Original Articles

N-octanoyl dopamine treatment exerts renoprotective properties in acute kidney injury but not in renal allograft recipients

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ABSTRACT

Background. N-octanoyl dopamine (NOD) treatment improves renal function when applied to brain dead donors and in the setting of warm ischaemia-induced acute kidney injury (AKI). Because it also activates transient receptor potential vanilloid type 1 (TRPV1) channels, we first assessed if NOD conveys its renoprotective properties in warm ischaemia-induced AKI via TRPV1 and secondly, if renal transplant recipients also benefit from NOD treatment.

Methods. We induced warm renal ischaemia in Lewis, wild-type (WT) and TRPV1−/− Sprague–Dawley (sd) rats by clamping the left renal artery for 45 min. Transplantations were performed in allogeneic and syngeneic donor–recipient combinations (Fisher to Lewis and Lewis to Lewis) with a cold ischaemia time of 20 h. Treatment was instituted directly after restoration of organ perfusion. Renal function, histology and perfusion were assessed by serum creatinine, microscopy and magnetic resonance imaging (MRI) using arterial spin labelling (ASL).

Results. NOD treatment significantly improved renal function in Lewis rats after warm ischaemia-induced AKI. It was, however, not effective after prolonged cold ischaemia. The renoprotective properties of NOD were only observed in Lewis or WT, but not in TRPV1−/− sd rats. Renal inflammation was significantly abrogated by NOD. MRI-ASL showed a significantly lower cortical perfusion in ischaemic when compared with non-ischaemic kidneys. No overall differences were observed in renal perfusion between NOD- and NaCl-treated rats.

Conclusions. NOD treatment reduces renal injury in warm ischaemia, but is not effective in renal transplant in our experimental animal models. The salutary effect of NOD appears to be TRPV1-dependent, not involving large changes in renal perfusion.

Keywords: AKI, ischaemia, rat, renal transplantation, TRPV1

INTRODUCTION

Acute kidney injury (AKI) is recognized as a major complication in intensive care unit patients [1–4]. Approximately 13% of hospital-acquired AKI patients will progress to end-stage renal disease within 3 years [5]. Innovative interventions beyond supportive therapy are currently not available for AKI patients, emphasizing the clinical need for new therapeutic approaches.

Delayed graft function (DGF) is a manifestation of AKI with attributes unique to transplantation (Tx). The diagnosis of DGF is complicated by a variety of definitions based on a range of clinical criteria depending on the local transplant centre, region and country [6, 7]. Along with an increased use of marginal
of Laboratory Animals published by the National Academy of Sciences and were approved by the local authorities (Regierungsspräsidium Karlsruhe AZ: 35-9185.81/G—19/12, 35-9185.81/G—229/12).

Ischaemia-induced AKI and renal Tx

All surgical procedures were performed under general anaesthesia using ketamine (100 mg/mL; Ketamin®, Intervet Deutschland, Unterschleißheim, Germany) and xylazine (6 mg/mL; Rompun®, Bayer Health Care, Leverkusen, Germany) intraperitoneally. AKI was induced by clamping the left renal artery for 45 min in unilateral nephrectomized rats. For arterial spin labelling (ASL) measurements, the contralateral kidney was not removed but served as a non-ischaemic control.

Allogeneic and syngeneic renal Txs were performed as described previously [28, 29]. After explantation, donor kidneys were stored at 4°C in University of Wisconsin (UW) solution for 20 h, before Tx into bilaterally nephrectomized recipients.

Osmotic minipump (Alzet® 2ML1, 10 µL/h, Charles River Laboratories) filled with 40 µM NOD (Novaliq GmbH, Heidelberg, Germany) or NaCl (0.9%, Fresenius Kabi Deutschland, Bad Homburg, Germany) was implanted subcutaneously directly after induction of AKI or after Tx as described [28, 29]. Each group consisted of a minimum of eight animals. No immunosuppression was administered in the Tx model. Animals were treated according to the following scheme.

Acute kidney injury

Group 1: Control; ischaemia time 45 min; NaCl treatment for 5 days
1A: AKI in Lewis rats.
1B: AKI in WT sd rats.
1C: AKI in TRPV1−/− sd rats.
1D: AKI (left kidney) in Lewis rats, right kidney was not removed and served as a control for renal perfusion experiments.

