Kidney Injury Molecule-1 is an early non-invasive indicator for donor brain death-induced injury prior to kidney transplantation

Willemijn N. Nijboer1, Theo A. Schuurs1, Jeffrey Damman1, Harry van Goor2, Vishal S. Vaidya1, Jaap J. Homan van der Heide1, Henri G.D. Leuvenink1, Joseph V. Bonventre3, Rutger J. Ploeg1

1Surgery Research Laboratory & Department of Surgery
2Department of Pathology & Laboratory Medicine
4Department of Internal Medicine, Division of Nephrology
University Medical Center Groningen, University of Groningen
The Netherlands

3Renal Division, Department of Medicine,
Brigham and Women's Hospital, Harvard Medical School
Boston, MA 02115 USA

With more marginal deceased donors affecting graft viability there is a need for specific parameters to assess kidney graft quality at the time of organ procurement in the deceased donor. Recently, Kidney Injury Molecule-1 (Kim-1) was described as an early biomarker of renal proximal tubular damage. We assessed Kim-1 in a small animal brain death model as an early and non-invasive marker for donor derived injury related to brain death and its sequelae, with subsequent confirmation in human donors.

In rat kidney, real-time PCR revealed a 46-fold Kim-1 gene upregulation after 4h of brain death. In situ hybridisation showed proximal tubular Kim-1 localization, which was confirmed by immunohistochemistry. Also, Luminex assay showed a 6.6-fold Kim-1 rise in urine after 4h of brain death. In human donors, 2.5-fold KIM-1 gene upregulation and two-fold higher urine levels were found in DBD donors compared to living kidney donors. Multiple regression analysis showed that urinary KIM-1 at brain death diagnosis was a positive predictor of recipient serum creatinine 14 days (P<0.001) and 1 year (P<0.05) after kidney transplantation.

In conclusion, we think that Kim-1 is a promising novel marker for the early, organ specific and non-invasive detection of brain death-induced donor kidney damage.
- Introduction -

Kidney transplantation has become the therapy of choice in the treatment of end-stage renal failure. Nowadays, due to the persistent donor shortage more organs from living donors are used in transplantation. However, the majority of donor organs is still retrieved from heart beating donation after brain death donors (DBD donors). Living (un)related grafts are associated with better survival and lower rates of delayed graft function compared to kidneys retrieved from DBD (1;2). Also, brain death in the donor has been reported as a probable risk factor for developing vascular rejection (3).

In the past years it has become clear that this difference in success can largely be attributed to pathophysiological changes during the brain death process in the donor in addition to other factors as are, warm and cold ischemia times or HLA mismatches (4;5). Experimental and clinical studies have shown that brain death negatively affects hemodynamic stability and hormone regulation in the donor (6;7). Our group, as well as others, have demonstrated that brain death induces pro-inflammatory and pro-coagulatory responses in potential donor organs, enhancing the immunogenicity of the graft-to-be and affecting the allograft response in the recipient (8-10). Despite a better insight, the exact causes and mechanisms of brain death which leads to decreased organ viability have not been determined.

Given the lack of knowledge about the underlying processes responsible for the systemic changes, it is very difficult to predict outcome after transplantation. With older and more marginal donors, a better assessment of organ quality in the DBD has become very important. Obviously, it would be beneficial to know beforehand whether an organ is injured to such an extent that transplantation will not be successful. Unfortunately, in DBD kidney donors, plasma creatinine and creatinine clearance (either calculated by the Cockcroft-Gault equation or measured) are poor predictors of long term graft function and survival (11;12). This is especially important, as the criteria upon which donor organs are used have been extended in the last years to meet the shortage of donor organs (13). If a better prediction of transplantation success could be made in the donor, matching of organ and recipient characteristics could be enhanced. Also, strategies for donor management, organ preservation and immunosuppression in the recipient could be optimized, leading to better transplantation outcome.

