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ORIGINAL ARTICLE

Donor dopamine treatment in brain dead rats is associated with an improvement in renal function early after transplantation and a reduction in renal inflammation

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Keywords
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Summary

Brain death (BD) is associated with tissue inflammation. As dopamine treatment of BD donor rats reduces renal monocyte infiltration, we tested if this treatment affects renal function and inflammation in recipients. BD was induced in F344 rats and was maintained for 6 h in all experiments. Dopamine was given for 6 (DA6) or 3 h (DA3) from the onset of BD. Ventilated non-BD (NBD) and BD animals served as controls. Kidneys were transplanted into bilaterally nephrectomized Lewis recipients. Serum creatinine (s-crea) was measured and leukocyte infiltration was assessed 10 days after transplantation. One day after transplantation, s-crea was significantly reduced in recipients who received a renal allograft from dopamine treated BD or from NBD rats compared to BD vehicle ($P < 0.05$). Ten days after transplantation, the number of infiltrating monocytes was significantly lower in grafts obtained from dopamine treated and from NBD rats ($P < 0.05$). A reduced infiltration in these grafts was confirmed by Banff 97 classification. Cytokine-induced neutrophil-chemoattractant 1 and interleukin (IL)-6 mRNA expression were reduced in DA rats compared to BD controls. No difference for macrophage chemoattractant protein 1 and IL-10 were found. These findings may explain the salutary effect of donor dopamine treatment in renal transplantation.

Introduction

Although brain death (BD) is considered an important cause of pretransplantation allograft injury, a majority of renal allografts are still retrieved from deceased donors. Therefore, understanding the mechanisms causing tissue injury in BD donors and investigating possible strategies to overcome or prevent these harmful processes in BD are essential. As BD promotes inflammation in end-organs, affects hormone regulation and haemodynamic stability, it is generally accepted that this condition severely influences organ quality [1–3]. BD seems to be associated with a worse ischaemia/reperfusion injury after transplantation [4], although in large animals, this could not be demonstrated [5,6]. In the sequel of BD, a rapid upregulation of inflammatory mediators like interleukin (IL)-6 and tumour necrosis factor (TNF)-α occurs [7–10]. This might, in turn, result in the upregulation of an array of genes including selectins, fibrinogen and KIM-1 [11]. BD is considered a risk factor for organ dysfunction [12,13] and may accelerate acute rejection episodes [14–16].

As damaging process in organ allografts already occurs during BD, donor treatment may represent a genuine approach to improve organ quality. Several experimental studies have emphasized the applicability of this approach and unambiguously demonstrated its benefit on transplantation outcome. The use of agents that induce endogenous HO-1 expression [17] or the use of anti-inflammatory agents [18], i.e. P-selectin glycoprotein ligand (sPSGL) or steroids seems to be promising in this regard.

In two retrospective clinical studies, Schnuelle *et al.* [19,20] demonstrated that dopamine treatment of BD donors have a beneficial effect on delayed-graft function and long-term renal allograft survival. The favourable effect of donor dopamine treatment might be related to its anti-inflammatory properties, as dopamine inhibits the production of chemokines in renal tubular epithelial and endothelial cells [21]. In addition, dopamine treatment of BD rats reduces monocyte infiltration and significantly improves mean arterial blood pressure (MAP) and organ perfusion [22,23]. As clinical studies have shown that donor dopamine treatment positively affects delayed graft function and acute rejection, this study was conducted to address if dopamine treatment of BD donor rats can influence early renal function and renal inflammation after transplantation. To this end, we harvested renal allograft from BD Fisher rats and transplanted these in Lewis recipients. Renal function and histology were assessed in both the donor and the recipient.

Methods

Animals

Inbred male Lewis (LEW, $RT1^{1}$) and Fisher (F344, $RT1^{1vr}$) rats weighing 200–250 g were obtained from Charles River (Sulzfeld, Germany). Animals were kept under standard conditions and fed standard rodent chow and water *ad libitum*. All procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences and were approved by the local authorities (RP Karlsruhe, AZ 35–9185.81/27/04).

