Relationship between duration of brain death and hemodynamic (in)stability on progressive dysfunction and increased immunologic activation of donor kidneys

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Background. Consistent difference in graft survival after renal transplantation has been shown when cadaveric transplants are compared to the living related donor situation, in favor of the latter. Recently, evidence has been put forward that brain death has significant effects on the donor organ quality. In this study, we aimed to assess the relation between brain death–induced hemodynamic instability in combination with the duration of brain death on the function and immunogenicity status of potential donor kidneys.

Methods. In Wistar rats, short-term (1 hour) or long-term (6 hours) brain death in the presence or absence of hemodynamic stability was applied. Sham-operated rats served as controls (1 hour and 6 hours). Organ function was studied by monitoring serum creatinine, lactate dehydrogenase (LDH), lactate, and total protein content. Expression of cell adhesion molecules [intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)] and the influx of leukocytes in the kidney assessed the immunologic status of the kidney.

Results. Progressive organ dysfunction was most pronounced in hemodynamically unstable brain-dead donors reflected by increased serum creatinine levels. Regardless of hemodynamic status, a progressive inflammatory activation by cell adhesion molecule expression and an influx of leukocytes could be observed in kidneys of brain-dead rats compared with nonbrain-dead controls.

Conclusion. Brain death causes progressive kidney dysfunction. Also, inflammatory responses reflecting tissue injury are caused by brain death. When hemodynamic instability in the brain-dead donor is not corrected, kidney dysfunction is enhanced and immune activation occurs faster and is more profound. The observed changes may predispose the graft for additional ischemia/reperfusion injury during the transplant process and hence accelerate rejection of the graft after transplantation.

When results after transplantation of kidneys derived from living (un)related donors are compared with those obtained from brain-dead donors, a consistent inferior survival is observed in the latter [1–3]. Multiple factors possibly responsible for this difference in survival have been studied over the years. First, in the past, the superior human leukocyte antigen (HLA) matching in the living related donor combination has always been regarded as a prime factor for a better survival. HLA-identical siblings have always shown the best graft survival; however, when results of the non-HLA-identical living related transplant combinations were compared to the inferior-matched living unrelated combinations, graft survival was similar in both groups [1, 4]. Also, despite the higher level of HLA matching for cadaveric grafts compared to that of living unrelated donor combinations, transplantations of well-matched cadaveric kidneys are by far inferior to HLA mismatched living related kidney grafts. Another major factor and possibly as important is the quality of the donor kidney after preservation. The difference in cold ischemia time between living (un)related and cadaveric transplantation has been considered as a cause for the difference in transplant results. Long cold ischemia time promotes delayed graft function [5, 6] and organs with delayed graft function show more acute rejection episodes and a greater likelihood of developing chronic transplant dysfunction than organs with initial good function [7–9]. However, no significant effect of the duration of cold ischemia could be shown with preservation times up to 24 to 28 hours [5, 6, 10], the time frame for most cadaveric kidneys to be transplanted in the Eurotransplant Organ Sharing System.

A few years ago, the nonphysiologic state of brain death prior to organ retrieval was considered a potential cause for the inferior outcome after cadaveric vs. living donor transplantation [11–14]. Since then, the effects of brain death on donor organ quality have received considerable attention and a number of groups has attempted to better define its impact on short- and long-term graft function. Several authors as well as our group were able to demonstrate in animal models [11, 12, 15] and in humans [16] a significant immunologic activation of potential donor organs induced by brain death. In addition, animal experiments have now shown that brain death in the donor is responsible for an increased risk of ischemia/reperfusion injury and a decreased graft survival of the kidney and liver [14, 17].

The exact effects of brain death, however, and, in particular, its interdependence with brain death–induced hemodynamic instability have not been unraveled yet. In this study, we examined the effects of brain death as such, as well as the impact of the duration of brain death in relation to donor hemodynamic stability on function and immunologic status of potential donor kidneys.

METHODS

Design of the study

To study short- and long-term effects of brain death in the presence and absence of hemodynamic donor stability on kidney function and immunologic status, adult male Wistar rats were used (HSD.Cpb:WU, 300 to 350 g body weight). All animals received care in compliance with the guidelines of the institutional animal ethics committee according to the Experiments on Animals Act (1996) issued by The Netherlands Ministry of Public Health, Welfare, and Sports. Rats were randomly allocated to six groups.

Controls. Two groups of six rats each served as sham-operated nonbrain-dead controls and were killed 1 or 6 hours after onset of the experiment.

Brain-dead groups. In all rats of the other four groups, brain death was induced as described below. Rats were either killed 1 or 6 hours after the commencement of the brain-dead state. Two groups, the 1-hour and 6-hour so-called optimal (normotensive) donors ($N = 6$ and $N = 10$, respectively) received donor management (hemodynamic support to achieve normotension). The two other brain-dead groups did not receive donor management and remained hypotensive after brain-death induction, representing the 1 hour and 6 hour marginal (hypotensive) donors ($N = 5$ and $N = 7$, respectively).

Anesthesia and ventilation

Halothane anesthesia was used in all procedures until the moment of brain-death induction. Rats were intubated and then ventilated using a Medec MK 78 infant ventilator (Medec NL, Wormerveer, The Netherlands). A mixture of nitrous oxide and oxygen was used for a period up to 30 minutes after brain-death induction (stroke rate 60 per minute, peak inspiratory pressure 12 to 14 mm Hg, 40% inspiratory phase, 10% plateau). Subsequently, all rats were ventilated with 30% O_2 in air. The nonbrain-dead control groups remained anesthetized throughout the whole experiment.