Other approaches to assess organ viability are often based on post-transplant data, and not available prior to organ recovery and preservation. Therefore, we have searched for a marker that is able to better detect donor kidney damage prior to retrieval and that could predict transplantation success in terms of the likelihood of delayed graft function, long term function and graft survival.

Previously, we performed DNA microarray studies comparing transcriptional changes in kidneys from brain dead (normo- or hypotensive) versus living donor rats (14). One of the genes that was identified to be upregulated during brain death is Kidney Injury Molecule-1 (KIM-1 in humans, or Kim-1 in rodents). KIM-1 was first described by Ichimura et al in 1998 (15) and is a type 1 transmembrane protein. It structurally resembles mucosal ad-
dressin cell adhesion molecule 1 (MAdCAM-1) which is a member of the immunoglobulin
gene superfamily. KIM-1 has been reported to be very specific and upregulated in renal
tubular cells in a variety of injury models (16), in various human renal disease (17) and renal
cell carcinoma in man (18). The same group showed that upon injury, KIM-1's ectodomain is
actively cleaved and shedded into the lumen of the renal tubules to appear in the urine (19).
Urinary KIM-1 correlates significantly with tissue KIM-1 expression in protein overload
nephropathy (20). Also, the rise of KIM-1 levels in urine precedes any increase of serum
creatinine.

We estimated that these characteristics make KIM-1 a promising and elegant marker for the
detection of brain death-induced injury in a potential organ donor: not only specific for the
kidney and in particular for tubular injury, but also as an early and sensitive marker. The fact
that the ectodomain of KIM-1 is shed into urine obviously facilitates sampling in a clinical
setting.
In this study, we have analyzed the effect of brain death on the expression of Kim-1 and
evaluated its use as a new biomarker in organ donation and transplantation. We have used
our slow induction model of brain death in the rat, simulating the clinical situation of
cerebral injury due to hemorrhage. We have studied KIM-1 gene and protein expression
in the kidney and in urine at different intervals after induction of brain death using various
techniques. In addition, we report the first pilot with KIM-1 as a kidney injury marker in
brain death in man, including multiple regression analysis to study the effect of KIM-1 on
outcome after kidney transplantation.

- Materials and Methods -

Animal model
Adult male Fisher F344 rats (250-300 g) were used. Animals received care in compliance
with the guidelines of the local animal ethics committee according to the Experiments on
Animals Act (1996) issued by the Netherlands Ministry of Public Health, Welfare and
Sports.
Rats were randomly divided into five groups, each group consisting of six animals. Brain
death was induced as described previously (21). Briefly, animals were anesthetised using
isoflurane with a mixture of O₂/NO₂. A cannula was inserted in the femoral artery for blood
pressure monitoring. Animals were intubated via a tracheostomy and ventilated through-
out the experiment. Through a frontolateral borehole in the skull a no. 4 Fogarty catheter
(Edwards Lifesciences Co., Irvine, CA) was placed subdurally and slowly inflated (16 μl/
min) with saline using a syringe pump (Terufusion, Termo Co., Tokyo, Japan). Inflation of
the balloon was stopped during the subsequent sharp rise in blood pressure, which reflects
the autonomic storm at the beginning of brain death, and which typically took place after
30 min. Brain death was confirmed by the absence of corneal and pupillary reflexes and an
apnea test. From the beginning of brain death, animals received no more anesthesia. If blood pressure fell below 80 mmHg, it was restored by the administration of 10% hydroxyethylstarch (HAES-Steril 10%, Fresenius Kabi AG, Bad Homburg, Germany). Temperature was monitored rectally and kept constant. Animals were kept brain dead for 30 minutes, 1 hr, 2 hrs or 4 hrs. At the end of the brain death period, a laparotomy was performed and blood and urine were collected from the aorta and the bladder. Serum and urine were snap frozen and stored at -80˚C until further analysis. Then, kidneys were flushed with saline and removed. Kidney tissue was partly snap frozen in liquid nitrogen and partly fixed in formalin and embedded in paraffin. Sham operated rats served as controls. These animals were cannulated and intubated as described before, but the subdurally placed Fogarty catheter was not inflated. After 30 minutes of ‘no-touch; a laparotomy was performed and blood, urine and kidneys were collected as described for the study groups.

