Complications and Their Prevention in Experimental Renal Transplantation in Rats

Badri Man Shrestha, John Haylor

Abstract

Objectives: Experimental rat models of renal transplant have played a pivotal role in renal transplant research. Both intraoperative and postoperative complications during donor nephrectomy and implantation in the recipient can be associated with significant morbidity and mortality. The aim of this paper is to discuss the incidence, pathophysiology, and prevention of complications that occurred in the process of establishment of a rat model of chronic allograft injury at our institution.

Materials and Methods: The complications observed while performing 67 consecutive donor nephrectomies and 61 renal transplants were recorded prospectively, and appropriate measures were taken to prevent these complications in the subsequent transplant procedures.

Results: Donor-related complications included failure of the kidney to clear of blood by the kidney perfusion solution and intraoperative deaths. The recipient-related complications included intraoperative hemorrhage, inadequately perfused kidneys with dusky appearance, congested and paralysed hind limbs, urine leak, necrosis of the kidneys, renal and bladder calculi formation, and death during and after kidney transplant.

Conclusions: Complications during donor nephrectomy and renal transplant can lead to significant loss of kidneys and animals. Proper recognition can allow appropriate measures to be taken to prevent these complications, thus achieving high-quality transplants and prolonged graft and animal survival.

Key words: Experimental transplantation, Graft loss, Mortality

Introduction

Experimental rat models of renal transplant (RT) have been instrumental in transplant research for the past 5 decades; these models have enhanced the understanding of transplant immunology, ischemia-reperfusion injury, acute and chronic rejections, and the effects of immunosuppressive agents on short- and long-term outcomes after RT. Several experimental rat models of RT have been described previously.1-4 Because of advantages of rat models of RT over other animal models, such as improved long-term survival, lower cost, simplicity of animal maintenance, less critical requirements for aseptic surgery, and well-established surgical techniques of vascular and ureteric anastomoses for RT, the model is commonly employed in transplant-related research.5 A rodent model of RT was first described in rat (Rattus norvegicus) in 1965 by Bernard Fisher and Sun Lee at the American College of Surgeons Meeting in Chicago in 1961, with subsequent publication in 1965.6 Microvascular techniques have undergone several modifications to establish blood flow through arterial and venous anastomoses between donor blood vessels and recipient systemic circulation and urinary tract drainage via anastomosis to the recipient urinary tract to achieve successful RT with minimum morbidity and mortality.7,8 The basic principles of RT in rat models for experimental studies are similar to those applied to human clinical transplantation.9,10 Complications after RT in rats not only compromise the outcomes but also prove fatal and add to expenses and time. It is paramount to have a clear understanding of the possible complications so that appropriate protocols can be followed to prevent them. In this paper, we reviewed the intraoperative and postoperative complications in donor and recipient rats observed during the process of establishing a Fisher-Lewis chronic
allograft injury rat model of RT at our institution with the objective of recommending possible strategies to prevent these complications.

**Materials and Methods**

**Animals**

Male inbred Fisher 334 (RT11v1) and Lewis (RT11) rats weighing 250 to 300 g were purchased from Harlan UK (Bicester, UK). All experiments were performed in accordance with the protocols of the Animals Scientific Procedures Act of 1986 and with approval from the UK Home Office. We used 128 rats (106 Lewis and 22 Fisher rats) for donor nephrectomy and RT during establishment of the rat model of chronic allograft injury. Rats used for study of anatomy and physiology (n = 47) were excluded from the analyses.

**Anesthesia**

Anesthesia was induced by isoflurane (5% in oxygen, 4 L/min), with animals maintained on isoflurane (3% in oxygen, 1 L/min) following a subcutaneous injection of buprenorphine (50 µg/kg). Core body temperature was measured with a rectal probe and maintained at 35°C to 37°C using a thermostat blanket (Harvard Apparatus UK; Cambridge, UK). Subcutaneous injection of normal saline (3 mL), in both the donor and recipient before commencement of surgery, was associated with intraoperative deaths due to hypotension. Hence, after a series of experiments, we developed a protocol in which normal saline (2 mL bolus followed by continuous infusion at 6 mL/h) was infused by cannulation of the internal jugular vein in the donor and recipient. This technical change led to improved graft and animal survival and satisfactory renal function.

