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Calcium ion concentration of machine perfusate predicts early graft function in expanded criteria donor kidneys

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Abstract Pulsatile preservation offers the advantage of pretransplant assessment of donor kidneys. Selected electrolyte concentrations of machine perfusate were measured over time in order to: (1) describe electrolyte changes in perfusate during the pulsatile preservation of expanded-criteria donor (ECD) kidneys, and (2) to assess the prognostic significance of these characteristics to early graft function. One hundred and fifty ECD kidneys were preserved in our laboratory between 1 January 1995 and 11 January 1997. ECD kidneys were defined as those requiring pretransplant biopsy. Kidneys were grouped by the presence or absence of delayed graft function (DGF), and perfusion parameters were measured every hour during pulsatile perfusion. All kidneys were preserved by continuous hypothermic pulsatile perfusion using Belzer II solution. Renal flow is decreased and renal resistance is increased in the presence of DGF in machine-preserved ECD kidneys. In addition, ionized calcium concentration of the machine perfusate is significantly elevated in the DGF group compared with the No DGF group (0.091 vs 0.054, $P = 0.0016$). The incidence of DGF is significantly lower in the ECD kidney. Among the pretransplant variables of donor characteristics, perfusion parameters and histology, perfusion parameters are highly predictive of early graft function. In addition, we found that ionized calcium concentration in the perfusate is significantly elevated in kidneys exhibiting DGF, which may have implications for assessing the suitability of donor kidneys for transplantation.

Key words Machine preservation · Marginal donor · Kidney · Calcium

Introduction

Recent efforts to improve access to cadaveric kidneys for transplantation have been directed at extending donor criteria. These expanded-criteria kidneys are procured from donors with hypertension, diabetes, advanced age, and elevated creatinine, among others. As a consequence, there has been greater emphasis on identifying pretransplant variables that might aid in determining the suitability of an expanded-criteria donor kidney (ECD) for transplant. Our laboratory utilizes continuous hypothermic pulsatile preservation (CHPP) of kidneys as the primary method of organ preservation. Unlike simple cold storage (SCS), CHPP allows the dynamic assessment and manipulation of the donor organ prior to transplantation. An additional benefit of CHPP is the ability to derive pretransplant information on perfusion characteristics that may have prognostic significance [1].

Our recent efforts in utilizing CHPP have focused on (1) describing hydrostatic and biochemical perfusion characteristics in ECD kidneys, and (2) assessing the predictive value of these characteristics in early graft function.

Materials and methods

All kidneys were procured from heart-beating donors and were preserved at the Organ Preservation Laboratory of The New York Presbyterian Hospital-Cornell Medical Center.

The following data on donor, perfusion, and post-transplant characteristics were collected: donor age (D age, years); final donor creatinine (D Cr, milligrams per deciliter); donor intraoperative urine output (U/O, milliliters); hypothermic perfusion time (PT, hours); cold ischemic time (CIT, hours); renal flow (FL, milliliters per minute per 100 g tissue weight); renal resistance (RES, millimeters of mercury per milliliters per minute per 100 g tissue weight); immediate function (IF), defined as recipient urine production exceeding 2000 ml during the first 24 h post-transplant; delayed graft function (DGF), defined as the need for hemodialysis within the first 7 days post-transplant; recipient discharge creatinine (R Cr, milligrams per deciliter); and 6-month graft function (GF, percentage).

Renal perfusion

Kidneys were perfused en bloc at 4.0 C and at 60 beats/min with 1 l of Belzer II perfusate (SUNY-Healthscience Center, Brooklyn, N. Y.) on Mox 100 organ-perfusion machines (Waters Instruments, Rochester, Minn.). The Mox perfusion machine provides a fixed-pressure system, which allows our staff to adjust the perfusion pressure as needed. All kidneys were perfused at a diastolic pressure below 60 mmHg. Our laboratory prefers to machine-perfuse kidneys en bloc in order to minimize the damage to perinephric vasculature that is associated with machine-perfusing kidneys separately. Perfusion characteristics (FL, RES, PT, $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Ca^{2+}]$, and pH) were measured when the kidneys were placed on the machine-perfusion system, every 30 min for the first 2 h of CHPP, and every hour thereafter throughout the period of CHPP. All chemical data were compared with a baseline assay of perfusate composition that had not circulated through the kidneys. All perfusion characteristics were standardized to 100 g of kidney tissue weight.