**Patient material**

From 2004, kidney biopsy specimens, blood and urine samples were routinely obtained during organ recovery procedures from a consecutive series of DBD (N=20) and living kidney donors (N=20). Donors whose kidneys were discarded for transplantation after retrieval were not included in this analysis. Biopsy specimens were collected just prior to organ perfusion using a 16G Acecut biopsy gun (TSK Laboratory, Tochigi, Japan). Specimens were cut in two halves. One half was immersed immediately in RNaLater (Ambion, Austin, TX, USA) until storage at -80˚C. The other half was embedded in Tissue-Tek (Sakura Finetek Europe, Zoeterwoude, The Netherlands) and snap-frozen in isopentane on dry ice (-80˚C). Serum and urine samples from the same donors were collected at two different time points. Baseline samples were collected at the time of brain death diagnosis; for living donors these samples were collected before donor nephrectomy (T0). Further, samples were collected during organ retrieval simultaneously with obtaining our biopsy specimens prior to cessation of heart beat and/or retrieval (T1). All samples were placed on ice; to urine samples 1 tablet of proteinase inhibitor cocktail (Roche) was dissolved. Blood samples were centrifuged and supernatants were stored at -80˚C until further analyses.

**Biochemical determinations in serum and urine samples**

In all human serum samples, creatinine, C-reactive protein (CRP) and lactate dehydrogenase (LDH) was determined in a routine fashion (Mega, Merck, Amsterdam, The Netherlands) to standardize serum values of parameters commonly used to assess kidney function in DBD donors during organ recovery. In urine samples of both rats and patients, urine creatinine levels were measured using the Jaffe reaction and total protein was measured using a modified Lowry’s protein assay (Biorad DC Protein Assay, Biorad, Hercules, CA, USA).

**Real-Time RT-PCR**

Total RNA was isolated from snap frozen cross-section kidney slices, using the SV Total RNA Isolation Kit (Promega, Madison, WI, USA) following the manufacturer’s instruc-
tions, including a DNase treatment step. RNA samples were verified for absence of genomic DNA contamination by performing RT-PCR reactions, in which the addition of reverse transcriptase was omitted, using β-actin primers. For animal tissue, 1 μg of RNA was then reverse transcribed into cDNA in a reaction volume of 20 μl using M-MLV Reverse Transcriptase (Invitrogen). For human tissue 130 ng of RNA was reverse transcribed using the Superscript II Reverse Transcriptase kit (Invitrogen). Gene-specific primers for KIM-1 were designed using the computer program Primer Express 2.0 (Applied Biosystems, Foster City, CA, USA) using published gene sequences. For rat, primer sequences were as follows:

forward primer 5'-AGAGAGAGCAGGACACGCTT-3' and reverse primer 5'-ACCCGTGGTAGTCCCAAACA-3'. For human tissue, primer sequences were 5'-CGTCCACCAGCAATGCTT-3' (forward) and 5'-TCTGCAGACTAGTGGTTTGTC-3' (reverse). Amplification and detection were performed with the Applied Biosystems 7900 HT Real Time PCR System (Applied Biosystems, Foster City, USA) using emission from SYBR Green (SYBR Green master mix, Applied Biosystems). All assays were performed in triplicate. After an initial activation step at 50 °C for 2 min. and a hot start at 95°C for 10 min., PCR cycles consisted of 40 cycles at 95°C for 15 sec. and 60°C for 60 sec. Specificity of qPCR products was routinely assessed by performing a dissociation curve at the end of the amplification program and by gel electrophoresis. Gene expression was normalized with the mean of β-actin mRNA content and calculated relative to controls using the relative standard curve method. Results were finally expressed as $2^{ΔCT}$ (CT threshold cycle).