**Protocol**

The surgeon (BMS) who performed the procedure was an experienced renal transplant surgeon. The left kidney was retrieved from the Fisher or Lewis donor rats and transplanted into Lewis rats, thereby generating the Fisher-Lewis allografts and Lewis-Lewis isografts. Of the 61 transplanted rats, 33 RTs were given cyclosporine (Novartis UK; Camberley, UK) at 5 mg/kg subcutaneously for 10 days, which was followed by right native nephrectomy. Transplanted rats were killed, and kidneys were harvested when an animal’s physical condition indicated the development of uremia, as reflected by decreased body weight and physical condition in accordance with Home Office Project License, or after 52 weeks. Rat postmortem examinations were carried out to establish the cause of deterioration and death.

**Renal transplant procedure**

Donor nephrectomy and recipient transplant procedures were conducted as described previously. In summary, after the left kidney, including aortic and inferior vena cava (IVC) conduits, was retrieved, the donor aortic and IVC conduits were anastomosed end-to-side with the recipient’s infrarenal abdominal aorta and IVC using continuous 10/0 Prolene sutures with the aid of an operating microscope. The donor’s ureter with a bladder cuff was anastomosed to the dome of the recipient’s bladder with continuous 5/0 Vicryl suture.

**Results and Discussion**

Our group conducted 67 donor nephrectomies and 61 RTs. The donor- and recipient-related complications are shown in Tables 1 to 3.

<table>
<thead>
<tr>
<th>Table 1. Intraoperative Complications in Donor Rats</th>
<th>No. of Rats</th>
</tr>
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<tbody>
<tr>
<td>Total donor nephrectomies</td>
<td>67</td>
</tr>
<tr>
<td>Deaths</td>
<td>5</td>
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<tr>
<td>Failure to perfuse</td>
<td>3</td>
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<table>
<thead>
<tr>
<th>Table 2. Intraoperative Complications in Recipient Rats</th>
<th>No. of Rats</th>
</tr>
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<tbody>
<tr>
<td>Total transplant recipients</td>
<td>61</td>
</tr>
<tr>
<td>Bleeding</td>
<td></td>
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<tr>
<td>Aorta</td>
<td>3</td>
</tr>
<tr>
<td>Inferior vena cava</td>
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</tr>
<tr>
<td>Narrowed inferior vena cava</td>
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</tr>
<tr>
<td>Difficulty in identifying lumen of renal vein</td>
<td>1</td>
</tr>
<tr>
<td>Congested and paralyzed hind limb</td>
<td>1</td>
</tr>
<tr>
<td>Intraoperative death</td>
<td>8</td>
</tr>
<tr>
<td>Dusky kidney</td>
<td>4</td>
</tr>
<tr>
<td>Too short renal vein for anastomosis</td>
<td>1</td>
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<tr>
<th>Table 3. Postoperative Complications in Recipient Rats</th>
<th>No. of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>11</td>
</tr>
<tr>
<td>Urine leak</td>
<td>3</td>
</tr>
<tr>
<td>Congested and paralyzed hind limb</td>
<td>2</td>
</tr>
<tr>
<td>Weakness of hind limbs</td>
<td>1</td>
</tr>
<tr>
<td>Unwell</td>
<td>1</td>
</tr>
<tr>
<td>Anuric postnephrectomy (necrosed kidneys)</td>
<td>7</td>
</tr>
<tr>
<td>Vesical calculi</td>
<td>2</td>
</tr>
<tr>
<td>Thrombosed kidney</td>
<td>1</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>1</td>
</tr>
<tr>
<td>Wound infection, dehiscence, and incisional hernias</td>
<td>0</td>
</tr>
<tr>
<td>Visceral injury and peritonitis</td>
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Donor-related complications

Donor deaths
Intraoperative deaths of the donors were multifactorial regarding cause. Five donor rats died during the nephrectomy procedure, which was attributed to hypotension and anesthesia overdose. The hypotension was likely to have resulted from hypovolemia due to inadequate fluid replacement because only 3 mL of normal saline was given subcutaneously. Significant fluid loss can occur from the exposed intestinal surface, which can lead to hypovolemia. Compression of IVC, caused by the Czerny retractor and leading to reduced venous return to the heart and reduced cardiac output, was an important cause of hypotension. Replacement of the heavy Czerny retractor with a West self-retaining retractor avoided compression of IVC and the intestine.

In the initial experiments, a heating board was used to keep the rats warm, which did not include facility temperature regulation. The rectal temperature had dropped to as low as 34°C; however, at the point of contact of the body surface to the heating pad, the temperature was as high as 40°C. We then exchanged the heating board for a normothermic heating pad that could be set to a desired temperature level during both donor nephrectomy and RT in the recipient rats. This allowed the core body temperature to be maintained between 36°C and 37°C. The bowel was covered with swabs soaked in warm saline to prevent fluid and heat loss.