Group 2: NOD; ischaemia time 45 min; NOD treatment for 5 days
2A: AKI in Lewis rats.
2B: AKI in WT sd rats.
2C: AKI in TRPV1−/− sd rats.
2D: AKI (left kidney) in Lewis rats, right kidney was not removed and served as a control for renal perfusion experiments.

Transplantation

Allogeneic: Graft from Fisher donors transplanted into bilaterally nephrectomized Lewis recipients.

Group 3: Allo-Tx—control; post Tx treatment with NaCl for 7 days.
Group 4: Allo-Tx—NOD; post Tx treatment with NOD for 7 days.
Syngeneic: Graft from Lewis donors transplanted into bilaterally nephrectomized Lewis recipients.
Group 5: Syn-Tx—control; post Tx treatment with NaCl for 7 days.
Group 6: Syn-Tx—NOD; post Tx treatment with NOD for 7 days.

Renal function
Renal function was assessed by serum creatinine (s-crea) and serum urea (s-urea), measured on Days 0, 1, 3 and 5 (and 7 in Tx models) after surgery. S-crea and s-urea are expressed as mean in mg/dL ± SD.

Immunohistochemistry
The kidneys were harvested on Day 5 after AKI and on Day 7 after Tx, and immunohistochemical staining was performed as described previously [30]. Quantification of ED1 staining was performed by morphometric analysis using the software (Cell F 5.1, Olympus Soft Imaging Solutions, Münster, Germany). For each section, 20 random high magnification (×400) microscopic fields were analysed; the results are expressed as percent ED1-stained area per microscopic field ± SD.

Real-time quantitative polymerase chain reaction
Total RNA was isolated from renal tissue using Trizol- Reagent (Life Technologies, Inc., Rockville, MD, USA). After DNase treatment, (RNase free DNase I, Ambion, Woodward, Austin, TX, USA) 1 µg of total RNA was reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies). TaqMan™ real-time polymerase chain reaction was used for quantitative measurement of mRNA expression of CXCL1 (Rn00578225_m1), E-selectin (Rn00594072_m1), ICAM (Rn00564227_m1), IL-6 (Rn99999017_m1) and VCAM1 (Rn00563627_m1). All samples were measured in triplicate. Gene expression was normalized to the housekeeping gene -actin (Rn006677869_m1) and expressed as fold change relative to control native kidney and calculated with the ΔΔCT method [31].

Live cell calcium imaging
Preparation of acutely dissociated dorsal root ganglion (DRG) neurons and imaging were done as described previously [17]. A cell was considered an excitable neuron and then evaluated only when significantly responding to application of capsaicin and/or depolarization by high potassium solution (140 mM). As determined by live cell calcium imaging, neither capsaicin nor NOD (30 and 100 µM) were able to acti- vate TRPV1 in DRG isolated from TRPV1−/− sd rats, while this was clearly observed in WT sd rats (Figure 2).

Arterial spin labelling-magnetic resonance imaging
Renal perfusion was measured with magnetic resonance imaging (MRI) using a flow-sensitive alternating inversion recovery (FAIR)-ASL technique [32, 33]. In brief, to acquire a perfusion-weighted image, arterial blood is labelled by inverting its magnetization. After an inflow time (inversion time), which allows the labelled blood to flow into the tissue of interest, it is imaged with an appropriate readout sequence. When using FAIR, the labelling is alternating between a global inversion (tag image) and a slice-selective inversion (control image). Several tag–control pairs and additional M0 images, without prior labelling, are recorded. All ASL perfusion measurements were performed following the protocol and parameters described in Zimmer et al. [34]. A 3-T whole-body MR scanner (Magnetom Tim Trio, Siemens Healthcare Sector, Erlangen, Germany) was used. The total measurement time for 30 tag–control pairs and 30 M0 images was 9 min. ASL perfusion maps were calculated by analysing the acquired data with an in-house written MATLAB (Version 7.10, The MathWorks, Natick, MA, USA) script. Renal perfusion was expressed in mL/100 g/min.

Statistical analysis
Data are shown as means ± standard deviation (SD). For the calcium imaging, data are shown as means ± standard error of the mean (SEM). For comparison of two non-parametric independent groups, Wilcoxon rank sum test was applied. For comparison of two parametric independent groups, two-sample t-test was performed (JMP 10.0.0; SAS Institute, Inc., Cary, NC, USA). Statistical significance was defined as a P-value of <0.05.

