Donor Desmopressin Treatment Does Not Affect Transplant Outcome in the Fischer to Lewis Rat Renal Transplant Model

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Abstract

Objectives: Retrospective studies suggest that donor desmopressin (DDAVP) treatment improves renal transplant outcome. The present study tests the hypothesis that desmopressin neutralizes the graft's endothelium from proinflammatory angiopoietin 2 containing Weibel-Palade bodies in the donor, resulting in reduced Weibel-Palade body release at the time of reperfusion in the recipient.

Materials and Methods: Using rat models, we examined the influence of desmopressin treatment on the expression of vasopressin 2 receptors and adhesion molecules in brain-dead donors, with renal function examined in allogeneic recipients. The influence of desmopressin on the expression of adhesion molecules also was tested in vitro.

Results: Vasopressin 2 receptors were restricted to collecting ducts and distal tubules and only scarcely found in the renal vasculature. Vasopressin 2 receptor expression was down-regulated in brain-dead rats by desmopressin. Renal expression of vascular cellular adhesion molecule 1 and intercellular adhesion molecule 1 were significantly reduced in these rats. In contrast, angiopoietin 2 did not influence the expression of adhesion molecules in in vitro cultured endothelial cells after tumor necrosis factor α stimulation. Donor desmopressin treatment improved neither renal function nor histology in allogeneic renal transplant recipients.

Conclusions: Our data do not support the hypothesis that the clinically observed salutary effect of desmopressin is mediated by depletion of Weibel-Palade bodies in renal allografts.

Key words: Angiopoietin 2, Brain death, Kidney transplant, Vasopressin 2 receptor, Weibel-Palade bodies

Introduction

Endothelial cells are equipped with Weibel-Palade bodies (WPB), containing a multiplicity of bioactive molecules, to rapidly respond to changes in their microenvironment.1 Among these compounds are von Willebrand factor, P-selectin, interleukin 8, endothelin 1, and angiopoietin 2, which can directly induce a proinflammatory milieu after release. Angiopoietin 2 exhibits its proinflammatory properties by sensitizing endothelial cells to tumor necrosis factor α (TNF-α), thereby augmenting the expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cellular adhesion molecule 1 (VCAM-1) already at low TNF-α concentrations.2 Hence, the release of WPB may sustain the inflammatory cascades and contribute to migration of mononuclear cells,3 particularly under conditions where TNF-α is present (eg, sepsis or ischemia-reperfusion injury).

Desmopressin (1-deamino-8-D-arginine vasopressin; DDAVP), clinically used to treat diabetes insipidus, is known to induce WPB exocytosis in a vasopressin 2 receptor (V2R)-dependent manner.4 Interestingly, 2 retrospective studies showed that donor treatment with desmopressin is associated with reduced episodes of acute rejection, improved creatinine levels 1 and 3 years after transplant, and enhanced graft survival in renal allograft recipients.5,6 Why donor desmopressin treatment may have a salutary effect on transplant outcome is still elusive. We hypothesize that the beneficial effect

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of desmopressin might be explained by the fact that desmopressin depletes WBP from the endothelium of the donor graft. Hence, the release of WBP in the recipient at the time of reperfusion is low. In keeping with the proinflammatory properties of angiopoietin 2, this might be advantageous to prevent sustained inflammation after reperfusion.

To test this hypothesis, we first analyzed the effects of cold preservation and rewarming on WBP exocytosis in vitro using a static cold preservation model of cultured endothelial cells. Second, we assessed whether angiopoietin 2 acts in concert with TNF-α to increase the expression of ICAM-1 and VCAM-1 in endothelial cells. Third, because the release of WBP by desmopressin occurs in a V2R-dependent manner, we assessed V2R expression in renal allografts of brain-dead rats and whether this expression is influenced by desmopressin. Finally, we assessed whether desmopressin treatment of brain-dead donor rats influenced long-term renal function and histology in allogeneic rat recipients.

Materials and Methods

Animals
Inbred male Fischer 344 (RT1 haplotype) rats were used as graft donors, and inbred male Lewis (RT1 haplotype) rats were used as graft recipients. All rats weighed between 180 and 200 g and were purchased from Harlan Winkelmann (Borchen, Germany). 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 (Regierungsrätschium Karlsruhe, Karlsruhe, Germany).

