Urol Res (1996) 24:211–216

ORIGINAL PAPER

S. Salomon · D. Steinbrüchel · B. Nielsen · E. Kemp

Hamster to rat kidney transplantation: technique, functional outcome and complications

Received: 10 November 1995 / Accepted: 26 February 1996

Abstract Hamster to rat kidney transplantation has only recently been introduced as model of concordant xenografting. The kidney model offers unique possibilities for studying both immunological and functional aspects of xenografts as opposed to the widely used heterotopic heart model. This article provides a detailed description of surgical technique as well as data on functional outcome and complications. The renal artery with a small segment of the aorta is sutured end-to-side to the abdominal aorta of the recipient, and the renal vein is anastomosed end-to-side to the inferior vena cava. The urinary system is reconstructed by bladder-to-bladder anastomosis. Xenografts will maintain close to normal serum-creatinine levels for 2–3 days, after which they are rejected. Complications occurred in 22% of xenografts. Postrenal obstruction due to severe hematuria or ureter stenosis was the most frequent problem encountered.

Key words Rats · Hamster · Microsurgery · Kidney transplantation · Renal function · Xenotransplantation

Introduction

Heterotopic heart transplantation from hamster to rat is a widely used model of so-called concordant xenotransplantation [2, 15]. The mechanism of rejection has been investigated [14, 16, 20, 24], and various treatment protocols have been tested [12, 21, 25]. The hemodynamics of heterotopically transplanted hearts are, however, greatly disturbed [8]. The left side of the heart is bypassed, resulting in intraventricular thrombosis, subendocardial ischemia and the risk of infarction [13, 22]. Disturbed hemodynamics may consequently lead to unspecific reactive changes difficult to distinguish from rejection-specific changes upon subsequent histological examination. Also, evaluation of graft function is essentially qualitative, and recipient survival is not dependent on xenograft performance. Data on long-term graft survival may therefore need to be interpreted with some reservation.

To be able to study the mechanism of xenograft rejection in a hemodynamically unaltered model in which animal prosperity and survival is dependent upon xenograft function, we have explored a new model in which hamster kidneys are transplanted into bilaterally nephrectomized rats [17, 18]. The model has only recently been reported [9, 10]. No detailed account for the surgical procedure has been published until now.

In this paper, we provide a complete description of the surgical technique, functional outcome and complications we experienced in our first 59 consecutive experiments using the technique described.

S. Salomon (✉) · D. Steinbrüchel · B. Nielsen · E. Kemp
Laboratory of Nephropathology, Institute of Pathology, Odense University Hospital, DK-5000 Odense C, Denmark

Materials and methods

Animals

Outbred, male, golden hamsters (SPF, Mollegaard Breeding Center, Skensved, Denmark) weighing 100–160 g and inbred, male, Lewis rats (SPF, Mollegaard Breeding Center, Denmark) weighing 180–334 g served at random as donors and recipients, respectively. The animals were kept under standard laboratory conditions with free access to food (Altromin 1434) and tap water.

Instruments and sutures

A basic set of microvascular instruments were used (S&T Scandinavia, Hägersten, Sweden) including one pair of No. 5 jeweller's forceps (JF-5), dissection scissors (SCD-15 R-8), adventitia scissors (SAS-15 R-8) and a curved forceps/needle holder (FRC-15 RM-8). Bipolar coagulation or an ophthalmic cautery (C-line, Xomed-Treace,

Jacksonville, USA) were used to control minute bleeding. Arteriotomy was performed with a microsurgical blade (Keisei, S&T, Scandinavia). 2-0 Cotton ligatures (Davis&Geck, Danbury, USA) served as temporary vascular clamps during reimplantation. Vascular anastomosis was performed with a 10-0 monofilament nylon on a 70-μm, 3/8-taper-point needle (Davis&Geck). For bladder-to-bladder anastomosis, a monofilament 8-0 nylon suture mounted on a 145-μm, 3/8-cardiopoint needle was used (Davis&Geck). 6-0 Silk was used for ligation.

