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Ischemic postconditioning in human DCD kidney transplantation is feasible and appears safe

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Summary

Ischemic postconditioning may improve outcome after kidney transplantation. We performed a pilot study to assess feasibility and safety of ischemic postconditioning in human donation-after-circulatory-death kidney transplantation. Twenty patients were included. Primary outcome was rate of serious adverse events. Secondary outcomes were incidence of DGF and renal function at 3 months. Data were compared to a historical control group (n = 40). Furthermore, we performed a paired kidney analysis using the contralateral kidney (n = 11). Donor age and serum creatinine were higher in the experimental group compared with historical control: 57.7 (20–71) vs. 51.5 (24–74) years (P = 0.01) and 79 (40–156) μmol/l vs. 64 (25–115) μmol/l (P = 0.047). Postconditioning could be applied all times. One complication, a venous tear, occurred related to postconditioning. The experimental group experienced more DGF (85% vs. 63%) (P = 0.07). Serum creatinine at month 3 was 166 (109–331) μmol/l vs. 159 (81–279) μmol/l (P = 0.71). Paired kidney analysis showed no significant differences in DGF (72.2% vs. 54.5%, P = 0.66) and serum creatinine 199 (90–473) μmol/l vs. 184 (117–368) μmol/l (P = 0.76). This is the first report of applying IPoC in human kidney transplantation. Although IPoC is feasible and appears to be safe, no benefit in terms of reduced DGF or better renal function was observed (Dutch trial registry number NTR 3117).

Introduction

Ischemia reperfusion injury (IRI) is considered to be the main cause of delayed graft function (DGF) which frequently complicates deceased donor kidney transplantation (KT) [1]. DGF increases the length of hospital stay and transplantation costs and is associated with acute rejection, and possibly, chronic transplant loss [1–3].

The possibility to create resistance against ischemic injury through 'organ conditioning' is an area of increasing interest. Several studies have demonstrated that ischemic postconditioning (IPoC), defined as rapid, intermittent interruptions of blood flow at the onset of reperfusion, can reduce myocardial infarct size by as much as 40% in animals [4–6]. Beneficial effects of IPoC have also been observed in humans with acute myocardial infarction and after cardiac surgery [7–10]. Even more recently, ischemic conditioning was shown to provide cardioprotection and a better prognosis in patients undergoing coronary artery bypass surgery [11]. In addition, IPoC has been studied extensively in animal models of renal IRI (reviewed in [12]). In these experiments, robust protection against IRI was seen after IPoC with significantly better renal function and lower expression of inflammatory and oxidative damage markers [12].

As a result of the continuing organ shortage, the use of kidneys from extended-criteria donors (ECD), including organs from donation-after-circulatory-death (DCD) donors, has increased [13]. The use of ECDs is associated with inferior transplantation outcomes [1]. Interventions

to reduce the extent of IRI could lead to the more widespread acceptance of organs from ECDs and may be of considerable benefit to transplant recipients. Therefore, the '*P*rotection against IRI by *I*PoC in *N*on-heart beating *K*idney transplantation (PINK) trial' was performed to study the feasibility and safety of IPoC in human DCD donor KT.

Materials and methods

Study design

The PINK study was an open-label, one-arm, single center, proof-of-principle, pilot study with the aim to investigate the feasibility, safety, and potential benefit of IPoC after DCD donor KT. Because this was the first study of IPoC in human KT and no data on its feasibility, safety, or efficacy with regard to renal function were known beforehand, we chose to conduct a nonrandomized pilot study. DCD kidney transplant recipients were studied because these are the kidneys with the most extensive ischemic damage due to the extra warm ischemia time after circulatory arrest. It was hypothesized that most profit by IPoC could be gained in this group. We chose to include only 20 patients because of safety concerns and did not consider it ethical to include a larger number of patients. Given the lack of clinical data on the potential benefit of IpoC on renal recovery, it was not possible to perform a power calculation at the start of the study.