Operative procedure

A laparotomy was performed through a midline incision for implantation of a telemetric device for continuous registration of mean arterial pressure (MAP) and heart rate (Chronic Use TA11PA-c40 Implant) (Data Science, Int., St. Paul, MN, USA). The tip of the telemetry implant was subsequently inserted into the abdominal aorta just proximal to the bifurcation. A catheter was introduced in the bladder after which the bladder and abdomen were closed.

Brain-death induction

Through a frontolateral trepanation (1×1 mm with a dental drill), a balloon catheter (Fogarty 14G) (Baxter Health Care Corp., Irvine, CA, USA) was introduced in the extradural space with the tip pointing caudally. Inflating the balloon for 1 minute increased the intracranial pressure, thereby inducing rapid progressive brain injury and leading to immediate brain death. A sharp rise and then a subsequent drop of blood pressure and heart rate defined initiation of brain death. The state of brain death was confirmed 30 minutes after induction of brain death by the absence of corneal reflexes and by an apnea test.

Donor management

As indicated in the design of the study, rats in the so-called optimal donor groups received donor management. Normotension in these groups was obtained by Gelofusin infusion (Vifor Medical SA, Basel, Switzerland) in the tail vein at a rate of 0.5 mL per minute. A MAP ≥ 80 mm Hg was considered normotensive [18]. In these two groups, treatment started 15 minutes after brain-death induction allowing a period of hemodynamic stabilization after brain-death induction. If the infusion of Gelofusin was insufficient to maintain normotension, norepinephrine was administered in a dose of 0.01 μg/mL. Animals in the 1-hour and 6-hour marginal donor groups were not treated for hypotension during the whole study period. Control animals received 0.5 mL Gelofusin intravenously immediately after sham operation.

Sampling procedure and biochemical determinations

Just prior to termination of the experiment, a relaparotomy was performed to collect serum for biochemical analysis and to take biopsies from the kidney. Tissue samples were snap-frozen in isopentane (−80°C).

Table 1. Primary antibodies used in this study for immunohistochemical analysis of rat kidney tissue sections

Clone	Reactive with	Source
5F10	VCAM-1	Dr. R. Lobb, Cambridge, Massachusetts
1A29	ICAM-1	Dr. M. Miyasaka, Osaka, Japan
OX1	CD45	European Collection of Animal Cell Cultures
R73	T-cell receptor	Dr. Hunig, Wurzburg, Germany
OX8	CD8	European Collection of Animal Cell Cultures
0X35	CD4	European Collection of Animal Cell Cultures
HIS48	PMN	Department of Histology, Groningen University, The Netherlands
NKR-P1A	NK cells	Pharmingen, San Diego, California
ED-1	Naive Mφ	Serotec, Amsterdam, The Netherlands
ED-2	Tissue Mφ	Serotec, Amsterdam, The Netherlands

Abbreviations are: VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecules; PMN, polymorphonuclear cells; NK, natural killer.

Blood samples were analyzed in a routine fashion. As an indicator of loss of kidney function, serum creatinine concentration was used. Serum lactate dehydrogenase (LDH) was used as an indicator of general (dys)function of the donor. Lactate concentration was determined to identify the occurrence of acidosis. Total protein content of the serum was used to analyze hemoconcentration or hemodilution (Mega Merck, Darmstadt, Germany).

Immunohistochemical analysis of kidney tissue

Snap-frozen kidney tissue was cryostat-cut at 6 μm. As changes in endothelial cell activation status are considered significant factors for success or failure of the graft [19, 20], endothelial expression of cell adhesion molecules [intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)] was studied. Endothelial expression of these cell adhesion molecules facilitates leukocyte recruitment. This leukocyte recruitment was analyzed by staining for CD45 (leukocyte-common antigen). Leukocytes in the glomeruli and interstitium were differentiated by staining for the T-cell receptor, CD8, CD4, polymorphomononuclear cells (PMNs), natural killer (NK) cells, circulating macrophages (ED-1), and resident (mature) macrophages (ED-2). See Table 1 for primary antibodies used.

After thorough washing, the sections were incubated with appropriate horseradish peroxidase–conjugated secondary antibodies (Dako, Glostrup, Denmark) and color was developed using 3-amino-9-ethylcarboxide (AEC)/H_2O_2. Sections were counterstained using Mayer's hematoxylin (Merck, Darmstadt, Germany). Routine hematoxylin and eosin staining was also performed on tissue sections of all rats.

Quantitation of immunohistochemical staining was assessed by light microscopy. VCAM-1 and ICAM-1 staining was scored as negative (no staining) or weakly (+), moderately (++), or strongly positive (+++). Microscopic examination and comparison were always performed with biopsies simultaneously stained. Three individuals independently scored all samples. Quantitation of stained cells in the sections stained for CD45, T-cell receptor, CD8, CD4, PMNs, NK cells, and macrophages was performed by counting the mean number of positive cells per 25 glomeruli and for the interstitium by counting the mean number of positive cells per 25 areas of 0.025 mm^2 in the cortex at 400× magnification.

Statistical analysis

All results are expressed as mean ± standard error of the mean (SEM). Statistical analysis comparing different treatment modalities was performed using the Mann-Whitney U test with $P < 0.05$ considered significant. Statistical analysis comparing different time points was performed using the Kruskal-Wallis test with $P < 0.05$ considered significant.