Anesthesia and perioperative care

Rats were anesthetized with Hypnorm^{vet} (fluanisone 10 mg/ml, fentanyl 0.135 mg/ml, Janssen) 0.03 ml/100 g i.m. supplemented with diazepam 0.2 mg/100 g i.p. [23]. Near the end of the transplantation, anesthesia often became shallow, which was judged by pinching a foot. In these instances, nitrous oxide:oxygen (1:2) was added until the end of the procedure. Hamsters were anesthetized by pentobarbital 5 mg/100 g i.p. supplemented with nitrous oxide:oxygen (1:2) on an open mask.

Animals were maintained at normal body temperature on a thermostatic heat pad throughout the operation, and warm isotonic saline was used for irrigation. To compensate for fluid loss, all animals received 10 ml isotonic saline injected subcutaneously before surgery. After surgery, recipients were similarly hydrated to 5% above preoperative body weight and allowed to recover in a 27 °C cabinet.

Clean surgical technique was used. The animals were shaved with electrical clippers, and the skin was cleaned twice with isopropanol before entering the abdomen. Instruments were soaked in isopropanol before use.

Transplantation procedure

The description of the transplantation procedure is divided into: (1) graft preparation, (2) recipient preparation, (3) vascular anastomosis and (4) reconstruction of the urinary system. In short, the renal artery together with a small segment of the aorta was sutured end-to-side to the abdominal aorta of the recipient, and the renal vein was anastomosed end-to-side to the inferior vena cava. The urinary tract was reconstructed by bladder-to-bladder anastomosis.

1. Graft preparation

The abdominal cavity was entered through a long midline incision. The intestines including the stomach were deflected to the right, wrapped in gauze and drenched with warm saline to protect from heat loss and prevent drying. The left hepatic lobes were gently retained to the left hypochondrium by moist cotton wool. Care was taken not to interfere with diaphragmatic movements, which may cause asphyxia to the experimental animal. The left kidney was then fully exposed.

The infrarenal aorta was mobilized by digital dissection, and all side branches including the spermatic vessels were ligated and cut. The renal vein was exposed by sharp dissection of the surrounding fat and lymphatic tissue. Minute bleeding may occur from small lymphatic blood vessels, but is readily controlled by gentle compression. The left suprarenal and spermatic veins, which invariably drain into the left renal vein, were ligated and cut. The kidney was mobilized by sharp dissection of the perinephric fat, leaving the suprarenal gland untouched at the upper pole.

The left ductus deferens was divided to expose the ureter at its full length. The ureter was mobilized by sharp dissection along the periureteral connective tissue, while care was taken not to traumatize the ureter or its delicate vasculature. To free the bladder, the right ureter and the urethra were ligated en bloc and cut.

The mobilized infrarenal aorta was ligated distally, and a polythene catheter (OD 0.76 mm, ID 1.22 mm, Portex, Berck Sur Mer, France) mounted on a syringe was inserted into the aorta. Blood flow to the kidney was arrested by ligating the aorta superior to the left renal artery, and the graft was flushed in situ with 3–5 ml ice-cold citrate/mannitol hyperosmolar perfusion solution (calcium citrate 37.4 mM, sodium citrate 38.3 mM, mannitol 198 mM, magnesium phosphate 84.0 mM). If dissection had been performed correctly, the graft paled off instantaneously, ensuring a warm ischemic time of less than 1 min. The hypertonic perfusion solution entered the circulation via the renal vein and caused circulatory arrest in the donor. The graft was excised by dividing the renal vein close to the vena cava and the aorta 2 mm inferior to the origin of the renal artery. The kidney was preserved in ice-cold perfusion solution while the recipient was prepared.

2. Recipient preparation

The abdominal cavity was entered, and the gut was deflected and protected as in the donor. The stomach was not mobilized in the recipient. The left renal pedicle including the ureter was ligated and cut, and the kidney was excised. The abdominal aorta and vena cava were mobilized en bloc over a distance of approximately 10 mm by blunt dissection. The left iliolumbar vessels, which may originate at varying positions were, ligated and cut if they were interfering. The freed aortic and caval vascular bundle was clamped by two 2-0 cotton ligatures pulled taut but not tied. With a microsurgical blade, a slit was made in the aorta 1.5 times the diameter of the aortic segment of the kidney graft. In a slightly more inferior position, an elliptical incision was made in the vena cava. The vessels were emptied and rinsed by careful intraluminal irrigation to remove blood and clots.