All adult patients who were admitted to the Renal Transplant Unit of the Erasmus MC, University Medical Center Rotterdam, the Netherlands, to undergo DCD donor KT were asked to participate in the study. Because the primary aim of this study was to assess the feasibility of the IPoC procedure, we wanted the study to reflect everyday clinical practice as much as possible. Therefore, there were no exclusion criteria other than recipient age <18 years. All patients gave written informed consent. Ethical approval for the study was obtained from the Medical Ethical Committee of the Erasmus MC (number MEC-2011-067; NL 34987.078.11, version 4, 19 April 2011). The study was monitored by an independent Data Safety Monitoring Board. The PINK study was registered in the Dutch Trial Registry (Nederlands Trial Register, www.trialregister.nl), number TC-3117 (20 October 2011).

Control groups

Two control groups were studied. The first consisted of those patients who received the contralateral kidney (i.e., the other kidney originating from the same donor as the donor of the patient participating in the PINK trial). This paired-kidney control group was included to study any potential benefits of IPoC without the need to overcome differences in donor characteristics. Data of the patients in this control group were retrieved anonymously through the Dutch Transplant Registry at three months post-transplantation. Approval to use these data was given beforehand by the *Dutch Organ Transplantation Registry Committee*. When both kidneys were assigned to the Erasmus MC, both recipients were asked to participate in the trial and, in case of informed consent, both kidneys underwent IPoC.

As a second, historical, control group, we retrieved data from DCD donor KT recipients ($n = 40$) who were transplanted in our center immediately prior to the start of the PINK trial or, in case of deferred consent, during the run-in phase of the trial. These patients received the same immunosuppressive treatment and were operated by the same surgical team as the patients in the PINK trial. Recipients of a pediatric kidney were not included.

The donor characteristics were retrieved from the Eurotransplant Registry Database through the Eurotransplant form. This database contains extensive demographic, clinical, and laboratory data of the donors, and this anonymized information is always provided as part of routine clinical care to the transplant team at the time of kidney allocation. All donor kidneys were kept and transported in cold storage with HTK preservation solution.

Experimental surgical procedure

KT was performed according to standard protocol until reperfusion. The kidney was placed in the iliac fossa using an extraperitoneal approach. The external iliac vein (EIV) was clamped with an atraumatic, curved vascular clamp (Dietrich clamp), the external iliac artery (EIA) with two straight noncrushing vascular clamps (Dardik clamps). Standard end-to-side vascular anastomoses were made on the EIV and EIA using running Prolene 5-0/6-0 sutures. Heparin was administered locally at the end of the anastomoses to prevent thrombus formation. After reperfusion, the Dietrich clamp was removed from the vein and placed along the EIA, surrounding the arterial anastomosis (clamp positioned proximally and distally from the anastomosis as illustrated in Fig. 1a). After 1 min of reperfusion, this clamp was closed for 1 min. This was repeated two times (Fig. 1b). Clamping and opening times were monitored using a timer. Fluid regimen and perioperative medication were followed according to the local protocol [14].

During transplantation, three biopsies were taken with a 4-mm biopsy punch for further investigation, after which the biopsy sites were stuffed with hemostatic gauze (Surgicel) and oversewn with PDS 4-0. No biopsies were taken when patients were receiving oral anticoagulants or P2Y12-receptor inhibitors.