Experimental protocol

Before induction of BD, donor animals were anaesthetized with ketamine (Ketanest, Pfizer, Karlsruhe, Germany; 100 mg/kg intraperitoneally) and xylazine (Rompun, BayerVital, Leverkusen, Germany; 6 mg/kg intraperitoneally) and placed on a heating table to keep their body temperature constant. A 3F Fogarty catheter was inserted epidurally in an occipital burr hole and gradually inflated during 1 min with 200 μl of saline. The state of BD was verified by the occurrence of autonomic storm, the absence of corneal reflexes and by an apnoea test. All animals were mechanically ventilated by a tracheostoma with a rodent ventilator (Ugo Basile, Comerio, Italy). Systemic blood pressure MAP (mmHg) was continuously measured (6 h) in the donors using a femoral arterial catheter (Statham pressure transducer P23Db and a Gould pressure processor; FMI, Ober-Beerbach, Germany). Anaesthetized, non-brain-dead ventilated donor animals served as controls. Recipients were anaesthetized with enflurane (Ethrane; Aca Mueller/Adag Pharma, Gottmadingen, Germany). Experiments were performed in the allogeneic Fisher–Lewis rat model. Animals were divided into five groups. Donor animals were treated intravenously by microinjection pumps (CMA/100, CMA/Microdialysis, Solna, Sweden) according to the following scheme:

Group 1: Fisher donor rats were ventilated and treated intravenously with NaCl 0.9% for 6 h [non-BD (NBD) group].

Group 2: Brain death was induced in Fisher donor rats. BD lasted 6 h; the animals were ventilated and treated with NaCl 0.9% (BD group).

Group 3: Brain death was induced in Fisher donor rats. BD lasted 6 h; the animals were ventilated and treated with NaCl 0.9% and HES (hydroxy ethyl starch) to normalize blood pressure [BD normotensive (BD-normot) group].

Group 4: Brain death was induced in Fisher donor rats. BD lasted 6 h; the animals were ventilated and treated for 3 h with 10 μg/min/kg dopamine [23] [dopamine treated group (DA3)].

Group 5: Brain death was induced in Fisher donor rats. BD lasted 6 h; the animals were ventilated and treated for 6 h with 10 μg/min/kg dopamine [dopamine treated group (DA6)].

Infusion of dopamine and control solutions started at the beginning of BD induction. In each group, the kidney was harvested after 6 h, flushed with 1 ml of cold UW solution and transplanted in allogeneic bilaterally nephrectomized Lewis rats. The transplantation was performed as previously published [24–26]. No immunosuppression was administered. Each group consisted of a minimum of six animals.

Renal function

Renal function was assessed both in donors and in recipients by serum creatinine. In the recipients, serum creatinine was measured on days 0, 1, 3, 5, 8 and 10 after transplantation, while in the donors, serum creatinine was measured before induction of BD and at the end of the BD period.

Immunohistochemistry

Renal grafts were harvested 10 days after transplantation. The upper pole of the kidney was frozen in liquid nitrogen and the remaining part fixed in 10% buffered formalin solution. Serial sections (4 μm) of paraffin embedded tissue were fixed in 10% neutral buffered formalin for immunohistochemical staining. The sections were extensively washed with phosphate-buffered saline (PBS) and subsequently treated with 3% hydrogen peroxide. Endogenous biotin activity was blocked using the Avidin blocking kit (Vector, Burlingame, CA, USA). Monocytes and macrophages were detected by ED1 [monoclonal mouse anti-rat (Linaris Biologische Produkte GmbH, Bettingen, Germany)] and by major histocompatibility complex (MHC) class II expression (F-17–23–2, monoclonal mouse anti-rat; Linaris). Incubations of primary and secondary antibodies were sequentially applied and to the sections for 1 h. After each incubation step, the sections were extensively washed with PBS. Standard avidin–biotin complex staining was performed according to the manufacturer's instructions (ABC kit; Vector). After addition of 3,3′ diaminobenzidine substrate and washing, the sections were counterstained with haematoxylin. Evaluation of ED1 and MHC class II positive cells was performed in a blinded fashion at 400× magnification. At least six animals per group and 20 fields per section were analysed.

Light microscopy

Paraffin sections were stained with haematoxylin–eosin, periodic acid-Schiff, and trichrome. A minimum of 20 microscopic fields per graft were assessed. Histological grading was performed according to the Banff '97 classification [27]. Sections were blindly evaluated and graded by a renal pathologist (R.W.). Histological evaluation and grading included transplant glomerulopathy, tubulointerstitial fibrosis, tubular atrophy and vasculopathy. The histological grading scale was from 0 to 3 (0 = not present, 1 = mild alteration, 2 = moderate alteration and 3 = severe alteration).

Histomorphometric analysis

Haematoxylin–eosin stained sections were used to determine glomerular size.

The glomerular volume (Vg) was calculated from the mean planar area of glomeruli of which the glomerular tuft and the macula densa could be seen. At least ten glomeruli per section were evaluated. Mean glomerular area (Ag) was estimated by the surface calculating tool of analySIS. Volume was calculated according to the Weibel and Gomez method [28,29]:

$$Vg = Ag^{3/2} * \beta / d$$

in which the shape coefficient of the sphere (β) is 1.38 and the size distribution of the glomeruli (d) is 1.01 representing the size assuming a 10% coefficient of variation of the caliper diameter.

