The alloantigen-independent factors brain death and cold ischemia
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Chapter 3: The Additional Detrimental Effects of Cold Preservation on Transplantation Associated Injury in Kidneys from Living and Brain-Dead Donor Rats

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ABSTRACT

Background Brain death and cold preservation are major alloantigen-independent risk factors for transplantation outcome. The present study was conducted to assess the influence of these factors on transplantation associated injury independently or in combination.

Methods Brain death (BD) was induced in F344 rats. Renal grafts were harvested after 6 hrs and either directly transplanted in unilateral nephrectomized Lewis recipient or subjected to 24 hrs of cold preservation in UW solution before implantation. Allografts obtained from living donor (LD) rats were also subjected to cold preservation or not. DNA damage was assessed before implantation by TUNEL staining. Ten days after transplantation renal histology was performed according to Banff 97 classification. The expression of cytokines and adhesion molecules were analysed by quantitative PCR.

Results Cold preservation significantly increased the number of TUNEL positive cells in renal allografts. Ten days after transplantation histology revealed a higher degree of tubulitis and vasculitis scores when the grafts were subjected to cold storage. Vasculitis was aggravated when the graft was obtained from BD donors. BD, but not cold preservation alone, was associated with papillary necrosis. This was more frequently observed after cold preservation. Immunohistology showed an increase of MHC class II+ cells after cold preservation. The combination of BD and cold preservation revealed a higher degree of VEGF and IL-10 expression.

Conclusions Our Study emphasizes that cold ischemia time should be limited when renal allografts from brain-dead donors are transplanted.
INTRODUCTION

Kidney transplantation is the treatment of choice for the majority of patients with end-stage renal disease. In the face of the increasing shortage of donor organs, the number of renal transplantsations using allografts form living donors has increased substantially over the past decade. Nevertheless, the majority of renal allografts are still retrieved from deceased donors. Yet, transplantation outcome is poor compared to that of renal allografts obtained from living donors [1, 2].

It has been well established that in the sequel of brain death, perturbation of organ function and structure develops, possibly as a consequence of hemodynamic instability, hypotension, hypothermia, and increased inflammation [3] [4-8]. Clearly, this will influence organ quality and hence affect long-term graft survival. Evidence has culminated in various experimental brain death models, that organs derived from such animals are prone to accelerated rejection after heart or renal transplantation [9-11]. Because brain death is a potential risk factor for transplantation outcome, there is an increasing urge to improve organ quality of deceased donors by means of donor treatment. The use of steroids [12], soluble P-selectin glycoprotein ligand (sPSGL) [12] or dopamine [13-16] are only a few examples that have shown a beneficial effect in this context.

Apart from brain death, organs from deceased donors are further injured by prolonged cold preservation, another alloantigen-independent factor which may in turn lead to functional deterioration [17, 18]. Prolonged cold storage is associated with poor graft outcome [17, 18] as it significantly affects organ quality by increasing tissue necrosis or apoptosis during organ preservation and reperfusion [19-22]. Hypothermia also impairs the endothelial barrier function, thus resulting in parenchymal oedema and haemorrhage [23]. Aside from donor age, brain death and cold ischemia time have such a profound influence on graft outcome, they are likely the most important donor variables that might explain the difference in long-term graft survival between allografts from living versus deceased donors.

Although a number of studies already have documented the single influence of cold ischemia time or brain death on transplantation outcome, only in a few of these, have a combination of brain death and cold ischemia been investigated [24, 25]. Therefore, in the present study we addressed to what extent brain death and cold ischemia, have a synergistic effect on organ damage, tissue inflammation and graft histology after renal transplantation.
METHODS

Animals

Inbred male Lewis (LEW, RT1\(^{1}\)) and Fisher (F344, RT1\(^{1\text{vr}}\)) rats weighing 200 to 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 brain death, donor animals were anesthetized 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 epidural in an occipital burr hole and gradually inflated during 1 minute with 200 µL of saline. The state of brain death 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 (mean arterial pressure [MAP], mm HG) was continuously measured (6 hrs) in the donors by a femoral arterial catheter (Statham pressure transducer P23Db and a Gould pressure processor, FMI, Ober-Beerbach, Germany). Six hours post brain death, the left kidneys were harvested and either persevered for 24 hours in UW-solution or transplanted directly into recipients. Anesthetized living donor animals served as controls. Recipients were anesthetized with enflurane (Ethrane; Aca Mueller/Adag Pharma, Gottmadingen, Germany). Experiments were performed in the allogeneic Fisher-Lewis rat model. Animals were divided into 4 groups.