Renal biopsy

For this study, ECD kidneys were defined as all kidneys requiring a renal biopsy. Renal biopsy is performed at the request of a surgeon at any of the nine transplant centers in the New York City region, most commonly on the basis of one or more of the following donor characteristics: advanced donor age (more than 55 years), elevated donor creatinine (more than 1.5 mg/dl), donor history of hypertension (more than 10 years) or diabetes (insulin-dependent). Biopsy data were based upon frozen-section evaluations of pre-transplant core biopsies because only this information is available prior to transplantation. Biopsy data were evaluated by a pathologist at a single transplant center and included: degree of tubular interstitial scarring (TIS, percentage), arterial fibrous narrowing (IFN, percentage), and glomerulosclerosis (GS, percentage).

Table 1 Prognostic significance of selected variables (mean ± SEM) in predicting early graft function (unpaired student's t-test)

	DGF ($n = 48$)	No DGF ($n = 102$)	P value
Donor characteristic			
D age (y)	52.2 ± 5.2	49.9 ± 3.8	NS
D Cr (mg/dL)	1.2 ± 0.6	1.0 ± 1.5	NS
U/O (mL)	315 ± 60	360 ± 200	NS
Perfusion characteristic			
PT (h)	12	12	NS
CIT (h)	23.3 ± 3.2	24.9 ± 3.9	NS
FL (ml/min/100 g)	107.7 ± 5.6	150.4 ± 4.5	0.02
RES (mm Hg/FL)	0.39 ± 0.08	0.24 ± 0.05	0.009
[Na+] (mM/100 g)	99.2 ± 7.8	94.1 ± 9.9	NS
[Cl–] (mM/100 g)	27.9 ± 2.4	31.2 ± 8.0	NS
[K+] (mM/100 g)	34.1 ± 5.1	29.4 ± 3.8	NS
[Ca++] (mM/100 g)	0.091 ± 0.06	0.054 ± 0.06	0.0016
pH	7.37 ± 0.06	7.30 ± 0.94	NS
Biopsy feature			
TIS (%)	10.1 ± 3.1	8.4 ± 4.2	NS
IFN (%)	22.1 ± 5.1	20.0 ± 12.6	NS
GS (%)	2.3 ± 4.1	2.0 ± 5.6	NS
Outcome characteristic			
IF (%)	62%	89%	0.002
R Cr (mg/dL)	2.4 ± 0.4	1.9 ± 0.6	NS
GF (%)	81 ± 5.2	86 ± 3.2	NS

Perfusate analysis

The following biochemical components of perfusate were measured throughout CHPP with an Omni 4 Multianalyte system (AVL Medical Instruments, Atlanta, Ga.): $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Ca^{2+}]$, and pH. All chemical analyses were standardized to 100 g of tissue weight. The Omni 4 Multianalyte System is a fully automated, self-calibrating, biochemical assay module that provides immediate analytical results from a sample of machine perfusate. For each measurement of perfusion characteristics, a 0.5-cm^3 aliquot of perfusate is drawn from the perfusion chamber, analyzed by the Omni 4 Multianalyte System, and is available for evaluation within 30 s. Our laboratory prefers utilizing this system because of the assay's accuracy, reliability, and relative low cost.

Statistical analysis

All data are expressed as mean value ± SEM unless otherwise noted. Statistical analysis was performed by Statview 4.5 (Abacus Concepts, Berkeley, Calif.) Two-tailed paired and unpaired Student's t-test, ANOVA, and correlation coefficients were utilized where appropriate. A P-value of less than 0.05 was considered statistically significant.

Results

Between January 1995 and October 1997, 150 ECD cadaveric kidneys were machine perfused in our laboratory and transplanted at one of the New York regional transplant centers.