RESULTS

NOD treatment improves renal function after warm ischaemia
NOD treatment significantly improved renal function in Lewis rats after induction of warm renal ischaemia (s-crea NOD versus NaCl): Day 1—2.42 ± 0.36 versus 3.02 ± 0.52, P = 0.0406; Day 3—1.04 ± 0.60 versus 3.52 ± 1.70, P = 0.0038; Day 5—0.46 ± 0.10 versus 2.04 ± 1.63, P = 0.0032; (s-urea NOD versus NaCl): Day 3—198.11 ± 106.64 versus 503.05 ± 231.94, P = 0.01; Day 5—89.75 ± 32.69 versus 387.44 ± 293.78, P = 0.0054 (Figure 1A). While NaCl-treated rats displayed a significant body weight loss, this was not observed with NOD-treated rats (Figure 1B). Also, the numbers of infiltrated ED1+ cells assessed at Day 5 were significantly decreased in NOD-treated rats (Figure 1C and D) (fold change TNF-α: 1.16 ± 0.4 versus 0.69 ± 0.19; fold change IL-6: 6.60 ± 2.3 versus 10.52 ± 3.91; not significant; P = 0.0128; NaCl versus NOD treatment). There was no difference in cytokine mRNA expression except for TNF; IL-6 was not statistically significant.

The salutary effect of NOD treatment in ischaemia-induced AKI is mediated via TRPV1
We assessed the role of TRPV1 activation in the renoprotective properties of NOD by using TRPV1−/− sd rats. To assure that TRPV1 responses were absent in the TRPV1−/− sd rats, DRG cells were isolated from WT sd and TRPV1−/− sd rats and subsequently stimulated with either capsaicin or NOD. As determined by live cell calcium imaging, neither capsaicin (10 and 100 µM) nor NOD (30 and 100 µM) were able to activate TRPV1 in DRG isolated from TRPV1−/− sd rats, while this was clearly observed in WT sd rats (Figure 2).
Warm ischaemia-induced AKI was induced in WT sd and TRPV1−/− sd rats as described for Lewis rats. Impairment of renal function was observed to a similar extent in NaCl-treated WT and TRPV1−/− sd rats, indicating that the absence of TRPV1 does not influence renal function in the course of AKI (Figure 3A). While NOD treatment was significantly improving renal function in the WT sd rats (Figure 3A, graph to the left), it was not effective in TRPV1−/− sd rats (Figure 3A, graph to the right). Loss of body weight 5 days after AKI was significantly lower in NOD-treated WT sd rats when compared with NaCl treatment. This was not observed in TRPV1−/− sd rats (Figure 3B). Irrespective of treatment, TRPV1−/− sd rats displayed less infiltration of ED1+ cells when compared with WT sd rats. Only in WT sd rats, NOD treatment significantly reduced the amount of infiltrated ED1+ cells (Figure 3C and D). No difference were observed in cytokine expression in renal tissue 5 days after AKI between the NOD-treated and control group, except for IL-6, which was significantly higher in treated WT sd rats (Table 1) and TRPV1−/− sd rats. Cytokine expression was significantly lower in the TRPV1−/− sd rats when compared with WT sd rats (Figure 3E).
**FIGURE 2**: NOD does not activate DRG from TRPV1−/− rats. Live cell calcium imaging was performed on DRG isolated from WT sd and TRPV1−/− sd rats. Images were recorded at baseline and after stimulation of DRG with 30 and 100 µM NOD, capsaicin 10 µM (1) for WT and 100 µM (2) for knockout, 140 mM potassium chloride and 10 µM ionomycin subsequently. The ratio 340/380 at 510 nm is a measure proportional to free intracellular calcium. The images are false colour-coded according to the amount of intracellular calcium (graph at the top where warmer colours indicate increasing calcium values). The graphs at the bottom show the mean trace ± SEM of at least 60 regions of interest (ROI) with each ROI containing one cell. SEM is depicted as dotted grey line.
FIGURE 3: The renoprotective effect of NOD is abrogated in TRPV1−/− sd rats. (A) s-crea (mg/dL) from WT sd (graph to the left) and TRPV1−/− sd rats (graph to the right) were assessed on Days 0, 1, 3 and 5 after induction of AKI. The animals were treated with NOD (bold line) or NaCl (dotted line) via osmotic minipumps over the whole experimental period. s-crea was significantly lower in NOD-treated rats on all days of measurements in the WT rats, whereas in the TRPV1−/− sd rats no differences were found between NOD- and NaCl-treated rats. s-crea/WT: *P = 0.038 (Day 1), **P = 0.002 (Day 3), **P = 0.004 (Day 5). s-crea/TRPV1−/− sd: P = 0.267 (Day 1), P = 0.722 (Day 3), P = 1.0 (Day 5). (B) Body weight was measured 5 days after AKI in WT sd and TRPV1−/− sd rats. The results are expressed as mean percent body weight loss relative to the body weight immediately before AKI induction. In WT sd rats, NOD treatment significantly mitigates body weight loss (P = 0.0041). No difference was found in TRPV1−/− sd rats. (C) Representative sections obtained from kidneys of NaCl- or NOD-treated WT and TRPV1−/− sd rats are depicted. (D) Renal infiltrated ED1+ cells were assessed by immunohistochemistry. Quantification of ED1+ was performed by quantitative morphometric analysis. For each animal, at least two sections were evaluated using 10 randomly chosen high power (×400 magnification) microscopic fields. The results are expressed as mean percent ED1-stained area per microscopic field ± SD. (E) The mRNA expression for different inflammatory cytokines was performed by qPCR on renal tissue harvested 5 days after AKI induction. Result expressed as relative quantity ± SEM relative to untreated native kidney.
Is renal perfusion influenced by NOD?

