

Comparison of 2 Devices in Pigs To Induce Hypothermia in Laparoscopic Orthotopic Kidney Transplant

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Abstract

Objectives: To laparoscopically compare the effectiveness of 2 cooling devices for renal hypothermia and investigate the feasibility of laparoscopic orthotopic kidney transplant using a pig model.

Materials and Methods: Eight pigs were divided into 2 groups of 4 animals each. Laparoscopic nephrectomy and autotransplant were performed first on only the right kidney. One week later, these procedures were performed on the left kidney, while the first transplanted autograft was removed. After 1 more week, the left autograft was removed for observation. In 1 group, the silicon tube cage was used to induce hypothermia during laparoscopic orthotopic kidney transplant (silicon tube cage group), and in the other group, the plastic bag jacket was used to induce hypothermia during laparoscopic orthotopic kidney transplant (plastic bag jacket group).

Results: Two pigs in the silicon tube cage group survived for 7 days after the second autotransplant with serum creatinine levels of 210 $\mu\text{mol/L}$ and 1010 $\mu\text{mol/L}$. One pig in the plastic bag jacket group survived for 5 days. The mean surface temperature

of the grafts was maintained at $9^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and $12^{\circ}\text{C} \pm 3^{\circ}\text{C}$ in the silicon tube cage and plastic bag jacket groups ($P = .166$). Three of 6 plastic bag jacket devices were ruptured by stitches or instruments. The mean venous and arterial anastomotic times in the silicon tube cage group were significantly shorter than were those in the plastic bag jacket group.

Conclusions: Our study shows that the silicon tube cage may be a reliable renal cooling device for use in laparoscopic kidney transplant and indicates the feasibility of laparoscopic orthotopic kidney transplant in pigs.

Key words: Kidney, Heterotopic, Orthotopic transplant, Laparoscopy, Hypothermia devices

Introduction

Minimally invasive surgical techniques, such as laparoscopic techniques, and the more advanced da Vinci surgical system, have revolutionized the field of urologic surgery. Laparoscopic donor nephrectomy transplant was pioneered by Ratner¹ in 1995 and is now considered to be the procedure of choice for live-donor kidney transplant.² However, the laparoscopic kidney transplant technique has not been fully developed.

A few experimental³⁻⁵ and clinical laparoscopic^{6,7} or robot-assisted renal transplants have been performed in recent years.⁸⁻¹¹ The hindrance in laparoscopic kidney transplant is not only that of technical demand, but also of intraoperative renal hypothermia, which has not yet been solved. On the other hand, the reported cases of laparoscopic or robot-assisted kidney transplant were performed heterotopically into the iliac fossa. Thus far, laparoscopic orthotopic kidney transplant (LOKT) has not been attempted.

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In light of these data and of our extensive experience in both kidney transplant and laparoscopic urologic surgery, we performed LOKT using a pig model with 2 kinds of renal cooling devices developed by our team and tested the efficacy of these devices for renal hypothermia and feasibility of LOKT.

Materials and Methods

Transplant was conducted on 8 white males pigs (weight, 25-35 kg). Fasting and water deprivation were enforced in pigs 12 hours before the operation. This protocol was approved by the research committee and the institutional animal care and use committee within our hospital.

Cooling device

Device 1: Silicon tube cage

Silicon tubes taken from Travenol infusers commonly used in clinics were used to form a cooling device. The needle heads were removed from the silicon tubes and the ends were cut off, and the heads and ends were connected together and elongated. Based on the general anatomic size of the pig kidney to find a model, such as a bottle, the connected silicon tube surrounded the model and extended spirally, and then was fixed by several parallel banding threads to form a cagelike device. The kidney could be positioned within the cage (Figure 1).

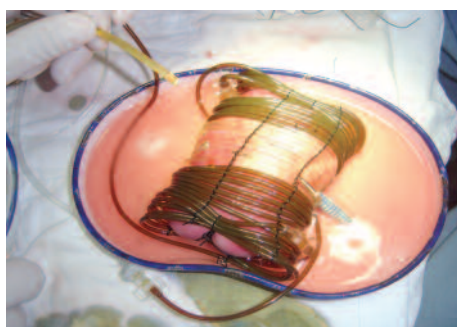


Figure 1. Silicon tube cage. Silicon tubes form a cagelike device. Ice water from a reservoir placed at a height of 100 cm can be infused into 1 end and effused from the other

Device 2: Plastic bag jacket

A common plastic bag (approximately 12 × 20 cm) was used to create a cooling device in the form of a plastic bag jacket. One silicon tube was inserted 10 cm into the bag, and another was inserted 5 cm from the opening of the bag. The tubes were tightly tied

together with the opening to seal the bag. The bottom was then deepened, forming a 2-layer jacket with an open mouth. After the kidney was positioned inside the bag through the open mouth, the open edge of the jacket was closed and secured around the hilum using silk thread ligation (Figure 2).