Brain death
Fischer 344 donor rats were anesthetized with ketamine (Ketanest, Pfizer; 100 mg/kg intraperitoneally) and xylazine (Rompun, BayerVital; 6 mg/kg intraperitoneally) and placed on a heating table to maintain body temperature. To induce brain death, a 3F Fogarty catheter was gradually inflated for 1 minute with 200 μL of saline after positioning in an occipital burr hole. Brain death was confirmed by absence of corneal reflexes, by apnea test, and by occurrence of autonomic storm. All animals were mechanically ventilated by a tracheostoma with a rodent ventilator (Ugo Basile, Comerio, Italy). Monitoring of systemic blood pressure (mm Hg) in organ donors was performed by a femoral arterial catheter (Statham pressure transducer P23Db and a Gould pressure processor; FMI, Ober-Beerbach, Germany). Animals received fluid replacement to stabilize blood pressure over the 6-hour brain death period.

Desmopressin treatment and renal transplant
Three hours after induction of brain death, 1 group of donor rats (12 rats/group) received desmopressin (intravenous bolus of 0.4 μg/kg; Ferring Pharmaceuticals Inc., Suffern, NY, USA); the other group received intravenous saline (vehicle treatment). Six hours after brain death induction, the left kidney was explanted from the Fischer 344 donor rats and then flushed with and stored for 1 hour at 4°C in University of Wisconsin solution. Urine was collected from the bladder by catheterization, and the right kidney was recovered for quantitative polymerase chain reaction and histology. Recipients were anesthetized with ketamine (Ketanest, Pfizer; 100 mg/kg intraperitoneally), xylazine (Rompun, BayerVital; 6 mg/kg intraperitoneally), and isoflurane (Ethrane; Aca Mueller/Adag Pharma, Gottmadingen, Germany). The left donor kidney was then transplanted into these uninephrectomized Lewis recipients, as previously described.7-9 Immunosuppression (5 mg/kg cyclosporine) was given subcutaneously for 14 days. The right native kidney was removed from the recipient after 2 weeks. During the 24-week follow-up, kidney function and urine parameters were measured every 4 weeks. After 24 weeks, isoflurane overdose was used to kill recipient rats, and grafts were recovered for histologic investigation. We used neither antibiotics nor other drugs perioperatively during our study. All procedures were performed under aseptic conditions.

Immunohistology
Immunohistochemistry for ED1-positive monocytes and class II major histocompatibility complex (Mhc) was performed as described previously (ED1 antibody from Linaris, Dossenheim, Germany; class II Mhc from Acris, Herford, Germany).10,11 Positive cells were expressed by measuring positive field of view (μm²) or percent grading using cell^F software from Olympus Europe (Hamburg, Germany).
Immunohistochemistry for V2R (V2R antibody from Acris, Herford, Germany) and aquaporin 2 (Abcam, Cambridge, UK) was performed similarly to ED1 and class II Mhc. Vasopressin 2 receptor and aquaporin 2 expression levels were assessed by examination of least 10 microscopic fields by 2 blinded independent individuals using a semiquantitative scale (0 = absent, 1 = slightly positive, 2 = positive, 3 = strongly positive).

RNA isolation, cDNA conversion, and quantitative polymerase chain reaction analyses
RNA was isolated from snap frozen upper poles of the donor kidneys using TRIzol reagent, and then 1 μg of total RNA was reverse transcribed into cDNA. Quantitative polymerase chain reaction was performed on a real-time polymerase chain reaction platform (AB7900HT) using TaqMan probes for ICAM-1 (Rn00564227_m1), VCAM-1 (Rn00563627_m1), and E-selectin (Rn00594072_m1) (Applied Biosystems, Foster City, CA, USA). All samples were normalized for an equal expression of glyceraldehyde 3-phosphate dehydrogenase.

Cell culture
Human umbilical vein endothelial cells and cell growth medium were purchased from Promocell (Heidelberg, Germany). Human umbilical vein endothelial cells were cultured in T25 flasks (Greiner, Frickenhausen, Germany) coated with 1% gelatin (Fluka, Neu-Ulm, Germany).