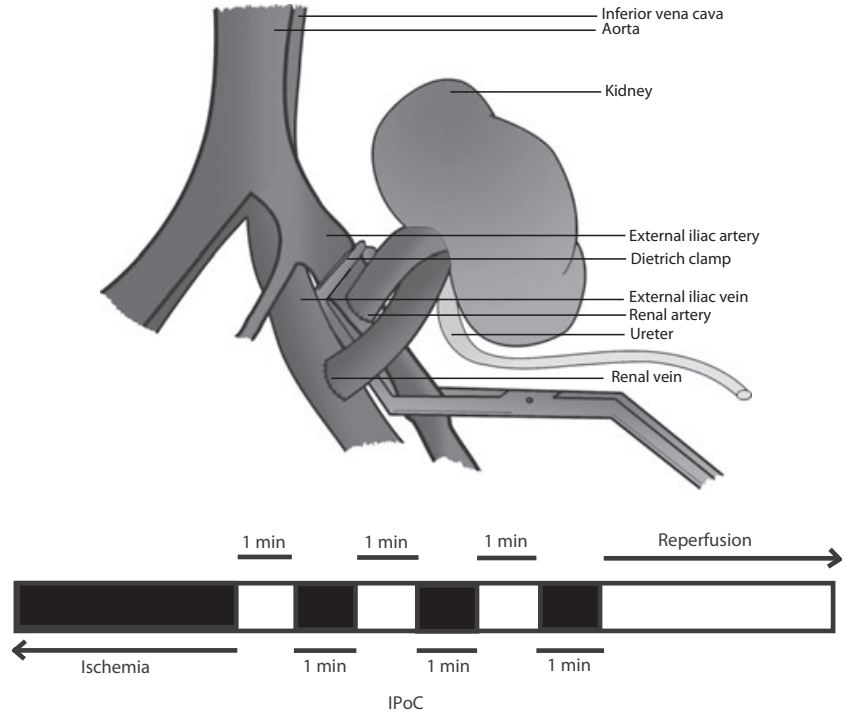

Figure 1 Placement of the Dietrich clamp during IPoC. (a) Before reperfusion, the Dietrich clamp is placed along the external iliac vein. At reperfusion, this clamp is removed from the vein and placed along the external iliac artery and clamped after 1 min of reperfusion. (b) The IPoC algorithm. After 1 min of reperfusion, the external iliac artery is clamped for 1 min. This is repeated two times, after which final reperfusion follows.

IPoC algorithm

The IPoC algorithm of three times 1 min was chosen based on experience in human IPoC studies and the principle of metabolic rate. IPoC in human cardiology studies involved comparable algorithms, ranging from 3× 30 s to 4× 1 min. An algorithm of 3× 1 min gave better results compared with 3× 30 s [10,15,16]. The principle of metabolic rate is based on the fact that small animals have a higher metabolic rate, and therefore, optimal algorithms have been reported to be short and repetitive. Larger species have slower metabolic rates and appear to need longer clamping times. Conclusive evidence on this hypothesis is lacking, however. Of note, the ischemia–reperfusion cycles used in this trial, as well as in other postconditioning studies, are in general of shorter duration than those employed in ischemic *pre*conditioning [12,17–19].

Immunosuppressive protocol and postoperative medication

All patients received induction therapy with 20 mg basiliximab (Simulect®; Novartis Pharma, Arnhem, the Netherlands) on days 0 and 4. No antithymocyte globulin induction therapy was given. All patients received tacrolimus (Prograf®; Astellas Pharma, Leiderdorp, the Netherlands) aiming for predose concentrations of 10–15 ng/ml in weeks 1–2, 8–12 ng/ml in weeks 3 and 4, and 5–10 ng/ml thereafter. In addition, all patients were treated with mycophenolate mofetil (MMF; Cellcept®; Roche Pharmaceuticals, Woerden, the Netherlands) in a starting dose of 1000 mg twice daily. Methylprednisolone was given for the first three days in a dose of 100 mg intravenously. Thereafter, prednisolone was given orally in a starting dose of 20 mg once daily and subsequently tapered 5 mg per day at month 3 post-KT. All patients received unfractionated heparin for the first 4 days postoperatively. Patients at risk of CMV infection received valganciclovir prophylaxis, and all patients received trimethoprim-sulfamethoxazole prophylactically.

Endpoints

The primary aim of this study was to investigate the feasibility and safety of IPoC. As such, data on all (serious) adverse events and length of hospital stay were collected prospectively for the first 3 months following transplantation. Serious adverse events were defined as events with the following consequences: death, life-threatening, need for surgery, leading to permanent disability/incapacity, and the need for hospital admission or prolongation of hospital stay. To assess any possible beneficial effects of IPoC, we collected data on renal function (serum creatinine and

estimated GFR according to the MDRD formula [20]) and the incidence and length of DGF (defined as the need for dialysis within the first week after transplantation). These parameters were used as secondary endpoints.

Statistical analysis

Categorical data are presented as numbers (percentages) and were compared by chi-square tests. Continuous data are presented as means with range. Parametric data were compared using Student's independent t-test and nonparametric data with Mann–Whitney *U*-test. All the analyses were performed using IBM Corp. Released 2011. IBM SPSS Statistics for Windows, version 20.0 (Armonk, NY, USA:IBM Corp).

