Acute ischemic injury to the renal microvasculature in human kidney transplantation

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Kidney transplantation is inevitably associated with ischemia and reperfusion injury. Depending on the severity of injury, 20–80% of recipients of deceased donor kidneys require dialysis treatment in the first week after transplantation, which is referred to as delayed graft function (26). This condition complicates patient management and is associated with a 40% increased rate of graft loss in kidneys from donors after brain death (45). Liberal use of donations after cardiac death greatly expands the number of available donor kidneys and may even eliminate transplant waiting lists (37). However, kidneys from these donors suffer extensive ischemic injury from circulatory arrest until organ preservation, which almost invariably leads to delayed graft function after transplantation. More importantly, up to 15–25% of these kidneys will never regain function, unnecessarily exposing recipients to the risks of major surgery and immunological sensitization (43).

Adequate reperfusion is essential for functional recovery of donor kidneys and prevents ongoing ischemic tissue injury after revascularization. In rodent models of ischemic acute kidney injury, it has been demonstrated that the peritubular microcirculation suffers endothelial injury and functional impairment after reperfusion (5, 44), which has recently been observed in humans as well (13, 21, 31). The endothelium is covered by the glyocalyx, a dynamic network of proteoglycans and glycoproteins that determines vascular permeability, transduces shear stress to the endothelium, and prevents interaction of leukocytes and platelets with the vascular wall (29). Loss of endothelial glyocalyx integrity after ischemia and reperfusion has been observed in experimental models (8, 24, 27) and in patients undergoing aortic surgery (28). Degradation of the endothelial glyocalyx by infusion of hyaluronidase causes capillary perfusion defects in rodents (7). Taken together, ischemic injury to endothelial cells and glyocalyx of peritubular capillaries may play a major role in the pathophysiology of acute kidney injury by reducing tissue perfusion and propagating inflammation in the reperfused kidney.

In the current manuscript, we studied the human renal microcirculation after clinical kidney transplantation by direct visualization of cortical peritubular capillaries and by measuring renal arteriovenous gradients of the main constituents of the endothelial glyocalyx. We found that ischemically injured kidneys from donors after cardiac death (DCD) were characterized by reduction of capillary blood flow and loss of glyocalyx integrity compared with living donor kidneys that suffered minimal ischemia. These findings form the basis for development of novel interventions to prevent ischemic acute kidney injury. Ischemia-reperfusion injury; endothelial glyocalyx

METHODS

Study design. In this observational study, eight consecutive recipients of ischemically injured DCD kidneys were compared with eight recipients of living donor kidneys that suffered minimal ischemic injury. All patients received dialysis therapy and were 18 yr of age or older. The kidneys were recovered from separate donors between 16 and 60 yr of age to reduce the possibility of perfusion abnormalities as a result of small pediatric grafts or atherosclerotic blood vessels. At transplantation, renovascular resistance, microvascular perfusion, and endothelial integrity and activation were assessed immediately after reperfusion. Thereafter, patients were observed for 10 days after transplantation to measure early graft function. The study was approved by the local institutional review board (MEC 07–2-025), and all patients gave written informed consent for participation in the study.

Kidney transplantation. Living donor kidneys (n = 8) were recovered by open mini-incision donor nephrectomy and were cold-stored
in HTK preservation solution (Dr. F. Köhler Chemie, Bensheim, Germany). DCD kidneys were recovered after in situ perfusion for uncontroled donation after failed cardiopulmonary resuscitation in the emergency department \( n = 3 \) or after rapid laparotomy and direct aortic cannulation for controlled donation after scheduled withdrawal of supportive treatment in the intensive care unit \( n = 5 \) \((33)\). DCD kidneys were preserved by cold storage in HTK solution or by machine perfusion (LifePort; Organ Recovery Systems, Des Plaines, IL).