3. Vascular anastomosis (Figure 1a–d)

The graft was preserved in a cooling coil to prevent it from rewarming in the recipient during reimplantation [11]. The coil was manufactured from a stainless steel tube (diameter 1.5 mm) bent cold to completely encircle the graft. By means of simple hydrostatic pressure, cold water was driven through the coil to cool the embedded transplant. The aortic segment of the transplant was anchored with two stay sutures at the upper and lower corners of the aortic slit. The circumference of the aorta-aorta anastomosis was thus roughly divided into two equal halves, in which the left side was preferably kept a little shorter than the right in order to prevent any through stitches. The vessels were then anastomosed by two semicircular running sutures (Fig. 1a, b). The renal vein and vena cava were anastomosed employing the same basic technique. Since the arterial anastomosis restricts access to the left side of the venous anastomosis, this side was sutured from within the lumen (Fig. 1c). The right side was conventionally completed from the adventitial surface (Fig. 1d). Before tying the last stitch, the anastomosis was irrigated intraluminally to expand the suture line and to prevent stenosis when the cotton ligatures were removed, the graft immediately became vivid and began to produce urine. Sparse bleeding may be controlled by gentle compression with moistened gauze for a few minutes to allow platelets to seal the anastomosis.

Because running sutures provide better primary hemostasis than interrupted ones, only four and five stitches are needed on each side of the arterial and venous anastomosis, respectively. It is important to place the corner stitches in close proximity, because bleeding upon revascularization is most likely to stem from these sites. The venous vascular wall is very fragile and will readily collapse. It may therefore be difficult to identify the cut edge when