We assessed if the renoprotective effect of NOD was associated with improved renal perfusion. Renal cortical perfusion in the ischaemic kidney was measured 5 days after warm ischaemia-induced AKI using ASL. The non-ischaemic kidney served as a control. Renal cortical perfusion was significantly lower in the ischaemic kidney, as reported previously [34]. NOD treatment did not influence cortical perfusion either in the ischaemic, or in the non-ischaemic control, kidney (Figure 4).

NOD does not affect renal function in NOD-treated transplant recipients

We next assessed if the renoprotective effect of NOD treatment was also noted after prolonged cold ischaemia and Tx NOD treatment of the recipients did not significantly influence renal function, yet there was a trend observed towards a minor improvement (Figure 5A). Similarly, NOD did not influence body weight loss, renal inflammation (Figure 5B–D) or cytokine mRNA expression (data not shown). To exclude the possibility that an anti-Fisher allo-response or intrinsic strain differences were underlying the failure of NOD to act renoprotectively, syngeneic renal Txs were also performed. Similar as observed in the allogeneic Tx model, recipient NOD treatment was not renoprotective (Figure 5E) nor did it influence body weight (Figure 5F). This was unlikely due to the susceptibility of TRPV1-expressing sensory nerves to prolonged cold preservation since isolated DRG that were either or not subjected to cold preservation reacted in a similar fashion to both NOD and capsaicin (Figure 5G).

DISCUSSION

In the present study, we further explored the renoprotective effect of NOD in the setting of warm ischaemia-induced AKI in more details and assessed if renal allograft recipients may also benefit from NOD treatment. The major findings of this study are as follows. First, NOD treatment improves renal function in warm ischaemia-induced AKI, even when treatment is started after renal ischaemia. Improved renal function was not associated with large changes in renal perfusion. Secondly, the renoprotective properties of NOD are completely lost in TRPV1−/− sd rats. Thirdly, NOD treatment of renal transplant recipient rats does not improve renal function. TRPV1 responsiveness was not lost as a consequence of cold preservation since both NOD and capsaicin were able to activate DRGs that were subjected to cold preservation.

