Urological Research 3, 87-90 (1975)

Renal Transplantation in the Rat

I. Studies Concerning the Ureteral Anastomosis with Special Reference to the End-to-end Technique

L. Frödin and A. Engberg

Department of Clinical Research II, Department of Surgery, University Hospital, Uppsala, Sweden

Received: May 20, 1975

Summary. End-to-end anastomosis of the rat ureter over an in-dwelling silastic splint gives good primary results and a low frequency of complications. This technique has been used when reconstructing the ureter in situ and in renal transplantation.

Key words: Renal transplantation, ureteral anastomosis, rat kidney.

Introduction

Renal transplantation in the rat is now an established technique (4). Once the art of microsurgery is mastered, the use of rats provides several advantages for research studies of rejection and preservation, such as cost, uniformity and the availability of inbred strains. Thus far the rat has been used mostly for studies of rejection phenomena. Observations of complications due to technical short-comings are scarce. Concerning the surgical procedure, the ureteral anastomosis seems to be the most crucial point (10, 9).

The technique of anastomosing the ureter over an in-dwelling silastic splint was reported by Owen (11) and has also been used by others with good results (2, 7). The purpose of our investigation was to find out if this technique results in infections of the urinary tract or otherwise affects the structure and function of the kidney.

Material and Methods

For an isolated study of the ureteral anastomosis the ureter was divided and reconstructed in situ (I). The technique was also utilized in a small series of renal grafts (II).

I. Reconstruction in situ. Material. Male Sprague-Dawley rats (n = 42) weighing 250-330 g were used. The rats received a standard laboratory diet (210 Anticimex, Sweden) and were kept under standardized conditions (automatic-controlled light, temperature 20-24° C and two rats per cage). Body weight was controlled daily. Prior to operation, the animals had free access to food and water.

Methods. Ether on an open mask was the preferred anaesthetic procedure. Surgical technique was clean but not sterile. The abdomen was opened by a midline incision and the left kidney was removed. The right ureter was divided approximately one mm distal to the ureteropelvic junction. The ureter was then reconstructed end-to-end over a silastic splint. The splint (0.12 inch ID, 0.25 inch OD, Dow Corning Cooperation Medical Products, Division Midland, Michigan, U.S.A.) was 6-12 mm in length, cut obliquely at both ends and heat sterilized (120° C, 90 min). Two or four interrupted nylon (8-0 Ethicon) stitches were placed with the aid of magnifying glasses (Jena 2 x). The midline incision was closed with two layers of continuous 3-0 silk sutures.

Thirty animals survived the first three postoperative days and were divided in three groups of 10 each and sacrificed after 14 (group I A), 30 (group I B) and 60 days (group I C), respectively. The animals were anaesthetized with ether on an open mask and opened in the previous midline incision. Urine for bacterial culture was aspirated from the dome of the

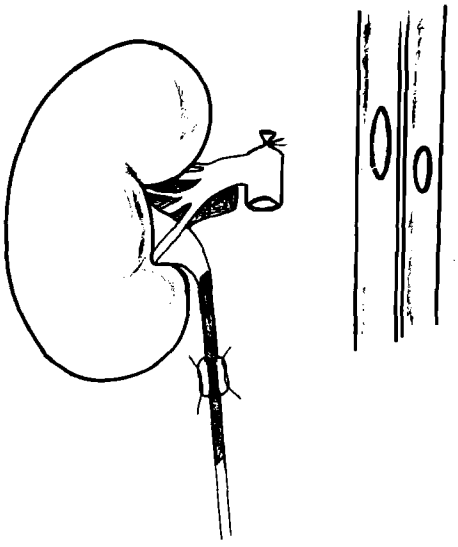

Fig. 1. Illustration of the ureteral anastomosis over an in-dwelling silastic splint