Figure 2. Plastic bag jacket. A plastic drainage bag formed a cooling device. The kidney was positioned inside the 2-layered bag jacket. The open edge of the jacket was closed and secured around the hilum using silk thread ligation

So that the cooling apparatus completely enveloped the kidney and induced hypothermia, near-freezing ice water was continuously infused from a reservoir placed at a height of 100 cm into 1 end of the tube and effused from the other.

Study design

Eight pigs were randomly divided into 2 groups of 4 animals each. Both kidneys of the animals in the silicon tube cage group (STC group) were retransplanted under renal hypothermia induced by STC device. Retransplant in the plastic bag jacket group (PBJ group) took place under renal hypothermia induced by the PBJ device. Renal surface temperature also was monitored using thermocouples and recorded (Thermal Detector, DT-613, Shenzhen, China) during the last 4 trials in each group. Thermocouples were interposed between the surface of the kidney and the device on both sides of kidney, taking care not to damage the graft. Laparoscopic nephrectomy and autotransplant was first performed on the right kidney, leaving the left kidney untouched. One week later, these procedures were performed on the left kidney while the first transplanted autograft was removed. After 1 more week, the left autograft was removed for observation. Thus, every pig in both groups underwent the same experimental procedures, and every transplanted autograft was observed for 7 days. The 2 devices were used by turns. Each

operation was performed by the same surgeon. Similar surgical techniques were used on both side operations.

Surgical technique

Under general anesthesia, the pig is positioned in a lateral decubitus position, and a midline incision (7-8 cm) is made. The surgeon's hand creates a retroperitoneal space by blunt dissection. To open the renal capsule and perirenal tissue, trocars are guided into the retroperitoneal space. The protocol for trocar insertion is as follows: a 10-mm trocar is placed in the flank just upon or slightly below the kidney. The camera is often used through this port. A second 5-mm trocar is placed in the flank above the iliac crest. A third 3-mm trocar is placed in the lower quadrant. If necessary, a fourth 3-mm trocar is placed 3 to 4 cm above the third. After placement of the trocars, the wound is temporarily closed. After identification and careful dissection of the ureter, renal artery, and renal vein, the ureter is clipped distally and divided. The renal artery and vein are isolated and divided using an endoscopic scissors. The wound is loosened and opened, and the kidney is removed manually. Five minutes before dissection of the renal artery and vein, 1500 IU of heparin is administered intravenously. The kidney is perfused with perfusion solution at 0°C to 4°C and a pressure of about 80 cm H₂O, then the renal artery and vein are extended 2 to 3 cm by angioplasty with an alcohol-processed pig corpse artery or artificial vessel.

After the kidney is placed into the cooling device (tested and sterilized before use) with blood vessels on the outside of the device, ice water is perfused into the silicon tube. The flow rate is maintained at 5 to 10 mL/min until revascularization. The graft with the cooling device is brought into the operative field through the midline incision and properly positioned for anastomosis. The renal vein is anastomosed in a continuous manner, end-to-end to the native renal vein, with 5-0 or 6-0 nonabsorbable sutures. The renal artery is anastomosed end-to-end to the native renal artery with running nonabsorbable 6-0 sutures (Figure 3). The 2.8-mm laparoscopic instruments are used for vessel anastomoses (3.0-mm trocar inserted through a 5.0-mm trocar). After the anastomoses tested negative for leaks, the kidney is revascularized. Three minutes before unclamping, 100 mg of

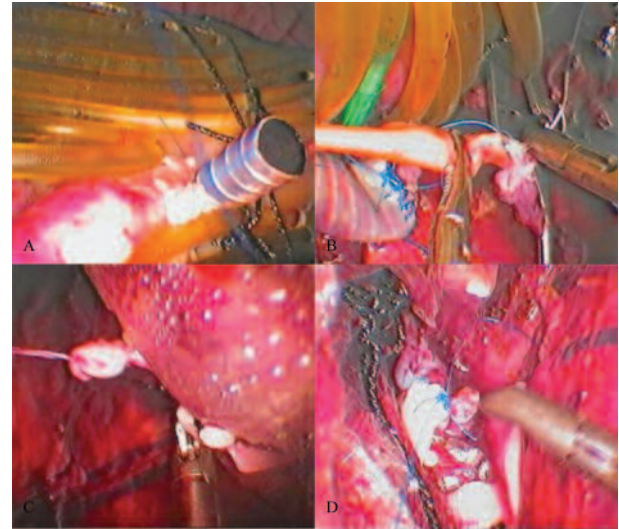


Figure 3. (A, B) Silicon tube cage in the operation (the device was effective as shown by the presence of condensation, or “sweat,” on its surface). The renal artery was anastomosed in a continuous manner, end-to-end to the native renal artery, with 5-0 or 6-0 nonabsorbable sutures. (C, D) Plastic bag jacket in operation (shown as sweat also). The renal vein was anastomosed end-to-end to the native renal vein with nonabsorbable running 5-0 sutures

furosemide and 10 mg of mannitol are administered intravenously. The cooling device is cut and removed, and the wound is finally closed. Endostenting of the end of the ureterocutaneostomy is performed to assure complete diversion of urine. Graft removal is performed by open surgery.