Results

Study population

Donors

The donor characteristics are shown in Table 1. Eighteen donations resulted in 20 KTs in the experimental group (PINK) (Fig. 2). All four kidneys of two donors were allocated to our center and subsequently underwent IPoC according to the PINK protocol. The outcome of these kidney transplantations was considered independent, and therefore, the donor characteristics were analyzed as four individual donors. Compared with the PINK group, historical control group donors were significantly younger [57.7 (20–71) vs. 51.5 (24–74) years; $P = 0.01$]. Also, a significant difference in serum creatinine (last recorded before death) and eGFR was observed: [79 (40–156) μmol/l vs. 64 (25–115) μmol/l ($P = 0.047$)] + [100 (39–197) vs. 126 (60–268) ml/min per 1.73 m^2 ($P = 0.04$)], respectively, in favor of the historical control group. Furthermore, significantly more massive atherosclerosis was reported by the procurement surgeons in the PINK group kidneys (25% vs. 3%; $P = 0.007$). This means that the general quality of the kidneys in the historical group was better than those that underwent IPoC. It was not possible to create a matched case–control group based on age, most likely because of the multitude of donors >55 years in the PINK group. Throughout the Netherlands, there is a trend toward an increasing

Table 1. Donor characteristics. Categorical data are expressed as number (%). Continuous data are expressed as mean (range).

	PINK group ($n = 20$)	Historical control group ($n = 40$)	*P*-value	Paired kidney cohort ($n = 11$)
Age (years)	57.7 (20–71)	51.5 (24–74)	**0.01**	53.4 (20–71)
>55 years	17 (85)	15 (37.5)	**0.03**	7 (63.6)
Gender (female/male)	8 (40):12 (60)	16 (40):24 (60)	1.00	5 (45): 6 (55)
Height (cm)	176 (144–190)	174 (152–195)	0.47	174 (144–190)
Weight (kg)	74.1 (48–95)	78.9 (46–130)	0.29	70.2 (48–90)
BMI	24.0 (21–27)	25.9 (16–40)	0.11	23.2 (21–27)
Cause of death				
Neurological	10 (50)	23 (58)	0.38	6 (55)
Cardiac	4 (20)	7 (18)		1 (9)
Respiratory	4 (20)	2 (5)		2 (18)
Trauma	0 (0)	4 (10)		0 (0)
Not specified	2 (10)	4 (10)		2 (18)
Hypotension	5 (25)	8 (20)	0.66	2 (18)
Creatinine* (μmol/l)	79 (40–156)	64 (25–115)	**0.05**	67 (43–156)
eGFR (ml/min per 1.73 m^2)	100 (39–197)	126 (60–268)	**0.04**	110 (39–148)
Diuresis (ml in last hour)	128 (50–300)	193 (33–1100)	0.36	127 (50–250)
Atherosclerosis			0.099	
None	6 (30)	14 (36)	0.65	6 (55)
Moderate	9 (45)	24 (62)	0.23	4 (36)
Massive	5 (25)	1 (3)	**0.007**	1 (9)
Storage solution				
HTK	16 (80)	32 (80)	1.00	10 (91)
UW	4 (20)	8 (20)		1 (9)
WIT^1(min)	17 (9–36)	17 (7–28)	0.97	14 (10–22)
CIT (min)	879 (495–1380)	868 (400–1500)	0.87	–
WIT^2 (min)	27 (16–40)	24 (14–41)	0.13	–
TIT (min)	923 (541–1415)	909 (431–1549)	0.83	–

HTK, histidine-tryptophan-ketoglutarate; UW, University of Wisconsin; WIT^1, first warm ischemia time; CIT, cold ischemia time; WIT^2, second warm ischemia time; TIT, total ischemia time.

*Serum Creatinine: last measured prior to circulatory arrest.

Bold values represent significance.

number of donors >55 years of age over the last few years. In 2011, 50% of kidney donors were >55 years, compared with 45% in 2010, with especially an increase in donors older than 65 years (14% in 2010, 26% in 2011) (Dutch Transplantation Foundation, www.transplantatiestichting.nl). To prevent time bias, we decided not to use data from DCD KT before 2009.

First warm ischemia time (WIT^1), cold ischemia time (CIT), second warm ischemia time (WIT^2) and total ischemia time (TIT) were not significantly different between donors of the historical control group and donors of the PINK group (Table 1). Of note, the three IPoC cycles of 1 min of warm ischemia–reperfusion were not considered when calculating these ischemia times.

