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Short-term outcome of kidney transplants from non-heart-beating donors after preservation by machine perfusion

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Abstract In this study, the short-term outcome of renal transplants from non-heart-beating donors (NHBD) preserved by machine perfusion (MP) is evaluated and compared to preservation by cold storage (CS). Twenty-two NHBD kidneys were procured during 1993 and 1994 after in situ perfusion with histidine-tryptophan ketoglutarate and preserved by continuous perfusion using University of Wisconsin organ preservation solution for MP as a perfusate. Between 1980 and 1992, 57 NHBD kidneys were procured and preserved by CS. Donors in the MP group sustained increased first warm ischemia times (WIT1) ($P < 0.1$) and recipients in the MP group suffered longer anastomosis time, worse HLA-DR mismatch, and more initial use of cyclosporin as immunosuppressant; all these factors are known to be deleterious to short-term outcome. Despite these unfavorable conditions, delayed function (DF) rate was decreased in the MP group, although not significantly. However, when considering only kidneys with WIT1 $\geq$ 45 min, short-term outcome was significantly better in the MP group ($P < 0.05$). We conclude that MP is superior for the preservation of NHBD kidneys, especially after prolonged warm ischemia.

Key words Non-heart-beating donors · Kidney transplantation · Short-term outcome · Machine perfusion · Delayed graft function

Introduction

In the search for ways to reduce the organ shortage, non-heart-beating donors (NHBD) are recognized as a valuable source of organs for transplantation. After irreversible cardiac arrest and the subsequent diagnosis of death, organs can be procured and used for transplantation, especially kidneys since they are known to tolerate ischemia well [1]. The University Hospital Maastricht, has had an NHBD program for over a decade and procures and transplants kidneys from NHBD [2]. Analysis of the transplantation outcome with these renal grafts showed a high incidence of delayed function (DF) due to acute tubular necrosis (ATN) when compared to grafts from heart-beating donors (HBD) [3]. From experimental data, it is known that preservation of ischemically damaged kidneys by machine perfusion (MP) is superior to simple cold storage (CS) [4, 5]. In order to reduce the DF rate of kidney grafts from NHBD we recently implemented MP as the preferred method of preservation. In this study, the results of 22 renal grafts from NHBD preserved by MP were evaluated and retrospectively compared with grafts from NHBD preserved by CS.

Materials and methods

Patients dying after irreversible circulatory arrest in the emergency room or ICU, were considered potential NHBD when meeting the following criteria: circulatory arrest no longer than 30 min excluding the time of effective resuscitation and a total resuscitation

Table 1 Donor and recipient data. Results are given as mean ± SEM or numbers (%) (*CS* cold storage preservation, *MP* machine perfusion preservation, *WIT1* first warm ischemia time, *CIT* cold ischemia time, *CyA* cyclosporin A)

	CS	MP	*P* value
Period	1980–1992	1993–1994	
Cases	57	22	
Donor data			
Age (years)	40.7 ± 2.3	45.8 ± 3.7	0.21
Creatinine (μmol/l)	101.6 ± 4.9	111.6 ± 9.4	0.35
WIT1 (min)	33.1 ± 2.9	52.0 ± 7.9	0.10
Recipient data			
Age (years)	44.9 ± 1.7	49.0 ± 3.1	0.19
CIT (h)	31.5 ± 1.1	30.2 ± 1.2	0.5
Anastomosis time (min)	36.2 ± 2.0	40.7 ± 3.4	0.05
Graft number	1.2 ± 0.1	1.1 ± 0.1	0.8
HLA-DR mismatch	0.3 ± 0.1	0.6 ± 0.1	0.03
CyA immunosuppression	35 (38 %)	18 (82 %)	0.05

Table 2 Short-term outcome up to 3 months posttransplantation. Results are given as mean ± SEM or numbers (%) (*DF* delayed function, *mo* months)