Our study is in good agreement with previous publications on the protective properties of TRPV1 activation in the setting of I/R injury in kidney [18, 35], lung [20] and heart [36] models. Nonetheless, the finding that NOD treatment did not improve kidney function in renal allograft recipient rats was unexpected. In our previous studies on NOD and AKI, NOD was applied as bolus therapy shortly before and directly after induction of warm ischaemia-induced AKI. Because we intuitively reasoned

Table 1. Quantification of mRNA for various cytokines was done in renal tissue obtained 5 days after AKI

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>WT sd rats</th>
<th>TRPV1−/− sd rats</th>
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<tr>
<td></td>
<td>NaCl</td>
<td>NOD</td>
</tr>
<tr>
<td>CxCL1</td>
<td>6.43 ± 1.29</td>
<td>7.36 ± 1.02</td>
</tr>
<tr>
<td>E-SEL</td>
<td>2.65 ± 0.20</td>
<td>2.77 ± 0.23</td>
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<tr>
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<td>21.81 ± 1.94</td>
<td>41.06 ± 5.42*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>2.29 ± 0.26</td>
<td>2.44 ± 0.19</td>
</tr>
<tr>
<td>VCAM</td>
<td>0.49 ± 0.03</td>
<td>0.49 ± 0.04</td>
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</table>

Results are expressed as relative quantity ± SEM relative to untreated native kidney (*P = 0.0094; **P = 0.0062, ***P = 0.0096).
FIGURE 5: NOD treatment of renal allograft recipients does not improve renal function. (A) s-crea (mg/dL; graph to the left) and s-urea (mg/dL; graph to the right) were assessed on Days 0, 1, 3, 5 and 7 after Tx of allogeneic Fischer renal allografts in Lewis recipients. The renal allografts were subjected to 20 h of cold preservation in UW solution. The recipients were treated with NOD (bold line) or NaCl (dotted line) via osmotic minipumps over the whole experimental period. No differences in renal function between the groups were noted. s-crea: P = 0.494 (Day 1), P = 0.129 (Day 3), P = 0.470 (Day 5) and P = 0.086 (Day 7). s-urea: P = 0.119 (Day 1), P = 0.494 (Day 3), P = 0.939 (Day 5) and P = 0.939 (Day 7). (B) Body weight loss was measured on Day 7 after allogeneic renal Tx. The result is expressed as mean body weight loss in percent relative to the body weight on Day 0. No differences in loss of body weight were found in both Tx models. (C) Renal infiltrated ED1+ cells were assessed by immunohistochemistry. Quantification of ED1+ was performed by quantitative morphometric analysis. For each animal, at least 10 sections were evaluated using 20 randomly chosen high power (×400 magnification) microscopic fields. The results are expressed as mean percent ED1-stained area per microscopic field ± SD. (D) Representative sections obtained from kidneys of the NaCl- or NOD-treated recipient rats. (E) s-crea (mg/dL; graph to the left) and s-urea (mg/dL; graph to the right) were assessed on Days 0, 1, 3, 5 and 7 after Tx of syngeneic Lewis renal allografts in Lewis recipients. The renal allografts were subjected to 20 h of cold preservation in UW solution. The recipients were treated with NOD (bold line) or NaCl (dotted line) via osmotic minipumps over the whole experimental period. No differences in renal function between the groups were noted. s-crea: P = 0.713 (Day 1), P = 0.6 (Day 3), P = 0.96 (Day 5) and P = 0.792 (Day 7). s-urea: P = 0.227 (Day 1), P = 0.431 (Day 3), P = 1.0 (Day 5) and P = 0.674 (Day 7). (F) Body weight loss was measured on Day 7 after syngeneic renal Tx. The result is expressed as mean body weight loss in percent relative to the body weight on Day 0. No differences in loss of body weight were found in both Tx models. (G) Live cell calcium imaging was performed on freshly isolated DRG or on DRG that were subjected to 18 h of cold preservation. DRGs were subsequently stimulated with 30 µM NOD, 10 µM capsaicin, 140 mM potassium chloride and 10 µM ionomycin. Mean traces ± SEM of 200 ROI with each ROI containing one cell is depicted. SEM is depicted as dotted line.
that pretreatment of the recipient would not influence the function of the donor allograft, we first demonstrated that pretreatment per se is not required for the renoprotective effect of NOD in the warm ischaemia-induced AKI model. Hence both the AKI and Tx models were similar in the sense that treatment was started after the inciting event. It could be argued that cold preservation of renal grafts causes more damage to the kidney when compared with warm ischaemia; therefore, NOD might not be protective under the former condition. Yet this was not reflected by s-crea levels, which even showed a trend towards a faster recovery of renal function in the Tx, when compared with the warm ischaemia-induced AKI model. As this was observed in both syngeneic and allogeneic donor recipient combinations, intrinsic strain differences in susceptibility to tissue ischaemia also cannot explain these results. We did not study the expression of TRPV1 in renal tissue after cold storage and thus, it cannot be excluded that cold storage affects the expression of TRPV1 and consequently the efficacy of NOD to improve renal function. However, cold storage of isolated DRG did not impair the efficacy of capsaicin or NOD to activate TRPV1.