Light cycler polymerase chain reaction

Grafts from ventilated NBD-, NaCl treated and dopamine treated BD rats were investigated 10 days after transplantation. Snap-frozen tissue samples were homogenized using a Polytron homogenizer (IKA Labortechnik/Fischer Scientific, Wohlen, Switzerland). 500 ng of total RNA was reverse-transcribed into cDNA according to the manufacturer's instructions, using the first Strand cDNA Synthesis Kit. cDNA was diluted in 20 μl DEPC-treated water and stored at −80 °C until use. Specific DNA standards were generated by PCR amplification of cDNA, purification of the amplified products and quantification by spectrophotometry. Light cycler PCR of cDNA specimen and DNA standards were conducted in a total volume of 25 μl, containing 2 μl FastStart DNA Master SYBR GreenI, 10 pmol of each forward and reverse primer and 2 mM $MgCl_2$. Primer sequences were as follows: cytokine-induced neutrophil-chemoattractant 1 (CINC-1) (forward: 5′-AGT TTG AAG GTG ATG CCG C-3′, reverse: 5′-GGA CAC CCT TTA GCA TCT TTT G-3′), interleukin 6 (IL6) (forward: 5′-GAT ACC ACC CAC AAC AGA CCA G-3′, reverse: 5′-GCC ATT GCA CAA CTC TTT TCT C-3′), macrophage chemoattractant protein 1 (MCP-1) (forward: 5′-CAG ATG CAG TTA ATG CCC CA-3′, reverse: 5′-CCT GCT GCT GGT GAT TCT CTT-3′) and interleukin 10 (IL-10) (forward: 5′-TAC CTG GTA GAA GTG ATG CCC C-3′, reverse: 5′-AAT CGA TGA CAG CGT CGC A-3′). The amplification profile consisted of 2 min at 50 °C and 5 min at 95 °C followed by 45 cycles of amplification, each cycle consisting of denaturation at 95 °C for 15 s, annealing for 20 s at 55 °C and extension for 30 s at 72 °C. Standard curves were generated in all experiments. PCR efficiency was assessed from the slopes of the standard curves and was found to be between 90% and 100%. Linearity of the assay could be demonstrated by serial dilution of all standards and cDNA. All samples were normalized for an equal expression of GAPDH.

Statistical analysis

Numerical data are expressed as mean ± standard deviation. For immunohistological parameters, renal function, PCR-analysis and histomorphometric analysis, statistical analysis was performed using the Kruskal–Wallis test with

option for multiple comparisons (StatsDirect 2.2.8; Altrincham, UK). For analysis of the blood pressure data, two-way ANOVA was applied. For analysis of light microscopy, Fisher's exact test was used. For survival analysis, Kaplan–Meier, Logrank and Wilcoxon tests were applied. A *P*-value of less than 0.05 was considered significant.

Results

Influence of dopamine on mean arterial pressure and renal function in the donor

Brain death induced profound haemodynamic alterations, which were characterized by an initial increase in MAP, followed by a sharp decline leading to persistent hypotension. Although in NBD animals MAP gradually declined during 6 h of ventilation, there was a significant difference in MAP observed between BD and NBD in the first 3 h (Fig. 1a). MAP in BD animals was completely normalized by installation of dopamine during this period or by infusion with HES (BD-normot). Cessation of dopamine infusion slightly decreased MAP compared to animals that were continuously treated with dopamine over the whole BD period (Fig. 1b and c).

Serum creatinine increased during BD in all donors. The rise in serum creatinine was not specific for BD, as it also occurred in ventilated not BD donors. Serum creatinine was not significantly influenced by dopamine in the BD donors. (Fig. 1d).

