The RIP1-Kinase Inhibitor Necrostatin-1 Prevents Osmotic Nephrosis and Contrast-Induced AKI in Mice

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ABSTRACT

The pathophysiology of contrast-induced AKI (CIAKI) is incompletely understood due to the lack of an appropriate in vivo model that demonstrates reduced kidney function before administration of radiocontrast media (RCM). Here, we examine the effects of CIAKI in vitro and introduce a murine ischemia/reperfusion injury (IRI)-based approach that allows induction of CIAKI by a single intravenous application of standard RCM after injury for in vivo studies. Whereas murine renal tubular cells and freshly isolated renal tubules rapidly absorbed RCM, plasma membrane integrity and cell viability remained preserved in vitro and ex vivo, indicating that RCM do not induce apoptosis or regulated necrosis of renal tubular cells. In vivo, the IRI-based CIAKI model exhibited typical features of clinical CIAKI, including RCM-induced osmotic nephrosis and increased serum levels of urea and creatinine that were not altered by inhibition of apoptosis. Direct evaluation of renal morphology by intravital microscopy revealed dilation of renal tubules and peritubular capillaries within 20 minutes of RCM application in uninjured mice and similar, but less dramatic, responses after IRI pretreatment. Necrostatin-1 (Nec-1), a specific inhibitor of the receptor-interacting protein 1 (RIP1) kinase domain, prevented osmotic nephrosis and CIAKI, whereas an inactive Nec-1 derivate (Nec-1i) or the pan-caspase inhibitor zVAD did not. In addition, Nec-1 prevented RCM-induced dilation of peritubular capillaries, suggesting a novel role unrelated to cell death for the RIP1 kinase domain in the regulation of microvascular hemodynamics and pathophysiology of CIAKI.


Contrast-induced AKI (CIAKI) is the consensus name for what was formally called contrast-induced nephropathy or radiocontrast-induced AKI.1–3 CIAKI is a common and potentially serious complication4 after the administration of contrast media,5–7 especially in patients who are at risk for AKI, and is the most common cause of iatrogenic, inpatient, drug-induced AKI,3,8,9 with outstanding implications for patients with diabetes.1 CIAKI was recognized as the third commonest cause of hospital-acquired renal failure accounting for 11% of the cases10 even before magnetic resonance imaging contrast media were found to be associated with nephrogenic systemic fibrosis. Preclinical research thus far has failed to unravel the underlying pathophysiology of CIAKI.

Programmed cell death (PCD) was used synonymously with apoptosis until regulated necrosis (RN) was discovered.11 Apoptosis has been proposed to contribute to CIAKI12–14 and asialoerythropoietin was recently demonstrated in this context to prevent CIAKI.15 Apoptosis is a process

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Figure 1. Regular doses of RCM do not induce cell death in TKPTS cells, MMCs, and glENDs. (A) TKPTS cells are left untreated or are treated with 50 μl/ml standard RCM (Imeron) for the indicated time points. Rapid nuclear RCM uptake is visualized by light microscopy followed over a period of 25 minutes. (B) Greyscale quantification of A and Supplemental Figure 1, A and B, after application of RCM. (C) TKPTS cells, MMCs, and glENDs are left untreated or treated with 50 μl/ml RCM for the indicated time periods. Jurkat cells serve as

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positive controls for induction of apoptosis after stimulation with 100 ng/ml anti-Fas mAb. The classic apoptosis markers cleaved caspase-3 (upper blot) and full-length PARP-1 versus cleaved PARP-1 (central blot) indicate the induction of apoptosis in Jurkat cells, but not in any of the remaining cell lines tested. GAPDH serves as a loading control. (D and E) Cells are stimulated as in C for the indicated time periods. Positivity for the apoptosis-marker annexin V (D) and the necrosis marker 7-AAD (E) are depicted. Again, Jurkat cells serve as a positive control that is known to undergo secondary necrosis 8 hours after induction of apoptosis. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Figure 2. Regular doses of RCM do not induce cell death in freshly isolated primary renal tubules. (A) Primary murine proximal renal tubules are left untreated or are treated with 50 μl/ml RCM for the indicated time points. RCM uptake into the tubular compartment is visualized by light microscopy and followed over a period of 25 minutes. (B) Greyscale quantification of A and Supplemental Figure 3, A and B, after application of RCM. (C) Primary isolated murine renal tubules are left untreated (cold) or treated with 100 ml/ml RCM for the indicated time periods. Tubules incubated in hypoxia for 60 minutes followed by 60 minutes of reoxygenation serve as positive controls. LDH release is measured and is shown as the percentage of overall LDH present in the whole tubules. (D) Primary renal tubules treated as in C are stained for positivity of propidium iodide. No statistically significant difference is observed in untreated versus RCM-treated tubules after 240 minutes of incubation. DAPI, 4',6-diamidino-2-phenylindole; PI, propidium iodide.
Figure 3. Blockade of apoptosis does not protect from RCM-induced osmotic nephrosis or AKI (CIAKI). (A) Eight-week-old male C57Bl/6 mice undergo sham surgery or bilateral renal pedicle clamping 24 hours before intraperitoneal injection of either PBS or the pan-caspase inhibitor zVAD followed by intravenous injection of RCM. Renal sections stained with periodic acid–Schiff are shown at magnifications of 200-fold and 400-fold. Ischemia-reperfusion damage is not significantly altered in any of the groups (B), whereas quantification of
that is characterized by the activity of caspases that cleave hundreds of intracellular proteins to ultimately cause membrane blebbing, nuclear fragmentation, and regulated cellular shrinkage as a consequence of their proteolytic activity.16,17 Within this process, caspases are capable of cleaving NFs like poly(ADP-ribose)-polymerase (PARP)-family proteins.18 PARP-1 has also been demonstrated to elicit a necrotic phenotype in kidney cells and therefore exhibits a subroutine of the RN.19,20 It was suggested that tubular cell death by caspase-3–mediated apoptosis substantially contributes to the overall pathogenesis of CIAKI,14,15 and one report investigated the activation of the cell death molecules PARP, Bad, and BIM.14 On the basis of these findings, the currently proposed model ascribes apoptosis a major pathophysiologic function in CIAKI.12,13