After obtaining of negative cross-matches, kidneys were transplanted by end-to-side anastomoses of the renal artery and vein to the common or external iliac artery and vein. Heparin was not routinely administered, and none of the patients received blood transfusions during surgery. Recipient hemodynamic management was targeted at a central venous pressure of 10–15 mmHg during transplant surgery. Renal artery blood flow was measured at 5 and 30 min after reperfusion using perivascular flow probes (Transonic Systems, Ithaca, NY). Immunosuppression was started before surgery and consisted of corticosteroids, tacrolimus and mycophenolic acid, or sirolimus. In the first 10 days after transplantation, graft function was assessed daily by measuring creatinine clearance using 24-h urine collections.

Sidestream darkfield imaging. The cortical peritubular microcirculation was directly visualized by \( \times 5 \) magnification by sidestream darkfield imaging (MicroScan, Amsterdam, The Netherlands) at a frame rate of 25 Hz and a resolution of 720 \( \times \) 576 pixels. After reperfusion, the renal capsule was removed from the kidney over an area of \( \sim 2 \text{ cm}^2 \). Subsequently, the imaging probe was manually positioned on the kidney using saline irrigation to obtain a bloodless surface. At 5 and 30 min after reperfusion, three continuous image sequences of 20 s were digitally stored. At each time point, stable and sharp fragments of \( \sim 5 \text{s} \) were selected for off-line analysis. The imaging studies were done by the same investigator.

Characteristics of the cortical peritubular microcirculation were quantified using Automated Vascular Analysis software (MicroScan). After calibration and image stabilization by linear transformation, blood vessels were manually identified in an averaged frame and the vascular density was calculated. Blood vessel diameter was defined as the median diameter of the red blood cell column. For blood vessels up to 25 \( \mu \text{m} \) in diameter, blood flow velocity was measured by manual tracing of leukocytes and plasma gaps along the vessel centerline in space-time diagrams \((11)\). Centerline velocity was multiplied by 0.7 to account for the velocity distribution over the cross-sectional area of the vessels \((35)\). Assuming a cylindrical shape of the blood vessels, volumetric blood flow in each vessel was calculated as \((\pi/4) \times (\text{diameter})^2 \times \text{blood flow velocity}\). Total microvascular blood flow was calculated as the sum of the individual vessels. The analyses were done by a single investigator who was blinded to donor type.

Glycocalyx dimensions were quantified by measuring erythrocyte column width at \( \sim 200–300 \) sites in the peritubular microcirculation in each video fragment using Image-Pro Plus software (Media Cybernetics, Bethesda, MD). Infrequently, erythrocytes are able to transi- siently penetrate the endothelial glycocalyx, contributing to the dynamic range of erythrocyte column width. Reduced dynamic range of erythrocyte column width was therefore interpreted as a reduction of glycocalyx dimension. At each measurement site, erythrocyte column width was automatically determined from 50–\( \mu \text{m} \) radial line-intensity profiles at 20 locations distributed over a 10–\( \mu \text{m} \) longitudinal distance in 40 sequential frames, resulting in 800 measurements of erythrocyte column width. The dynamic range of erythrocyte column width was determined at each measurement site as the difference between median and maximal erythrocyte column width (50th and 99th percentiles of erythrocyte column width distribution, respectively). Glyco- calyx dimensions were compared by calculating differences in the median dynamic range of erythrocyte column width of differently sized microvessels (maximal erythrocyte column width between 10 and 25 \( \mu \text{m} \), classified into 1-\( \mu \text{m} \) intervals).

