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Protection From Second Warm Ischemic Injury Using a Thermal Barrier Bag in Kidney Transplantation

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Background. Second warm ischemic injury during vascular anastomosis not only adversely affects immediate posttransplant function but also affects long-term patient and graft survival. We developed a pouch-type thermal barrier bag (TBB) composed of a transparent, biocompatible insulation material suitably designed for kidneys and conducted the first-in-human clinical trial. **Methods.** A living-donor nephrectomy was performed using a minimum skin incision procedure. After back table preparation, the kidney graft was placed inside the TBB and preserved during vascular anastomosis. The graft surface temperature was measured before and after vascular anastomosis using a noncontact infrared thermometer. After completion of the anastomosis, the TBB was removed from the transplanted kidney before graft reperfusion. Clinical data, including patient characteristics and perioperative variables, were collected. The primary endpoint was safety, which was assessed by evaluating adverse events. The secondary endpoints were the feasibility, tolerability, and efficacy of the TBB in kidney transplant recipients. **Results.** Ten living-donor kidney transplant recipients with a median age of 56 y (range, 39–69 y) were enrolled in this study. No serious adverse events related to the TBB were observed. The median second warm ischemic time was 31 (27–39) min, and the median graft surface temperature at the end of anastomosis was 16.1 °C (12.8–18.7 °C). **Conclusions.** TBB can maintain transplanted kidneys at a low temperature during vascular anastomosis, which contributes to the functional preservation of transplanted kidneys and stable transplant outcomes.

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Kidney transplantation (KT) is the gold-standard treatment for end-stage kidney disease. It provides a superior quality of life and reduces morbidity and mortality compared with dialysis;[1-3] however, limited availability of donor grafts is a major drawback.[4] To address the organ shortage, KTs from brain-dead extended criteria donors (ECDs)[5] and donation after circulatory death (DCD) donors[6] are currently accepted.[7] The quality of organs procured from deceased donors is an important factor determining graft survival and function in KT. Although the most deleterious factor is donor age, second warm ischemic injury during vascular anastomosis adversely affects both immediate posttransplant function and long-term patient and graft survival after KT.[8,9] Possible molecular mechanisms include the generation of reactive oxygen species, induction of apoptosis, and stimulation of innate and adaptive immune systems.[10,11] To reduce the incidence of second warm ischemic injury, efforts should be directed at reducing procedural time for vascular anastomosis or keeping the organ cooler during this period. However, it has been pointed out that the former may result in technical complications and does not seem feasible.[12] Although little is known about the actual values of temperature variations of the kidney during the first or second warm ischemic period, experimental studies indicate that renal metabolic activity resumes at 15 °C to 18 °C[13-15] after cold storage. Various techniques for preventing organ

warming before reperfusion in clinical settings range from simple surface cooling methods to organ placement in an ice slush bag.[15-20] However, no effective and standardized cooling methods have been established. We recently developed a pouch-type thermal barrier bag (TBB; Organ Packet, Screen Holding Co, Ltd, Japan), which is composed of a transparent and biocompatible insulating material that is suitably designed for the kidneys.[21] In this novel study, we conducted a first-in-human clinical trial to evaluate the safety, feasibility, tolerability, and efficacy of the TBB at the Hiroshima University Hospital.

MATERIALS AND METHODS

Patients

Between October 2021 and June 2022, 10 patients underwent living-donor KT at Hiroshima University Hospital. Clinical data, including donor and recipient characteristics, such as age, sex, relationship, primary disease, dialysis period, and perioperative variables, were collected.

Ethics Approval

This study was approved by the Hiroshima University Certified Review Board (E-2014–0921) and registered with the Japan Registry of Clinical Trials (jRCTs062210034). This trial was designed and conducted in accordance with the tenets of the Declaration of Helsinki. All patients provided written informed consent before enrollment in the study.

TBB

TBB is a highly elastic, pouch-type device designed for organ protection and thermal insulation (Figure 1A). TBBs comprise a uniquely developed low-hardness styrene elastomer gel with an elongation rate of approximately 1000% and a melting point of ≥200 °C, which has been tested for biological safety (patent no. P2021–40938A, Japan). TBBs are available in 2 sizes (medium and large), and the choice of size is based on the dimensions of the kidney graft (medium size: 85–115 mm, large size: 105–135 mm).