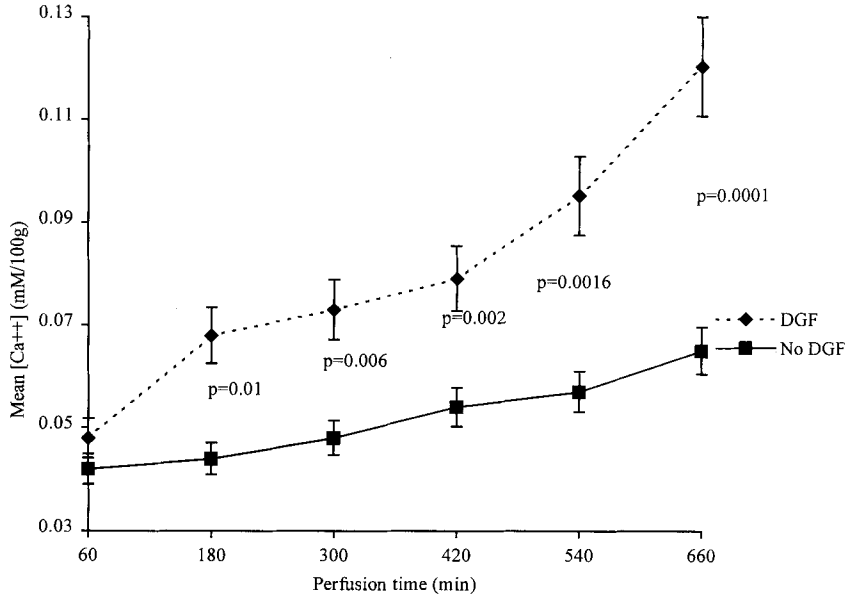

Fig. 1 Mean ionized calcium concentration of perfusate versus time (ANOVA; *DGF* delayed graft function)

Table 2 Correlation coefficients of selected variables (significant correlation is considered an absolute r-value > 0.60)

[Ca++]	r-value	RES	r-value	FL	r-value
FL	–0.79	FL	–0.86	DGF	–0.69
RES	0.84	DGF	0.73	GF	0.73
DGF	0.89	GF	–0.81		
GF	–0.86				

Prognostic value of selected variables in predicting early graft function

The data were divided into two groups based on the presence or absence of DGF (Table 1). Thirty-two percent (48/150) of the group exhibited DGF. There were no significant differences between the groups when donor characteristics were compared (D age, D Cr, U/O). Similarly, biopsy data (TIS, IFN, GS) did not differ significantly between the DGF and No DGF groups. Selected perfusion characteristics did not differ between the two groups (CIT, $[Na^+]$, $[Cl^-]$, $[K^+]$, and pH). However, significant differences in three perfusion characteristics existed between the two groups. Renal flow (FL) was decreased by 29% (107.7 ± 5.6 vs 150.4 ± 4.5; $P = 0.02$), renal resistance (RES) was increased by 38.5% (0.39 ± 0.08 vs 0.24 ± 0.05; $P = 0.009$), and ionized calcium concentration of the perfusate $[Ca^{2+}]$ was increased by 40.7% (0.091 ± 0.06 vs 0.054 ± 0.06; $P = 0.0016$) in the presence of DGF. Among the outcome characteristics, no significant differences existed between the groups when R Cr and GF were considered. A significant difference existed between the groups when IF was compared (62% for DGF vs 89% for No DGF; $P = 0.002$).

Correlation coefficients of selected variables

A correlation matrix was constructed to compare selected variables and to determine their *r*-values (Table 2). Among the perfusion characteristics, a strong correlation existed between RES and FL ($r = -0.86$), $[Ca^{2+}]$ and FL ($r = -0.79$), and $[Ca^{2+}]$ and RES ($r = 0.84$). Similarly, the following outcome characteristics were highly correlative: DGF and FL ($r = -0.69$), GF and FL ($r = 0.73$), DGF and RES ($r = 0.73$), GF and RES ($r = -0.81$), DGF and $[Ca^{2+}]$ ($r = 0.89$), and GF and $[Ca^{2+}]$ ($r = -0.86$).