Donor dopamine treatment is associated with a better renal function in recipients

The rise in serum creatinine 1 day after transplantation was significantly less in dopamine treated and NBD groups compared with vehicle treated BD rats [1.7 ± 0.9 vs. 0.8 ± 0.4 (DA3) and 0.8 ± 0.2 (DA6), vehicle treated versus dopamine treated BD animals $P < 0.05$]. Although there was also a trend for better renal function at day 3 and 5 after transplantation, this did not reach statistical significance because of the large standard deviation in the BD group. Serum creatinine in recipients which received

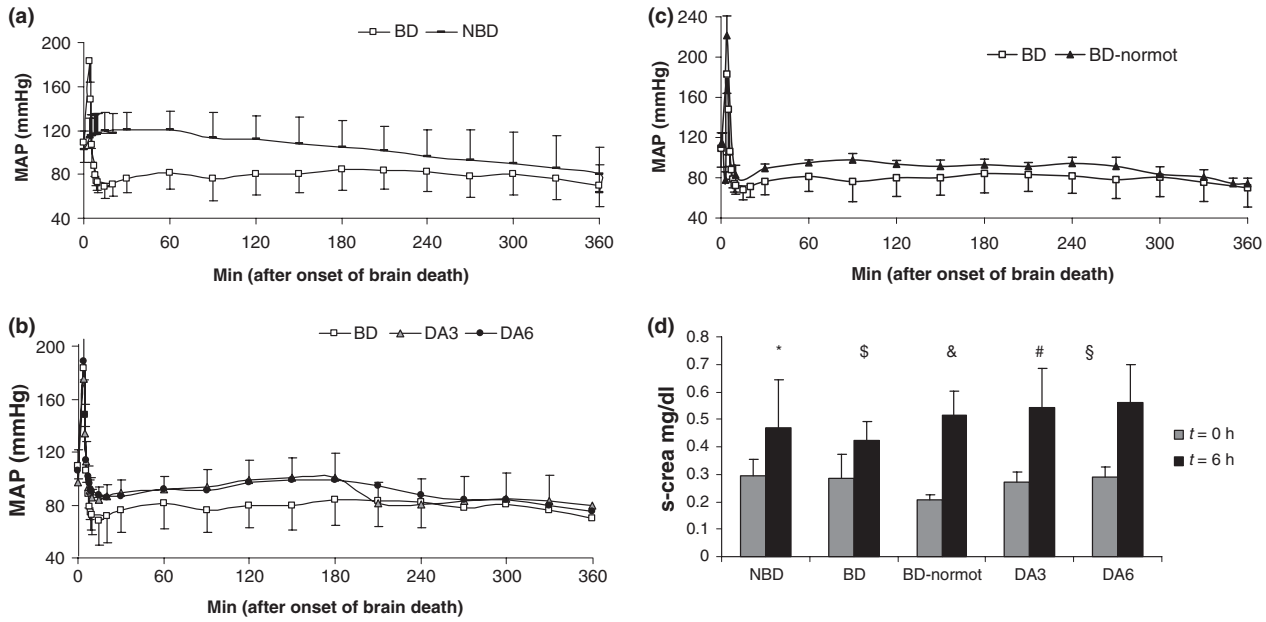

Figure 1 (a) BD vehicle (open squares) in comparison to NBD group (black line). Although in non-BD animals MAP gradually declined during 6 h of ventilation, there was a significant difference in MAP observed between BD and NBD in the first 3 h. The results are expressed as MAP (mmHg) of at least four animals in each group. (b) Hemodynamic changes in BD donor rats. MAP was recorded as described in the method section. Dopamine treatment during BD (closed circles) significantly improved MAP compared to NaCl treated BD rats (DA6 versus BD, $P < 0.05$). After cessation of dopamine treatment (DA3, grey triangles) MAP was not different from the NaCl treated BD rats (open squares). The results are expressed as MAP (mmHg) of at least four animals in each group. (c) BD vehicle (open squares) in comparison to BD normotensive group (closed triangle). HES-infusion stabilized blood pressure during BD. The results are expressed as MAP (mmHg) of at least four animals in each group. (d) Serum creatinine (mg/dl) before and 6 h after brain death induction in brain death donor rats or before and 6 h after intubation in the living controls (NBD). Serum creatinine levels increased during 6 h in all groups significantly (NBD, $t = 0$ vs. $t = 6$: $^*P < 0.05$; BD, $t = 0$ vs. $t = 6$: $\$P < 0.01$; BD-normot, $t = 0$ vs. $t = 6$: $\&P < 0.01$; DA3, $t = 0$ vs. $t = 6$: $\#P < 0.01$; DA6, $t = 0$ vs. $t = 6$: $\S P < 0.01$). Each group consisted of five to seven animals. Data are shown as means ± standard deviation.