Recipients

Between November 2011 and June 2012, 22 patients underwent DCD KT in our center. Twenty patients gave written informed consent and were included in the PINK trial. Two patients declined to participate and were included in the historical control group. The 20 recipients included in this study, received 20 kidneys from 18 donors. The recipient characteristics are depicted in Table 2. One patient died before the end of the follow-up due to unknown causes (Fig. 2).

In 11 cases, the contralateral kidney was transplanted elsewhere (Fig. 2); two times, the contralateral kidney was allocated to a recipient living outside the Netherlands, and therefore, no data on the contralateral kidney could be retrieved; in two cases, the contralateral kidney was not accepted for transplantation and was discarded; in one case, the donor had a single kidney. Two times, both kidneys were allocated to our center. Thus, four recipients participating in this study received a kidney originating from the same two donors. As a result, data on 11 control patients receiving a contralateral kidney were available for analysis. We only had permission to retrieve data on DGF incidence and serum creatinine at month 3 of the contralateral kidney recipients (see below). Therefore, no formal comparison of the recipient characteristics with the PINK group could be performed.

The characteristics of the 40 KT recipients in the historical control group are depicted in Table 2. No statistically

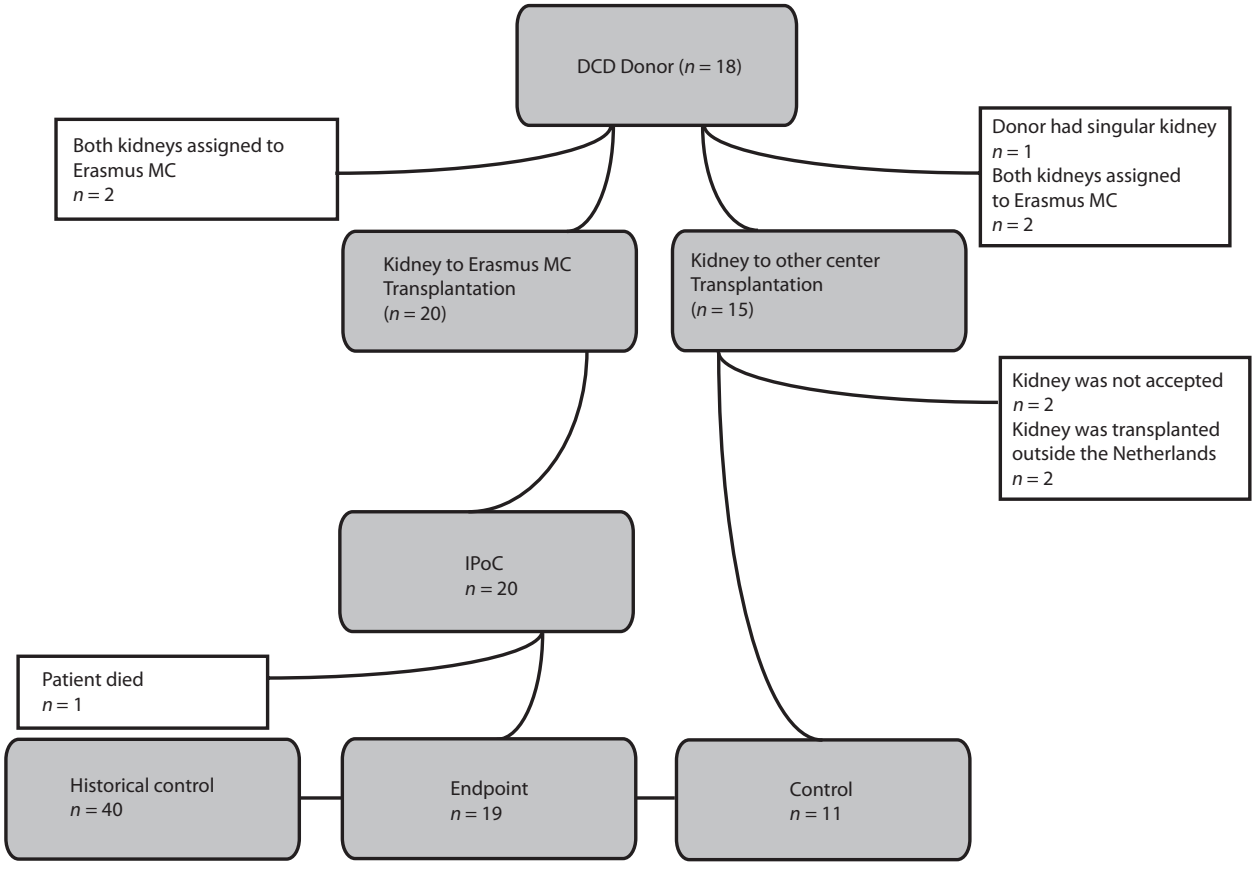

Figure 2 Flowchart of the PINK trial. Twenty patients were included and underwent DCD KT from 18 donors. One patient died before the end of our follow-up. 20 KT in the experimental group corresponded with 11 KT of the contralateral kidneys. For the historical control group, we took our most recent cohort of patients, which underwent DCD KT in our center. Recipients of a pediatric donor kidney were excluded.

Table 2. Recipient characteristics: PINK versus historical control.

	PINK group (n = 20)	Historical control group (n = 40)	P-value
Age	57.0 (31–75)	55.0 (22–76)	0.59
Gender (female/male)	3 (15):17 (85)	12 (30):28 (70)	0.21
Height (cm)	173 (155–200)	170 (140–195)	0.45
Weight (kg)	83.9 (49–118)	75.3 (42–118)	0.098
BMI (EH)	28.0 (19.5–39.5)	25.7 (16–38)	0.10
Ethnicity			
Caucasian	15 (75)	22 (55)	0.51
African	2 (10)	5 (12.5)	
Asian	3 (5)	9 (22.5)	
North-African/Arab	0	3 (7.5)	
Other	0	1 (2.5)	
Primary kidney disease			
Hypertensive nephropathy	2 (10.0)	9 (22.5)	0.63
Diabetic nephropathy	3 (15.0)	9 (22.5)	
Glomerulonephritis	3 (15.0)	5 (12.5)	
Polycystic kidney disease	5 (25.0)	3 (7.5)	