Measurement of glycoproteins in plasma. Systemic blood was drawn from a radial artery catheter at the start of surgery, just before reperfusion and at 5 and 30 min after reperfusion. Renal venous blood was drawn at 5 and 30 min after reperfusion. Blood was immediately transferred to EDTA tubes and centrifuged at 900 \( g \) at 4°C for 10 min. Plasma was kept on ice until storage at \(-80^\circ \text{C} \). Plasma syndecan-1 and heparan sulfate concentrations were measured using enzyme immunoassays (Diaclone Research, Besancon, France and Seikagaku, Tokyo, Japan, respectively) according to the manufacturer’s instructions. Before measurement of heparan sulfate concentrations, samples were treated with actinase E to digest plasma proteins (Sigma-Aldrich, St. Louis, MO). Measurements from blood sampled during surgery were adjusted for hemodilution using hemoglobin and hematocrit to calculate relative increases in plasma volume \((10)\). Renal fluxes of syndecan-1 and heparan sulfate were calculated by multiplying the arteriovenous concentration differences with total renal plasma flow which was measured simultaneously and adjusted for kidney weight.

Statistical methods. Continuous variables were expressed as means with SE and categorical variables as percentages. For continuous variables, differences between groups were compared with independent sample \( t \)-tests and differences within groups with paired sample \( t \)-tests. For categorical variables, differences between groups were compared with Fisher exact tests. Correlations between continuous variables were assessed with Pearson correlation coefficients and with multivariable linear regression. Results with \( P < 0.05 \) were considered statistically significant.

RESULTS

Patient characteristics. The effects of ischemia and reperfu- sion on the human renal microcirculation were studied by comparing DCD kidneys \( n = 8 \) with living donor kidneys \( n = 8 \). Transplant characteristics of the two study groups are presented in Table 1. Baseline characteristics of the two types of organ donors were comparable. As intended by study design, warm and cold ischemia times were significantly longer for DCD kidneys than for living donor kidneys. Furthermore, recipients of DCD kidneys were significantly older, had spent more time on dialysis, and received grafts with increased weight compared with recipients of living donor kidneys. Other transplant characteristics were similar between the two study groups.

Kidney function. All kidney transplantations were technically successful and eventually resulted in cessation of dialysis therapy. After transplantation, 6 (75%) DCD kidney recipients
Table 1. Transplant characteristics

<table>
<thead>
<tr>
<th></th>
<th>Living Donors (n = 8)</th>
<th>DCD Donors (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>40 (4)</td>
<td>45 (5)</td>
<td>0.44</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>5</td>
<td>4</td>
<td>0.61</td>
</tr>
<tr>
<td>Serum creatinine, µmol/l</td>
<td>91 (4)</td>
<td>93 (21)</td>
<td>0.91</td>
</tr>
<tr>
<td>Hypertension (yes)</td>
<td>0</td>
<td>2</td>
<td>0.20</td>
</tr>
<tr>
<td>Recipient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>44 (4)</td>
<td>60 (2)</td>
<td>0.005</td>
</tr>
<tr>
<td>Dialysis time, yr</td>
<td>1.4 (0.4)</td>
<td>5.2 (0.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cold ischemia, h</td>
<td>2.4 (0.1)</td>
<td>22 (1.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anastomosis time, min</td>
<td>34 (6)</td>
<td>39 (3)</td>
<td>0.48</td>
</tr>
<tr>
<td>Preservation (cold body storage/machine)</td>
<td>8</td>
<td>5/3</td>
<td>0.20</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>172 (14)</td>
<td>229 (17)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data are presented as means (SE) or as counts and are compared with independent sample t-tests or Fisher exact tests. DCD, donation after cardiac death; HD, hemodialysis; PD, peritoneal dialysis; MA, mycophenolic acid.

Table 2. Renal macrovascular perfusion

<table>
<thead>
<tr>
<th></th>
<th>Living Donors (n = 8)</th>
<th>DCD Donors (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP at reperfusion, mmHg</td>
<td>70 (4)</td>
<td>66 (3)</td>
<td>0.45</td>
</tr>
<tr>
<td>CVP at reperfusion, cmH2O</td>
<td>15 (3)</td>
<td>10 (1)</td>
<td>0.10</td>
</tr>
<tr>
<td>Resistance at 5 min, mmHg·ml·min⁻¹·100 g⁻¹</td>
<td>0.25 (0.07)</td>
<td>0.25 (0.05)</td>
<td>0.99</td>
</tr>
<tr>
<td>Resistance at 30 min, mmHg·ml·min⁻¹·100 g⁻¹</td>
<td>0.16 (0.03)</td>
<td>0.26 (0.09)</td>
<td>0.27</td>
</tr>
<tr>
<td>Tacrolimus concentration at reperfusion, μg/l</td>
<td>24 (3)</td>
<td>10 (3)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Data are presented as means (SE) and are compared with independent sample t-tests. MAP, mean arterial pressure; CVP, central venous pressure.