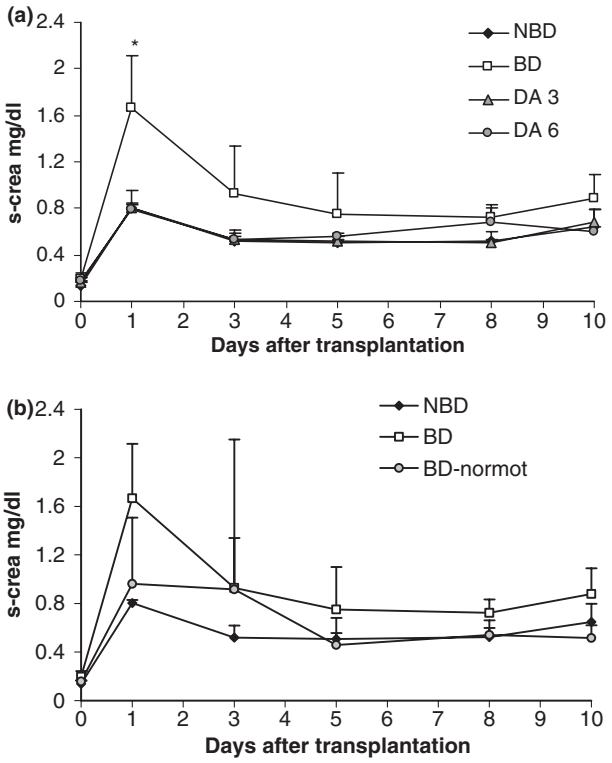

Figure 2 (a) Serum creatinine (mg/dl) on day 0, 1, 3, 5, 8 and 10 after transplantation. Dopamine treatment in brain dead donors significantly improved renal function in the recipients when compared to BD (BD versus DA3 and DA6, $*P < 0.05$). Each group consisted of five to seven animals. Data are shown as means ± standard deviation. (b) The normotensive BD group showed a tendency for reduced serum creatinine, but this did not reach statistical significance. Data are shown as means ± standard deviation.

a graft from dopamine treated BD donors did not significantly differ from that of recipients receiving a renal allograft from NBD animals (0.8 ± 0.5). Recipients receiving a graft from BD donors who were treated with HES showed a tendency for a decreased serum creatinine, but this was not statistically significant (1.7 ± 0.9 vs. 1.0 ± 0.6, BD versus BD-normot, P = NS) (Fig. 2).

Banff-classification

Light microscopic analysis according to the Banff 97 classification revealed a higher tubulitis and interstitial inflammation score in renal allografts obtained from BD animals (BD/BD-normot. versus NBD: $P < 0.05$, Table 1). Tubulitis and interstitial inflammation was reduced in the DA3 and DA6 treated groups, although in the latter group, this did not reach statistical significance [vehicle treated versus dopamine treated (DA3) BD animals: $P < 0.05$, Table 1].

Table 1. Histological scores according to the Banff '97 classification of allografts 10 days after transplantation.

Banff*	NBD (n = 7)	BD (n = 7)	BD-normot (n = 5)	DA3 (n = 6)	DA6 (n = 6)
i 0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
i 1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
i 2	4 (57.2)	0 (0)	0 (0)	4 (66.6)	2 (33.3)
i 3	3 (42.8)	7 (100)	5 (100)	2 (33.3)	4 (66.6)
t 0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
t 1	4 (57.2)	0 (0)	0 (0)	4 (66.6)	2 (33.3)
t 2	3 (42.8)	7 (100)	5 (100)	2 (33.3)	4 (66.6)
t 3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
v 0	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.6)
v 1	6 (85.7)	7 (100)	3 (60)	6 (100)	5 (83.3)
v 2	0 (0)	0 (0)	2 (40)	0 (0)	0 (0)
v 3	1 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)

Living (NBD) or brain death (BD) donors treated with saline or with HAES respectively (BD-norm). In addition, two groups of brain dead donors were treated with dopamine over 3 (DA3) and 6 h (DA6) after onset of brain death.

The results are expressed as the number of animals with a particular Banff '97 score; in parentheses, the proportion of animals with this score in each group is calculated and expressed as %. NBD and DA3 had significant less mononuclear cell interstitial inflammation and tubulitis ($P < 0.05$).

*Banff classification: i, mononuclear cell interstitial inflammation; t, tubulitis; v, intimal arteritis. The numbers represent the severity scale: 0, absent; 1, mild alteration; 2, moderate alteration; 3, severe alteration.

Donor dopamine treatment is associated with a reduction in monocyte infiltration in the recipient's graft

In accordance with the Banff classification, the number of ED1 positive monocytes in renal allografts obtained from BD animals was significantly higher than that obtained from NBD animals (39 ± 7 vs. 28 ± 5 ED1 positive cells, BD versus NBD animals, $P < 0.01$). Donor dopamine treatment significantly reduced the number of ED1 positive monocytes [39 ± 7 vs. 28 ± 6 (DA3) and 30 ± 5 (DA6), vehicle treated versus dopamine treated BD animals, $P < 0.05$] (Fig. 3).