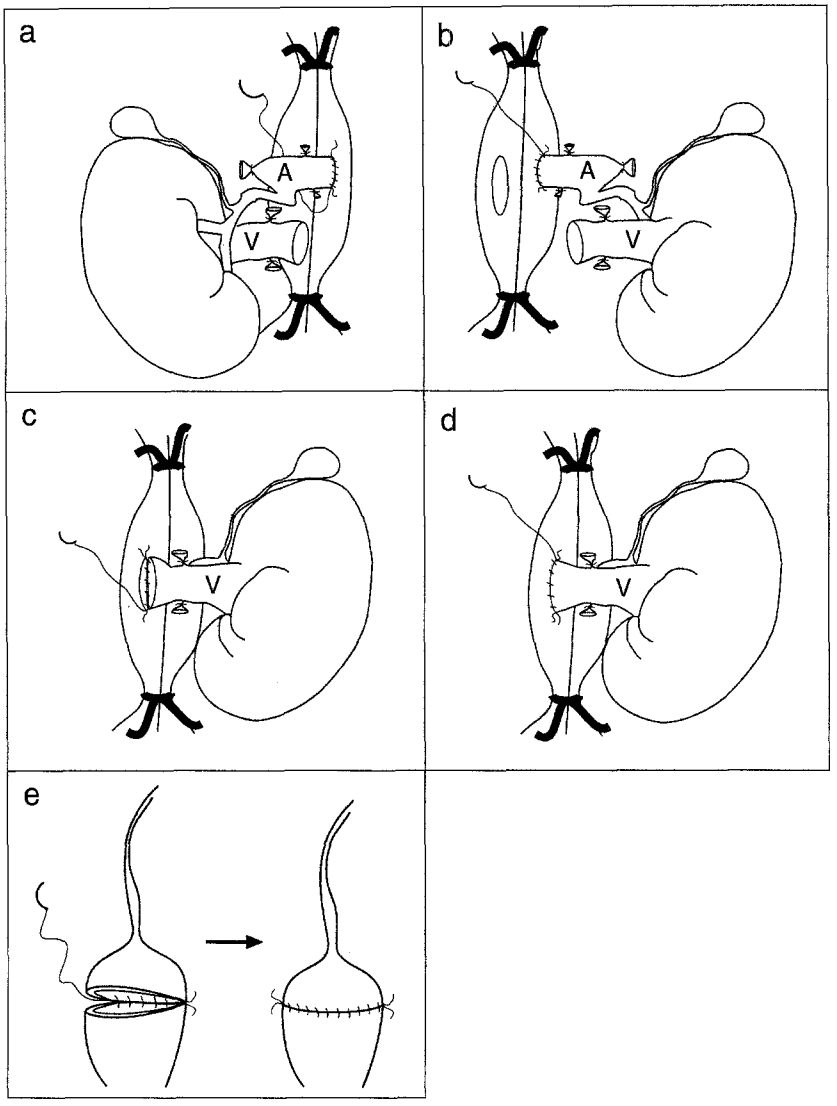

Fig. 1 Left side of the arterial anastomosis sutured with the graft deflected to the right (**a**). Arterial anastomosis completed with the graft deflected to the left (**b**). Left side of the venous anastomosis sutured from the intimal side (**c**). Venous anastomosis completed from the adventitial side (**d**). Bladder patch of the donor anastomosed to the dome of the recipient bladder by an extramucosal running suture (**e**)

placing the stay sutures. This problem is best solved by flooding the abdominal cavity, which will cause the vein to open and the cut edge to clearly present itself.

4. Urinary tract reconstruction (Fig. 1e)

The graft bladder was cut so to leave a patch one-third the size of the intact bladder with the ureter at its center. Minute bleeding from the ureteric artery was cautiously coagulated with care taken not to interfere with the ureterovesical junction. The dome of the recipient bladder was excised to match the patch. This was preferably done using cautery to prevent bleeding from the cut edge. The ureter was untwisted by inspection of the ureteric vessels, which should parallel the ureter. The patch was fixed with two stay sutures, and a bladder-to-bladder anastomosis was fashioned with two semicircular extramucosal running sutures. Finally, the right native kidney was removed, and the abdominal wound was closed in two layers.

Evaluation

Fifty-nine consecutive xenotransplants using the described technique were studied. Renal function was evaluated by the serum (s)-creatinine increase during the first 24 h (Δs-creatinine = s-creatinine day 1 minus day 0). The s-creatinine increase was correlated to total ischemic time, anastomotic time and donor-recipient weight ratio to determine the influence of different ischemic times and donor-recipient weight ratios on the functional outcome of the xenografts. The spontaneous course of rejection was determined by daily s-creatinine analysis in 11 unmodified recipients. For comparison, the s-creatinine increases in nine bilaterally nephrectomized rats were analyzed.

A detailed surgical journal was kept. All animals were thoroughly examined at death, or when sacrificed, and complications were recorded.

Statistics

Correlations were assessed by Spearman's rank correlation test (r_s). P values < 0.05 were considered significant.

Results

The median anastomotic time was 35 min (range 22–80 min), and the median total ischemic time was

Table 1 Complications in 59 kidney xenotransplants

Complication	*n*	%	Number of animals with affected graft function during the first 24 h
Venous thrombosis	1	1.7	1
Severe hematuria with postrenal obstruction	7	11.8	5
Ureteric leakage	1	1.7	1
Ureteric stenosis	2	3.4	0
Peroperative hemorrhage[a]	1	1.7	1
Surgical infection	1	1.7	0
Total	13	22.0	8

[a] Animal died within the first 24 h after surgery

90 min (range 52–160 min). The median donor-recipient weight ratio was 0.55 (range 0.40–0.78).

Eight out of 59 xenotransplanted animals had surgical complications clearly affecting renal function during the first 24 h and were excluded from functional analysis (Table 1). The median increase in s-creatinine during the first 24 h in the remaining 51 xenotransplants was 108 μmol/l (range 27–335 μmol). The median increase in the nine bilaterally nephrectomized rats was 267 μmol/l (range 235–340 μmol). Seven xenotransplanted rats (12%) had creatinine values within the latter range.