Postoperative management

Pigs were intravenously supplemented with 0.5 to 1.0 L of 0.9% sodium chloride solution after the operation. Free access to tap water was allowed, and standard food was offered on the first postoperative day. Antithrombotic therapy was provided with 1 shot of heparin (3000 IU) daily. Antibiotic treatment consisted of perioperative and postoperative administration of 500 mg of intramuscular ampicillin daily. Animals that survived were killed by an anesthetic injection on postoperative day 7, after removal of the transplanted graft under general anesthesia. In addition, postoperative serial creatinine measurements, urine volume, animal survival, renal histologic examination results, and surgical complications were recorded.

Statistical analyses

Data are expressed as mean ± standard deviation. The *t* test was used to assess the statistical significance of the 2 group differences. Statistical analyses were performed with SPSS software (SPSS:

An IBM Company, version 17.0, IBM Corporation, Armonk, New York, USA). Values for $P < .05$ were considered significant.

Results

One graft in the STC group had a double renal artery and double vein, and 1 graft in the PBJ group had a double renal artery and normal vein. In the first operation on the right kidney, all grafts posttransplant seemed to be nonfunctional with no signs of diuresis; however, an immediate and viable blood supply was seen based on the appearance of a bright red color in 3 grafts after revascularization (Figure 4). In the STC group, 1 pig died of a possible overdose of anesthetics immediately after the operation. In the PBJ group, 1 pig died suddenly on the second day posttransplant. Autopsy results showed stenosis in both the renal artery and vein. One autotransplant was abolished because the blood vessel and graft were destroyed during laparoscopic nephrectomy; hemorrhage occurred, and the procedure was immediately converted to open surgery. Seven days later, the second operation was performed. Despite the lack of urine produced by the transplanted autografts, all pigs that survived the first transplant operation were prepared for the second laparoscopic nephrectomy and autotransplant.



Figure 4. The kidneys were revascularized with sufficient reperfusion

All second operations on the left kidney (3 in each group) were successfully completed. Two pigs in the STC group immediately urinated after the operation (Figure 5), but 1 stopped urinating 5 days later. The 2 pigs survived for 7 days after second autotransplant with serum creatinine levels of 210 $\mu\text{mol/L}$ and 1010 $\mu\text{mol/L}$ (baseline, 135 $\mu\text{mol/L}$), before killing. One pig with anuria died on the fourth day posttransplant. One pig in the PBJ group began urinating within 3 hours after reperfusion, but its urination attenuated and disappeared in 3 postoperative days. The pig survived for 5 days, and its maximum urine volume



Figure 5. Endostenting of the end of the ureterocutaneostomy was performed to assure complete diversion of the urine. The pig urinated from the tube after the operation

was 200 mL on postoperative day 1. Two pigs with anuria showed signs of impending death at postoperative days 2 and 3 and underwent exploration under general anesthesia before killing.

The mean surface temperature of the grafts was maintained at $9^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and $12^{\circ}\text{C} \pm 3^{\circ}\text{C}$ in the STC and PBJ groups ($P = .166$). Three of 6 PBJ devices were ruptured by stitches or instruments. The mean transplant operative time and the venous and arterial anastomotic times in the STC group were significantly shorter than those in the PBJ group. Mean blood loss during the transplant operation was 180 ± 60 mL in the STC group and 245 ± 9 mL in the PBJ group ($P = .027$). Autopsy results showed 3 artery stenoses (3/7) and 2 vein stenoses (2/7) in the STC group, and 3 artery stenoses (3/6) and 3 vein stenoses (3/6) in the PBJ group. Thrombi were found in all of these strictured vessels. Histopathologic examination of the autografts demonstrated near-normal renal architecture in 1 that survived and acute tubular necrosis in the others. In the PBJ group, incisional herniation developed in 1 pig and wound infection occurred in another. One pig in the STC group had a blood vessel injury (Table 1).