RESULTS

Effects of brain death and donor management on blood pressure and heart rate

The effects of brain death and donor management on the hemodynamic stability are shown in Figure 1. Before brain-death induction, no differences in heart rate and MAP were observed between any of the groups. In all rats exposed to brain-death induction, a steep rise in heart rate and MAP was recorded. Within 10 minutes after brain-death induction, MAP decreased to a hypotensive level of less than 80 mm Hg. The animals in both marginal donor groups received no Gelofusin infusion and consequently remained hypotensive, with mean blood pressures of 55 ± 5.3 mm Hg after 1 hour and 52 ± 9.9 mm Hg after 6 hours of brain death. Before infusion therapy, no differences were seen in MAP or heart rate between the marginal and optimal donor groups. In the optimal donor groups, normotension was achieved in all animals with fluid administration within 5 to 7 minutes after starting Gelofusin infusion. This resulted in a significantly increased MAP compared with the marginal donor group. The mean volume of Gelofusin infused in the 1-hour and 6-hour optimal donor group was respectively 3.3 ± 0.6 mL and 10.1 ± 1.4 mL. No norepinephrine administration was necessary to
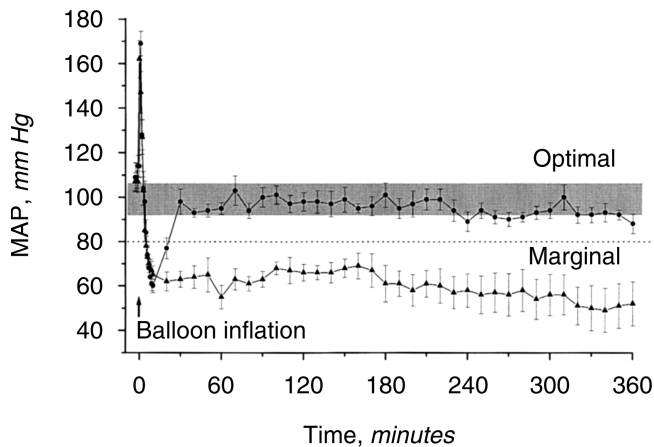
Fig. 1. **Changes in mean arterial pressure (MAP) in rats after induction of brain death are shown: immediate hypertension followed by hypotension.** Next, due to the absence or presence of donor management either hemodynamic instability persists (marginal donors) or hemodynamic stability is achieved (optimal donors). MAP is expressed as mean ± SEM. The shaded area shows the normal range of MAP in nonbraindead control rats. The cut-off point of hypotension (<80 mm Hg) is represented by the dotted line.

Fig. 2. **Creatinine concentration reflecting kidney (dys)function in non-brain-dead control rats, marginal, and optimal brain-dead donors 1 hour and 6 hours after brain-death induction.** All results are expressed as mean ± SEM. [a]$P < 0.05$ compared to 1 hour; [b]$P < 0.05$ compared to controls; [c]$P < 0.05$ compared to optimal donors.

maintain normotension in the 1-hour brain-death group. In the optimal 6-hour group, seven of ten rats needed additional support with norepinephrine infusion to remain normotensive.

In the brain-dead rats, diabetes insipidus occurred and diuresis increased with duration of brain death from $0.8 ± 0.3$ mL/kg/hour in the first hour to $3.7 ± 1.4$ mL/kg/hour after 6 hours. In the nonbrain-dead control rats, diuresis remained stable between 0.70 and 1.20 mL/kg/hour.

Effects of brain death and hemodynamic stability on serum parameters

Results of biochemical determinations are shown in Table 2. Serum creatinine concentration was significantly increased in the 1-hour and 6-hour marginal donor groups compared with controls. In optimal donors, the serum creatinine concentration was increased after 6 hours of brain death only. The increase in creatinine concentration in optimal donors was significantly lower compared to that in marginal donors (see Fig. 2). Serum parameter LDH was corrected for hemodilution or hemoconcentration as assessed by the total protein content of the serum. In all groups, the LDH concentration was elevated at 1 hour after operation. In the sham-operated controls, the LDH concentration returned to normal within 6 hours after operation. In marginal donors, the LDH concentration increased to a higher level in the 6-hour brain-death group, indicating progressive loss of cell viability. In optimal donors, the LDH concentration remained elevated and was significantly higher 6 hours after o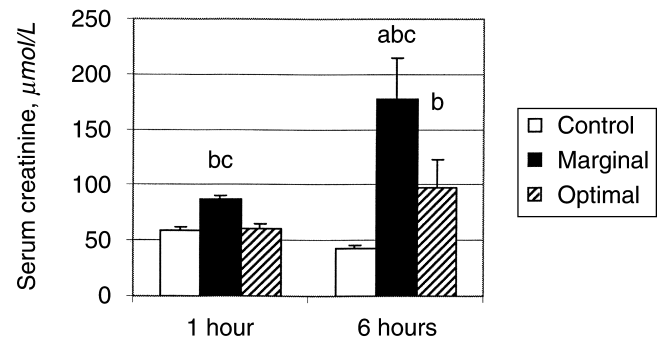nset of brain death than in controls. The increase in concentration in marginal donors was significantly higher than in optimal donors. Lactate concentrations in marginal donors were significantly increased at all time points compared to optimal donors and controls.

Immunohistochemistry of kidney tissue

Tables 3 and 4, as well as Figure 5, show the results of the immunohistochemical analysis of the kidney glomeruli and interstitium with respect to vascular activation status and leukocyte infiltration pattern.

Endothelial cell activation. Neither in kidneys from control rats, nor in kidneys retrieved from marginal and optimal donors, VCAM-1 expression was visible in the microvessels lining the proximal and distal tubuli. Only after 6 hours of brain death under both normotensive and hypotensive conditions, some reactivity (scored as weak) within the glomeruli could be detected. Likely, this staining was associated with glomerular blood vessel endothelium.

In normal rat kidney tissue, ICAM-1 expression was low in glomerular endothelium (scored as weak, exemplified in Figure 5 1-hour and 6-hour control biopsies). Similarly, in marginal as well as in optimal donors, glomerular endothelium expression of ICAM-1 was scored as weak (Fig. 5). In contrast, especially the microvasculature, lining of the tubuli was more strongly expressing ICAM-1 in both the marginal and optimal donor kidneys (both moderate and strong staining patterns of interstitial vasculature are exemplified in Fig. 5, hypotensive and normotensive kidneys). This staining pattern was observed throughout the kidney. Also, endothelium covering larger venules stained moderately to strongly positive for ICAM-1 throughout the kidney. Arterial endothelium was always negative for ICAM-1 expression (larger venule and artery staining patterns are not shown).

