic blocking agent, 3 mg/kg body weight, was given intravenously at least 60 min and heparin, 100 IU/animal, 15 min before excision of the kidney (6).

The operation was performed under clean but not sterile conditions. The abdomen was opened through a midline incision and the left kidney was exposed. The left ureter was divided about 10 mm from the renal pelvis, and the kidney was freed completely, except for the renal artery and vein, which, however, were separated from each other and from surrounding fat. The aorta and inferior vena cava were then closed off simultaneously with a specially constructed clamp (3), and the renal vein was divided before its junction with the vena cava. Through a small incision in the aorta a polyethylene catheter (PP 50, ID 0.58, OD 0.96 mm, Portex Ltd, Heath, Kent, U.K.) filled with perfusion solution was inserted into the renal artery and fixed in position with a ligature. The kidney was removed from the abdomen after a warm ischaemia time of 5 min or less, after which the arterial catheter was connected to a perfusion unit.

Perfusion

The perfusion unit is illustrated in Fig. 1. It consists of a large vessel containing ice-water for cooling, a bottle containing perfusion solution, a lucite micropuncture cup which holds the kidney during perfusion, and a pump (Variomatic LKB Medical AB, Stockholm, Sweden). The perfusion solution was driven through a bacterial filter (Millipore SA, France, 0.22 μm). The perfusion pressure was recorded continuously with a Statham P23AC transducer. The perfusion flow was 1.3 ml/min. For perfusion a modified Collins' solution with the addition of human albumin (AB KABI, Stockholm, Sweden) to a concentration of 5% was used (6). The perfusion time was 20 min. If the rinsing of the kidney was inadequate or the perfusion pressure at the end of the perfusion higher than 40 mm Hg, the kidney was discarded.

Hypothermic Storage

The kidneys were stored in the perfusion solution but without albumin at +4°C. The storage times in these series were 2, 12, and 16 h for the Sprague-Dawley rats, 16 and 20 h for the Lewis x Brown Norway F_1 rats.

Transplantation

All animals were anaesthetized with Inactin^R intraperitoneally in a dose of 120 mg/kg body weight except in the survival experiments, in which ether on an open mask was used. The animal was placed on an operating table specially

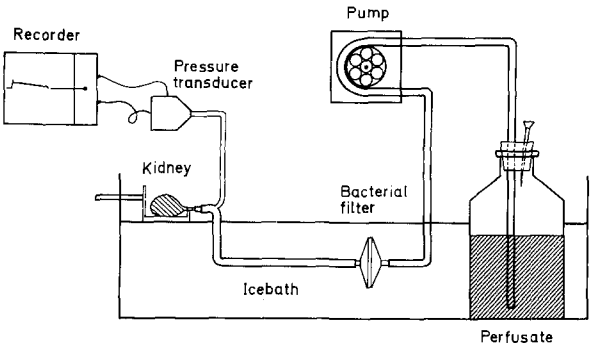

Fig. 1. Schematic drawing of the perfusion unit

constructed for micropuncture experiments. The body temperature was kept constant with a servo-regulated heating pad. The transplantation was performed with the aid of an operating microscope at a magnification of 10-16 x. A midline incision was used to allow good access to both kidneys. In acute experiments the right kidney was removed prior to transplantation, which was always on the left side. The left kidney was then dissected free together with its blood vessels. The renal artery and vein were occluded with a small vascular clamp (ca 20 mm long and 2 mm wide) close to the aorta and the vena cava and the ureter was divided at the level of the renal pelvis, and the kidney removed. Care was taken to avoid bleeding from the ends of the blood vessels due to inadequate placing of the clamp, as this could easily give rise to thrombosis in the anastomosis area.

During suturing of the renal vessels the kidney was placed in a modified micropuncture cup equipped with a double bottom, the inner one consisting of a silver plate. Cooling was achieved in two ways: Firstly, ice-water circulated between the two bottoms, and secondly, the kidney was superperfused with recirculating ice-cold physiological saline. The superperfusate was continuously sucked off by means of a suction tube in the bottom of the cup. By this technique the temperature of the kidney could be maintained at +5°C during the transplantation. A 1-mm thick plate of polystyrene foam isolated the cup from the animal. By means of a holder attached to the operating table the micropuncture cup could be adjusted to a suitable position for performance of the vascular anastomoses. This operation was begun by trimming the ends of the arteries and dilating them with a blunt needle (diameter ca 1 mm).

For the arterial anastomosis two everted stay sutures of 9-0 monofilament (round needle, diameter 150 μm, 3/8 circle) were used. Between these stay sutures, 4-5 single sutures of 10-0 monofilament were placed on both the dorsal and ventral wall (needle diameter 70 μm). For suturing the posterior wall, the artery was rotated 180° by means of the stay sutures.