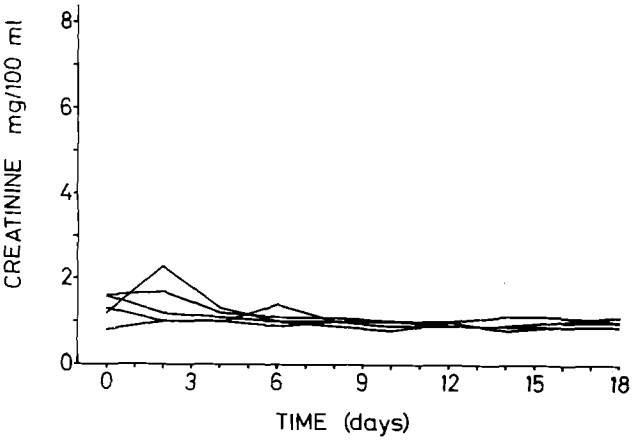

Fig. 2. The serum creatinine level for 18 days after renal transplantation is plotted for each of the animals

bladder into a sterile syringe. Blood was drawn from the aorta for serum creatinine analysis. After careful inspection of the anastomotic area, the kidney was excised for histological studies. Slices 1-2 mm thick were fixed with formalin, paraffin-embedded and stained with haematoxylin-eosin. Bacterial cultures were performed after serial dilution in test tubes. Serum creatinine was analysed using the Folin-Jaffe method (coefficient of variation 5%) (8).

II. Renal transplantation. Material. Twelve male Lewis X Brown Norway rats (LBN F1 hybrids) weighing 250-350 g, were used (Microbiological Associates Inc., Walkersville, Maryland). This strain was chosen to avoid rejection phenomena.

Methods. All donor animals were anaesthetized with Inactin ^{R}, 120 mg/kg B.W. (5 ethyl - 5 (methyl, propyl) thiobarbiturate, Chem. Fabrik Promonta GmbH, Hamburg) and operated on a thermistor controlled heating pad after pretreatment with phenoxybenzamine (3 mg/kg B. W.) and heparin (100 IU). The recipient was anaesthetized with ether on an open mask. The abdomen was entered through a long midline incision and the two kidneys were excised. Ten minutes after interruption of the renal blood flow, the left kidney was removed from the donor and immediately immersed in cold saline. The donor ureter was divided one mm distal to the ureteropelvic junction and sutured end-to-end to the right ureter of the recipient over an in-dwelling silastic catheter as in I using four separate 9-0 nylon stitches (Fig. 1). The vascular anastomoses were performed within an average of 28 min (range 25-32 min). Further details of the technique of renal transplantation are published elsewhere (5).

The animals were sacrificed 21 days after the transplantation. In the meantime they were weighed daily. Blood tests were drawn from the medial angle of the eye and serum creatinine was determined every other day. Autopsy was performed with the same technique as in I, with the exception that the renal grafts were fixed in vivo and prepared for light microscopic

Table 1. Autopsy findings from 30 experimental animals with end-to-end ureteral anastomosis. Figures represent the number of animals

Group	n	Compensatory hypertrophy	Anastomosis		Hydronephrosis			Dislocation of splint
			no stenosis	stenosis	none	slight	severe	
I A	10	5	9	1	9	-	1	9
I B	10	10	8	2	8	2	-	9
I C	10	10	10	-	10	-	-	8

studies (3). Light microscopic studies were performed on one μ thin Epon embedded sections stained with toluidine blue. Urinary specimens and creatinine were analysed as in I.

Results

I. Reconstruction in situ. Only one out of thirty animals had a severe stenosis of the ureter with advanced hydronephrosis and pyelonephritis verified by the histological studies. In two animals minimal stenosis was found, along with mild hydronephrosis and some indication of an interstitial nephritis. These animals had sterile urine. The in-dwelling catheter, it remained at the anastomotic site in only four cases; in the others it was dislocated into the renal pelvis or the distal part of the ureter. This phenomenon did not seem to interfere with the urinary flow (Table 1).

Only five animals were found to have bacteriuria. One of them was the animal with the ureteric stenosis and the severe hydronephrosis. In the other four cases, the bacteriuria was the only pathological finding and there were no signs of hydronephrosis nor any histological changes indicating infection.

A serum creatinine of 2.09 mg/100 ml was found in one rat (the one with severe hydronephrosis). In all the others serum creatinine was normal (below 1.0 mg/100 ml).

II. Renal transplantation. One rat died two days after the renal transplantation due to occlusion of the renal vessels. The other five survived. Nothing was found indicating an abnormality of the ureteral anastomosis. The silastic catheter remained at the anastomotic site in all cases. The vascular anastomoses were intact with the exception of the above mentioned rat. The histological picture did not deviate from the one seen in normal animals. None of the animals were found to have bacteriuria. The serum creatinine showed in one case a transient but moderate elevation the first three postoperative days followed by a rapid normalization (Fig. 2).