Cytokine expression in the grafts 10 days after transplantation

To investigate if the reduced renal inflammation in allografts obtained from dopamine treated BD animals was associated with a change in cytokine expression, IL-6, IL-10, MCP-1 and CINC-1 mRNA expression was assessed in these grafts. Although there was a tendency that IL-6 mRNA was decreased in renal allografts in the DA3 group, this did not reach statistical significance [IL-6/GAPDH ratio: 9 ± 5 vs. 4 ± 2, vehicle versus dopamine (DA3) treated BD animals, $P = 0.06$] (Fig. 4a). Likewise, MCP-1 mRNA expression between the groups was not

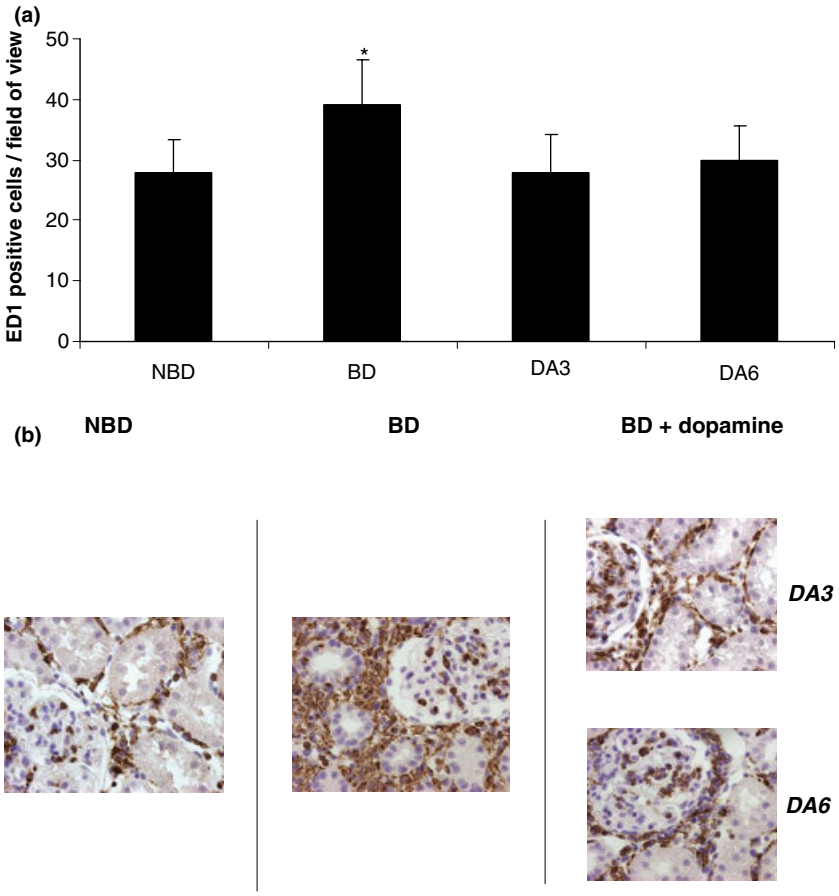

Figure 3 (a) Analysis of ED-1 positive cells in renal allografts. Living donors (NBD) and brain death donors (BD) were treated with saline. Brain death donors of group 4 and 5 were treated additionally with dopamine over 3 or 6 h from the onset of brain death. Renal allografts were transplanted into Lewis recipients. Ten days after transplantation, the transplanted grafts were collected and analysed. The number of ED1 positive cells was reduced in the grafts from DA3 (28 ± 6) and DA6 (30 ± 5) donors compared to BD vehicles (39 ± 7) (DA versus BD, $P < 0.05$). The results are expressed as mean number of positive cells per field of view ± standard deviation. At least 120 fields of view were analysed comprising six to seven animals per group. Analysis was performed using a magnification of 400×. (b) Representative immunohistological staining for ED1+ cells in renal allografts collected 10 days after transplantation. ED1 expression obtained from NDB, BD, DA3 and DA6 is depicted. Original magnification: 400×.

significant [MCP-1/GAPDH ratio: 2406 ± 210 vs. 2003 ± 726, vehicle versus dopamine (data not shown)]. Also, the increased IL-10 mRNA expression in the dopamine treated group did not reach statistical significance [IL-10/GAPDH ratio: 116 ± 25 vs. 131 ± 58, vehicle versus dopamine (data not shown)]. In contrast, CINC-1 mRNA expression was significantly decreased in the DA3 treated group [CINC-1/GAPDH ratio: 80 ± 25 vs. 34 ± 15, vehicle versus dopamine (DA3) treated BD animals, $P < 0.05$]. CINC-1 mRNA expression in this group was similar to that of the NBD group (CINC-1/GAPDH ratio: 38 ± 35) (Fig. 4b).