**Group 1:** LD-Cp, grafts from living Fisher donors (LD) directly transplanted (-Cp) in unilaterally nephrectomized Lewis recipients

**Group 2:** LD+Cp, grafts from living Fisher donors (LD) subjected to 24 hours of cold preservation (+Cp) before transplantation in unilaterally nephrectomized Lewis recipients

**Group 3:** BD-Cp, grafts from brain death Fisher donors (BD) directly transplanted (-Cp) in unilaterally nephrectomized Lewis recipients

**Group 4:** BD+Cp, grafts from brain death Fisher donors (BD) subjected to 24 hours of cold preservation (+Cp) before transplantation in unilaterally nephrectomized Lewis recipients.
No immunosuppression was administered. Each group consisted of a minimum of six animals. Kidneys were harvested 10 days after transplantation. The upper poles were frozen in liquid nitrogen, and the remaining part was fixed in 10% buffered formalin solution.

**TUNEL-Staining**

TUNEL staining was performed according to the manufacturer’s instructions (Roche Diagnostics, Mannheim, Germany). In brief, tissue sections were washed twice with phosphate-buffered saline after fixation and incubated for 15 min at 70°C in permeabilization solution (0.1 mM sodium citrate). Hereafter the sections were extensively washed and incubated for 30 min at 37°C with 50 µL of reaction mixture in humid chambers. In each experiment and for each staining a negative control was included by omitting dUTP transferase from the reaction mixture. TUNEL positive cells were detected by immunofluorescence microscopy using a fluorescein isothiocyanate filter.

**Histology and Immunohistochemistry**

Paraffin embedding of harvested grafts was performed using routine procedures. Paraffin sections (4 µm) were fixed in 10% neutral buffered formalin. For histology the sections were stained with hematoxylin-eosin, periodic acid-Schiff, and trichrome. A minimum of 20 microscopic fields were assessed for each graft. Histologic grading was performed according to the Banff ’97 classification [26], reporting on renal transplant biopsies. Sections were evaluated in a blinded fashion and graded by a renal pathologist (R.W.). Histologic evaluation and grading included transplant glomerulopathy, tubulointerstitial fibrosis, tubular atrophy, and vasculopathy. The histologic grading scale was from 0 to 3 (0=not present, 1=mild alteration, 2=moderate alteration, and 3=severe alteration).

For immunohistochemical staining the sections were first rinsed in phosphate-buffered saline followed by blocking of endogenous peroxidase (3% hydrogen peroxide) and biotin (Avidin blocking kit, Vector, Burlingame, CA, USA). The sections were incubated for 1 hr with ED1 monoclonal antibody for detection of monocytes and macrophages or with F-17–23–2 monoclonal antibody directed against major histocompatibility complex (MHC II) or with anti-CD3 monoclonal antibody for detection of T-cells (all from Linaris GmbH, Wertheim, Germany). Hereafter the sections were incubated with species-specific secondary antibodies for 1 hr. Sections incubated with murine or rabbit IgG were used as negative controls. Standard avidin-biotin complex staining was performed according to the manufacturer’s
instructions (ABC kit, Vector Burlingame, CA, USA). After addition of the 3,3’
diaminobenzidine substrate the sections were counterstained with hematoxylin, dehydrated in
graded ethanol and xylene and mounted in Permount. ED1, MHC II and CD3 positive cells
were counted in a blinded fashion under the microscope using a 400× magnification. A
histological grading scale was used for quantification (0=no positive cells present, 1=less than
25 positive cells/microscopic field, 2=25 to 50 positive cell/microscopic field, and 3= more
than 50 positive cells/microscopic field). At least seven or eight animals per group and 20
microscopic fields were evaluated.