There were no correlations between the s-creatinine increase and anastomotic time ($P > 0.05$) (Fig. 2a), between the s-creatinine increase and total ischemic time ($P > 0.05$) (Fig. 2b) or between the s-creatinine increase and donor-recipient weight ratio ($P > 0.05$) (Fig. 2c). Indeed, the distributions showed a completely random pattern: considerable s-creatinine increases are seen in experiments with both short and long ischemic times and in experiments with both high and low donor-recipient weight ratios.

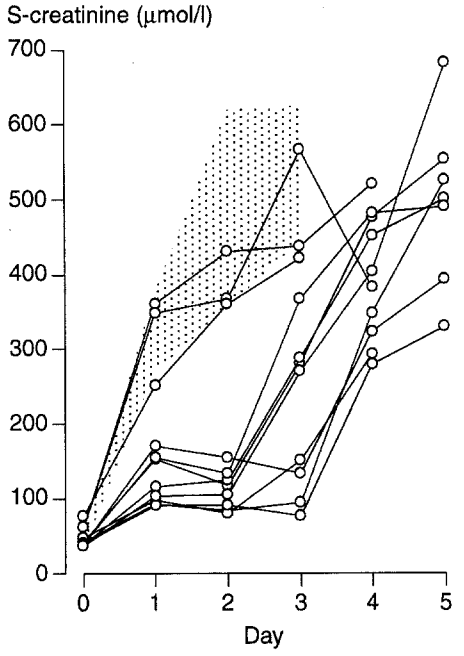

Fig. 3 Course of serum creatinine in 11 rats with spontaneously rejecting hamster kidneys. *Shaded area* s-creatinine range in bilaterally nephrectomized controls ($n = 9$)

The course of xenograft rejection in 11 unmodified recipients is shown in Fig. 3. Eight animals maintained close to normal s-creatinine levels for 2 or 3 days, after which xenograft function deteriorated rapidly. Three animals had s-creatinine levels within the range of bilaterally nephrectomized controls.

Thirteen complications were recorded out of 59 transplantations (22%) and are listed in Table 1. Severe

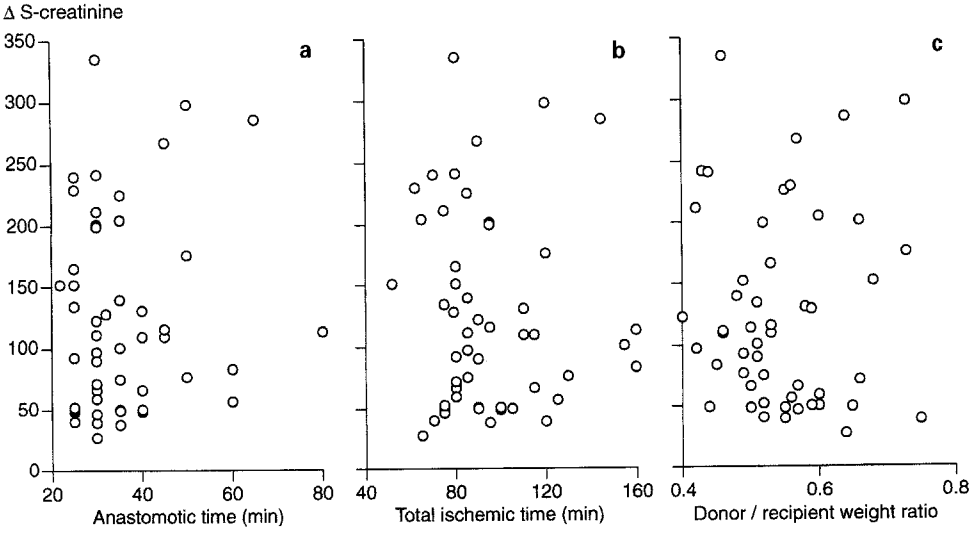

Fig. 2 Serum creatinine increase during the first 24 h correlated to anastomotic time (**a**), total ischemic time (**b**) and donor/recipient weight ratio (**c**)

hematuria with subsequent infravesical obstruction was the most frequent problem, and usually developed within the first postoperative 24 h. There were no anesthetic deaths.