Assessment of angiopoietin 2 concentrations and lactate dehydrogenase release
Human umbilical vein endothelial cells were stimulated for 1 hour with 100 μM N-Octanoyl dopamine (NOD) or left untreated. Plates were washed with phosphate-buffered saline and stored for 8 or 24 hours at 4°C in phenol red-free medium. Supernatants were then collected and assessed for angiopoietin 2 concentrations and lactate dehydrogenase release. Fresh medium was added, and the plates were rewarmed for 24 hours at 37°C and 5% CO₂. The supernatants were collected, and angiopoietin 2 concentrations were assessed according to manufacturer’s protocol (human angiopoietin 2 Quantikine ELISA kit, catalog No. DANG20, R&D Systems, Minneapolis, MN, USA). Lactate dehydrogenase assays were performed as recommended by the manufacturer (Roche Diagnostics, Mannheim, Germany). All experiments were performed in triplicate, and the results are expressed as mean angiopoietin 2 concentration or mean optical density at 490 nm ± standard deviation.

Western blot analyses
Human umbilical vein endothelial cells were stimulated for 4 hours with TNF-α (10 pg/mL) or angiopoietin 2 (2 μg/mL), either alone or in combination. Cells treated with 10 ng/mL TNF-α were used as positive control. The cells were lysed and processed for blotting as described previously. Blots were then incubated with antibodies against VCAM-1 (BBA19; R&D Systems, Wiesbaden-Nordenstadt, Germany), ICAM-1 (sc-8439; Santa Cruz Biotechnology, Heidelberg, Germany), or glyceraldehyde 3-phosphate dehydrogenase (ab9484; Abcam). Membranes were then washed and incubated with an appropriate horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology). The blots were developed by chemiluminescence using Western Lightning enhanced chemiluminescence (Perkin-Elmer, Waltham, MA, USA) and visualized by Chemismart-5100 (PEQLAB Biotechnologie, Erlangen, Germany).

Statistical analyses
Data are shown as means ± standard deviation or median. Differences between groups were assessed by t tests. For analysis of light microscopy, the Cochran-Armitage trend test was applied. Statistical significance was defined as P < .05. Statistical analysis was performed with GraphPad Prism5 (La Jolla, CA, USA) or SAS statistical software (SAS Institute Inc, Cary, NC, USA).

Results
Expression of vasopressin 2 receptors in renal tissue of brain-dead rats
Because desmopressin depletes WBP from the endothelium in a V2R-dependent manner, we first assessed V2R expression in renal grafts from brain-dead rats. Expression of V2R measured in brain-dead or ventilated rats that were not brain dead was not significantly different and was mostly restricted to the renal papilla in collecting ducts and to the cortex/medulla in convoluted/connecting tubules (Figure 1A and 1B). Vasopressin 2 receptor expression was scarcely found in peritubular
capillaries, and was completely absent in the glomerular vasculature and larger renal arteries (Figure 1C to 1F). Brain-dead animals that were treated with desmopressin not only showed a significant decrease in V2R expression in different renal compartments but also a diminished expression of aquaporin 2 (Figure 2).

Functionality of donor desmopressin treatment in brain-dead rats
In concordance to the desmopressin-induced change in V2R expression in collecting ducts and its biologic effects in promoting water reabsorption, urine collected from the bladder 6 hours after brain death clearly showed the expected effect of desmopressin, as indicated by significantly increased urea, creatinine, potassium, and osmolarity levels compared to vehicle-treated animals (Figure 3A). In desmopressin-treated rats, mean arterial blood pressure was not significantly different compared to untreated rats (Figure 3B), albeit the latter group required more fluid for stabilization of blood pressure over the 6-hour brain death period (data not shown).

The expression of VCAM-1 and ICAM-1 in renal tissue of desmopressin-treated donor rats was significantly decreased compared to vehicle-treated rats. We found no significant differences in expression levels of E-selectin (Figure 3C). Although the expression of some adhesion molecules were
decreased in the desmopressin-treated donor rats, there was no difference in the amount of infiltrated mononuclear cells as assessed by morphometric analysis on paraffin sections of these renal allografts (data not shown). Twenty-four weeks after transplant into allogeneic recipients, infiltration of class II Mhc- and ED1-positive cells was also not different between the groups (ED1 showed 4929 μm²/1.29% surface vs 7123 μm²/1.86% surface, \( P = .13 \); class II Mhc showed 4888 μm²/1.47% surface vs 6972 μm²/1.79% surface, \( P = .38 \)) (Figure 4A).