Leukocyte infiltration. A significant increase in infiltrating leukocytes (i.e., CD45-positive cells) in glomeruli of all brain-dead rats was observed (Fig. 3). In the mar-

Table 2. Biochemical serum parameters reflecting organ dysfunction in nonbrain-dead control rats, hypotensive (marginal), and normotensive (optimal) brain-dead donors 1 hours and 6 hours after brain-death induction

		Control		Marginal		Optimal	
		1 hour	6 hours	1 hour	6 hours	1 hour	6 hours
Na$^+$	*mmol/L*	138 ± 0.5	140 ± 0.7^a	139 ± 2.2	139 ± 1.6^c	140 ± 0.9	145 ± 1.7^{a,b}
K$^+$	*mmol/L*	5.2 ± 0.2	4.9 ± 0.2	6.3 ± 0.4	9.6 ± 1.2^{a,b,c}	5.9 ± 0.3	5.9 ± 0.5
Creat	*μmol/L*	59 ± 2.6	43 ± 2.2^a	87 ± 3.1^{b,c}	178 ± 37^{a,b,c}	60 ± 5.8	98 ± 25^b
LDH	*U/L*	1589 ± 240	410 ± 82^a	1357 ± 350	3037 ± 1008^b	1603 ± 256	1660 ± 421^b
Lactate	*mmol/L*	3.4 ± 0.4	1.2 ± 0.1	4.7 ± 0.7^c	4.9 ± 1.1^{b,c}	2.2 ± 0.7	2.2 ± 0.8

LDH is lactate dehydrogenase. Values are mean ± SEM. Serum parameter LDH is corrected for hemodilution or hemoconcentration as assessed by the total protein content of the serum.
^{a} $P < 0.05$ compared to 1 hour; ^{b} $P < 0.05$ compared to controls; ^{c} $P < 0.05$ compared to optimal donors

Table 3. Immunohistochemical analysis of mean antigen expression of glomeruli associated endothelial cell activation reflected by intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (−, no staining; +, weakly; ++, moderate; +++, strong) and cellular infiltrate (number of antigen-positive cells per 10 glomeruli) in kidney tissue of controls and animals subjected to brain death for 1 hours or 6 hours

	Control		Marginal		Optimal	
	1 hour	6 hours	1 hour	6 hours	1 hour	6 hours
VCAM-1	−	−	−	+	−	+
ICAM-1	+	+	+	+	+	+
CD45	13.5 ± 1.5	14.9 ± 1.5	36.7 ± 4.8^{b,c}	47.6 ± 5.8^{b,c}	19.3 ± 2.8	21.6 ± 1.7^b
T-cell receptor	1.7 ± 6.6	1.1 ± 0.1	3.4 ± 0.6^b	4.4 ± 0.5^{b,c}	2.2 ± 0.3	1.6 ± 0.4
CD8	2.4 ± 0.6	2.1 ± 0.2	4.6 ± 0.7	7.3 ± 1.4^{b,c}	3.1 ± 1	2.6 ± 0.3
CD4	8 ± 0.8	16.4 ± 2.8^a	14.5 ± 2.8^{b,c}	9.4 ± 2.9^{b,c}	6.4 ± 1.4	1.9 ± 0.3^{a,b}
Polymorphomononuclear cells	11.7 ± 1.7	9.2 ± 1.3	23.3 ± 4.9^{b,c}	40.1 ± 5.2^b	14.7 ± 1.9	31.6 ± 4.4^{a,b}
Natural killer cells	5.3 ± 4.1	1.7 ± 0.4	3.8 ± 0.5^b	4.5 ± 1.1	2.3 ± 0.4	1.9 ± 0.4
ED-1	14.2 ± 0.9	20.3 ± 1.8^a	26.4 ± 2.9^b	28.6 ± 3.5^b	18.1 ± 2.0	25.6 ± 2.0^a
ED-2	9 ± 1	9.4 ± 0.7	15.4 ± 2.1^b	22.4 ± 3.4^b	12.0 ± 1.2	20.3 ± 2.2^{a,b}

Values are mean number of 25 studied glomeruli at 400× magnification ± SEM.
^{a} $P < 0.05$ compared to 1 hour; ^{b} $P < 0.05$ compared to controls; ^{c} $P < 0.05$ compared to optimal donors. See **Methods** section for details of grading.

Table 4. Immunohistochemical analysis of mean antigen expression of endothelial cell activation by intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (−, +, ++, +++) and antigen-positive cellular infiltrate (number of antigen-positive cells per 10 microscopic fields) in the kidney cortical interstitium of controls and animals subjected to brain death for 1 hour or 6 hours