**In Situ Hybridisation**

In situ hybridisation was performed on 5 μm paraffin rat kidney tissue sections. Probes were made by subcloning Kim-1 PCR product into the pCRII-TOPO vector (Invitrogen) and labeling the probes using a DIG RNA labelling kit (Sp6/T7) (Roche, Mannheim, Germany) following the manufacturer’s instructions.

Tissue sections were depaaffinized and air-dried for 10 minutes. Slides were treated with Triton X-100 (Sigma-Aldrich, St Louis, MO, USA), followed by proteinase K (5 μg/ml in TBS) (Roche) at 37°C for 10 minutes. After washing with phosphate buffered saline (PBS), slides were incubated with 10 ng/100 μl DIG labelled probe (anti-sense or sense) in a hybridization solution containing 50 μl 100x Denhardt’s solution overnight at 55°C. After washing, slides were treated with 2 U/ml RNase T1 (Sigma-Aldrich) in 1 mM EDTA (pH 8) and 2x SSC at 37°C for 30 minutes. Positive cells were visualized with anti-DIG labelled alkaline phosphatase (Roche) for one hour at 37°C in 0.1 M maleic acid buffer containing 0.15 M NaCl, 1% blocking buffer and 2% normal sheep serum. Staining reaction was performed for 48 hours at 4°C with nitro-blue tetrazolium (NBT) and 5-bromo,4-chloride, 3-indolyolphosphate (BCIP) in 1 ml AP buffer (pH=9.0) containing 50 mM MgCl2 and 0.01 M levamisole.

Localisation and quantitation of staining was assessed by light microscopy in sham treated and 4h brain dead rats. At 400x magnification, 10 microscopic fields of the outer medulla/
inner cortex region of each tissue section were quantified using computerized image analysis (Advanced QUIPS, Leica Imaging Systems, Cambridge, UK). Sections were projected on a video screen and the KIM-1 positive parts in the outer medulla/inner cortex were outlined manually. Kim-1 positive areas were calculated as a proportion of total surface, with exclusion of glomeruli and blood vessels.

**Immunohistochemistry**

Immunohistochemistry was performed on 5 μm paraffin rat kidney tissue sections. Tissue sections were deparaffinized and antigen retrieval was done by overnight incubation in 0.1 M Tris-HCl buffer (pH 9.0) at 80°C. Next, they were stained with primary polyclonal antibody rabbit KIM-1 R9 (22) using an indirect immunoperoxidase technique. After thorough washing, the sections were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies (Dako, Glostrup, Denmark). The reaction was developed using 3,3-diaminobenzidine tetrahydrochloride /H₂O₂. Sections were counterstained using Mayer’s hematoxylin solution (Merck, Darmstadt, Germany). Negative antibody controls were performed. Localization of immunohistochemical staining was assessed by light microscopy.

**Protein Detection in Urine**

For quantification of Kim-1 protein in rat urine, urine samples were measured using microsphere-based Luminex xMAPTM technology (Liangos 2007). This technique is an adaptation of the recently developed and validated sandwich ELISA assay (23). For quantification of human KIM-1 protein in urine, a sandwich ELISA was used as described previously (24). Levels were normalized using urine creatinine values, to account for the dilution effect due to brain death-induced diabetes insipidus.

**Statistical Methods**

Statistical analysis was performed using the computer program SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). Results are expressed as arithmetic means ± standard error of the mean. Statistical comparisons between unpaired groups were performed using the Mann Whitney test.

Further, independent predictors of renal function in the kidney recipients of the tested donors were assessed using stepwise multiple regression analysis. Three multiple linear regression models were developed, examining serum creatinine in the recipient at 14 days, 3 months and 1 year after transplantation as the dependent variables. The independent variables in each model were restricted to variables known during organ recovery, and included donor age and gender, duration of brain death, duration of hospital stay, cause of brain death (trauma, cerebrovascular incident (CVA) or other), serum creatinine, LDH, and CRP during admission and at T0 and T1, urinary creatinine, total protein and KIM-1 at T0 and T1. All differences were considered to be significant at P<0.05 and all the statistical tests were two-tailed.
- Results -

Gene expression of Kim-1 in the rat donor kidney

To quantify Kim-1 gene expression during brain death, real-time quantitative RT-PCR was performed. A profound increase of Kim-1 gene expression was found 4h after induction of brain death compared to sham operated control rats (46.1 fold induction ± 11.9 vs 1.0 ± 0.5; P < 0.01, see Figure 1). At the other, earlier time points, no difference in gene expression was seen between brain dead and control animals.