Assessment of Banff classification revealed only mild signs of chronic allograft nephropathy, with no significant difference between the groups (data not shown). Renal function, measured by serum creatinine or urea levels, did not differ between the desmopressin-treated and vehicle-treated rats (creatinine level of 0.55 vs 0.58 mg/dL, \( P = .81 \); urea level of 60.8 vs 62.6 mg/dL, \( P = .88 \) after 24 weeks). Similarly, no difference was found for proteinuria and albuminuria during follow-up (proteinuria of 2.21 vs 2.52 g/L, \( P = .68 \); albuminuria of 1.42 vs 1.85 g/L, \( P = .57 \) after 24 weeks) (Figure 4B).

### Release of Weibel-Palade bodies during static cold storage and rewarming

We first tested the hypothesis that WPB exocytosis is impaired during rewarming when endothelial cells are damaged as a consequence of static cold storage but not when damage is prevented. Because N-octanoyl dopamine is more effective in preventing cold-inflicted damage of endothelial cells than dopamine, the cells were either or not pretreated with 100 μM NOD for 1 hour. Cells that were subjected to 8 hours of cold storage and subsequently rewarmed for 24 hours clearly released WPB as assessed by angiopoietin 2 (Figure 5) and von Willebrand factor (data not shown) concentrations in the cell supernatants. No significant difference between NOD-treated cells and untreated cells was observed. In contrast, WPB exocytosis was significantly impaired in cells that were subjected to 24 hours of cold storage, as only low concentrations of angiopoietin 2 could be measured after 24 hours of rewarming. This was partly restored in NOD-treated cells. The ability to release WPB seems to be inversely related to cell damage occurring during cold storage, as indicated by lactate dehydrogenase release measured immediately before rewarming (Figure 5).

### Synergistic effect of angiopoietin 2 and TNF-α in human umbilical vein endothelial cells

We next assessed whether angiopoietin 2 indeed acts synergistically in endothelial cells to increase...
ICAM-1 and VCAM-1 expression levels after TNF-α stimulation. Angiopoietin 2 and TNF-α concentrations were based on dose-response experiments (Figure 6A) and were similar to those shown by Fiedler and associates (0.01 ng/mL). We found that both VCAM-1 and ICAM-1 expression was induced in human umbilical vein endothelial cells by TNF-α in a dose-dependent manner, demonstrating the cell’s ability to respond to this cytokine (Figure 6A). However, neither a low concentration of TNF-α alone nor the combination of TNF-α and angiopoietin 2 had any effect on the expression of these adhesion molecules (Figure 6B).

Discussion

Although desmopressin is regularly administered to the vast majority of brain-dead organ donors who have diabetes insipidus, there are conflicting data as to whether desmopressin influences transplant outcomes in renal allograft recipients.5,6,12,13 Recently, in a group of 27 donors, Nijboer and associates reported that donor desmopressin treatment after brain death decreased the risk of rejection and improved serum creatinine levels 1 and 3 years after transplant.6 These data are in line with our retrospective multicenter cohort study, which included 487 renal allografts recovered from deceased heart-beating donors and transplanted at 60 European centers.5 Interestingly, donor desmopressin treatment only improved graft survival if cold ischemia time was short or if the donor was also treated with dopamine. Because dopamine protects the graft’s vasculature against cold-inflicted injury,9,14-18 it is conceivable that the effect of donor desmopressin treatment may only be observed in situations in which the graft’s vasculature is not compromised by static cold storage (ie, in grafts with short cold ischemia times or in grafts recovered from dopamine-treated donors). Because desmopressin increases angiopoietin 2 secretion via exocytosis of WPB from endothelial cells and angiopoietin 2 may act in concert with TNF-α to increase the expression of adhesion molecules, we hypothesized that the beneficial effect of donor desmopressin may occur through depletion of WPB from the graft’s vasculature, resulting in low angiopoietin 2 secretion in the recipient at the time of organ reperfusion.