	Control		Marginal		Optimal	
	1 hour	6 hours	1 hour	6 hours	1 hour	6 hours
VCAM-1	−	−	−	−	−	−
ICAM-1	+	+	++	++	++	++
CD45	16.3 ± 3.5	23.3 ± 3.1	38.6 ± 8.2^b	53.9 ± 5.8^b	21.8 ± 1.9	33.3 ± 3.5
T-cell receptor	11.7 ± 9.6	14.8 ± 1.6	21.5 ± 4.1	20.3 ± 1.9^b	12.7 ± 1.7	14.3 ± 1.1
CD8	23.1 ± 1.5	23.3 ± 2.2	27.5 ± 2.8^c	29.7 ± 2.2^{b,c}	18.7 ± 1.9	15.7 ± 1.3^b
CD4	9.2 ± 7.3	12.6 ± 9.9^a	12.7 ± 38.4	11.4 ± 26.5	8.1 ± 12.8	7.5 ± 11.5^b
Polymorphomononuclear cells	5.2 ± 0.8	3 ± 0.3	8.5 ± 1.2^c	16.8 ± 1.4^{a,b,c}	4.1 ± 0.8	8.2 ± 1.4^{a,b}
Natural killer cells	10.9 ± 1.9	9.7 ± 2.2	9.1 ± 2.7	11.7 ± 2.6	13.8 ± 2.4	12 ± 1.3
ED-1	14 ± 1.4	12.3 ± 1.9	27 ± 1.2^{b,c}	25.8 ± 1.9^b	18.3 ± 2.4	21.9 ± 1.9^b
ED-2	6.2 ± 0.5	6.9 ± 0.7	17.4 ± 1.9^{b,c}	20.9 ± 1.9^{b,c}	11.3 ± 0.8^b	14.5 ± 1.2^{a,b}

Values are mean number of antigen positive cells per 25 microscopic fields at 400× magnification ± SEM.
^{a} $P < 0.05$ compared to 1 hour time point; ^{b} $P < 0.05$ compared to pertinent controls; ^{c} $P < 0.05$ compared to optimal donors. See **Methods** section for details of grading.

ginal donor groups, the number of infiltrating leukocytes was already significantly increased 1 hour after induction of brain death, and the accumulation increased with duration of brain death. In the optimal donor groups, after 6 hours of brain death, the mean number of leukocytes per glomerulus was increased. Compared to marginal donors, the increase in leukocyte infiltrates was lower in optimal donor kidneys. Leukocyte infiltrates in the glomeruli of marginal brain dead donors were composed of PMNs (i.e., HIS48-positive cells) and CD8-positive T lymphocytes (see Fig. 3). In contrast, in optimal donors, only an influx of PMNs in the glomeruli had oc-
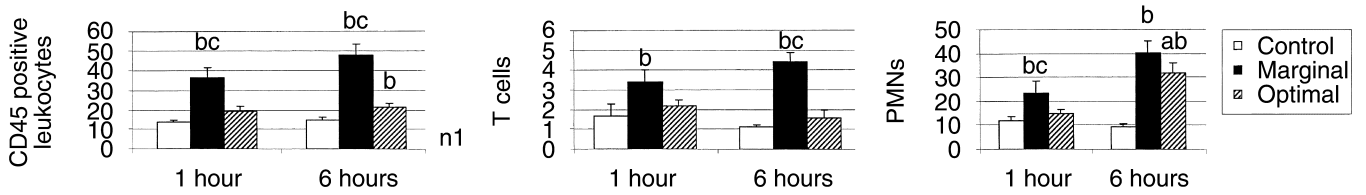
Fig. 3. Quantitative immunohistochemical analyses of expression of cellular infiltrate per 10 glomeruli in kidney tissue of control animals and animals subjected to brain death for 1 hour and 6 hours. $^aP < 0.05$ compared to 1-hour time point; $^bP < 0.05$ compared to pertinent controls; $^cP < 0.05$ compared to optimal donors.
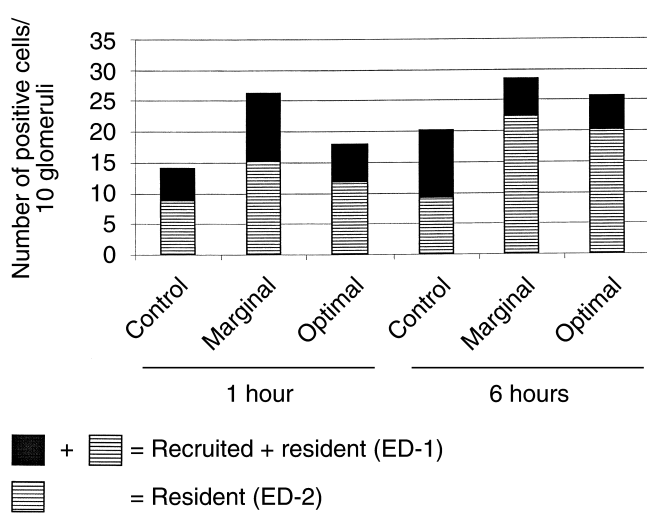

Fig. 4. Immuhistochemical analysis of the presence of monocytes and macrophages in the glomeruli of potential donor kidneys. ED-1 stains antigen in all monocytes and macrophages, thereby reflecting residential cells and cells recruited from the circulation, whereas ED-2 stains resident macrophages alone.
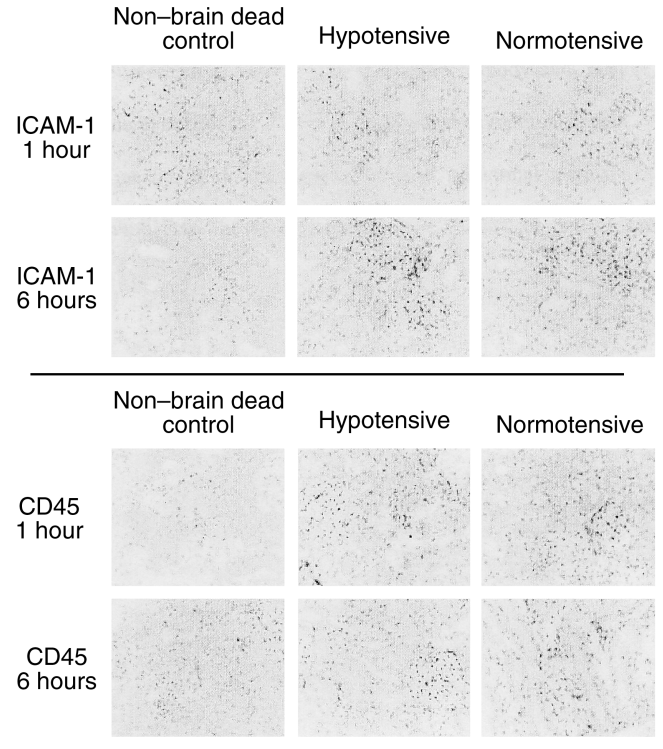