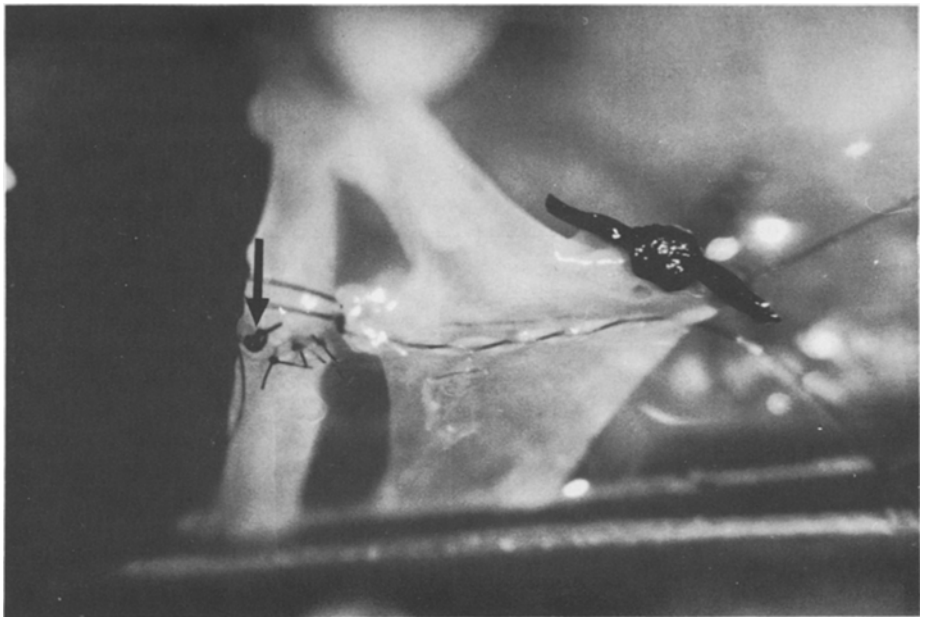

Fig. 2. Photograph of the vascular anastomosis before recirculation. To the left is the renal artery, with an arrow indicating a stay suture. To the right is the venous anastomosis, with a ligature of 5-0 silk around the testicular vein

The venous anastomosis was sutured with continuous 9-0 monofilament on the ventral and dorsal walls, but here the dorsal wall could be sutured without rotation (Fig. 2). One end of the suture was left untied so that the anastomosis could be widened when the renal vein was filled with blood. The cooling procedure was discontinued before the circulation was restored. Less than one minute of light compression at recirculation usually was sufficient to seal a bleeding anastomosis. Only occasionally was additional clamping for a haemostatic suture necessary.

The ureter was anastomosed end-to-end with three to four single 9-0 sutures over a 7-10 mm long silastic catheter (ID 0.51 mm, OD 0.94 mm or ID 0.30 mm, OD 0.64 mm, Dow Corning Corp, Medical Products Division, Midland, Michigan, USA), which was left in situ in the survival experiments (5). The kidney was fixed to the posterior abdominal wall by a suture through the perirenal fat at either pole. In the acute experiments the kidney was left in the micropuncture cup and the ureter was cannulated with a silastic catheter.

In the survival experiments the animals were weighed and blood samples taken on postoperative days 2, 4, 8 and 16. Serum creatinine concentration was determined photometrically (Slope Fotometer 211, Analys Instrument AB, Stockholm, Sweden).

RESULTS

The intrarenal temperature during suturing of the renal vessels was measured with needle thermistors in six experiments and lay between +4 and +6°C throughout.

In a series of 89 transplantations no failure due to the arterial anastomosis occurred. However, in 2 cases, sutures had to be applied to arrest bleeding from the anastomosis.

Three experiments failed due to bleeding from the venous anastomosis and two experiments due to venous thrombosis.

The handling of the ureter gave rise to problems in 5/53 animals. In 4 of these blood clots had formed in the indwelling silastic catheter and in the fifth animal the catheter had perforated the ureteric wall. The clotting of the catheters might have been due to the fact that in three of the animals the right kidney was left in place - the urine flow from the transplanted kidney would then be smaller than if the right kidney had been removed (12).

Neither the arterial nor the venous anastomosis showed signs of constriction. In order to measure this objectively a metal cannula with an outer diameter of 200 μm was introduced into the renal artery in one experiment via a lumbar artery as described by Källskog et al (10). With the tip of the cannula placed proximal and distal to the anastomosis, a maximum fall in pressure of 2 mm Hg was recorded, i.e. a negligible pressure drop. The operation times for the vascular anastomoses were about 30-40 min, and for complete transplantation just over one hour.