	CS	MP	*P* value
Cases	57	22	
Immediate function	15 (26 %)	8 (36 %)	
Delayed function	34 (60 %)	11 (50 %)	0.67
Primary non-function	8 (14 %)	3 (14 %)	
Urine output (ml/1st 24 h)	836.9 ± 154.1	1465.2 ± 540.2	0.66
Duration of DF (days)	17.2 ± 1.7	18.9 ± 2.8	0.63
Number of dialyses	6.0 ± 0.8	5.5 ± 1.2	0.84
Creatinine 1 mo (μmol/l)	338.5 ± 46.1	324.5 ± 69.4	0.58
Creatinine 3 mo (μmol/l)	194.2 ± 13.8	264.8 ± 44.8	0.39

time not longer than 2 h. The upper age limit was considered to be 65 years and the potential donors were not known to suffer from kidney disease, uncontrolled hypertension, or metastasizing malignancies. Patients with signs of sepsis or intravenous drug abuse were excluded. All donors became available either after an unsuccessful attempt at cardiopulmonary resuscitation (so called NHBD cat II) or sustained final cardiac arrest after withdrawal of ventilatory support (NHBD cat III) [6]. After consent, an in situ perfusion procedure using a double-balloon-triple-lumen cooling catheter (Porgès AJ 6516), according to a technique described before, was performed [7]. Afterwards, the kidneys were procured in the operating room. The organs were preserved by either CS, using Euro-Collins (EC), histidine-tryptophan ketoglutarate (HTK), or University of Wisconsin (UW) CS solutions, or by MP in a Gambro-PF3B perfusion machine using UW machine preservation solution as a perfusate [8]. The in situ flush out was performed using the respective CS solution or HTK in all MP cases. During MP, flow characteristics and enzyme loss were recorded to evaluate the viability of the kidney. Because of these parameters, among others, kidneys were discarded and not used [9]. Kidneys transplanted within the Eurotransplant area were included and data were collected by approaching the transplant centers.

Short-term posttransplant function was classified as: (a) immediate function (IF), i.e., immediate life-sustaining renal function without posttransplant dialysis, (b) DF, i.e., renal function that ultimately was life-sustaining but required one or more dialysis sessions, and (c) primary non-function (PNF), i.e., renal function failed and the patient was never without dialysis. Renal function was recorded as serum creatinine at 1 and 3 months after transplantation; DF was quantified by urine output during the first 24 h posttransplant, the duration of DF (number of days), and the number of dialysis sessions required.

Because WIT1 tended to be longer in the MP group, the results of transplantation with kidneys with prolonged warm ischemia, i.e., WIT1 of 45 min or more, were analyzed separately. The results are given as mean ± SEM. Statistical analysis employed the chi-squared test with Yates' correction or Fisher's exact test, the Mann-Whitney U-test, and the Kruskal-Wallis one-way ANOVA test for differences between the groups, as appropriate. A *P* value of less than 0.05 was considered significant.

Results

Between July 1993 and December 1994, 22 kidneys were recovered from 14 NHBD, preserved by MP, and subsequently transplanted. In the control group, 57 kidneys from NHBD were transplanted after preservation by CS in the period 1980–1992. Causes of death showed more brain trauma in the CS group while more patients died after myocardial infarction in the MP group ($P = 0.001$). Data concerning donor and recipient factors are summarized in Table 1. Donor age was 40.7 ± 2.3 years in the CS group and 45.8 ± 3.7 years in the MP group ($P = 0.21$); last serum creatinine level of the donors was 101.6 ± 4.9 and 111.6 ± 9.4 μmol/l, respectively ($P = 0.35$). Kidneys in the CS group sustained a first warm ischemia time (WIT1), the time elapsing between cardiac arrest and the start of in situ cooling, of 33.1 ±2.9 min vs 52.0 ± 7.9 min in the MP group ($P = 0.10$). Forty-two (74 %) organs were cold stored in EC, 14 (24 %) in HTK, and 1 (2 %) in UW; in the MP group, one pair of kidneys was initially flushed with EC.

Mean cold ischemia time (CIT) was 31.5 ± 1.1 h for cold-stored grafts and 30.2 ± 1.2 h for machine perfused grafts ($P = 0.50$). Recipient data in either group were comparable for age, sex, number of the graft, and peak level of panel-reactive antibodies. In the MP group, the mean HLA-DR mismatch was 0.6 ± 0.1 vs 0.3 ± 0.1 in the CS group ($P = 0.03$). Mean anastomosis time was shorter in the CS group (36.2 ± 2.0 min vs 40.7 ± 3.4 min, $P = 0.05$).

Short-term posttransplant outcome is summarized in Table 2. Results were comparable for both groups, reflected by a similar immediate posttransplant urine output and equal duration of DF, number of dialysis sessions, and kidney function at 1 and 3 months.