Reflux/obstructive nephropathy/ congenital	1 (5.0)	3 (7.5)	
Unknown	2 (10.0)	2 (5)	
Other	4 (20.0)	9 (22.5)	
HLA mismatch			
0	1 (5)	3 (7.5)	1.00
1–3	11 (55)	20 (50)	
4–6	8 (40)	17 (42.5)	
First transplantation*	14 (70)	32 (80)	0.85
Re-transplantation	5 (25)	8 (20)	

*In the PINK group, one first transplantation was pre-emptive; in the historical control group, three pre-emptive transplantations were performed. Recipient characteristics of the paired kidney analysis are not listed, as data of the contralateral kidney were retrieved anonymously. Categorical data are expressed as number (%). Continuous data are expressed as mean (range).

significant differences existed in any of the characteristics between the historical control group and patients that underwent IPoC.

Feasibility

The IPoC procedure was considered easy to perform and implement by the transplant surgeons and could be applied in all cases. In two cases, the EIA was clamped with two straight noncrushing vascular (Dardik) clamps (one proximal and one distal of the arterial anastomosis) during IPoC because of too little space to place an atraumatic, curved vascular (Dietrich) clamp. There is no significant change in procedure when using two Dardik clamps instead of a Dietrich clamp, except for the necessity of performing two actions instead of one. In four cases, there was a need to deviate from the IPoC algorithm, three times due to bleeding from the anastomosis. The first reperfusion period was therefore prolonged (i.e., the first reperfusion period of the IPoC algorithm varied from 1.5 to 3.5 min). After that, the IPoC protocol was applied as dictated. In one case, absence of visible flow in the renal artery occurred at initiation of reperfusion, for which the renal artery was reconstructed immediately, and IPoC was applied afterward. No difficulties were reported regarding clamping atherosclerotic arteries.

Safety

Perioperative adverse events

One major complication occurred, related to the IPoC procedure; the renal vein was injured while removing the Dietrich clamp. There was 800 ml blood loss and an additional 10 min WIT while reconstructing the venous anastomosis. Other perioperative complications, not considered a result of IPoC, included one technical arterial anastomosis problem for which the anastomosis was revised, two cases of venous bleeding, and two cases of prolonged hypotension during surgery. The caudal EIA was clamped 10 min during one of these transplantations to guarantee maximal perfusion to the kidney graft. Unfortunately, a lower pole infarction was evident a few days after transplantation.