The courses of the serum creatinine concentrations in bilaterally nephrectomized rats receiving kidneys exposed to varying periods of cold isch-

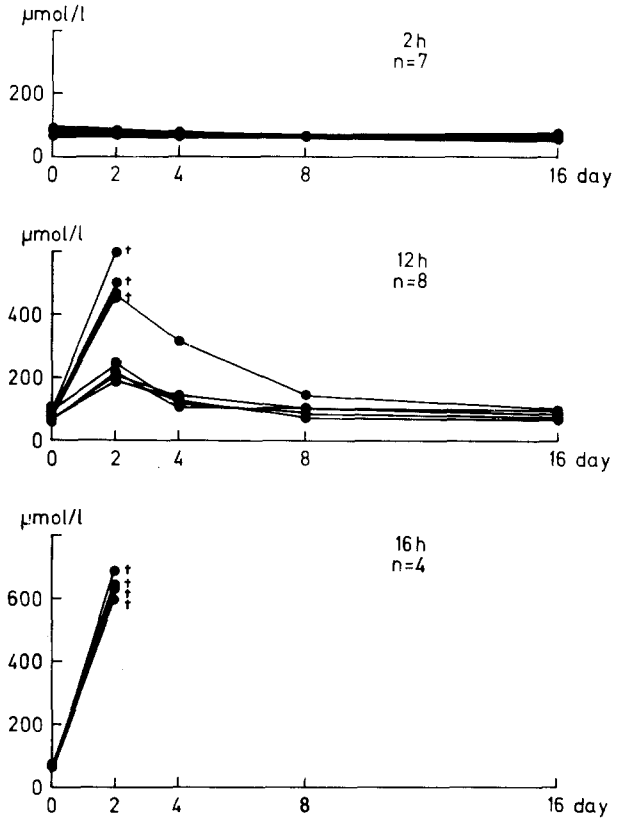

Fig. 3. Postoperative serum creatinine values in bilaterally nephrectomized Sprague-Dawley rats transplanted with kidneys with a cold ischaemia time of 2, 12 and 16 h

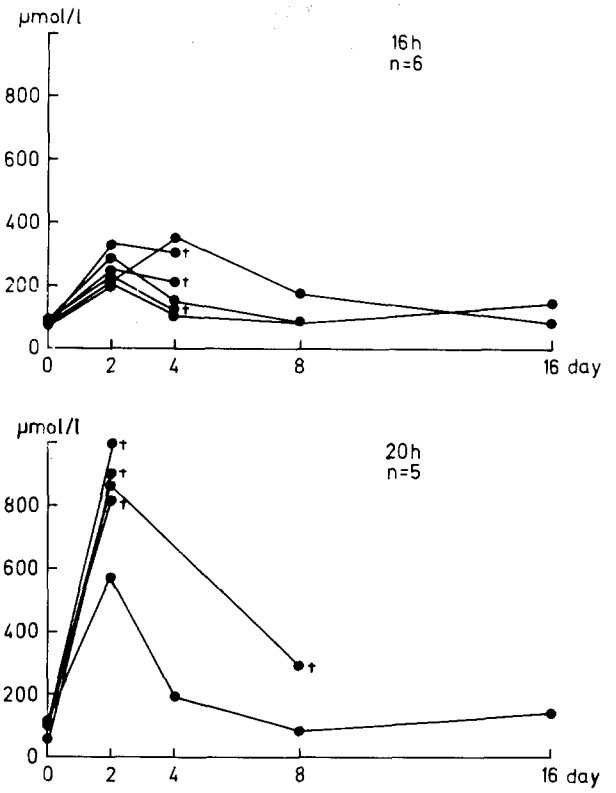

Fig. 4. Postoperative serum creatinine values in Lewis x Brown Norway F1 hybrids transplanted with kidneys with a cold ischaemia time of 16 and 20 h

aemia are shown in Figs. 3 and 4. Figure 3 gives the results for the experiments on 19 Sprague-Dawley rats that received kidneys exposed to 2, 12 and 16 h of cold ischaemia. After 2 h of cold ischaemia no creatinine increase was noted, and all rats survived an observation period of at least 16 days.

After 12 h of cold ischaemia a moderate increase in serum creatinine was observed on day 2. Three of the 8 rats in this group died, and the survivors showed decreasing creatinine values.

Four rats that had received kidneys exposed to 16 h of cold ischaemia all exhibited an increase in serum creatinine concentration on day 2 and died on the second or third day postoperatively.