In the separate analysis (concerning prolonged ischemia time defined as WIT1 of 45 min or more), 17 kidneys (30 %) in the CS group sustained prolonged warm ischemia (WIT1 59.7 ± 4.3 min) vs 10 kidneys (45 %) in the MP group (WIT1 89.0 ± 6.2 min,

Table 3 Donor and recipient data for kidneys with WIT1 of 45 min or more. Results are given as mean ± SEM or numbers (%)

	CS	MP	*P* value
Period	1980–1992	1993–1994	
Cases	17	10	
Donor data			
Age (years)	42.4 ± 3.9	52.6 ± 5.2	0.08
Creatinine (μmol/l)	114.6 ± 8.6	121.4 ± 5.4	0.44
WIT1 (min)	59.7 ± 4.3	89.0 ± 6.2	0.002
Recipient data			
Age	44.8 ± 3.8	51.7 ± 4.2	0.27
CIT (h)	30.8 ± 2.2	29.6 ± 1.9	0.56
Anastomosis time (min)	29.9 ± 2.3	47.5 ± 4.1	0.001
Graft number	1.3 ± 0.2	1.2 ± 0.1	1.0
HLA-DR mismatch	0.3 ± 0.1	0.5 ± 0.2	0.22
CyA immunosuppression	13 (76 %)	8 (80 %)	0.83

Table 4 Short-term outcome of kidneys with WIT1 of 45 min or more. Results are given as mean ± SEM or numbers (%)

	CS	MP	*P* value
Cases	17	10	
Immediate function	4 (23 %)	5 (50 %)	
Delayed function	13 (76 %)	3 (30 %)	0.02
Primary non-function	0 (0 %)	2 (20 %)	
Urine output (ml/1st 24 h)	959.5 ± 323.5	1734.8 ± 998.0	0.7
Duration of DF (days)	17.2 ± 2.4	12.7 ± 1.8	0.42
Number of dialyses	4.8 ± 0.9	5.3 ± 1.3	0.93
Creatinine 1 mo (μmol/l)	408.6 ± 76.9	197.3 ± 26.3	0.05
Creatinine 3 mo (μmol/l)	197.4 ± 23.0	163.3 ± 14.9	0.5

$P = 0.002$). Results are depicted in Table 3. All donor data were comparable; for recipient data again the anastomosis time was different (CS group 29.9 ± 2.3 min vs MP group 47.5 ± 4.1 min, $P = 0.001$).

In Table 4, the posttransplant outcome of kidney transplants with prolonged warm ischemia is summarized. It is shown that this outcome was better in the MP group when compared to the CS group ($P = 0.02$), reflected by a lower serum creatinine level at 1 month ($P = 0.05$); serum creatinine at 3 months showed statistically no significant difference ($P = 0.5$). The length of DF and the number of dialyses required were the same.

Discussion

Although kidneys from NHBD have been used ever since the first transplantation, ways have to be found to ameliorate the ischemic damage through optimal methods of preservation and minimization of reperfusion injury, in order to develop this valuable source of transplantable organs. Studies on the transplantation results with kidneys from NHBD show DF rates as high as 60–75 %, almost twice as high as for grafts from HBD [3, 10–15]. This delayed onset of renal function is unfavorable as DF masks the signs of early acute rejection, complicates the postoperative treatment, and the reinstitution of dialysis has a negative impact on the patient's psyche. Consequently, hospital stay will be prolonged and costs increased [16, 17]. The effect of DF on graft survival is controversial; some believe DF is associated with reduced graft survival, while others suggest that DF only in combination with acute rejection is responsible for a worse transplant outcome [18–23]. In spite of doubled DF rates, long-term graft and patient outcome did not differ between recipients of NHBD and HBD kidneys [3].

To reduce DF rates we introduced MP in 1993 as the method of preservation for NHBD kidneys using the UW preservation solution for MP as a perfusate. Nowadays, CS is accepted as the method of choice for the preservation of renal grafts; the advantages of preservation by continuous cold perfusion are most pronounced for prolonged preservation times and for kidneys from marginal donors with ischemic damage [4, 24]. Recent experimental work at our laboratories showed that preservation of ischemically damaged kidneys by MP was superior to preservation by CS. MP counteracts the intrarenal vascular resistance induced by ischemia, providing better reperfusion and delivering oxygen and nutrients to and removing waste products from the organ during preservation. This resulted in better survival of the animals and better preservation of the microcirculatory integrity [5, 25]. The superiority of MP has been confirmed in clinical transplantation on several occasions [26–29] and, recently, impressive results were reported with NHBD after preservation by MP. Kidneys were from NHBD cat III only and sustained considerably less ischemia than our series and showed merely 19–22 % DF rate [30, 31]. In the present analysis, the DF rate in the MP group was 50 % compared to 60 % in the CS group.