and none (0%) of the recipients of living donor kidneys required temporary continuation of dialysis treatment (P = 0.01). Kidney function in the first 10 days after transplantation was assessed by daily measurements of creatinine clearance from 24-h urine collections (Fig. 1). On each day, creatinine clearance of DCD kidneys was significantly lower than that of living donor kidneys (P < 0.05). These findings indicate that the extensive ischemic injury suffered by DCD kidneys was associated with a profound reduction in graft function in the early postoperative period.

Renal macrovascular perfusion. At reperfusion of the transplanted kidney, mean arterial pressure and central venous pressure in DCD kidney recipients were similar to those in recipients of living donor kidneys (P = 0.31 and P = 0.66, respectively). Taken together, ischemic injury to donor kidneys did not affect the renal macrovascular resistance to reperfusion.

Cortical peritubular microcirculation. Peritubular capillaries in the renal cortex were directly visualized by sidestream darkfield imaging at 5 and 30 min after reperfusion. Transplanted kidneys typically showed continuous flow through the vast majority of blood vessels (video 1; all supplemental material for this article is available on the Journal web site). In some cases, however, several nonperfused capillaries with stagnant or oscillating blood flow were observed (video 2). To compare the cortical microcirculation between kidney types, peritubular microvascular perfusion was quantified off-line. The cortical area, vascular density, and fraction of vessel length that could be analyzed were similar for the video fragments of DCD and living donor kidneys (Table 3). At 5 min after reperfusion, mean blood vessel diameter in DCD kidneys was significantly smaller than that in kidneys from living donor kidneys (P = 0.99 and P = 0.27, respectively). Recipients of living donor kidneys were loaded with immunosuppressive drugs before transplantation and therefore had higher tacrolimus levels at reperfusion than DCD kidney recipients. Since calcineurin inhibitors have been reported to cause renal vasoconstriction (25), we used multivariable linear regression to account for tacrolimus concentrations at reperfusion.

Table 3. Cortical peritubular microcirculation

<table>
<thead>
<tr>
<th></th>
<th>Living Donors (n = 8)</th>
<th>DCD Donors (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min After reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical area, mm²</td>
<td>0.60 (0.02)</td>
<td>0.50 (0.06)</td>
<td>0.20</td>
</tr>
<tr>
<td>Vessel length analyzed, %</td>
<td>85 (1)</td>
<td>87 (2)</td>
<td>0.30</td>
</tr>
<tr>
<td>Vascular density, mm²/m²</td>
<td>16 (1.2)</td>
<td>15 (2.1)</td>
<td>0.62</td>
</tr>
<tr>
<td>Mean blood vessel diameter, μm</td>
<td>12 (0.4)</td>
<td>11 (0.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean blood flow velocity, μm/s</td>
<td>244 (24)</td>
<td>275 (40)</td>
<td>0.56</td>
</tr>
<tr>
<td>Total microvascular blood flow, pl/min</td>
<td>87 (6)</td>
<td>51 (9)</td>
<td>0.007</td>
</tr>
<tr>
<td>30 min After reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical area, mm²</td>
<td>0.57 (0.05)</td>
<td>0.59 (0.03)</td>
<td>0.72</td>
</tr>
<tr>
<td>Vessel length analyzed, %</td>
<td>86 (2)</td>
<td>88 (2)</td>
<td>0.40</td>
</tr>
<tr>
<td>Vascular density, mm²/m²</td>
<td>16 (1.9)</td>
<td>16 (1.4)</td>
<td>0.88</td>
</tr>
<tr>
<td>Mean blood vessel diameter, μm</td>
<td>11 (0.8)</td>
<td>11 (0.5)</td>
<td>0.33</td>
</tr>
<tr>
<td>Mean blood flow velocity, μm/s</td>
<td>356 (57)</td>
<td>337 (54)</td>
<td>0.82</td>
</tr>
<tr>
<td>Total microvascular blood flow, pl/min</td>
<td>108 (33)</td>
<td>68 (8)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Data are presented as means (SE) and are compared with independent sample t-tests. Peritubular blood vessels ~25 μm in diameter were analyzed.
At 30 min after reperfusion, asterisks denote statistical significance. Minimal, and maximal glycocalyx dimensions relative to living donor kidneys (REF) were measured during reperfusion. Data are presented as median, interquartile range, minimal, and maximal glycocalyx dimensions to living donor kidneys at 30 min after reperfusion. Asterisks denote statistical significance.