The results of the experiments on Lewis x Brown Norway hybrids obtained on transplantation of kidneys exposed to 16 and 20 h of cold ischeamia are presented in Fig. 4.

Two animals in the 16 h group died from acute pyelonephritis, caused by an infected perfusion solution. These were excluded from the results. For the other animals in this group a maximum creatinine increase of 350 µmol/l was noted on the second day, followed by a decrease. Three of the 6 animals in this group died 7-9 days after transplantation. The cause of death was not established with certainty at post-mortem examination, but dehydration due to massive polyuria might have been present.

In the 20 h group one rat was excluded because of infection. The remaining rats in this group had an increase of the creatinine values to over 550 µmol/l on day 2. Four of the 5 rats in this group died.

DISCUSSION

Studies of preservation problems in which rats were used as experimental animals have previously been reported from this laboratory (5-8). In these, a transplantation technique described by Lee (11) was employed. This technique implies occlusion of the circulation to the lower half of the body during the transplantation, since the vessels are anastomosed end-to-side (aortic segment - aorta and renal vein - vena cava). When the clamp is released undesirable metabolic and circulatory phenomena may appear. A method introduced by Daniller et al (2) and described in detail by Fabre, Lim & Morris (4), in which the renal vessels are anastomosed end-to-end, was therefore used in the present investigation. Our ex-

perience with this technique, from over 200 transplantations in rats, about a hundred of which are reported in this article, is very favourable - the complications are few and the technique is comparatively easy to learn. Rats offer certain advantages over dogs as experimental animals in preservation research, i.e. they can be obtained as inbred strains and are cheaper. By analysis of every detail, so that each stage could be carried out identically operation after operation, good reproducibility of the results was obtained despite the complexity of the model. The donor animals were always pretreated with heparin to avoid thrombosis or deposition of fibrin in the kidney. Heparin combined with phenoxybenzamine was found necessary to achieve good rinsing of blood with hypothermic perfusion (6).

The dissection at excision of the donor kidney will be less extensive when the aortic segment is not used. The application of a special clamp (similar to a pediatric Satinsky clamp) over the aorta and vena cava facilitates catheterization of the artery, and also reduces the risk of air entering the renal vascular system. The shortest possible duration of warm ischaemia in this model seems to be about 2-3 min. A perfusion solution well established for clinical and experimental use - a modified Collins solution with 5 per cent human albumin, was used for the hypothermic perfusion. A different perfusion solution might possibly have raised the viability limits, but it was not a purpose of the present investigation to compare the effects of alternative perfusion solutions.

By the construction of a holder, in which the donor kidney was kept cool during the insertion procedure, several advantages were gained. Additional ischaemic injury during this procedure was avoided, the kidney was protected from trauma and dehydration, and the operation did not need to be hurried. The time taken for operation corresponds relatively well with those in techniques not employing cooling devices (4).

Central haemodynamics were affected very little in this experimental model. On restoration of the circulation an initial and negligible blood pressure decrease occurred and was already fully compensated after about a minute. Cardiac output, determined by the microsphere technique, was normal one minute after recirculation of the kidney (13).

In the majority of experiments Sprague-Dawley rats, which are histoincompatible, were used. Preformed antibodies may appear in these rats and influence acute experiments, but this risk is considered relatively small (H. Wigzell, personal communication). Sprague-Dawley rats are probably all similar genetically; Daniller et al (2) reported that rejections of renal transplants were unusual in this rat strain. The normal serum creatinine concentrations after transplantation in the group with 2 h of cold ischaemia is evidence against the occurrence of rejection episodes in the initial post-transplantation period.

During one period preceeding the presented experiments vascular thrombosis, especially on the arterial but also on the venous side, was very frequent. The explanation for this was probably that the experimental animals were infected. The greatest care in the planning of experiments with respect to the quality of the animals and to the animal housing hygiene must therefore be recommended. The viability limits vary between different strains of animals. This may be due to genetic differences, but dissimilarities in fostering and feeding may also be of influence. Thus, results concerning a certain duration of ischaemia from one laboratory cannot be reliably compared with those for the same duration from another laboratory.

In one series of experiments, with a short period of cold ischaemia, kidneys were transplanted without perfusion and without pretreatment with phenoxybenzamine. Heparin was the only pretreatment given and after excision, the donor kidney was immediately placed in the cooling cup. The circulation was restored about one hour after excision of the donor kidney. Kidneys treated in this way showed the same functional characteristics as kidneys which were pretreated with phenoxybenzamine and perfused hypothermically (14, 15). This supports the view that the experimental procedure per se does not damage the kidney.

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