Apart from donor type (cadaver vs living donor, and NHBD vs HBD) and the preservation mode, several other factors have been identified as risk factors for ATN and DF. Retransplantation, warm ischemia time, type of preservation solution, preservation time, and number of HLA-DR mismatches have all been supposed to worsen early posttransplant function [3, 16, 17, 23, 32, 33]. In this analysis several of these factors were studied and some appeared to be statistically significant different between the study group and historical controls. For the machine-perfused kidneys, the anastomosis time was longer as well as the WIT1; the evident worse match on HLA-DR antigens in the MP group further deteriorates the early posttransplant function of these grafts. The results of the comparison between the CS and MP groups, therefore, may be biased to the detriment of the MP group concerning transplant outcome. However, although considered to be more profoundly

damaged than the NHBD kidneys in the CS group, the grafted kidneys preserved by MP showed equal short-term outcome when compared to grafts preserved by CS. In a previous analysis, we showed that the elevation of serum creatinine at 1 month posttransplant when compared to HBD, was transient and was neutralized at 3 months, not affecting long-term graft outcome [3].

In the last few years, increased efforts have been made to identify potential NHBD and it was noticed that in our program more NHBD cat II were accepted after long resuscitation efforts. This fact is reflected by the increased number of donors dying after myocardial infarction and unsuccessful resuscitation; at present about half in the MP group (period 1993–1994) against one-third in the CS group (period 1980–1992). The more recently effectuated donors may, therefore, have suffered essentially more ischemic damage while all kidneys procured were preserved by MP. Kidneys in the historical control group on the contrary, may be less damaged while all were preserved by CS. The separate analysis of NHBD grafts with WIT1 longer than 45 min, indeed showed that almost half of the kidneys in the MP group suffered prolonged WIT1 and that even within this subgroup WIT1 evidently was increased (89.0 ± 6.2 and 59.7 ± 4.3 min for MP and CS group, respectively; $P = 0.002$). In the prolonged ischemia group, the other donor and recipient data were more comparable in the CS and MP groups, thus minimizing their influence. Although in the subgroup kidneys preserved by MP still sustained more warm ischemic damage than their CS counterparts, the short-term results were significantly improved by MP. This improvement was reflected by a better serum creatinine level at 1 month. The number of days before kidney function was life sustaining, as well as the number of dialyses needed, did not differ among the groups, implying that MP did not effect the severity of DF once it had occurred. Using cyclosporin (CyA) in the immediate posttransplant period is known to prolong the duration of DF [17]. The length of DF is important since prolonged DF was observed to be detrimental to graft survival in NHBD kidney transplants [34]. In the MP group, more patients received CyA (82 % vs 63 %, $P = 0.05$) and this may oppose the positive action of perfusion preservation. The use of ATG as induction immunosuppression and the delayed introduction of CyA until acceptable kidney function is established, could decrease the duration of DF [33].

A very simple and effective way to reduce the ischemic damage to the organs in NHBD is to reduce the WIT1. Since in an opting-in legislation consent is needed to start in situ cooling, WIT1 sometimes is very long while the gap between diagnosis of death and starting the donation procedure is bridged by cardiac massage and artificial ventilation. A presumed-consent law, or at least legal consent to start in situ cooling if the relatives cannot be approached immediately, will reduce ischemic damage considerably and, thus, improve transplant results.

NHBD kidneys and their recipients need special attention with regard to preservation and postoperative treatment. High DF rates are of major concern and must be reduced to improve graft prognosis. The CS group in this analysis suffered less severe ischemic attacks than the MP group. Nevertheless, short-term outcome was at least equal in both groups; for kidneys that sustained prolonged ischemia, however, MP was superior despite more unfavorable donor conditions. To verify these results and study the impact of MP on long-term outcome, a retrospective study with matched controls, along with a prospective study, is currently in process. We conclude that continuous cold perfusion is superior to simple cold storage as the preservation mode for NHBD kidneys, especially after prolonged warm ischemic periods.

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