Living donors ($P = 0.02$). Since mean microvascular blood flow velocity was comparable, the smaller diameter resulted in a significant 42% reduction of total blood volume flowing through the peritubular capillary network of DCD compared with living donor kidneys ($P = 0.007$). At 30 min after reperfusion, this difference in volumetric blood flow persisted but was no longer statistically significant ($P = 0.24$). Renal perfusion pressure at the time of imaging was comparable between the two study groups (data not shown). These findings indicate that ischemic acute kidney injury is associated with reduced cortical microvascular blood flow in the early reperfusion period.

**Endothelial glycocalyx.** The luminal surface of the capillary endothelium is covered by the glycocalyx, a dynamic network of proteoglycans and glycoproteins that contributes to endothelial function and microvascular perfusion (7, 29). To investigate whether the glycocalyx was disrupted by ischemia and reperfusion, we measured red blood cell exclusion zones in the cortical peritubular microcirculation of transplanted kidneys using sidestream darkfield imaging. At 5 min after reperfusion, red blood cell exclusion zones in DCD kidneys were 0.16 ± 0.06 μm smaller than those in living donor kidneys ($P = 0.02$, Fig. 2), indicating significant loss of glycocalyx in ischemically injured kidneys. Although glycocalyx thickness increased during the early reperfusion period ($P < 0.05$), differences between the two kidney types persisted until 30 min after reperfusion, but were no longer statistically significant at that time ($P = 0.20$).

Degradation of the endothelial glycocalyx was confirmed by measuring renal arteriovenous gradients of the main glycocalyx constituents syndecan-1 and heparan sulfate. At 5 min after reperfusion of DCD kidneys, concentrations of syndecan-1 and heparan sulfate in the transplant renal vein were higher than those in arterial blood ($P = 0.02$ and $P = 0.06$, respectively, Fig. 3). In contrast, no arteriovenous differences in syndecan-1 and heparan sulfate concentrations were observed in living donor kidneys ($P = 0.38$ and $P = 0.78$). The renal flux of syndecan-1, but not of heparan sulfate, was significantly greater in DCD kidneys than in kidneys from living donors ($2.6 ± 1.0$ vs. $0.2 ± 0.5$ μg·min$^{-1}$·100 g$^{-1}$, $P = 0.02$ for syndecan-1, and $57 ± 32$ vs. $114 ± 85$ μg·min$^{-1}$·100 g$^{-1}$, $P = 0.59$ for heparan sulfate). Moreover, creatinine clearance on the first day after transplantation was inversely correlated to the renal flux of syndecan-1 at 5 min after reperfusion ($R = -0.77$, $P = 0.001$). Renal arteriovenous differences of syndecan-1 and heparan sulfate had dissipated at 30 min after reperfusion (data not shown). Taken together, these findings provide strong evidence for glycocalyx disruption in the cortical peritubular microcirculation of ischemically injured kidneys.