Quantitative PCR
Snap-frozen tissue samples were homogenized using a Polytron homogenizer (IKA
Labortechnik/Fischer Scientific, Schwerte, Germany). 500 ng of total RNA was reversed
transcribed into cDNA according to the manufacturer’s instructions, using the 1st 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 VEGF, IL10, VCAM,
ICAM-1, E-selectin, IL-6 and TNFα forward and reverse primers and 2mMgCl₂. Primer
sequences were as follows: VEGF (forward 5’ TTC ATC ATT GCA GCA GCC C 3’,
reverse: 5’ CAG CTA TTG CCG TCC AAT TGA 3’), IL10 (forward: 5’TAC CTG GTA
GAA GTG ATG CCC C 3’, reverse: 5’AAT CGA TGA CAG CGT CGC A 3’), VCAM 1
(Applied Biosystems (AB), Foster City, CA, USA; material number Rn00563627_m1),
ICAM-1 (AB; material number Rn00564227_m1), E-selectin (AB; material number
Rn00594072_m1), IL6 (AB; material number Rn99999011_m1) and TNFα (AB; material
number Rn99999017_m1). The amplification profile consisted of 2 minutes at 50°C and 5
minutes at 95°C followed by 45 cycles of amplification, each cycle consisting of denaturation
at 95°C for 15 seconds, annealing for 20 seconds at 55°C and extension for 30 seconds 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. IL-10 and VEGF PCR were performed
by light cycler, all other PCR were performed on a taqman platform.
Statistical Analysis

Numerical data are expressed as mean ± standard deviation. For immunohistological parameters statistical analysis was performed using Fisher’s exact test (StatsDirect 2.2.8, Aswell, UK). For analysis of light microscopy, Fisher’s exact test was applied. For PCR-analysis Kruskal-Wallis test with option for multiple comparisons was applied. A P-value of less than 0.05 was considered as significant.
RESULTS

DNA damage

To study if BD and or cold ischemia affected organ quality before implantation, we assessed DNA damage in renal allografts by means of TUNEL staining. In renal allografts from LD only a few TUNEL positive cells were detected (1±3). This was slightly increased in allograft from BD donors (3±4). When renal allografts were subjected to cold preservation the number of TUNEL positive cells increased significantly both in LD (12±6) and BD (32±10) donors, compared to allografts that were not subjected to cold preservation (LD-Cp vs. LD+Cp: P<0.05; BD-Cp vs. BD+Cp: P<0.01). Significantly more TUNEL positive cells were found in BD donors compared to LD (Fig 1).

Fig. 1 TUNEL positive cells in renal allograft directly before transplantation. Note that BD alone does significantly affect apoptosis, although it clearly aggravates apoptosis when the grafts were subjected to cold preservation. Original magnification: 400x.
**Cold ischemia enhances renal infiltration after transplantation**

We next assessed severity of inflammation response 10 days after transplantation in renal allografts obtained from all groups. When the allografts were not subjected to cold preservation (Cp), there was a tendency for an increased number of infiltrated ED1+ cells in grafts obtained from brain dead (BD) donors compared to grafts from living donors (LD) (Table 1A, LD-Cp vs BD-Cp \( P=NS \)). Prolonged cold preservation (24 hrs in UW solution) increased the inflammatory response, in both LD and BD, although in the former this was not statistically significant (Fig 2 and Table 1, LD-Cp vs LD+Cp, \( P=NS \), BD-Cp vs BD+Cp, \( P<0.05 \)).

<table>
<thead>
<tr>
<th>Histologic grading scale*</th>
<th>LD-Cp (n=7)</th>
<th>LD+Cp (n=7)</th>
<th>BD-Cp (n=7)</th>
<th>BD+Cp (n=8)</th>
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<tr>
<td>0</td>
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<td>5 (71.4)</td>
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<td>2 (28.6)</td>
<td>5 (71.4)</td>
<td>3 (42.9)</td>
<td>8 (100)</td>
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</tbody>
</table>

**Table 1A: Immunohistological analysis for ED1 positive cells.**

*: histological grading was assessed as described in the materials and methods section (0= no positive cells present, 1= less than 10 positive cells/microscopic field, 2=10 to 25 positive cells / microscopic field, and 3= more than 25 positive cells/ microscopic field). At least six animals per group and 20 microscopic fields were evaluated. **: numbers in parenthesis represents the % of animals with a given score.