Fig. 5. Microscopic analysis of immunocytochemical staining of relevant primary antibodies are shown in liver tissue of nonbrain-dead control rats, marginal, and optimal brain-dead donors after 1 hour and 6 hours, respectively. Abbreviations are: ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

curred. In the interstitium, a virtually similar response was observed with the exception that an increased amount of CD45-positive cells was detected in the normotensive brain-dead rats. The increase in T lymphocytes in the interstitium of marginal donors consisted of CD8-positive cells. The number of PMNs was increased in marginal donors after 1 hour of brain death and increased further with duration of brain death, whereas in optimal donors only after 6 hours of brain death the amount of PMNs was significantly increased. No significant difference in the amount of NK cells was detected in any of the groups in the glomeruli or the interstitium.

The mean number of resident monocytes and macrophages per glomerulus has been shown to be between one and two [21], which corresponds well with the observed numbers in our nonbrain-dead controls. In the kidneys of hypotensive brain-dead rats, a significant increase in monocytes and macrophages (i.e., ED-1–positive cells) [22] was observed in the glomeruli (Fig. 4). In normotensive brain-dead donors, the total number of monocytes and macrophages was not significantly in-

creased compared to nonbrain-dead controls. The number of ED-2–positive macrophages was, however, significantly increased after 6 hours of brain death. In the interstitium an increase in ED-1–positive cells was found after 1 and 6 hours of brain death in both marginal and optimal donors. Also, the amount of ED-2–positive cells was significantly increased in both groups compared to nonbrain-dead control rats.

DISCUSSION

A few years ago, we and others hypothesized that brain death in the donor could be an important factor contributing to the decreased graft survival of cadaveric organs

compared with those of living donors. Since then, a number of studies have reported the effects of brain death on donor organ quality and have attempted to better understand the pathophysiologic changes that occur during this agonal phase [14, 17, 23–27]. It has become clear now that brain death has a detrimental effect on donor organ viability and renders it more susceptible to ischemia/reperfusion injury followed by a lower graft survival [14]. Thus, means to modulate brain death–related injury is becoming important as Pratschke et al [28] showed for the first time.

To unravel the complex phenomenon of brain death, we have focused in this study on the effect of the duration of brain death and the influence of hemodynamic stability in the organ donor on the cellular activation and function of potential donor kidneys.

Hemodynamic stability and brain death

Hemodynamic instability is inevitably encountered during brain death. After the onset of brain death and a temporary burst of sympathetic activation, parasympathetic stimuli prevail. This leads to a neurogenic shock in the potential organ donor. During neurogenic shock, hypotension with hypoperfusion in the donor has detrimental effects on posttransplant kidney function [6, 29]. Therefore, in the clinical setting of donor management, normotension is maintained as good as possible by fluid administration and vasoactive drugs. Until now, experiments that have studied the effects of brain death, however, have mostly been performed without correction of brain death–induced hypotension. These experiments have therefore not been able to unravel the effects of brain death as such and its interdependence with hypotension. In the current study, we addressed this issue by administering hemodynamic support to a subset of brain-dead donor rats. To our knowledge, so far, only one other group has made adaptations to their experimental brain death model to reach normotension [30]. By increasing the time to fully inflate the intracranially placed balloon catheter, less profound hemodynamic alterations occurred during the induction of brain death, leading to persistent normotension without hemodynamic support. This model, however, does not reflect the hemodynamic alterations observed in human donor studies in our hospital. In 95% of our human brain-dead donors, the exact time of brain death can be traced by the abrupt hemodynamic changes and the (increased) need for hemodynamic stabilization immediately thereafter, exactly as we have observed in our animal model as well. We feel that our brain death set-up in the animal as reported here, therefore, mimics more closely the clinical situation.

Effects of brain death on the biochemical status of the donor

Progressive kidney dysfunction is induced by the sequence of events resulting from brain death as shown by the increased creatinine levels. Normotension after inducing brain death, as in the optimal donors, reduces the severity of kidney dysfunction, but does not fully prevent it (43 µmol/L vs 178 µmol/L vs 98 µmol/L creatinine in controls, hypotensive, and normotensive brain-dead rats, respectively). High creatinine levels in the donor are an unfavorable sign for graft viability as they are associated with inferior graft survival rates compared to grafts from ideal donors [5, 31]. In addition, brain death in the donor causes time-dependent general dysfunction, as indicated by the elevated LDH concentration, being again more pronounced in hemodynamic unstable brain-dead donors.

The lactate serum levels were significantly increased in hypotensive brain-dead animals only. These data are in accordance with those reported by Novitzky et al [32]. In their baboon experiments, no hemodynamic support was given during brain death and similar elevated lactate levels were measured. Based on the current data, we can state that the observed increase in serum lactate concentration is an effect of concomitant hypotension and not of brain death itself.