**Histological assessment of peritubular capillary endothelium.** Since ischemic acute kidney injury was associated with reduced blood flow in the peritubular microcirculation and with shedding of the endothelial glycocalyx, we studied endothelial cell injury and activation in peritubular capillaries in renal biopsies taken at 10 min after reperfusion. In tissue sections stained for the endothelial cell markers CD31 and CD34, the endothelial lining of the peritubular capillaries was uninterrupted without evidence of endothelial denudation. To assess for endothelial cell activation, tissue sections were stained for P-selectin and the staining intensity was scored semiquantitatively. P-selectin staining intensity of DCD kidneys was similar to living donors, which generally suffer the most extensive ischemic cell injury and activation in peritubular capillaries in renal injury, had significantly greater staining intensity than the remainder of the donor kidneys ($1.3 ± 0.4$ vs. $0.6 ± 0.3$, $P = 0.23$, Fig. 4). Interestingly, the subset of DCD kidneys from uncontrolled donors, which generally suffer the most extensive ischemic injury, had significantly greater staining intensity than the remainder of the donor kidneys ($2.3 ± 0.7$ vs. $0.6 ± 0.2$, $P = 0.04$). These findings indicate that ischemic acute kidney injury...
Kidney transplantation is inevitably associated with renal ischemia and reperfusion, leading to a transient reduction in glomerular filtration rate due to tubular obstruction, afferent arteriolar vasoconstriction, and transtubular backleak of ultrafilterate (34). Furthermore, renal ischemia and reperfusion results in upregulation of genes involved in cellular injury and repair (particularly genes regulating oxidative stress and apoptosis) and inflammation (particularly toll-like receptors, complement components, chemokines, and adhesion molecules). In animal studies, each of these pathways has been shown to contribute to ischemic acute kidney injury. Despite these advances, specific pharmacological interventions to protect the human donor kidney from ischemia and reperfusion injury have not been found. It is therefore important to further study the pathophysiology of ischemic acute kidney injury to guide the development of more effective therapies.

In the current study, we report on the effects of acute ischemic injury on the renal microcirculation in human kidney transplantation. We took advantage of the unique accessibility of the kidney during transplant surgery to perform invasive renal hemodynamic measurements in the early reperfusion period. Two groups of donor kidneys were compared with highly different levels of ischemic injury but with similar baseline characteristics: 1) DCD kidneys that suffer extensive ischemic injury from circulatory arrest until organ preservation, and 2) kidneys from living donors that suffer minimal warm and cold ischemic injury.

The creatinine clearance of DCD kidneys was much lower than that of kidneys from living donors in the first 10 days after transplantation, illustrating the impact of acute ischemic injury on early graft function. Despite the profound reduction in glomerular filtration rate, total renovascular resistance of DCD kidneys was similar to that of living donor kidneys in the early reperfusion period. This finding seems to contradict previous publications that have shown a correlation between volumetric renal blood flow and early graft function (1, 2, 22). However, such studies are scarce and typically did not adjust for perfusion pressure and kidney weight, which may result in a confounded impression of renal hemodynamics.

In contrast to the similarity of macrovascular characteristics between DCD and living donor kidneys, microvascular perfusion of DCD kidneys was significantly reduced by 42% in the early reperfusion period. Since vascular density and blood flow velocity of the study groups were comparable, the reduction in microvascular perfusion was most likely accounted for by the significantly smaller blood vessel diameter of DCD compared with living donor kidneys. These findings are supported by previous reports of abnormalities in peritubular capillary perfusion after renal ischemia and reperfusion in rats and in human kidney transplantation (3, 5, 13, 31, 44). Moreover, microvascular dysfunction after renal ischemia and reperfusion is associated with permanent loss of up to 50% of peritubular capillaries in rats, predisposing the kidney to interstitial fibrosis and tubular atrophy (4, 16). Since sidestream darkfield imaging has a penetration depth of 300–500 μm (12), our microcirculatory measurements were confined to the cortical peritubular microcirculation at the renal surface. As the proximal tubular epithelium in the outer medulla is considered to be most vulnerable to ischemia and reperfusion injury, studies of the medullary microcirculation in human kidney transplantation may be performed as new imaging techniques become available (32).