Figure 1 Kim-1 gene expression in the rat kidney. Gene expression was normalized using β-actin mRNA content and calculated relative to controls using the relative standard curve method. Results are expressed as fold induction, with sham group values fixed at 1. Differences were tested using the Mann-Whitney test.

To study the localization of Kim-1 gene expression in the kidney, in situ hybridization of Kim-1 mRNA was performed. After 0.5h and 1h hour of brain death, as well as in sham operated animals, hardly any Kim-1 mRNA staining could be detected. After 2h of brain death, 1 out of 6 rats showed a weak staining for Kim-1 mRNA, while after 4h of brain death, 4 out of 6 animals showed a strong staining for Kim-1 mRNA. Kim-1 mRNA was primarily localized in those parts of the tubuli which are present in the outer medulla extending into the inner cortex, in proximal tubular cells. As expected in staining mRNA, staining seemed to appear dispersed throughout the entire cell (Figure 2A and B).
Kim-1 protein in the rat kidney

To analyze the localization of Kim-1 protein in the kidney immunohistochemistry was performed. From the 4 rats that had shown a strong staining for Kim-1 mRNA after 4h of brain death, 3 rats also showed a positive staining for subsequent Kim-1 protein formation in the kidney. At earlier points in time, as well as in sham operated controls, no Kim-1 protein expression was seen. Tissue sections were not morphometrically quantified, as staining was weak, compared to in situ hybridization. More importantly, Kim-1 protein expression co-localized with gene expression as seen with in situ hybridisation. The Kim-1 protein was detected at the apical side of tubular epithelial cells (Figure 2C and D). To test whether Kim-1 expressing cells are dedifferentiated in the brain dead rat, a staining for vimentin was performed. No vimentin staining in Kim-1 positive areas in the kidney was found.

Figure 2  Kim-1 localisation in the rat kidney. Tissue sections are shown at 100x magnification. Localisation of gene expression is shown by in situ hybridisation in a sham operated animal (A) and a 4h brain dead animal (B). Kim-1 protein expression is shown in sham operated animals (C) and after 4h of brain death (D).
Shed Kim-1 protein in rat urine

To quantify Kim-1 protein in urine, the Kim-1 levels shed were measured by Luminex ELISA. Kim-1 levels were normalized for the abundance of creatinine from the same sample. When urine from sham operated animals was compared to that of brain dead animals a significant elevation of Kim-1 protein levels was found in the urine of brain dead animals (Figure 3). After 4h of brain death, relative Kim-1 levels were 35.5 ng/mmol creatinine ± 22.1 as compared to 5.4 ng/mmol creatinine ± 1.1 in sham operated counterparts (P<0.05).

Figure 3  Kim-1 protein in rat urine. Kim-1 was measured by Luminex ELISA and normalized with urinary creatinine to account for dilution caused by diabetes insipidus in brain death. Differences were tested using the Mann-Whitney test.

Assessment in human donors

Following our experiment in the rat model we studied the expression of the KIM-1 gene in human kidney biopsy specimens using qPCR. Demographics of the human donors are listed in Table 1. KIM-1 gene expression was found to be upregulated 2.5-fold in DBD donors compared to living donors (P=0.002) (Figure 4).
Table 1  Characteristics of human kidney donors.