The number of MHC class II positive cells in the grafts obtained from BD or LD donors was also increased after prolonged cold preservation. However, no significant difference was observed in the number of MHC class II positive cells between BD and LD when the grafts were not subjected to cold preservation (Table 1B, BD-Cp vs BD+Cp \( P<0.001 \); LD-Cp vs LD+Cp \( P<0.05 \); LD+Cp vs BD+Cp \( P<0.05 \), LD-Cp vs BD-Cp \( P=NS \)). The number of infiltrating T-cells was not different amongst the groups (Data not shown).
Chapter 3 – Effects of Cold Preservation and Brain Death in Renal Transplantation

<table>
<thead>
<tr>
<th>histologic grading scale*</th>
<th>LD-Cp (n=7)</th>
<th>LD+Cp (n=7)</th>
<th>BD-Cp (n=7)</th>
<th>BD+Cp (n=8)</th>
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<td>5 (71.4)</td>
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<td>3 (42.9)</td>
<td>1 (14.3)</td>
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Table 1B: Immunohistological analysis for MHCII positive cells.

*: histological grading was assessed as described in the materials and methods section (0= no positive cells present, 1= less than 10 positive cells/microscopic field, 2=10 to 25 positive cells / microscopic field, and 3= more than 25 positive cells/microscopic field). At least six animals per group and 20 microscopic fields were evaluated. **: numbers in parenthesis represents the % of animals with a given score.

Fig 2: Representative immunohistological staining for ED1+ cells in renal allografts collected 10 days after transplantation. ED1 expression in allografts from LD-Cp, LD+Cp, BD-Cp and BD+Cp is depicted. Original magnification: 400x.
**Banff-Classification**

Histologic scores according to the Banff 97 classification also revealed that the inflammatory response was significantly increased in both LD and BD donors when the grafts were subjected to cold preservation (Table 2). This was reflected by a higher tubulitis (t) and vasculitis (v) score (t: BD-Cp vs BD+Cp: \( P < 0.0001 \); v: BD-Cp vs BD+Cp: \( P < 0.0001 \), t: LD-Cp vs LD+Cp: \( P < 0.05 \); v: LD-Cp vs LD+Cp: \( P = 0.05 \)). No difference in the degree of interstitial (i) infiltration between the groups was found. BD seems to aggravate cold preservation damage particularly in vessels as higher vasculitis scores were observed in these grafts, while tubulitis scores were not significantly different (v: LD+Cp vs BD+Cp: \( P < 0.01 \), t: LD+Cp vs BD+Cp: \( P = \text{NS} \)) (Table 2; Fig. 3A). In light microscopy we could also demonstrate the occurrence of papillary necrosis. This was observed in 20% and 25% of the animals in the BD-Cp and BD+Cp group respectively, but was not found in the LD groups (Fig. 3B). Papillary necrosis occurred after transplantation, since it was not observed directly after BD (data not shown).

<table>
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<th>BD+Cp (n=8)</th>
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<td>i 2</td>
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<td>i 3</td>
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**Table 2: Histologic scores according to the Banff’97 classification.**

*: severity of interstitial inflammation (i), tubulitis (t) and intimal arteritis (v) is indicated by the grading scale (0=not present, 1=mild alteration, 2=moderate alteration, and 3=severe alteration). **: numbers in parenthesis represents the % of animals with a given score. Cold
preservation resulted in significantly more severe tubulitis in both donor types (-Cp vs +Cp, P<0.05). The combination of BD and cold preservation resulted in significantly more severe vasculitis (LD+Cp vs BD+CP, P<0.01).

Fig 3A: Representative hematoxylin-eosin staining of renal allografts collected 10 days after transplantation. Note that cold preservation results in more severe tubulitis in both types of donors (panels to the left). Cold preservation also resulted in more severe vasculitis, particularly in the BD group (panels to the right). Original magnification: 400x.
Fig. 3B: Overview of a renal papilla in LD (panels to the left) and BD (panels to the right) donors. While in LD papilla necrosis was not observed, in 20% of the BD animals this occurred after renal transplantation. An example of papilla necrosis is depicted (panel to the right). Original magnification: 20x upper panels, 100x lower panels.