Failure of perfusion of donor kidney
Clearance of the donor kidney of all blood from the kidney is vital for preservation and restoration of transplant function. Three donor kidneys failed to perfuse and remained dusky in appearance when perfused in situ with kidney perfusion solution; therefore, these were discarded. This was due to thrombosis of vessels in the renal cortex related to hypotension. Unlike in human RTs, it is not easy to perfuse the rat kidney ex vivo due to the renal vessels being narrow; hence, proper cannulation of the aorta and perfusion in situ is of paramount importance. Transplanting a poorly perfused dusky-looking kidney leads to a nonfunctioning kidney with high risk of vascular thrombosis (Figure 1).

Recipient-related complications

Bleeding from the aorta and inferior vena cava
A large arteriotomy in the recipient’s abdominal aorta compared with the donor aortic lumen and an arteriotomy with ragged edges were associated with significant blood loss from the anastomosis site. Small bleeds were satisfactorily controlled with application of Surgicel (Ethicon, West Somerville, NJ, USA) and pressure for 5 minutes. A large venotomy of the IVC and a ragged edge of the vein were also associated with significant blood loss. Gentle handling of the transplanted kidney was essential because excessive traction led to disruption of arterial and venous anastomoses and significant hemorrhage.

Bleeding from minor vessels could be controlled with diathermy in the donors and recipients. There was an increased risk of reactionary hemorrhage from diathermized branches of the aorta because of the higher arterial pressure leading to dislodgement of blood clot from the end of the diathermized artery. Hence, these were ligated. Diathermy coagulation of bleeding from the aorta should be avoided because this can weaken its wall, leading to aneurysm formation and subsequent rupture or a stenosis. Bleeding from vascular anastomosis was arrested by applying pressure with a cotton swab for about 1 minute or by use of Surgicel (Ethicon) and Gelfoam.

Figure 1. Donor Nephrectomy

(a) Normal kidney before perfusion with kidney perfusion solution. (b) Kidney with dusky appearance in the donor, which failed to clear with perfusion fluid. (c) Kidney after perfusion with University of Wisconsin solution.
As an alternative, Fibrin glue (Baxter) can also be used. 

**Narrowed inferior vena cava following venous anastomosis**
The use of large sections of the IVC during venous anastomosis, particularly at the superior and inferior corners, led to significant stenosis of the IVC. This compromised venous drainage from the transplanted kidney, leading to venous congestion and the dusky appearance of the transplanted kidney with distended renal vein. The narrowing of the IVC compromised the venous return from the lower limbs, which led to intense venous congestion in the lower half of the body. Decreased venous return due to IVC stenosis was an important cause of hypotension and postoperative death.

**Poor reperfusion of the allograft (dusky kidney)**
When the vascular clamps are released after completion of vascular anastomoses, restoration of normal perfusion and color should occur (Figure 2a). Four kidney allografts remained dusky in color, indicating poor perfusion. This could have resulted from hypotension in the recipient from various causes, including hypovolemia due inadequate fluid replacement, blood loss, or fluid loss due to evaporation from the exposed surface of the intestine.

Stenosis or occlusion of the lumen of the renal artery or the vein at the anastomosis site can result in inadequate perfusion or venous drainage, respectively. After completion of the arterial anastomosis, microvascular clamps should be applied to the donor aortic segment as close to the anastomosis as possible to prevent formation of thrombus within the conduit. Torsion of the renal artery or renal vein at the hilum can lead to a similar outcome.

Poorly perfused kidneys with dusky appearance (Figure 2b) in the recipient led to nonfunction, as evidenced by anuria and death after right native nephrectomy.

**Intraoperative assessment of renal transplant perfusion**
The restoration of normal blood flow and color immediately after RT has an important prognostic value. Hammad and associates were the first to report the use of laser Doppler flowmetry for the measurement of renal cortical perfusion after RT in Lewis rats. Under baseline conditions, renal cortical perfusion in the donor and recipient kidneys were similar in coefficient variabilities of 11% and 12%, respectively. There was a progressive increase in renal cortical perfusion during the first 60 minutes after RT, with a return to baseline values 2 weeks later.

Yang and associates demonstrated that successful and reproducible renal apparent diffusion coefficient maps can be obtained in normal and transplanted rat kidneys by using spin echo diffusion-weighted magnetic resonance imaging at 7 T. The group concluded that spin echo diffusion-weighted magnetic resonance imaging can be a noninvasive tool for monitoring blood perfusion and early graft rejection after kidney transplant.