KT Procedure

Living-donor nephrectomy was performed using a muscle-splitting minimum skin incision procedure.[22] Immediately after procurement, the kidney graft was flushed and stored in static cold. The first warm ischemic time is the time between the clamping of the renal artery and the initiation of cold perfusion during the procurement procedure, after which the cold ischemic time (CIT) begins. In cases of 2 renal arteries, a single ostium side-to-side anastomosis was performed during bench surgery. After back table preparations, the kidney graft was placed inside the TBB with an outlet for vessels, and the organ was preserved in this device during vascular anastomosis. The kidney graft was implanted in the iliac fossa of the recipient using an extraperitoneal approach with vascular anastomosis to the iliac vessels (Figure 1B). The average graft surface temperature was measured at the upper, middle, and lower poles of the graft and recorded before and after vascular anastomosis, using a noncontact infrared thermometer (FLIR E54; Teledyne FLIR, Wilsonville, OR; Figure 1C). The surface temperature of the TBB was recorded every 5 min during the vascular anastomosis (Figure 1D). After completion of the anastomosis, the TBB was manually slid out of the transplanted kidney and removed before graft reperfusion. The second warm ischemic time (WIT2) is equivalent to the anastomotic time, which is the time between the end of the cooling period of the graft and the recirculation in the recipient. Finally, ureter–bladder anastomosis was conducted using the modified Lich-Gregoir method.

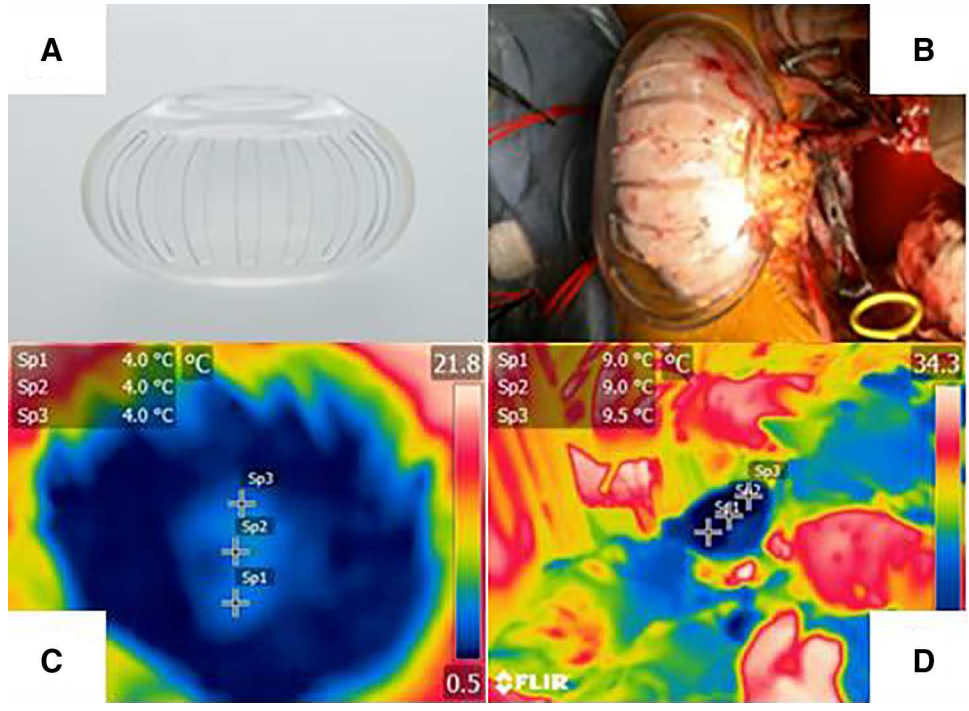

FIGURE 1. A, Appearance of the TBB. B, Appearance of the TBB during vascular anastomosis. C, The average graft surface temperature was measured at the upper, middle, and lower poles of the graft, using a noncontact infrared thermometer, and recorded before vascular anastomosis. D, The surface temperature of the TBB was recorded every 5 min during the vascular anastomosis using a noncontact infrared thermometer. TBB, thermal barrier bag.