Effects of brain death on the immunologic status of the kidney

Adhesion molecules play a pivotal role in modifying the immunologic status of the donor organ [33]. Brain death induces adhesion molecule expression as shown by the expression of ICAM-1 on proximal tubules in biopsies of brain-dead rats. A similar expression pattern has previously been described during episodes of inflammation in the kidney [34, 35]. Takada et al [11] also described activation of cell adhesion molecules in rats after 6 hours of brain death by staining for P- and E-selectin. In human renal biopsies derived from brain-dead donors, Koo et al [16] and Fuggle et al [36] also found induction of cell adhesion molecule expression. High levels of proximal tubular ICAM-1 expression in 62% of the cadaver donor kidneys was observed, whereas tubular ICAM-1 expression on biopsies of living related donors was absent [16]. In contrast to our findings in rats, however, Koo et al, Fuggle et al, and Rice et al [37] observed constitutive VCAM-1 expression on Bowman's capsule in all the renal biopsies analyzed. High levels of proximal tubular VCAM-1 were detected in cadaveric biopsies, while living related biopsies remained negative in all cases. Also, our preliminary data of human donor renal biopsies show endothelial cell activation as assessed by E-selectin staining (unpublished data). Mild to moderate E-selectin expression could be observed in intertubular capillaries of proximal and distal tubules in 5 of 29 (17%) kidney biopsies taken after explantation from living donors. None of these biopsies showed strong expression of E-selectin. In contrast, in 15 of 30 kidney bi-

opsies taken from brain-dead donors, moderate to strong staining could be observed.

Leukocyte recruitment into the kidney occurred in our experiments presumably facilitated by up-regulated cell adhesion molecule expression. A significant increase in infiltrating leukocytes (i.e., CD45-positive cells) was observed in glomeruli and interstitium of the potential donor kidneys of hemodynamic unstable brain-dead donors. Also, in normotensive brain-dead rats, an increased amount of CD45-positive cells was observed in the glomeruli after 6 hours of brain death. Hemodynamic instability in the donor was accompanied by an additional influx of CD8-positive T lymphocytes in both glomeruli and interstitium. This finding seems to contradict data reported by Takada et al [11], who concluded that brain death induced an influx of CD8-positive T cells in kidney tissue. In their interesting study, however, immunohistochemical analysis of hypotensive brain-dead rats were compared to those of hypotensive nonbrain-dead controls. It was assumed that the T-lymphocyte recruitment, which was not observed in hypotensive controls, was an effect of brain death. By subtracting the observed effects seen in the hypotensive nonbrain-dead control rats from those of hypotensive brain-dead animals, however, synergistic effects of brain death and hypotension could not be distinguished.

This study shows that brain death has definite effects on organ function and on the immunologic status of the donor kidney prior to organ retrieval. The immunologic changes observed relate well to those observed by Lu et al [38] after injury of the potential graft. In this respect, recognition of injury, together with recognition of specific nonself alloantigens, is required for allograft rejection. The first step of injury leading to rejection is considered to induce expression of adhesion molecules and subsequent recruitment of inflammatory cells into the graft as observed in the kidney biopsies of this study. The next phase toward allograft rejection, in which the influx of T cells is assisted by dendritic cells and costimulatory signaling, cannot be shown since in this study no transplant model was used. Considering the transplant results of Kusaka et al [17], however, it is likely that this immunologic reaction will prevail after transplantation leading to initiation of rejection. Furthermore, in the human renal biopsy study of Koo et al [16], with immunologic findings comparable to the data presented here, expression of pretransplant cell adhesion molecules in cadaver donor kidneys was significantly associated with early acute rejection following transplantation.

CONCLUSION

Brain death induces progressive organ dysfunction, which is more severe when hemodynamic instability is not corrected during this nonphysiologic phase prior to organ retrieval. Furthermore, brain death induces progressive immune activation by cell adhesion molecule expression and influx of leukocytes, primarily PMNs. The influx of inflammatory cells occurs faster and more pronounced when hypotension persists during brain death–related donor management. This study stresses the need for adequate hemodynamic stabilization of the brain-dead organ donor and suggests that the duration of the state of brain death should be kept as short as possible.

REFERENCES

1. TERASAKI PI, CECKA JM, GJERTSON DW, TAKEMOTO S: High survival rates of kidney transplants from spousal and living unrelated donors. *N Engl J Med* 333:333–336, 1995
2. HARIHARAN S, JOHNSON CP, BRESNAHAN BA, *et al*: Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med* 342:605–612, 2000
3. UNITED NETWORK FOR ORGAN SHARING (UNOS) 1999: Annual Report of the U.S. Scientific Registry of Transplant Recipients and the Organ Procurement and Transplantation Network, 1999
4. PLOEG RJ, PIRSCH JD, STEGALL MD, *et al*: Living unrelated kidney donation: An underutilized resource? *Transplant Proc* 25:1532–1534, 1993
5. KONING OH, PLOEG RJ, VAN BOCKEL JH, *et al*: Risk factors for delayed graft function in cadaveric kidney transplantation: A prospective study of renal function and graft survival after preservation with University of Wisconsin solution in multi-organ donors. *Eur Multicenter Study Group Transplant* 63:1620–1628, 1997
6. BOOM H, MALLAT MJ, DE FIJTER JW, *et al*: Delayed graft function influences renal function, but not survival. *Kidney Int* 58:859–866, 2000
7. GULANIKAR AC, MACDONALD AS, SUNGURTEKIN U, BELITSKY P: The incidence and impact of early rejection episodes on graft outcome in recipients of first cadaver kidney transplants. *Transplantation* 53:323–328, 1992
8. MATAS AJ: Acute rejection is a major risk factor for chronic rejection. *Transplant Proc* 30:1766–1768, 1998
9. MARCEN R, PASCUAL J, OROFINO L, *et al*: The effect of delayed graft function and early graft rejection on renal transplant outcome. *Transplant Proc* 30:1776–1777, 1998
10. OFFERMANN G: What is a reasonably short cold ischemia time in kidney transplantation? *Transplant Proc* 30:4291–4293, 1998
11. TAKADA M, NADEAU KC, HANCOCK WW, *et al*: Effects of explosive brain death on cytokine activation of peripheral organs in the rat. *Transplantation* 65:1533–1542, 1998
12. VAN DER HOEVEN JA, PLOEG RJ, POSTEMA F, *et al*: Induction of organ dysfunction and up-regulation of inflammatory markers in the liver and kidneys of hypotensive brain dead rats: a model to study marginal organ donors. *Transplantation* 68:1884–1890, 1999
13. PRATSCHKE J, KUSAKA M, WILHELM MJ, TILNEY NL: Chronic rejection: Effect of brain death and ischemia/reperfusion. *Graft* 1:34–36, 1998
14. VAN DER HOEVEN JAB, LINDELL S, VAN SCHILFGAARDE R, *et al*: Donor brain death reduces survival after transplantation in rat livers preserved for 20 hrs. *Transplantation* 72:1632–1636, 2001
15. VAN DER HOEVEN JAB, PLOEG RJ, POSTEMA F, *et al*: Induction of organ dysfunction and activation of inflammatory markers in donor