Glomerular volume

Ten days after transplantation, the glomerular volume was significantly larger in grafts from BD compared with grafts from NBD donors (BD versus NBD: 1.9 ± 0.2 vs. 1.54 ± 0.2 μm^3, $P < 0.01$). Donor dopamine treatment did not significantly influence glomerular volume [vehicle treated versus dopamine treated BD animals: 1.9 ± 0.2 vs. 1.76 ± 0.3 (DA3) and 1.73 ± 0.3 (DA6), Fig. 5].

Discussion

Brain death is considered an important donor associated risk factor, which influences organ quality [13,15,30]. Deterioration in organ quality may be related to a number of processes that can occur during BD, e.g. hypotension, reduced organ perfusion, hypothermia, coagulopathies and inflammation in end-organs [1,31,32]. As dopamine pretreatment in donors reduces inflammation in donor renal allografts [22,33], we investigated in this study graft outcome in recipients who received renal allografts from dopamine treated BD donors. The main findings of this study are the following. First, donor dopamine treatment improved mean arterial blood pressure, but it did not significantly influence renal function in the donor before harvesting. Second, renal function in the recipient was significantly better in rats receiving a renal allograft obtained from a dopamine treated BD donor compared with the control rats. Third, 10 days after transplantation, the number of graft infiltrating cells was significantly reduced in the donor dopamine treated group. This was reflected by lower Banff 97 tubulitis and interstitial inflammation score.

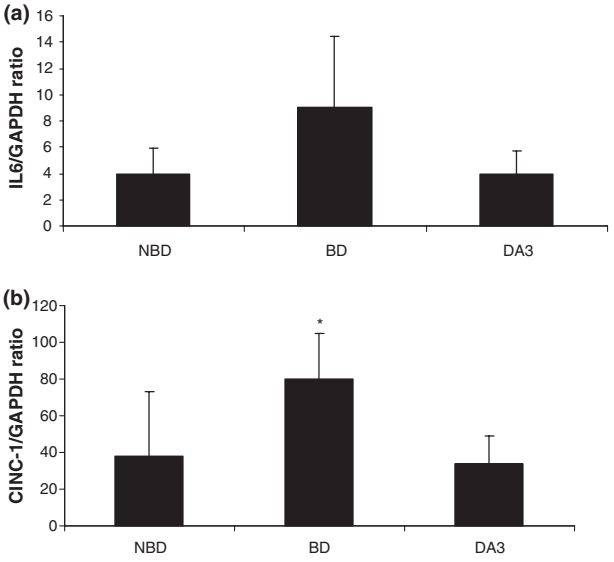

Figure 4 Quantitative PCR-analysis for IL-6 (a) and CINC-1 (b) gene expression in grafts of ventilated non-BD (NBD), NaCl treated brain death (BD) and dopamine treated brain death (DA3) animals 10 days after transplantation. The results are expressed as mean CINC-1/S16 and IL-6/S16 ratio ± SD. In each group, kidneys from four animals were analysed. CINC-1: BD versus DA, 80 ± 25 vs. 34 ± 15, $P < 0.05$. IL-6: BD versus DA, 9 ± 5 vs. 4 ± 2, P = NS; BD versus NBD, 9 ± 5 vs. 4 ± 2, P = NS.

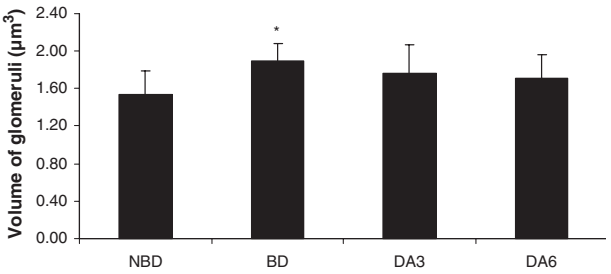

Figure 5 Enlargement of glomerular volume. Ten days after transplantation, glomerular volume in grafts from NBD animals was significantly less compared with grafts from BD rats (BD versus NBD: $P < 0.05$).