In the current study, we show for the first time that the endothelial glycocalyx is rapidly degraded after transplantation of ischemically injured kidneys. This novel finding is in line with previous observations on glycocalyx disruption by ischemia and reperfusion in animal models and in human cardiovascular surgery (8, 24, 27, 28). We used two complementary methods to measure the integrity of the endothelial glycocalyx in the early reperfusion period during human kidney transplantation. First, smaller red blood cell exclusion zones were observed in sidestream darkfield images of the peritubular capillaries of ischemically injured DCD kidneys. Second, renal arteriovenous gradients of syndecan-1 and heparan sulfate, the main constituents of the endothelial glycocalyx, were greater in DCD kidneys than in kidneys from living donors. Red blood cell exclusion zones increased and release of glycocalyx constituents decreased during the first half-hour of reperfusion, suggesting a rapid restoration of degraded endothelial glycocalyx. Together, these findings demonstrate that damage to the endothelial glycocalyx develops early in the course of renal ischemia and reperfusion.

Glycocalyx injury may have major implications for graft function. Disruption of the endothelial glycocalyx results in vascular permeability, interstitial edema, and endothelial cell swelling (15, 39, 42). In addition, the glycocalyx acts by transducing shear stress to the underlying endothelium, which in turn responds by production of the vasodilator nitric oxide.
Glycocalyx loss in ischemically injured kidneys is therefore expected to result in reduced nitric oxide production, which has indeed been observed in human kidney transplantations with delayed graft function (20). Impaired flow-induced vasodilation and capillary compression by interstitial edema as a result of glycocalyx degradation may both account for the smaller capillary diameters observed in DCD compared with living donor kidneys. Furthermore, the intact glycocalyx prevents direct contact of blood cells with the endothelium. Disruption of the glycocalyx uncovers adhesion molecules at the endothelial surface and increases leukocyte and platelet binding to the vascular wall (9, 41). In addition, several constituents of the glycocalyx (hyaluronan fragments, heparan sulfate, and biglycan) have been shown to activate innate and adaptive immunity by signaling through toll-like receptors when shed from the extracellular matrix (17, 30, 36, 38). Degradation of the endothelial glycocalyx may therefore contribute to the excessive inflammatory response following ischemia and reperfusion.

Whereas the endothelial glycocalyx was rapidly degraded after ischemic acute kidney injury, the integrity of the endothelial cells lining the peritubular capillaries was not affected by ischemic injury on histological assessment of renal biopsies. In contrast, endothelial denudation has been observed on electron microscopy of rat kidneys exposed to 45 min of ischemia and 4 h of reperfusion (5). It would therefore be of interest to examine the peritubular capillary endothelium in more detail using electron microscopy in future studies of human ischemic acute kidney injury. Finally, we observed increased P-selectin expression on peritubular capillaries of kidneys that suffered particularly severe ischemic injury, which may reflect either the early activation of endothelial cells or the rapid deposition of platelets in the peritubular microcirculation following ischemic acute kidney injury (18).

In conclusion, our study demonstrates that ischemically injured DCD kidneys are characterized by reduced microvascular blood flow and loss of glycocalyx integrity compared with a control group of intact kidneys from living donors. These findings may apply more generally to other conditions associated with ischemia and reperfusion injury such as acute myocardial infarction, stroke, and liver resection. Interventions aimed at increasing microvascular perfusion and restoring the endothelial glycocalyx may improve early graft function and enable expansion of the donor pool with kidneys that suffered prolonged ischemia.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