liver and kidney during hypotensive brain death. *Transplant Proc* 31:1006–1007, 1999

16. Koo DD, Welsh KI, McLaren AJ, *et al*: Cadaver versus living donor kidneys: Impact of donor factors on antigen induction before transplantation. *Kidney Int* 56:1551–1559, 1999

17. Kusaka M, Pratschke J, Wilhelm MJ, *et al*: Activation of inflammatory mediators in rat renal isografts by donor brain death. *Transplantation* 69:405–410, 2000

18. Rahimifar M, Tator CH, Shanlin RJ, Sole MJ: Effect of blood transfusion, dopamine, or normal saline on neurogenic shock secondary to acutely raised intracranial pressure. *J Neurosurg* 70:932–941, 1989

19. Adams DH: Adhesion molecules and liver transplantation: New strategies for therapeutic intervention. *J Hepatol* 23:225–231, 1995

20. Kelly KJ, Williams WW, Jr, Colvin RB, Bonventre JV: Antibody to intercellular adhesion molecule 1 protects the kidney against ischemic injury. *Proc Natl Acad Sci USA* 91:812–816, 1994

21. van Goor H, van der Horst ML, Atmosoerodjo J, *et al*: Renal apolipoproteins in nephrotic rats. *Am J Pathol* 142:1804–1812, 1993

22. Dijkstra CD, Dopp EA, Joling P, Kraal G: The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. *Immunology* 54:589–599, 1985

23. Mertes PM, Carteaux JP, Jaboin Y, *et al*: Estimation of myocardial interstitial norepinephrine release after brain death using cardiac microdialysis. *Transplantation* 57:371–377, 1994

24. Pratschke J, Wilhelm MJ, Kusaka M, *et al*: Brain death and its influence on donor organ quality and outcome after transplantation. *Transplantation* 67:343–348, 1999

25. Herijgers P, Leunens V, Tjandra Maga TB, *et al*: Changes in organ perfusion after brain death in the rat and its relation to circulating catecholamines. *Transplantation* 62:330–335, 1996

26. Wilhelm MJ, Pratschke J, Laskowski IA, *et al*: Brain death and its impact on the donor heart-lessons from animal models. *J Heart Lung Transplant* 19:414–418, 2000

27. Bittner HB, Kendall SW, Chen EP, Van Trigt P: The combined effects of brain death and cardiac graft preservation on cardiopulmonary hemodynamics and function before and after subsequent heart transplantation. *J Heart Lung Transplant* 15:764–777, 1996

28. Pratschke J, Kofla G, Wilhelm MJ, *et al*: Improvements in early behavior of rat kidney allografts after treatment of the brain-dead donor. *Ann Surg* 234:732–740, 2001

29. Grekas D, Alivanis P, Derveniotis V, *et al*: Influence of donor data on graft function after cadaveric renal transplantation. *Transplant Proc* 28:2957–2958, 1996

30. Pratschke J, Wilhelm MJ, Kusaka M, *et al*: A model of gradual onset brain death for transplant-associated studies in rats. *Transplantation* 69:427–430, 2000

31. Sola R, Guirado L, Lopez-Navidad A, *et al*: Renal transplantation with limit donors: to what should the good results obtained be attributed? *Transplantation* 66:1159–1163, 1998

32. Novitzky D, Cooper DK, Morrell D, Isaacs S: Change from aerobic to anaerobic metabolism after brain death, and reversal following triiodothyronine therapy. *Transplantation* 45:32–36, 1988

33. Solez K, Racusen LC, Abdulkareem F, *et al*: Adhesion molecules and rejection of renal allografts. *Kidney Int* 51:1476–1480, 1997

34. Kanagawa K, Ishikura H, Takahashi C, *et al*: Identification of ICAM-1-positive cells in the nongrafted and transplanted rat kidney—An immunohistochemical and ultrastructural study. *Transplantation* 52:1057–1062, 1991

35. Nakai K, Traynor FF, Isobe M: Time course and localization of intercellular adhesion molecule-1 induction in kidney allografts in mice. *Transplant Proc* 26:349–353, 1994

36. Fuggle SV, Sanderson JB, Gray DW, *et al*: Variation in expression of endothelial adhesion molecules in pretransplant and transplanted kidneys—Correlation with intragraft events. *Transplantation* 55:117–123, 1993

37. Rice GE, Munro JM, Corless C, Bevilacqua MP: Vascular and nonvascular expression of INCAM-110. A target for mononuclear leukocyte adhesion in normal and inflamed human tissues. *Am J Pathol* 138:385–393, 1991

38. Lu CY, Penfield JG, Kielar ML, *et al*: Hypothesis: Is renal allograft rejection initiated by the response to injury sustained during the transplant process? *Kidney Int* 55:2157–2168, 1999