In BD donors, serum creatinine was slightly increased at the end of the BD period. This was, however, not related to BD, as it was also observed in ventilated NBD rats. Although dopamine increases renal blood flow, glomerular filtration rate, urinary sodium and water excretion [34], serum creatinine did not change in the BD donors in our study. In comparison with grafts obtained from untreated BD donors, renal function was significantly better 1 day after transplantation when the grafts were obtained from dopamine treated BD or NBD donors. As renal function recovered in time, statistical significance disappeared, but there was still a trend for a better renal function in these two groups. We have chosen to include a group in which dopamine infusion was stopped after 3 h to investigate if 3 h of donor dopamine treatment was sufficient to influence renal function and inflammation in the recipient. We could already show that 3 h of dopamine treatment was able to reduce infiltration of inflammation cells in BD donor kidneys significantly [23]. Indeed, 3 h of dopamine treatment was sufficient to influence renal function and inflammation in the recipient in a beneficial way. In the human situation, early renal function has an enduring effect on the subsequent course after renal transplantation [35–37] and predicts a 5-year graft survival [38]. Improvement in early renal function by donor dopamine treatment may therefore significantly improve long-term graft prognosis [19,20].

It was surprising that some of the beneficial effects were observed for both dopamine treatment regimes, e.g. renal function in the recipients, while others, e.g. inflammation, were only seen for 3 or 6 h of dopamine infusion. Nevertheless, if both dopamine groups were pooled, statistical analysis also revealed that in the dopamine treated groups, there was significant less inflammation compared with the untreated BD group.

Hypotension may lead to reduced organ perfusion, tissue ischaemia and generation of reactive oxygen species (ROS) [13,39]. Thus, haemodynamic stabilization before organ procurement can improve organ quality by limiting ROS mediated organ damage. Moreover, haemodynamic stabilization seems to reduce organ inflammation during BD as was previously demonstrated [11–13,33,40]. Although it can be argued that improvement in blood pressure and organ perfusion largely contribute to the beneficial effect of donor dopamine treatment, in this study, we also demonstrated that other strategies to improve blood pressure during BD were less effective or did not influence graft infiltration as assessed by serum creatinine and Banff classification respectively. Banff 97 classification revealed lower tubulitis and interstitial inflammation scores in the donor dopamine treated groups compared with the BD normotensive group. These findings therefore indicate that donor dopamine treatment can affect transplantation outcome independently of its haemodynamic effect. This is in accordance with the clinical findings of Schnuelle *et al.* [41], demonstrating that the favourable effect of donor dopamine treatment was independent of donor blood pressure.

If improved haemodynamics only partially can explain the beneficial effect of donor dopamine treatment, then what other factors may be considered? First, dopamine might ameliorate ischaemia/reperfusion (I/R) injury as we have previously demonstrated [42]. Second, dopamine treatment reduces monocyte infiltration during BD and hence reduces the number of passenger leukocyte in the

graft [22,33]. These mobile cells migrate out of the graft into secondary lymphoid organs where they can initiate an immune response against the graft [43]. While amelioration of I/R injury reduces renal inflammation and improves renal function, reduction in the number of passenger leukocytes may decrease the response to the allogeneic kidney [44]. As tubulitis is a hallmark for acute interstitial rejection after renal transplantation in men, our data indicate that dopamine given to the donor may influence the process leading to acute interstitial rejection, as evidenced by a lower Banff tubulitis score.

Nevertheless, it remains to be further elucidated how exactly dopamine influences renal inflammation *per se*. Previously, we showed that dopamine has the propensity to inhibit IL-8 production in renal tubular epithelial cells [21,45]. In this study, we now demonstrate that CINC-1 expression, a rat homologue for IL-8, is significantly reduced 10 days after transplantation in the donor dopamine treated group. We also observed a tendency for reduced IL-6 and MCP-1 expression in these grafts. A reduction in chemokine expression might also contribute to a decreased inflammatory response in the transplanted renal allograft.

Glomerular enlargement in renal allografts is associated with inferior graft survival [28] and with renal allograft dysfunction [46]. Ten days after transplantation, glomerular size was significantly larger in grafts obtained from BD rats. Donor dopamine treatment, however, did not significantly influence glomerular size in these grafts.

This study demonstrates that donor dopamine treatment during BD may provide a benefit on graft survival both by improving early renal function after transplantation and by reducing renal inflammation. Our data are in concordance with the clinical studies of Schnuelle *et al.* [20], which found that donor dopamine usage was associated with improved renal function, less acute rejection episodes and improved long term graft survival. These data therefore justify a prospective randomized multi-centre study on the beneficial effects of donor dopamine usage in terms of transplantation outcome.

Authorship

SH: performed research, analysed data and wrote the paper. AR: performed research and analysed data. UG and FD: analysed data. CB and ZK: collected data. MAS, WJVS and PS: designed research. RW: analysed histological data. BAY: designed research and was involved in writing the paper.

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