Desensitization Protocol and Immunosuppressive Regimen

Preoperative desensitization was performed in ABO blood type–incompatible cases. Two weeks before transplantation, a single dose of rituximab (375 mg/m^2 body surface area) was administered to the patients. Subsequently, they received cyclosporine (target trough level: 80–100 ng/mL) and mycophenolate mofetil (20 mg/kg/d) and underwent 0 to 3 sessions of plasmapheresis until a 16-fold reduction in anti–blood group isoagglutinin titers was achieved. The basic immunosuppressive regimen after KT has been described previously.[23] Briefly, basiliximab was administered at a dose of 20 mg/d at the time of transplantation and on postoperative day 4. After transplantation, the regimen comprised cyclosporine, mycophenolate mofetil, and methylprednisolone, which was gradually tapered. The trough whole levels of cyclosporine were maintained between 200 and 250 ng/mL during the first few postoperative weeks and between 150 and 200 ng/mL thereafter.

Definitions and Other Laboratory Data

Serum creatinine levels were monitored until postoperative week 4. Delayed graft function (DGF) was defined as the need for dialysis within the first postoperative week. Surgical site infection was defined as infection of the skin or subcutaneous tissue surrounding a surgical wound. T cell–mediated rejection (TCMR) was defined as graft dysfunction, as evidenced by elevated serum creatinine levels in the absence of vascular or urinary complications or infection. Vascular and urinary complications were identified using Doppler ultrasonography. Clinical suspicion of TCMR was supported by the protocol mixed lymphocyte reaction assay, which can rigorously monitor rejection.[23] TCMR diagnosis was based on the Banff criteria in episode biopsies. The criteria for a positive diagnosis of urinary tract infections included microbial presence at a concentration of >10^4 CFU/mL of urine or >10^3 CFU/mL from culture analysis, presence of clinical signs and symptoms, and the use of antibacterial agents.

Statistical Analysis

Quantitative variables are expressed as medians and ranges. Correlations were assessed using Pearson's or Spearman's analysis as appropriate. Statistical analyses were performed using the JMP, version 16 (SAS Institute, Cary, NC). Statistical significance was set at a P value of <0.05.

RESULTS

Characteristics of the Patients

Ten living-donor KT recipients were enrolled in this study between October 2021 and June 2022. The median recipient age was 56 (range, 39–69) y, and the median BMI was 24.2 (21.5–25.9) kg/m^2. Seven of these patients underwent preemptive KT. Six patients received kidneys from unrelated donors, and 2 patients received kidneys from ABO-incompatible donors (Table 1).

Surgical Factors

The median graft weight was 189 (120–260) g, and the major axis of the graft was 11 (10.0–13.0) cm. Three of the 10 grafts had 2 renal arteries. Nine transplants were performed in the lower right quadrant. The median first warm ischemic time, CIT, and WIT2 were 3.5 (2–4) min, 37 (22–69) min, and 31 (27–39) min, respectively (Table 1). A positive correlation was noted between recipient age and WIT2 ($r = 0.65$; $P = 0.0407$). Higher graft weight was correlated with longer WIT2 ($r = 0.64$; $P = 0.0444$). Patients with 2 renal arteries had longer CIT ($P = 0.0039$) but shorter WIT2 ($P = 0.0151$). The median graft surface temperature at the start of the anastomosis was 5.2 °C (2.7–8.4 °C). During the anastomosis, the surface temperature of the TBB never exceeded 20 °C (Figure 2A). At the end of the anastomosis, the median graft surface temperature was 16.1 °C (12.8–18.7 °C). No correlation was observed between graft weight, WIT2, and graft surface temperature at the end of the anastomosis.

Adverse Events, Serious Adverse Events, and Infections

The kinetics of serum creatinine levels after KT are shown in Figure 2B. The median time to reach nadir serum creatinine levels was 13 (4–26) d. No serious adverse events related to the TBB were observed. No surgical site infections were noted after transplantation. Urinary tract infections developed in 2 of the 10 patients. No other significant infectious complications or DGF were discerned.

TABLE 1.

Characteristics of the patients and surgical factors

	Recipient										Blood loss	Adverse events		
Case no.	Age, y	Sex	Original disease	Recipient BMI	Graft weight (g)	Graft major axis (cm)	No. renal arteries	WIT1 (min)	CIT (min)	WIT2 (min)	(mL)	SSI	UTI	DGF
1	69	F	CGN	24.4	260	13.0	1	4	22	39	80	–	+	–
2	39	F	IgAN	24.1	184	11.0	2	3	63	31	50	–	–	–
3	48	F	MPG	21.9	160	11.0	1	4	34	30	70	–	+	–
4	65	F	NS	24.1	200	11.0	1	4	26	39	80	–	–	–
5	44	M	IgAN	21.5	120	10.0	2	4	69	28	65	–	–	–
6	51	M	ADPKD	24.3	160	11.0	1	2	22	30	25	–	–	–
7	59	F	IgAN	21.7	220	10.5	1	2	34	31	155	–	–	–
8	53	F	Unknown	24.7	160	11.0	1	3	40	33	50	–	–	–
9	60	F	Unknown	25.9	194	12.0	2	3	51	27	30	–	–	–
10	59	M	ADPKD	24.7	208	11.0	1	4	54	35	135	–	–	–

ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; CGN, chronic glomerular nephritis; CIT, cold ischemic time; DGF, delayed graft function; F, female; IgAN, immunoglobulin A nephropathy; M, male; MPG, mesangial proliferative glomerulonephritis; NS, nephrosclerosis; SSI, surgical site infection; UTI, urinary tract infection; WIT1, first warm ischemic time; WIT2, second warm ischemic time.

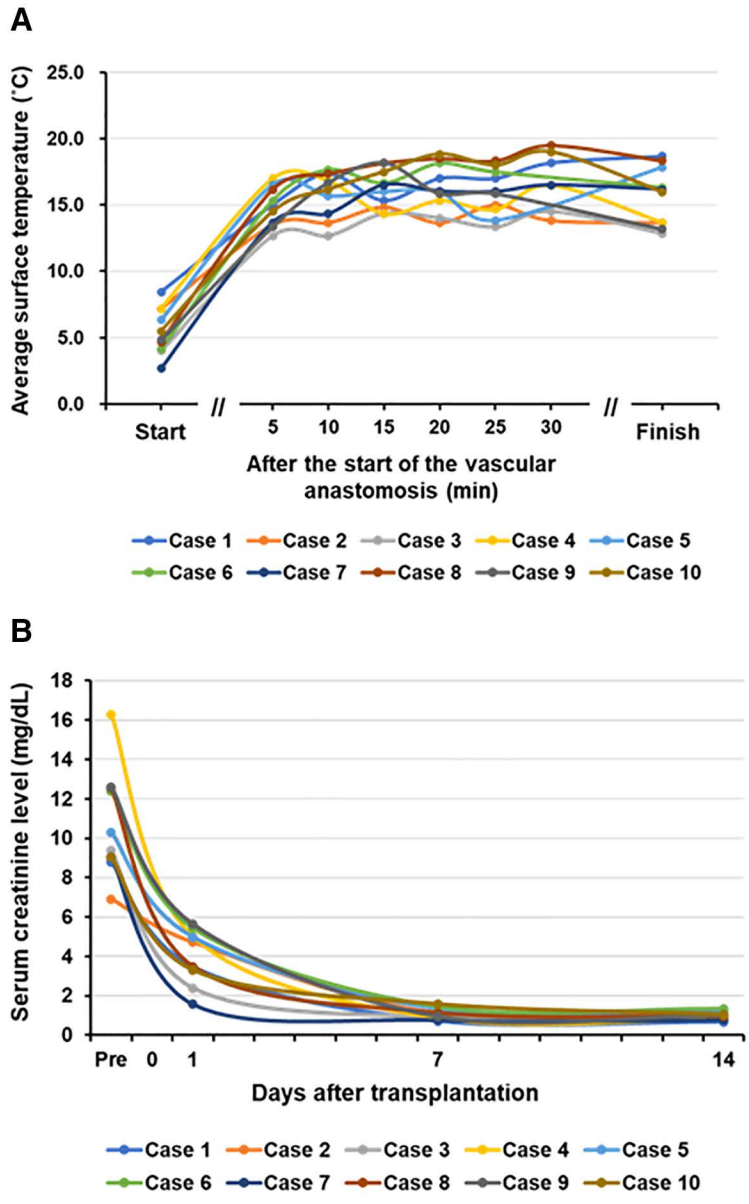

FIGURE 2. A, Kinetics of the surface temperature of the TBB during vascular anastomosis. B, Kinetics of serum creatinine levels for the first 14 d after kidney transplantation. TBB, thermal barrier bag.