Mean ionized calcium concentration of perfusate versus time

The mean calcium ion concentration of machine perfusate was measured over time and compared between the DGF and No DGF groups (Fig. 1). The rate of increase in perfusate $[Ca^{2+}]$ increased significantly in the presence of DGF (0.007 mM Ca^{2+}/100 g per hour vs 0.001 mM Ca^{2+}/100 g per hour; $P = 0.0016$)

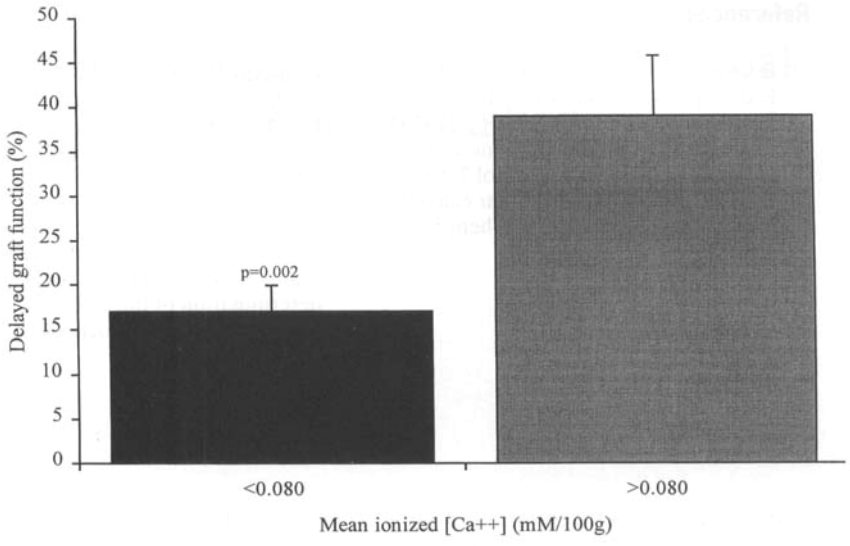

Fig. 2 Incidence of delayed graft function stratified by mean calcium ion concentration of perfusate after 12 h of hypothermic pulsatile perfusion (n = 150)

Incidence of DGF stratified by mean calcium ion concentration of perfusate after 12 h of pulsatile perfusion

The data were related by the incidence of DGF and the mean perfusate [Ca^{2+}] after 12 h of CHPP (Fig. 2). The grafts exhibiting No DGF (102/150, 68%) had corresponding mean perfusate Ca^{2+} levels below 0.080 mM/100 g. Similarly, the grafts that exhibited DGF (48/150, 32%) corresponded to mean [Ca^{2+}] of greater than 0.080 mM/100 g.

Discussion

Utilization of ECD kidneys has resulted in a greater emphasis on pre-transplant indicators of early graft function that might influence the decision to accept an ECD kidney for transplant. CHPP provides pretransplant information in evaluating the suitability of an ECD for transplant that is not available in the SCS kidney. Our data indicate that hydrostatic (FL and RES) and biochemical [Ca^{2+}]$_i$ perfusion characteristics are highly predictive of early graft function. Increasing FL and decreasing RES are associated with improved graft function. Intrinsically high resistance to flow within the graft may reflect preexisting parenchymal disease or acute tubular necrosis in the setting of donor management. Time-dependent elevated calcium ion concentration is highly predictive of early graft function. Alterations in calcium metabolism during hypothermia have been implicated in increased mitochondrial dysfunction [6, 8]. This dysfunction is characterized by calcium-induced activation of endogenous phospholipases present in mitochondrial membranes [2, 3]. Activated membrane phospholipases contribute to the release of free fatty acids and lysophosphatides, both of which can result in irreversible organelle and cellular membrane injury [2, 5]. Moreover, adenine nucleotide depletion is related to modulations in cellular calcium levels during hypothermic ischemia [3, 4, 7]. Inadequate substrates for ATP synthesis may inhibit cellular energy formation and limit graft viability upon reperfusion [8, 6]. Our data suggest that the degree of cell death and subsequent release of free ionized calcium into the circulating perfusate during CHPP is predictive of early graft function in ECD kidneys.

In summary, hydrostatic and biochemical perfusion characteristics are highly predictive of early graft function. Moreover, [Ca^{2+}] of machine perfusate is the most sensitive, preservation-related indicator of early graft function in our population. These results may aid in determining the suitability of an ECD kidney for transplantation.

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