<table>
<thead>
<tr>
<th>Donor type</th>
<th>DBD donors</th>
<th>Living donors</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>7/13</td>
<td>7/13</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.7±2.1</td>
<td>51.5±2.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Death: CVA</td>
<td>11</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Death: trauma</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Death: other</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Duration of brain death (min)</td>
<td>655±21</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hospital stay (h)</td>
<td>72.3±16.5</td>
<td>24*</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine: adm/T0/T1</td>
<td>111±26 / 72±7 / 61±6</td>
<td>- / 67.1±4.3 / 60.9±2.1</td>
<td>/ 0.6 / 0.4</td>
</tr>
<tr>
<td>Serum LDH: adm/T0/T1</td>
<td>409±83 / 292±66 / 212±30</td>
<td>- / 131.0±3.5 / 141.5±12.9</td>
<td>/ &lt;0.001 / 0.02</td>
</tr>
<tr>
<td>Serum CRP: adm/T0/T1</td>
<td>10.8±3.9 / 80.4±19.2 / 60.1±9.7</td>
<td>- / 5.4±0.3 / 5.5±0.3</td>
<td>/ &lt;0.001 / &lt;0.001</td>
</tr>
<tr>
<td>Creatinine in urine: T0/T1</td>
<td>3.5±0.9 / 2.8±0.6</td>
<td>7.7±1.2 / 4.5±0.9</td>
<td>0.002 / NS</td>
</tr>
<tr>
<td>Total protein in urine: T0/T1</td>
<td>13.7±3.7 / 11.3±2.4</td>
<td>5.4±0.6 / 6.8±1.1</td>
<td>NS / NS</td>
</tr>
<tr>
<td>KIM-1 in urine: T0/T1</td>
<td>204±47 / 538±137</td>
<td>246±54 / 243±73</td>
<td>NS / 0.012</td>
</tr>
</tbody>
</table>

* Living donors were routinely admitted 1 day before donor operation.
In addition, the amount of shedded KIM-1 was determined in urine samples taken from the same donors, collected before and during organ recovery. KIM-1 protein was measured in the urine using sandwich ELISA. At baseline (T0), no significant differences were found in urine KIM-1 levels between DBD and living kidney donors (DBD 203.7 ng/mmol creatinine ± 47 vs living 246.1 ng/mmol creatinine ± 54, NS). However, in the urine samples taken during organ recovery (T1) we found an increase in KIM-1 levels after the cerebral injury and the period of brain death as it rose to levels of 537.7 ng/mmol creatinine ± 137 in samples from DBD donors compared to 243.0 ng/mmol creatinine ± 73 in samples from living kidney donors (P<0.05) (Figure 5).

**Figure 5** KIM-1 protein in human urine. KIM-1 was measured using a sandwich ELISA. T0 living: sample taken at the beginning of living donornephrectomy; T0 BD: sample taken after diagnosis of brain death; T1 living / BD: sample taken just prior to organ retrieval and perfusion. Differences were tested using the Mann-Whitney test.
Next, multiple linear stepwise regression models were used to determine which donor factors in DBD donors had an independent effect on kidney function after transplantation. As we were primarily interested in variables which are known before organ retrieval, variables such as cold ischemia time were not included. For the analyses, one year-follow up from one kidney only from each DBD donor was randomly chosen. In this cohort, 4/20 recipients suffered from delayed graft function, and 1/20 recipients suffered from graft loss in the first year. Therefore, no models were made for these outcome variables. Instead, serum creatinine levels in recipients at 14 days, 3 months and 1 year after transplantation were taken as dependent variables. Outcomes are shown in Table 2.

Table 2  Multiple regression models for recipient kidney function after human kidney transplantation. Donor variables from Table 1 were entered in the analyses.