Cytokine expression
To assess if the cytokine profile was different amongst the groups, we performed quantitative PCR for VCAM-1, ICAM-1, E-selectin, TNFα, IL6, VEGF and IL-10. To this end total RNA was isolated from renal allotrafts 10 days after transplantation. No difference in the mRNA expression of VCAM-1, ICAM-1 was found between the groups. Although the mRNA expression of E-selectin was significantly higher in the BD-Cp group compared with the LD-Cp or LD+Cp groups, this was not found in the BD+Cp group (Table 3). Similar findings were observed for IL-6. There was a tendency for an increased TNFα mRNA expression when the grafts were subjected to cold preservation, but this did not reach statistical significance. Both VEGF and IL-10 mRNA expression were significantly increased in the BD compared to the LD groups. This was more pronounced after prolonged cold preservation (Table 3).
### Table 3: Quantitative PCR-Analysis for the expression of adhesion molecules and cytokines

<table>
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<tr>
<th>Gene</th>
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<th>LD+Cp</th>
<th>BD-Cp</th>
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<td>2.7±0.45</td>
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<td>307±94</td>
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</table>

*: The results are expressed as mean gene of interest/GAPDH ratio ± SD. In each group kidneys from 4 animals were analysed. **: P<0.05, LD-CP or LD+Cp vs BD-Cp  
***: P<0.01, LD-Cp or LD+Cp vs BD+Cp.
DISCUSSION

Many studies have clearly shown that brain death is a donor associated risk factor that negatively affects transplantation outcome [27-30]. There is also general consensus that prolonged cold preservation time significantly contributes to pre-transplantation injury as it results in tubular and vascular damage [17-19]. Although based on these findings, it can be anticipated that the combination of BD and cold preservation will lead to culmination of organ damage. There are, however, only a few studies that have investigated the combination of brain death and cold preservation on tissue damage after transplantation. Therefore we assessed to what extent BD, cold preservation or the combination of both affects post-transplant injury after renal transplantation.

The main findings of our study are that cold preservation, but not BD alone, increases DNA damage in renal tissue before implantation. BD augmented DNA damage when grafts were subsequently subjected to prolonged cold preservation. After transplantation renal inflammation was significantly higher in grafts that were subjected to cold preservation. BD aggravated inflammation as indicated by a higher score for MHC class II positive cells in these grafts. In particular, vasculitis but not tubulitis was significantly more severe in the BD+Cp compared to the LD+Cp group. BD, but not cold preservation alone, was associated with papillary necrosis. Papillary necrosis occurred after transplantation as it was not found directly after BD.

Prolonged cold preservation severely affects the vasculature and might impair the endothelial barrier function [31]. Endothelial barrier dysfunction is generally associated with edema formation, which might subsequently lead to vascular occlusion and secondary tissue ischemia. Although we did not investigate endothelial barrier dysfunction in the present study, we recently have shown in an isolated ventilated and perfused rat lung model that cold preservation severely affects the endothelial barrier resulting in profound edema formation upon lung perfusion, accompanied by an increase in mononuclear cell infiltration [32, 33]. We are aware that these results may not be entirely extrapolated to renal grafts, but they do indicate the vulnerability of the micro-vasculature towards cold preservation. We did not specifically address if DNA damage occurred in endothelial cells during cold preservation. However, based on the localisation of TUNEL positive cells, our data suggest that damage of peritubular capillaries might have occurred. Interestingly, BD alone did not affect DNA damage in renal tissue, but clearly augmented the number of TUNEL positive cells when these grafts were subjected to cold preservation. Due to hemodynamic imbalances
hypoperfusion of the kidney could occur [13]. Reduced perfusion could in turn make the renal tissue more prone to cold ischemic damage.

Interstitial inflammation was not different amongst the groups. In contrast, vasculitis, a hallmark for acute vascular rejection [26], was more severe in the BD+Cp group. This was not found for tubulitis, as the tubulitis scores between the BD+Cp and LD+Cp groups were not significantly different. BD alone also did not affect severity of vasculitis (LD-Cp vs BD-Cp, \( P=\text{NS} \)). In a retrospective study of Sanchez-Fructuoso [34], BD was found to be a risk factor for acute vascular rejection. It must be stressed that in the study of Sanchez-Fructuoso all renal grafts were subjected to hypothermic preservation. Therefore BD alone might not be a risk factor for vasculitis but only in combination with cold ischemia, as our data suggest. Recently, Mikhalski et al [35] showed that cold ischemia time is a risk factor for acute interstitial rejection when renal allografts were obtained from deceased donors. Cold ischemia time was not found to be a risk factor for acute interstitial rejection when grafts were obtained from living donor in the study of Simpkins et al [36]. These findings are not in agreement with the data from our model as for both living and BD donors cold preservation increased severity of tubulitis. It must be noted however, that in the study by Simpkins et al cold ischemia time was not more than 8 hrs, while in our study 24 hrs of cold preservation was applied. This might explain the conflicting results found in both studies.