Postoperative adverse events

A total of 129 adverse events occurred in the 20 PINK patients of which 47 were considered to be severe and 82 nonsevere (Table 3). Although there was a high rate of adverse events, in our experience this is not uncommon after KT. Most adverse events were considered not directly related to IPoC. In the supplementary Table S1, these adverse events are listed per patient. During follow-up, one patient died at home, one day after discharge, 13 days after transplantation. The cause of death was unknown because autopsy was not performed. One patient suffered from rejection, multiple peritonitis episodes (peritoneal dialysis had to be continued because of DGF), retroperitoneal abscesses and the previously mentioned kidney infarction, which finally resulted in nonfunction and transplant nephrectomy (Table S1).

Kidney allograft function

Hospital stay did not differ between PINK and historical controls with a mean of 17 (10–27) vs. 21 (10–148) days (P = 0.92). In the PINK group, 17 patients (85%) experienced DGF and required more than one postoperative dialysis treatment. The DGF incidence in the historical control group was 62.5% (P = 0.07). The mean DGF duration was identical for the PINK and historical control group: 19 (0–84) vs. 16 (0–136) days (P = 0.44). At 3 months post-transplantation, 15 (75%) patients had functioning

Table 3. Serious adverse events in the PINK group. Complications listed until 3 months after transplantation. Serious adverse events are complications with the following consequences: death, life-threatening, need for surgery, and need for hospital admission. All other complications are defined as adverse events.

(Serious) adverse events	*n*
Death	1
Graft failure	1
Delayed graft function	17
Rejection*	4
Acute tubular necrosis	4
Infection	22
Urological complications (other than urinary tract infection)	3
NODAT/dysregulation of pre-existing diabetes mellitus	11
Operation/intervention	7
Kidney infarction	1
Bleeding	8
Blood transfusion	5
Wound-related problems	6
Cardiac decompensation/fluid overload	6
Cardiac (other than cardiac decompensation)	5
CNI nephrotoxicity	3
BK virus nephropathy	1
Liver enzyme abnormalities	2
Thrombotic microangiopathy	1
Neurologic (other than CVA/TIA)	3
Hematologic (other than bleeding or thrombosis)	10
Diarrhea	3
Gastro-intestinal side effects (other than diarrhea)	2
Laboratory abnormalities (other than liver enzymes)	1
Gout	1
Other	1
Total	129

*One patient was treated twice for acute rejection.

transplants and were off dialysis. Three patients had persistent DGF and were still on dialysis. Two of these patients had received their second and third transplant, respectively, and their postoperative course was complicated by severe acute rejection (Banff type 2B with a humoral component), which necessitated therapy with depleting antibodies. In addition, at month 3, a fourth patient had restarted dialysis treatment after having recovered from DGF and after a very complicated post-transplant course, including, among others, thrombotic microangiopathy, type 2B acute rejection, and multiple infectious complications which eventually necessitated graft nephrectomy. As stated above, a fifth patient died before month 3 with a functioning transplant.

The mean serum creatinine and eGFR of the 15 PINK patients with functioning transplants at month 3 were 166 (109–331) μmol/l and 41 (17–63) ml/min per 1.73 m^2, respectively. At month 3, in the historical control group, 37 of the 40 patients (93%) had functioning renal transplants. Two patients were still on dialysis, and 1 patient had died because of pancreatitis. The serum creatinine at month 3 of these 37 patients was 159 (81–279) μmol/l and the eGFR was 42 (14–80) ml/min per 1.73 m^2. These differences were not statistically significant ($P = 0.71$ and $P = 0.78$, respectively). The percentage of patients who were alive and off dialysis at month 3 post-KT was not significantly different between the 2 groups ($P = 0.10$).

Paired kidney analysis

Next, we compared patients in the PINK study with the recipients of the contralateral kidneys. For this analysis, only 11 pairs were available (Fig. 2). In Table 1, the donor characteristics of the 11 pairs are listed. In the PINK group, 8 of 11 patients experienced DGF (72%), compared with 6 patients (55%) in the contralateral kidney group ($P = 0.66$). The duration of DGF was also not different between the two groups: 14 (0–84) vs. 8 (0–35) days ($P = 0.44$). The serum creatinine of the contralateral kidneys was 199 (90–473) μmol/l compared with 184 (117–368) μmol/l in the PINK cohort ($P = 0.76$).