DISCUSSION

Warm ischemic injury is an important modifiable factor affecting graft and long-term patient survival. However, prolongation of WIT2 may be unavoidable, depending on donor factors (number of vessels, vessel length, vascular disease, and other abnormalities) and recipient characteristics (large body mass index and vascular calcification). WIT2 is independently associated with DGF, as evidenced by a reported 5% increase in the risk of DGF development for every 1 min of anastomosis time.[8] Furthermore, prolonged WIT2 had detrimental effects on allograft histology and function for up to 3 y after transplantation.[8] It is biologically plausible that minor changes in WIT2 can affect long-term outcomes. To reduce the incidence of second warm ischemic injury, many hospitals adopt a simple surface cooling method. However, the posterior surface of the kidney in contact with the iliac fossa warms at a faster rate than the anterior surface and can easily reach ≥20 °C. Additionally, kidney temperature is inversely proportional to kidney size/weight.[24,25] Here, we applied a pouch-type TBB that could wrap the entire kidney during vascular anastomosis. The TBB prevents direct warming of the kidney graft by the patient's body temperature and by room temperature; consequently, the kidney graft is expected to remain at a low temperature during anastomosis in vivo. Lower temperatures can minimize mitochondrial injury and reduce the rate of ATP depletion and energy fluctuation. Thus, if the kidney graft is maintained at a low temperature by the use of the TBB, cellular metabolism is expected to be maintained at a low level, resulting in energy (ATP) savings. In addition, a recent report on a porcine kidney model demonstrated that TBB reduced tubular damage, indicated by decreased syndecan-1 expression compared with controls.[26] As the measurement of core temperature is not feasible in clinical studies,

kidney temperatures were measured using a noncontact infrared thermometer. Previous studies using TBB in a porcine kidney model showed that the core temperature of the kidney was lower than the surface temperature, suggesting that renal metabolic activity is suppressed by a sufficiently low surface temperature.[26] Here, we confirmed that TBB can maintain transplanted kidneys at a low temperature during vascular anastomosis and preserve the thermal threshold at the end of anastomosis.

Various cooling methods for reducing secondary warm ischemic injury have been tested in clinical and laboratory settings. These include topical/surface cooling techniques for the isolated kidney, immersion of the kidney in bags/stockinettes containing ice slush, kidney immersion in bags/stockinettes containing ice slush with or without an additional cold preservation solution, and the application of a shell/jacket containing a mechanical cooling system around the kidney. The "ice bag technique" is a relatively simple method for preventing second warm ischemic injury, with negligible preparation time/equipment, and manipulation of treatment protocols.[27] However, the elimination of WIT2 using this technique does not reduce the incidence or length of DGF.[27] Maintaining low-temperature conditions is not ideal because certain proteins denature at temperatures <2 °C, which significantly increases the risk of cold injury and frostbite.[28] TBB is composed of an ultrasoft elastomeric gel material that prevents kidney damage while maintaining high biocompatibility and insulation properties. No serious adverse events or surgical site infections related to the TBB were observed in this study. Some surgeons do not use these methods and prefer to suture the vessels as quickly as possible. However, this approach may lead to technical errors, excessive bleeding, and a poor learning experience for resident staff. The use of TBBs can reduce the time pressure associated with WIT2, minimize the risk of surgical complications, expand surgical training avenues, and facilitate robotic implantation. If WIT2 is expected to require more time owing to anatomical or technical issues, TBB is a better choice. The TBB potentially provided superior insulation compared with controls.[21,26] Besides its high thermal barrier effect, the TBB may also have physical benefits. The elasticity of the TBB, which adheres closely to the kidney, may be effective in preventing kidney tissue edema caused by warm ischemic injury. This hypothesis is currently highly speculative, requiring further study.

Although low temperatures maintain cellular metabolism at a low level, ensuring high ATP savings, temperatures under 2 °C might allow protein denaturation and damage to cell structure.[29,30] The TBB may prevent an excessive temperature drop or increase in the kidney graft. In the setting of deceased donor KT, the TBB may be beneficial in preventing tissue damage because of excessive cooling of kidney grafts during cold storage. This possibility requires further basic and clinical research.

The study results are limited by the study population size and short follow-up period. Additionally, although the effects of ischemic reperfusion injury are limited to living-donor KT, this phenomenon assumes importance in ECD and DCD. We plan to conduct a randomized controlled trial of ECD and DCD to determine whether TBB decreases DGF in KT. Furthermore, now that the safety of the TBB has been confirmed, long-term observational studies will be conducted to evaluate graft survival, function, rejection rates, and other complications.

CONCLUSIONS

TBB can maintain transplanted kidneys at a low temperature during vascular anastomosis, which contributes to the functional preservation of transplanted kidneys and stable transplant outcomes. Larger studies with TBB are necessary to determine whether the prevention of second warm ischemic injury decreases the incidence and length of DGF.

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