The renal pelvis, pelvi-ureteric junction and ureter are heavily innervated by TRPV1-positive sensory nerves located between the layers of smooth muscles and epithelia [37, 38]. The sensory nerve endings contain among others vasoactive neuropeptides, e.g. CGRP and SP, which are released upon TRPV1 activation [39, 40]. Because CGRP and SP are vasoactive neuropeptides, TRPV1-positive sensory nerve fibres may regulate local renal blood flow [41].

Mizutani et al. [25] have reported that denervation of primary sensory nerves before renal I/R aggravates the inflammatory response in the kidney and worsens renal function. Accordingly, they postulate that TRPV1-expressing sensory nerves are activated in the pathologic process of warm I/R-induced AKI and that such activation reduces acute renal injury by attenuating inflammatory responses. The finding that no significant difference in renal function impairment was found between WT and TRPV1−/− sd rats that were subjected to ischaemia-induced AKI and the finding that in kidneys of the latter rats consistently less infiltration of ED1+ monocytes/macrophages was present argue against this hypothesis. The expression of a number of chemokines was also reduced in the TRPV1−/− when compared with the WT sd rats, albeit that this might equally well be explained by a reduced number of infiltrated monocytes/macrophages. A recent study by Chen et al. [42] also demonstrated that TRPV1 activation improves AKI while the absence of TRPV1 or inhibition of these channels did not further deteriorate renal function in the setting of AKI. Although our data are in good agreement with this study, it remains to be assessed why the expression of pro-inflammatory cytokines are significantly reduced in TRPV1−/− rats. Cytokine expression has been addressed in the study by Chen et al. [42]. These findings also suggest that the inflammatory response in the course of AKI might not be a major critical factor that determines renal function. Also, despite the fact that in vitro studies clearly have demonstrated the anti-inflammatory properties of NOD [43], with the exception of a reduction in infiltrating ED1+ cells, NOD treatment did not have a major influence on the expression of pro-inflammatory cytokines in the kidney following AKI. In fact, we observed that the expression of IL-6 was increased by NOD in WT and TRPV1−/− sd as well as in Lewis rats, albeit that this did not reach statistical significance in the latter strain.

Although our study unambiguously demonstrates that the renoprotective effect of NOD in warm ischaemia-induced AKI is strictly dependent on TRPV1 expression, there are some limitations that warrant further studies. In such studies, also the influence of NOD on early inflammatory parameters needs to be implemented. At present, it is not clear why TRPV1 activation in warm ischaemia-induced AKI models translates into improved renal function. Even though capsaicin leads to vasodilation in the renal vasculature [41], we were not able to demonstrate differences in renal perfusion in NOD-treated rats using ASL. However, it cannot be excluded that NOD treatment slightly increases renal perfusion at a level not detectable by the ASL-MRI method [34]. Secondly, it is not clear why renal recipients do not benefit from NOD treatment. This seems not to be related to allogeneic differences, cold preservation or intrinsic differences in kidney from different strains, yet the role of renal denervation in NOD-mediated renoprotection and kidney expression of TRPV1 over time in AKI has not been addressed. Thirdly, renoprotective effect conveyed by NOD in the warm ischaemia-induced AKI model cannot be extrapolated to other causes of AKI. Although NOD treatment in pre- and post-bolus combination, and as a continuous treatment directly after the inciting event, shows a protective effect, it still needs to be assessed if NOD treatment would impart protection once the degree of injury is large and manifested by already increased s-crea levels. Apart from this, NOD improves renal function in allograft recipients when used in brain dead donors [14], yet whether this is also mediated by TRPV1 is currently not known. An intriguing question which arises is as to whether donor NOD treatment can improve organ quality of renal allograft with already impaired donor renal function.

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CONFLICT OF INTEREST STATEMENT

None declared.

(See related article by Landoni et al. Dopamine derivatives and acute kidney injury: the search for the magic bullet continues… and leads to new (magic?) targets. Nephrol Dial Transplant 2016; 31: 512–514)
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