According to our results, there are several arguments that do not support the formulated
hypothesis. First, desmopressin mediated WPB exocytosis in a V2R-dependent manner; however, V2R expression in the renal tissue of brain-dead rats was completely absent in glomeruli, renal arteries/arterioles and only scarcely found in peritubular capillaries. Our results demonstrate for the first time that V2R expression is not affected by the state of brain death and is comparable to that of non–brain-dead rats. Because of the absence of V2R in the endothelium, desmopressin-mediated WPB release in renal tissue is highly unlikely. However, it should be mentioned that Medina and associates showed desmopressin-mediated endothelium-dependent relaxation in isolated rings of human renal arteries, suggesting the presence of V2R in these arteries. Hence, we stained sections of human kidneys for V2R and could exclude interspecies differences in the renal V2R expression between human and rat (data not shown). Second, because brain death is generally considered to be a proinflammatory event, desmopressin-mediated WPB exocytosis in the donor may equally sustain tissue inflammation in end organs. In fact, desmopressin treatment of brain-dead donor rats significantly decreased ICAM-1 and VCAM-1 mRNA, suggesting that WPB release in the renal graft of desmopressin-treated brain-dead rats does not occur to a large extent. Finally, our animal donor model showed no beneficial effect by donor treatment with desmopressin, although desmopressin treatment was effective. Desmopressin not only changed the V2R expression in collecting ducts but also promoted water reabsorption, as demonstrated by significantly increased urine concentrations of urea, creatinine, potassium and osmolarity. However, 24 weeks after transplant into allogeneic recipients, renal function, infiltration of mononuclear cells, and proteinuria levels did not differ between groups.

In contrast to our animal model, our in vitro results are partially in line with our hypothesis. Our study clearly showed that the ability of endothelial cells to secrete angiopoietin 2 during rewarming was inversely associated with the extent of cell damage occurring during static cold storage. Although this might explain why the salutary effect of donor desmopressin treatment is only observed in grafts with short cold ischemia times or in grafts recovered from dopamine-treated donors, this study does not provide experimental evidence that angiopoietin 2 secretion during rewarming can be reduced by desmopressin treatment before static cold storage.

Because human umbilical vein endothelial cells do not express V2R, we were unable to address this point. It would be prudent to be cautious in suggesting that the negative findings of angiopoietin 2 on induction of adhesion molecules indicate an inferior role for WPB exocytosis in tissue inflammation. Indeed, the studies of Pinsky and associates have illustrated both the effect of WPB exocytosis in rodent cardiac allografts on long-term graft survival and the salutary effect of soluble P-selectin glycoprotein ligands on transplant-relevant inflammatory events, suggesting that prevention of WPB exocytosis could be an effective way to limit tissue inflammation.

**Limitations**

The potential benefit of donor desmopressin treatment might also be attributed to the reduced need of fluid replacement, thereby improving hemodynamic stability of potential organ donors. Because our hypothesis included a blood pressure-independent mechanism, we stabilized both groups with appropriate amounts of fluid to maintain sufficient organ perfusion. Therefore, we could not address whether hemodynamic stabilization played a role in the postulated effect of desmopressin treatment. It should also be mentioned that the administration of desmopressin 180 minutes after induction of brain death might be too late, as the half-life of vasopressin is less than 30 minutes.

**Conclusions**

In contrast to our retrospective clinical study, donor desmopressin treatment did not improve the long-term transplant outcomes in the Fischer to Lewis rat transplant model. To our knowledge, there are no experimental or other animal data available, with only 2 prospective randomized controlled studies performed on the use of desmopressin and vasopressin in human donors after brain death (Table 1). These studies were intended to assess the influence of donor treatment on early renal function in the recipient. Similar to our retrospective study, neither study showed benefits of desmopressin treatment on early graft function in kidney transplant recipients. The influence of desmopressin on long-term graft survival has not been prospectively assessed. Hence, it remains to be addressed by a prospective randomized controlled multicenter trial whether
donor desmopressin treatment indeed has a salutary effect on long-term transplant outcomes as suggested.5,6

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