Discussion

The rat will rapidly grow to twice the size of a hamster. This difference imposes microvascular difficulties due to unequal diameters of the renal vessels. End-to-end arterial anastomosis is likely to cause flow-limiting stenosis with subsequent damage to the transplant. Indeed, in our first 20 transplants (data not included), end-to-end anastomosis was attempted without success. Diuresis did not appear, and hypoperfusion was evident. When the method was altered to the end-to-side technique, function was immediate.

The hamster ureter is indeed a delicate structure and is not easily manipulated as in the rat [5]. Successful ureterovesical anastomosis, however, has been reported in hamster-to-hamster kidney transplantation [4]. In our hands, this strategy has only occasionally proven successful. In 21 experiments evaluating this technique (data not included), only 5 animals exhibited a satisfactory ureterovesical passage. Bladder-to-bladder anastomosis was therefore commenced and has worked fairly reliably.

Twelve percent of xenografted animals had s-creatinine increases within the range of bilaterally nephrectomized controls. Since all transplants were kept cold until revascularization, ischemic damage cannot explain this dysfunction. It could therefore be argued that these grafts had been subjected to hyperacute rejection mediated by high levels of preformed rat antibodies directed against xenogeneic epitopes in the hamster. In a previous study, however, we found only minor levels of xenoantibodies both before xenografting and after 24 h [7, 17]. These levels were independent of renal function. Alternatively, different quantities of xenogeneic epitopes could exist in outbred hamsters, and would explain the heterogeneity observed in renal function during the first 24 h. This hypothesis is under current investigation.

A 30-min normothermic ischemic insult has been shown to precipitate acute renal failure in rats [3, 11]. Ischemic injury may unpredictably alter graft immunogenicity [19], accelerate chronic rejection [26] and potentiate the nephrotoxic effect of cyclosporine A [1]. Also, the morphology of rejected hamster-to-rat xenografts may mimic infarction [13, 17]. For these reasons, control of ischemia is of utmost importance and was achieved by preventing the transplant rewarming during reimplantation. Accordingly, we found no correlation between renal function and anastomotic time even though the latter varied from 22 to 80 min.

Xenotransplantation has introduced a question of physiological compatibility between different species. In the hamster-to-rat combination, long-term survival after liver grafting has been reported [12], an indication that even complex metabolic pathways seem to operate across this species barrier. Recent experiences with the kidney model have been somewhat more disappointing [9, 10, 18]. Considering that only one kidney is transplanted into a bilaterally nephrectomized recipient twice the size of the donor, poor survival could be due to an inadequate amount of transplanted renal mass [6]. Within the weight ratios studied, however, we found no correlation between donor-recipient weight ratio and the s-creatinine increase during the first 24 h. Whether the amount of transplanted renal mass is crucial for long-term survival remains to be clarified.

Acknowledgements This work was supported by The Danish Foundation for the Advancement of Medical Science, The Ruth-Koenig-Petersens Fund for Kidney Research, The Research Fund of the Danish Kidney Association and Institute of Clinical Research, Odense University, Denmark.