**Intraoperative recipient deaths**
Eight recipient rats died intraoperatively from the reasons described in the donor section. One recipient rat died because of hemorrhage from the vascular anastomosis and generalized ooze after intravenous administration of heparin. Heparin was given to
prevent thrombosis of the limb vessels as paraplegia had occurred in the previous recipient. Thereafter, administration of heparin was abandoned. The blood glucose level was 3 mmol/L after death in one rat, indicating the presence of hypoglycemia. We changed our protocol and avoided overnight starvation of rats, allowing them to eat until 6 hours before surgery.

Paralyzed hind limbs posttransplant
One rat developed intense congestion of hind limbs during the venous anastomosis, and 3 rats developed venous congestion and weakness of hind limbs after recovery from anesthesia. We attributed this to ischemia of the spinal cord due to division of the lumbar arteries arising from the abdominal aorta during its mobilization and to ischemic paralysis of the limb muscles from prolonged clamping of the abdominal aorta in addition to thrombosis of the leg arteries and veins. Venous thrombosis of the major leg veins from prolonged IVC clamping or stenosis after venous anastomosis can lead to congested, painful, and weak legs (Figure 3).

In subsequent RTs, mobilizations of aorta and IVC were kept to the minimum. We decided to preserve all lumbar arteries arising from the aorta and lumbar veins draining into the IVC. These vessels were temporarily snagged with 6/0 Vicryl and released once vascular anastomoses were completed. We also decided to carry out the aortic anastomosis first. As soon as aortic anastomosis was completed, a microvascular clamp was applied to the aortic conduit and the aortic clamps were released, thereby restoring blood flow to the legs. After these changes, we did not observe any further paralysis of the hind limbs.

Urine leaks
Three rats with urine leaks looked unwell on the day after transplant. Laparotomy revealed presence of urine in the peritoneal cavity. Although no obvious source of urine leak was observed in 2 cases, the possible sources of leak could include the ureterovesical anastomosis site, ligated donor urethra, and right donor ureter. Other possible sources could include injury along the length of the donor ureter and necrosis of the bladder cuff and the ureter itself. Two cases had urine leak from the ureterovesical junction and necrosed bladder cuffs. To preserve the blood supply, the size of the donor bladder cuff should be kept as small as possible. It is paramount to preserve the ureteric blood supply by keeping the periureteric fat intact and paying attention to the details of the ureterovesical anastomosis. The ureteric anastomosis should be conducted without causing tension and ischemia at the anastomosis site, which occurs if large amounts of tissue are included in the anastomosis and a too tight anastomosis is performed. Placement of 2 stay sutures at the corners followed by either continuous or interrupted sutures without tension is recommended.
Ureteric stenosis
Narrowing or obstruction of the ureter can occur either along its course or at the ureteric anastomosis sites. Injury to the ureter and its blood supply leads to ischemic damage and scarring, causing partial or complete obstruction, resulting in dilatation of the pelvicaliceal system (hydronephrosis). Stenosis at the ureteric anastomosis due to a faulty surgical technique can have similar outcomes. The ureter can also be obstructed by blood clots from gross hematuria resulting from injury to the kidney during retrieval. Blood clots within the bladder can also cause retention of urine and hydronephrosis. Hydronephrosis leads to impaired renal function from cortical thinning, pyelonephritis, pyonephrosis, generalized sepsis, and death from renal and multiple organ failure. Prevention of ureteric obstruction can be achieved by paying attention to the details of the surgical techniques during donor nephrectomy and RT in the recipients. In 8 transplanted rats with necrotic kidneys and calculi in the kidney and bladder, it was not possible to exclude the possibility of ureteric obstruction.

Formation of calculi
Calculi can form in the urinary bladder after RT due to presence of a suture within the bladder, infection, and necrosis of ureter or donor bladder cuff. Calculi cause pyelonephritis and hydronephrosis and compromise transplant function, resulting in death from sepsis. Multiple vesical calculi were observed in the initial 2 transplants, in which postmortem examination revealed necrosed kidneys. In the presence of the aforementioned predisposing factors, vesical calculi can form as early as 2 weeks after RT, if the contralateral kidney remains functioning and produces urine (Figure 4b).

Similarly, stenosis of the ureteric anastomosis and stagnation of urine in a hydroureter can lead to formation of calculi. Presence of a ureteric stent can lead to precipitation of urine content and stone formation. In one study, 4 different types of sutures (silk, polyglycolic acid, polyglactin, and chromic catgut) were examined regarding their calculogenic properties after ureteric anastomosis, but no differences were observed in these properties.