<table>
<thead>
<tr>
<th>Recipient serum creatinine 14 days</th>
<th>Beta (Std.Error)</th>
<th>P-value</th>
<th>R^2-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor urinary KIM-1 at T0</td>
<td>0.106</td>
<td>&lt;0.001</td>
<td>0.995</td>
</tr>
<tr>
<td>Donor gender</td>
<td>49.975</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Donor urinary KIM-1 at T1</td>
<td>-0.022</td>
<td>0.033</td>
<td></td>
</tr>
</tbody>
</table>

Recipient serum creatinine 3 months

| Donor age                          | 1.102 (0.227)   | 0.008   | 0.854     |

Recipient serum creatinine 1 year

| Donor serum creatinine at admission | -0.369 (0.016)  | 0.002   | 0.997     |
| Cause of donor death: trauma vs non-trauma | 30.367 (2.250) | 0.005   |           |
| Donor urinary KIM-1 at T1           | 0.006 (0.001)   | 0.045   |           |
The major finding of this study is the distinct rise of *Kim-1* gene expression as well as protein shedding in the urine within 4h of brain death, while morphology of the epithelium is still well-retained and serum creatinine levels have changed only little, remaining within the normal range. Our results resemble those obtained with the folic acid nephrotoxic model of Ichimura et al (25) and suggest that *Kim-1* may be a very sensitive biomarker for detection of low-level renal injury or early stages of repair during brain death in donors.

The question arises how early in the injury/repair process of acute kidney damage in brain death *Kim-1* is expressed. Upon noxious stimuli, surviving tubular cells react with a complex process of dedifferentiation and proliferation, followed by regeneration with renewed differentiation (26). Ichimura et al showed (27) that *Kim-1* protein is upregulated in vimentin-positive tubular cells, with vimentin as a marker of dedifferentiation. Also, Kuehn et al showed *Kim-1* upregulation in dedifferentiated cells in murine polycystic kidney disease (28). In our experiment, we could not find any dedifferentiation as in *Kim-1* positive areas in the kidney.

The *Kim-1* upregulation in brain death could be a reaction to local ischemia in the kidney as *Kim-1* is known to increase upon ischemia/reperfusion injury (29). Also, the location of *Kim-1* expression makes this hypothesis likely, as proximal tubular cells are known to be very sensitive to ischemic damage (30). Although all animals were kept stable at a mean arterial pressure of 80 mmHg during the period of brain death, a short phase of low systemic blood pressure preceding the moment of herniation of the brain stem is a hallmark of brain death induction and might have contributed to *Kim-1* expression. One could also speculate that *Kim-1* in the kidney has a role in the inflammatory response that takes place during brain death, as *Kim-1* shows homology to MAdCAM-1, an adhesion molecule expressed in intestine and the nervous system which plays a role in leukocyte homing in intestinal inflammation (31;32).

To see whether our findings in the rat model are also present in the human situation we then investigated upregulation and shedding of KIM-1 in a consecutive series of regional donors retrieved by our team. We compared the results to those from living donor kidneys at our institution. This is the first study to report on KIM-1 shedding in the urine of in human DBD donors. As the brain death period in human donors typically amounts to over eight hours (33;34), the finding of increased KIM-1 levels could create a ‘window of opportunity’ for diagnostic and therapeutic measures during donor management and selection of recipients.

To investigate whether KIM-1 shedding in DBD donors had any predictive value for kidney function after transplantation, we built three multiple linear regression models. In these models, we chose to include only donor variables which can be known before actual organ recovery. To our surprise, KIM-1 at T0 turned out to be an independent positive predictor of serum creatinine at 14 days and 1 year after transplantation. Although KIM-1 levels at T0 do not differ between DBD donors and living donors on average, the difference within the group of DBD donors reflects the differences seen in transplantation success of DBD donor grafts.
Summarizing, in this study we have shown that Kim-1, which is known to be a specific marker for renal tubular damage, is substantially upregulated after 4 h of brain death in the rat model and is shed into the urine. In addition, patient data show for the first time that KIM-1 is also upregulated in human DBD organ donors compared to living kidney donors. KIM-1 measured at the time of brain death diagnosis is an independent predictive factor for short time kidney function after transplantation. On the basis of our experimental and first clinical findings we feel that further clinical study is justified to assess the efficacy of KIM-1 as a viability marker and predictor of outcome after donation and kidney transplantation.
References