Discussion

It is not unimaginable that kidney transplant can be performed using laparoscopic instruments. To date, there have been a few reports on laparoscopic^{6,7} or robotic-assisted⁸⁻¹¹ kidney transplant. However, intraoperative renal hypothermia is an important hindrance to laparoscopic kidney transplant. From its initial step, laparoscopic kidney transplant raises concerns regarding the effects of increased warm ischemia time on graft viability. Indeed, during

Table 1. Observation Data of the 2 Groups

Variables	STC group	PBJ group	P value
Animal number (n)	4	4	
LOKT number (n)	7	6	
Warm ischemia time (min)	3 ± 0.7	3 ± 0.5	.877
Cold ischemia time (min)	38 ± 5	37 ± 5	.729
Operation time (h)	5.2 ± 0.6	7.4 ± 0.4	.000
Vein anastomotic time (min)	57 ± 14	100 ± 11	.000
Artery anastomotic time (min)	46 ± 12	73 ± 5	.000
Mean blood loss (mL)	180 ± 60	245 ± 9	.027
Surface temperature of graft (°C)	9 ± 3	12 ± 3	.016
Device failure (n)	0	3	
ATN (n)	6	6	
Complications			
Artery stenosis (n)	3 (3/7)	3 (3/6)	
Vein stenosis (n)	2 (2/7)	3 (3/6)	
Incisional hernia (n)	0	1	
Wound infection (n)	0	1	
Vessel injury (n)	1	0	
Animal survival (d) mean*	(7, 3, 7) 5.6	(2, 3, 5) 3.3	

Abbreviations: ATN, acute tubular necrosis; LOKT, laparoscopic orthotopic kidney transplant; PBJ, plastic bag jacket; STC, silicon tube cage

*Animal survival was calculated from second autotransplant

construction of vascular anastomoses, graft temperature progressively increases because maintenance of a stable graft temperature is difficult to achieve laparoscopically. With no available ideal cooling technique, some laparoscopic or robotic-assisted kidney transplants have been experimentally or clinically performed under warm ischemia conditions without additional renal graft-cooling procedures by some professors who anticipate that this issue will be extensively debated, and who expects it to provide new impetus to research.^{4,7,9,10}

In the series by Meraney, intracorporeal hypothermia was achieved by intra-arterial perfusion of ice-cold solution through a 4-F balloon catheter,³ but the possible risks of vascular injury and issues of technical complexity were its drawbacks. Rosales and associates reported that renal hypothermia was maintained by means of ice and continuous transcutaneous irrigation with cold saline solution in their laparoscopic kidney transplant.⁶ This method would distract the attention of the surgeons by having to constantly suction the solution during this complex operation involving vascular reconstructions. In addition, cold irrigation would blur visibility of the vessels to be anastomosed, and the requirement of concurrent suction would decrease the level of pneumoperitoneum and thus decrease operative space.

We developed 2 devices for pure laparoscopic renal hypothermia and used them in recovery animal models. Our cooling devices are based on a

noninvasive concept with the potential for rapid application and rapid removal during laparoscopic kidney transplant, which is simple, inexpensive, and disposable. The principle behind the design of the 2 systems was to achieve hypothermia in the renal tissue by continuously circulating near-freezing ice water, maintained at a temperature as low as possible, across the surface of the kidney. There is no direct contact between the cooling agent and the organ surface. This decreases the likelihood of organ surface damage or excessive cooling. Both of the prototype devices in this study demonstrated an ability to cool the porcine kidney during the operation. However, of the 2 devices, the STC model may be preferred. Its structure is more stable and helpful for laparoscopic maneuvers and provides a reliable way to achieve a stable graft temperature laparoscopically. The PBJ must be enhanced to overcome its shortcomings: Easy expansion may hamper operative exposure during infusion; easy rupture by stitches or instruments may lead to leakage of ice water, potentially compromising continued hypothermia; and blurred visibility of the vessels and the requirement for concurrent suction may decrease the level of pneumoperitoneum.

Engraftment of a renal allograft into the iliac fossa^{12, 13} is a common technique used by almost all surgical teams with a high rate of success. Orthotopic renal transplant is seldom performed in clinical practice because of its relative difficulty in open surgery. Because more advanced instruments are available today, orthotopic renal transplant should be re-evaluated. Laparoscopic orthotopic kidney transplant, which encompasses the benefits of orthotopic renal transplant and maximizes the advantages of modern instruments, is worth further study. Of 13 completed LOKTs in our study, 5 had reliable artery and vein anastomoses, and 2 had life-supporting autograft function for days. This study comprises only initial practices. To our knowledge, this is the first report of successful LOKT. Although transplant operative time and venous and arterial anastomotic time were long, the rate of anastomotic stenosis was high, and too many cases of anuria occurred, it still may be concluded that LOKT in pigs may be possible.

In summary, our study showed that the silicon tube cage may be a reliable renal cooling device for use in laparoscopic kidney transplant and demonstrated the feasibility of LOKT in pigs. Our

report is of initial research only; our cooling devices are currently undergoing further development to enhance their efficiency. Further experience is needed to shorten the operation time of and decrease surgical complications in LOKT.

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