Necrosis of kidneys and bladder
Necrosis of the transplanted kidneys and bladder was observed in 7 rats after RT. Factors that predispose transplanted kidneys to necrosis are shown in Table 4. These variables were eliminated in the subsequent RTs, which resulted in successful outcomes with functioning transplants. Papillary necrosis is associated with severe cortical damage, which is usually attributed to preservation injury.

<table>
<thead>
<tr>
<th>Table 4. Factors That Predisposed Animals to Transplant Necrosis</th>
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<tbody>
<tr>
<td>• High hydrostatic pressure generated by syringe during perfusion of the donor kidney, damaging glomerular and intrarenal blood vessels and leading to thrombosis of intrarenal blood vessels and ischemic infarction</td>
</tr>
<tr>
<td>• Inadequate clearance of donor kidney of blood, leading to thrombosis and subsequent necrosis</td>
</tr>
<tr>
<td>• Inadequate reperfusion of the renal transplant kidney with blood from either intraoperative hypotension or vasospasm of the renal artery because of cold preservation</td>
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<tr>
<td>• Prolonged cold ischemia time, leading to severe ischemia-reperfusion injury</td>
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<td>• Torsion of the renal vascular pedicle during or after renal transplant</td>
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<tr>
<td>• Hypotension in the postoperative period</td>
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<tr>
<td>• Inadequate immunosuppression, leading to acute rejection</td>
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<tr>
<td>• Inadequate aseptic and antisepsic techniques, leading to infection of the kidney</td>
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</table>

Figure 4. (a) Necrosed Kidney, Ureter, and Bladder; (b)Opened Bladder Showing Multiple Calculi; (c) Necrosed Kidney
Papillary necrosis manifests with hematuria and impaired renal function. Massive papillary necrosis culminates in renal infarction (Figure 4, a and c). Histology of these kidneys showed extensive necrosis. Swabs taken of the necrotic kidneys grew Escherichia coli and coryneform bacteria. Culture of kidney perfusion fluid also showed coryneform bacteria, although having cultural characteristics different from those grown from the pus swabs.

Postoperative deaths
Postoperative deaths of the recipients occurred either in the immediate postoperative period (3 rats) or after right native nephrectomies (8 rats). The death of transplanted rats in the incubator during recovery was attributed to hypotension and hypothermia. Subsequent deaths were related to either urine leak, necrosed kidneys with uremia, bleeding from the arterial pseudoaneurysm at the anastomosis site, or progressive uremia. Hypotension and hypoxemia are the major causes of graft failure and animal death. Similar to our own experience, a continuous intravenous infusion of fluid through vein cannulation during surgery is helpful in maintaining hemodynamic stability and providing better outcomes.

Laparotomy-related complications
There were no incidences of wound infection, dehiscence, and incisional hernias after RT. Similarly, visceral injury and peritonitis were not observed after a major laparotomy during the RT procedure. In both donors and recipients, after start of anesthesia, the abdomen was shaved and painted with aqueous betadine solution. Co-amoxiclav (Augmentin, GSK, Middlesex, UK) was administered intraperitoneally to recipients at 25 mg just before the abdomen was closed. Surgical instruments were cleaned and kept in boiling water for 5 minutes. Sterile gloves were worn during the procedures.

Postoperative care
The transplanted rats were closely monitored for their general health. The general appearance, mobility, and progressive weight gain were indicators of good renal function. With the advancement of uremia, there was weight loss with ill-looking appearance in rats. The loss of urine-concentrating capacity of the renal tubules led to increased 24-hour urine output, which could have contributed to dehydration and loss of weight. These rats were killed in the initial experimental group; postmortem examination showed necrotic kidney, indicating ongoing sepsis. To reduce suffering, rats that appeared not well were humanely killed.

Final outcomes
In the initial experiments, most transplanted rats were not saved because of inconsistencies regarding the intraoperative care of donors and recipients. However, after problems identified in the initial experiments were addressed, the final group of transplanted rats (21 cases) experienced minimal complications, and long-term survival was achieved.

In conclusion, to establish a successful experimental rat model of RT, it is important to be familiar with the complications discussed above and adopt appropriate measures to prevent them. There is always a learning curve before achieving uncomplicated and successful RTs. Maintenance of a database incorporating detailed records of events that occur during and after RT is paramount; these should be audited on a regular basis, with modification of the protocol done as necessary for the prevention of these complications.

References