Although Banff classification and immunohistology scores revealed a significant difference between the groups, this was not reflected by quantitative PCR analysis when mRNA expression of inflammatory genes was assessed. This might be explained by the fact that only a minority of infiltrated mononuclear cells were present in the vessel wall or tubuli resulting in different Banff vasculitis and tubulitis scores respectively. The majority of infiltrating cells were present in the interstitium and no differences in Banff interstitial infiltration scores were detected between the groups. Nevertheless, immunohistology showed that significant more ED1 and MHC class II positive cells were present in the grafts obtained from the BD+Cp group. Given that interstitial infiltration scores where not different between the groups, our data suggest enrichment of monocytes in the BD+Cp group. Because the inflammatory genes that were studied are not specific for monocytes, the results obtained from the quantitative PCR and immunohistology might differ.

A comparative immunohistochemical analysis of pretransplant donor biopsies from deceased and living related donors (LRD) have revealed high expression levels of E-selectin, HLA-DR
antigens, ICAM-1 and VCAM-1 in biopsies from cadaveric kidneys, as opposed to that was found in biopsies from LRD kidneys [37]. Upregulation of inflammatory genes during BD have also been demonstrated by others using animal models [4, 7, 8]. This is compatible with our previous findings that BD increases the number of inflammatory cells in the kidney [13, 14]. Nevertheless, our present study revealed that 10 days after transplantation no large differences between the groups were found for the inflammatory genes that were tested. While VCAM-1 and ICAM-1 mRNA expression was similar in the BD and LD groups, there was an increased E-selectin mRNA expression in the BD-Cp but not in the BD+Cp group. Although it remains to be elucidated why this was not found in the latter group, endothelial damage as a consequence of vasculitis might underly this phenomenon.

With exception of VEGF and IL-10 no significant differences in mRNA expression for cytokines or growth factors were found between the groups. VEGF was strongly up-regulated in grafts from BD animals compared to grafts from LD donors. This was not influenced by cold preservation and did not correlate with the severity of vasculitis. VEGF is up-regulated by hypoxia in a variety of organs [38-40]. Hence the increased expression could be related to tissue ischemia. It has also been demonstrated, that acute brain trauma with consecutive sympathetic activation increases IL10 plasma levels [41, 42]. Ten days after transplantation IL10 expression was still increased in grafts from brain dead donor rats, probably as a response to renal inflammation.

Renal papillary necrosis can occur as a consequence of an ischemic process in the renal papilla [43, 44]. Interstitial inflammation may lead to compression of the medullary vasculature and thus predispose the vessels to ischemic damage. Also vasculitis compromises perfusion and hence can contribute to papillary necrosis. Since severity of vasculitis was higher in the BD+Cp group this might explain the higher incidence of papillary necrosis in this group. It remains to be addressed why papillary necrosis was only observed in grafts obtained from BD animals, even when the grafts were not subjected to cold preservation. One possible explanation might be related to hypoperfusion of the renal tissue during the state of BD, thereby predisposing the renal papillae to ischemic damage.

In conclusion, our study demonstrates that BD amplifies tissue damage before and after transplantation when renal allografts are subjected to prolonged cold ischemia. Because our study once more stresses the importance of minimizing cold ischemia time in grafts from deceased donors, strategies in donor management and/or organ procurement aimed to
minimize pre-transplant injury are warranted. This could be feasible by donor pre-
conditioning that limits brain death and cold preservation injury [15, 16, 45]. Alternatively,
changing organ allocation, e.g. local organ donors for local recipients, would significantly
reduce the time for cold preservation. While the later might be more difficult to implement for
reasons of matching, multi-organ donation and logistics, donor pre-conditioning seems to be a
more practical approach to overcome this problem.
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REFERENCES

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