References

1. Bia MJ, Tyler KA (1987) Effect of cyclosporine on renal ischemic injury. Transplantation 43:800
2. Chaline J, Cardoso J, Houssin D (1994) Organ xenografting between rodents: an evolutionary perspective. Transpl Int 7:216
3. Donohoe JF, Venkatachalam MA, Bernard DB, Levinski NG (1978) Tubular leakage and obstruction after renal ischemia: structural functional correlations. Kidney Int 13:208
4. Dubois MA, Haertig AG, Kuss R (1982) Renal transplantation in hamsters. J Microsurg 3:107
5. Frodin L, Engberg A (1975) Renal transplantation in the rat. I. Studies concerning the ureteral anastomosis with special reference to the end-to-end technique. Urol Res 3:87
6. Heemann UW, Azuma H, Tullius SG, Mackenzie H, Brenner BM, Tilney NL (1994) The contribution of reduced functioning mass to chronic kidney allograft dysfunction in rats. Transplantation 58:1317
7. Lillevang ST, Steinbrüchel DA, Kemp E, Kristensen T (1994) Sensitive detection of xenogeneic antibodies with the gel technique. Transplant Proc 26:1274
8. Mann FC, Priestly JT, Markowitz J, Yater WM (1933) Transplantation of intact mammalian heart. Arch Surg 26:219
9. Miyazawa H, Murase N, Demetris AJ, Nakamura K, Matsumoto K, Ye Q, Manez R, Todo S, Starzl TE (1994) Mechanisms of hamster kidney graft rejection in the rat. Transplant Proc 26:1205
10. Miyazawa H, Murase N, Demetris AJ, Matsumoto K, Nakamura K, Ye Q, Manez R, Todo S, Starzl TE (1995) Hamster to rat kidney xenotransplantation. Effects of FK 506, cyclophosphamide, organ perfusion, and complement inhibition. Transplantation 59:1183
11. Müller GH, Hohn U, Greger B, Schareck W, Hopt UT, Lauchart W (1989) Effect of anastomosis time and intraoperative organ cooling on primary function in renal transplantation. Transplant Proc 21:1239
12. Murase N, Starzl TE, Demetris AJ, Valdivia L, Tanabe M, Cramer D, Makowka L (1993) Hamster-to-rat heart and liver xenotransplantation with FK506 plus antiproliferative drugs. Transplantation 55:701

13. Nielsen B, Steinbrüchel DA, Laenkholm AV, Kemp E (1992) The histology at various stages of acute rejection in concordant xenogeneic heart transplantation. A sequential study in rodents. APMIS 100:249
14. Nielsen B, Steinbrüchel DA, Lillevang ST, Kemp E (1993) Evidence for a primarily humoral rejection mechanism in concordant xenogeneic heart transplantation. A sequential immunohistological study in a hamster-to-rat model. APMIS 101:587
15. Reding R (1988) Organ xenografts in rodents. In: Hardy MA (ed) Xenograft 25. Elsevier Science Publishers, Amsterdam, pp 39
16. Rosengard BR (1986) Differences in the pathogenesis of first-set allograft rejection and acute xenograft rejection as determined by sequential morphologic analysis. J Heart Transplant 5:263
17. Salomon S, Nielsen B, Starklint H, Steinbrüchel DA, Lillevang S, Kemp E (1996) Sequential morphological, functional and xenoreactive antibody analysis of hamster kidneys transplanted into unmodified rats. Transplant Proc 28:687
18. Salomon S, Steinbrüchel DA, Nielsen B, Lillevang S, Kemp E (1996) The effect of leflunomide, cyclosporine and splenectomy in two different organ systems of concordant xenotransplantation in rats. Transplant Proc 28:698
19. Schanzer H, Bramis J, Severin C, Ramachandar K, Taub R (1974) Effect of operative ischemia time on survival of rat renal allografts. Transplantation 18:417
20. Steinbrüchel DA, Nielsen B, Salomon S, Kemp E (1992) Sequential, morphological, and antidonor antibody analysis in a hamster-to-rat heart transplantation model. Transpl Int 5:38
21. Steinbrüchel DA, Nielsen B, Kemp E (1994) Treatment of hamster heart to rat xenotransplantation. Transpl Immunol 2:3
22. Steinbrüchel DA, Nielsen B, Salomon S, Kemp E (1994) A new model for heterotopic heart transplantation in rodents: graft atrial septectomy. Transplant Proc 26:1298
23. Svendsen P, Carter AM (1985) Influence of injectable anaesthetic combinations on blood gas tensions and acid-base status in laboratory rats. Acta Pharmacol Toxicol 57:1
24. Thomas FT, Marchman W, Carobbi A, Araneda D, Pryor W, Thomas J (1991) Immunobiology of the xenograft response: xenograft rejection in immunodeficient animals. Transplant Proc 23:208
25. Xiao F, Chong AS, Foster P, Sankary H, McChesney L, Koukoulis G, Yang J, Frieders D, Williams JW (1994) Leflunomide controls rejection in hamster to rat cardiac xenografts. Transplantation 58:828
26. Yilmaz S, Paavonen T, Hayry P (1992) Chronic rejection of rat renal allografts: II. The impact of prolonged ischemia time on transplant histology. Transplantation 53:823