Discussion

The results of this pilot study show that IPoC is feasible and appears safe in human KT. In all cases, the IPoC algorithm could be applied, albeit with some minor modifications of the first reperfusion period in certain patients. Furthermore, the surgeons considered the procedure not to be difficult and easy to implement.

One major complication (a venous tear due to manipulation of the Dietrich clamp) was directly related to the IPoC procedure, while another major complication (a case of lower pole infarction) was possibly related to the IPoC procedure. We feel that after completion of the learning curve, such complications can be avoided. Numerous other postoperative complications occurred (e.g., infections and wound healing problems), but this is not uncommon after KT, and these complications were not considered to be directly related to IPoC. Because a historical control group was studied and data on adverse events were therefore likely to be incomplete, we did not compare complications between the IPoC and the control group. The question whether IPoC is truly safe can only be answered by a randomized controlled trial. Nonetheless, although the incidence of complications directly attributable to IPoC was low in this study, their severity warrants caution. In our view, any future trials on IPoC should therefore include appropriate (interim) safety analyses. In addition, remote ischemic postconditioning should be considered as an alternative [21,22].

No beneficial effect of IPoC on the incidence of DGF or kidney function was demonstrated. In fact, the outcomes in the PINK group were numerically (although not statistically significantly) worse compared with our historical

control group. The most likely explanation for this observation is that there were considerable differences in donor characteristics between the two groups. Donors in the PINK group were older, had more atherosclerosis, and last measured renal function before donation was worse compared with the historical controls. This likely reflects the changing attitude of transplant physicians toward the acceptance of ECD kidneys in the Netherlands. A better matching for these baseline characteristics was attempted but considered not meaningful as this resulted in differences in yet other important characteristics. Most notably, historical controls who received kidneys with a comparable quality to that of the PINK trial participants were transplanted well before the start of the trial received different immunosuppressive treatment and were often operated by other surgeons.

In the paired kidney analysis, again no differences in renal outcomes were observed, although this analysis had little statistical power due to small patient numbers. From these results, it appears that our IPoC protocol is unlikely to ameliorate IRI to an important degree in DCD KT recipients. These findings are in line with a recent trial in human living-donor KT that did not show an improvement in early renal function after remote ischemic preconditioning [23]. The two control groups of the present study had several obvious shortcomings. However, the PINK trial was a first-in-human pilot study and was designed to serve as the basis of a future randomized controlled clinical trial and not intended to provide definitive answers on the benefits and complications of IPoC.

However, the potential of IPoC remains open to question. The robust protection against IRI in animal models warranted translation into human renal transplantation. The beneficial effect of IPoC is dependent on factors such as IPoC algorithm and index ischemia [24,25]. No consensus exists on the optimal IPoC algorithm, although it is commonly accepted that it should be in proportion with the subjects' metabolism [10,12,26,27]. Although the algorithm used in the present trial has been used previously in human cardiology studies, it is unknown whether it had the potential to provide beneficial effects in transplantation. Furthermore, in animal models applying IPoC to ameliorate renal IRI, only warm ischemia was induced, whereas in the clinical KT setting, a considerable period of CIT is inevitable. As IPoC appears to lose effectiveness when index ischemia is longer [24,25], it is imaginable that after prolonged CIT or even prolonged WIT alone, the damage to the kidney allograft is simply too extensive for IPoC to work. One option to address and possibly elucidate these uncertainties could be to conduct large animal KT experiments with DCD characteristics and considerable cold ischemia times to study the effect of different IPoC algorithms, ischemia times and window of opportunity [28–30].

In conclusion, IPoC is feasible and appears safe, but in this pilot study, no clinical benefit was present in terms of a reduced incidence of DGF or better renal function 3 months after transplantation. Although IPoC in its present form is not ready for a randomized controlled trial, we feel that further investigation of the IPoC concept should be considered.

Authorship

EKA, DAH and FJMFD: participated in design, in the performance of the research, in writing, and in data analysis. OCM: participated in design, in writing, and in data analysis. JAL: participated in the performance of the research, in writing, and in data analysis. WW and RWFB: participated in design and in writing. JNMI: participated in design, in the performance of the research, and in writing.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. All reported serious adverse events (SAEs) listed per patient.

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