Discussion

There are several methods to conduct the urine from the grafted rat kidney to the urinary bladder (for reference see 4). In our experience, all of these methods have produced complications in the form of leakage, stenosis, stone formation or infection after follow-up, except for the end-to-end anastomotic technique used in this study. We have also tried a new method using the donor ureter with a patch of the donor bladder implanted into the recipient bladder without the use of sutures. The results so far have not been better. The direct end-to-end anastomosis appears to produce superior results. With our technique of renal transplantation (5), the ureter passes down to the bladder in a curved fashion and this might prevent the splint from being dislocated. Furthermore, the end-to-end technique has been used in more than 30 rat renal grafts with the same good results. These animals were, however, not included in this study due to the fact that the donor kidneys were perfused in vitro with various solutions, which could interfere with the function of the graft (6). Few complications from the ureteral anastomosis were found in these animals. The large immediate postoperative mortality when the ureter was reconstructed in situ was due to urinary leakage. In all these animals only two stitches were used for the ureteral anastomosis. On the other hand, no urinary leak was observed with the use of four stitches. The dislocation of the silastic splint did not seem to interfere with the urinary flow.

It is interesting to note that only five animals had a positive urinary culture. In a pilot study of 20 animals, we used splints which were not sterilized and bacteriuria was more frequent. The rat seems to have an inherent resistance against infection and, if no obstruction to the urinary flow is present, these infections appear to disappear spontaneously. Inoculation of bacteria into the urinary tract simultaneously with urinary obstruction is, however, frequently used as a model for experimental pyelonephritis (1).

The LBN F1 hybrid rats were chosen for transplantation purposes for two reasons. First: they are compatible at the Ag-B locus, which is the most important locus in the kinetics of transplantation immunity (12). Second: a hybrid is usually more resistent than an individual from a highly inbred strain.

Conclusion

The ureteral anastomosis in rat renal transplantation may be constructed safely and simply over an in-dwelling silastic catheter. The technique provides good results and a low frequency of complications.

References

1. Cotran, R.S.: Experimental Pyelonephritis. The Kidney. C. Rouiller and A.F. Muller. New York and London: Academic Press 1969
2. Daniller, A., Buchholz, R., Chase, R.A.: Renal transplantation in rats with the use of microsurgical techniques: A new method. Surgery 63, 956 (1968)

3. Engberg, A., Eriksson, J. L. E.: Effects of dextran 40 on proximal renal tubule. Electron microscopic and cytochemical studies in the mouse. Acta chir. scand. 135, 263 (1969)
4. Fabre, J., Lim, S. H., Morris, P. J.: Renal transplantation in the rat: Details of a technique. Aust. N. Z. J. Surg. 41, 69 (1971)
5. Frödin, L.: Renal transplantation in the rat. Studies on the in vitro perfused and transplanted rat kidney. Acta Univ. Upsal. 193
6. Frödin, L., Engberg, A., Källskog, Ö., Wolgast, M.: Renal transplantation in the rat. IV. Cortical pressure gradients measured in vivo and during isolated perfusion with reference to renal function after transplantation. Scand. J. Clin. Lab. Invest. (in press)
7. Ippolito, R. J., Mahoney, R. J., Murray, I. M.: Renal transplantation between histoincompatible rats. I. Acute rejection and prolonged survival of reciprocal renal allografts in immunocompetent rats. Transplantation 14, 183 (1972)
8. Kingsley, G. R., Schaffert, R. R.: Creatinine. Standard Methods of Clinical Chemistry. M. Reiner. New York: Academic Press Inc. 1953
9. Klein, T. W., Gittes, R. F.: The Three-Kidney Rat: Renal Isografts and Renal Counterbalance. J. Urol. 109, 19 (1973)
10. Lameijer, L. D. F., Smith, J., Mowbray, J. F.: Effect of kidney perfusion on renal allograft survival in azothioprine-treated rats. Brit. J. exp. Path. 53, 130 (1972)
11. Owen, E. R.: Personal communication 1969
12. Palm, J., Black, G.: Interrelationship of inbred rat strains with respect to Ag-B and non Ag-B antigens. Transplantation 11, 184 (1971)

Doc. A. Engberg M. D.
Riddargatan 25/a
Sundsvall
Sweden