Apart from PARP-mediated RN, necroptosis, another RN pathway, is mediated by activation of the “necrosome” consisting of receptor-interacting protein kinases 1 and 3 (RIP1 and RIP3).11,21–23 Necroptosis involves all necrotic cellular hallmarks such as early loss of membrane integrity as well as rupture of the plasma membrane after cellular swelling. We recently described the functional relevance of both apoptosis and necroptosis in AKI.24,25 Here, we demonstrate that necrostatin-1 (Nec-1), a highly specific inhibitor of the RIP1 kinase domain, prevents CIAKI in a new and easy-to-use preclinical model for the in vivo analysis of CIAKI. Our model reliably mimics “osmotic nephrosis,” a pathologic feature that is typical of CIAKI in humans. In vitro and in vivo, we found that apoptosis is of minor pathophysiologic importance. Mechanistically, the data implicate RIP1 in the functional renal failure in vivo and provide evidence for the prevention of CIAKI by the RIP1 kinase inhibitor Nec-1 that also prevented the functional changes in the peritubular vasculature after RCM injection as demonstrated by intravital microscopy. Because of the outstanding specificity of Nec-1 that has been subject to extensive investigation,26–29 we consider it justified to conclude that a novel non-cell death role of RIP1 might account for the functional kidney failure in CIAKI. In addition, we introduce Nec-1 as a potential inhibitor of CIAKI.

RESULTS

Rapid Nuclear RCM Uptake in Kidney Cell Lines Does Not Induce Cell Death In Vitro

Previous reports suggested that kidney cells undergo PCD after application of diverse RCM.12–15,30 We aimed to investigate the direct influence of clinically used standard RCM (Imeron) on renal cells and initially demonstrated the direct uptake of RCM into murine proximal tubular cells (TKPTS), mesangial cells (MMCs), and glomerular endothelial cells (glENDs) upon incubation with 50 μl RCM/ml (Figure 1A and Supplemental Figure 1). Greyscale analysis of contrast phase identified a peak RCM concentration within 15 seconds and a regular decline within 25 minutes (Figure 1B). We further investigated RCM-induced cell death by evaluation of annexin V positivity and persistence of membrane integrity (exclusion of 7-AAD) upon medium (10 μl/ml) and high (50 μl/ml) concentrations of RCM. No significant induction of cleaved caspase-3 or PARP-1 was detected in comparison with anti-Fas-treated Jurkat cells that serve as an apoptotic positive control, suggesting that classic caspase-mediated apoptosis is not initiated after RCM application (Figure 1C and Supplemental Figure 2). Minimal levels of PARP1-cleavage in glENDs upon long Western blot exposure (Supplemental Figure 3A) did not correlate with any detectable cell death (Supplemental Figure 3, B and C). Consistent with this, annexin V positivity in FACS analysis was minimal (Figure 1D). To assess other necrotic-type cell death modalities, we applied 7-AAD that was excluded over time in all renal cell lines (Figure 1E) at both 10 and 50 μl/ml RCM. However, in accordance with previously published data13–15 and Supplemental Figure 3A, very high concentrations of RCM (250 μl/ml) did result in some annexin V positivity after 24 hours (Supplemental Figure 4A). With 400 μl RCM/ml, we found an increase in PARP-1 cleavage that was unaffected by the addition of the caspase-8 inhibitor zIETD, the pan-caspase inhibitor zVAD, TAT-crmA (a previously published fusion protein that utilized the viral caspase-8 inhibitor31,32), or the cyclophilin D inhibitor cyclosporin A (Supplemental Figure 4B). We conclude that only doses that are high enough to induce artifacts lead to significant amounts of cell death even when applied in cell culture for 24 hours or longer.

RCM Do Not Induce Cell Death in Freshly Isolated Renal Tubules

To transfer our in vitro data into an ex vivo setting, we treated freshly isolated proximal tubule segments with RCM. Comparable to the results in TKPTS cells, epithelial cell nuclei in the proximal tubule segments rapidly took up contrast media (Figure 2A). Similar results were obtained for thick ascending limb segments and segments from the distal convoluted tubules (Supplemental Figure 5). Greyscale analysis revealed similar RCM uptake kinetics in all tubular segments investigated (Figure 2B). Given the rapid direct RCM uptake, we investigated RCM-induced cell death as measured by lactate dehydrogenase (LDH) release (Figure 2C). Tubules treated for 60 minutes with hypoxia followed by 60 minutes
Figure 4. The kinase domain of RIP1 mediates osmotic nephrosis and CIAKI. (A) Eight-week-old male C57Bl/6 mice undergo sham surgery or bilateral renal pedicle clamping 24 hours before intraperitoneal injection of either PBS, the highly specific RIP1 kinase inhibitor Nec-1, or the inactive derivate of necrostatin-1 (Nec-1i) in the presence or absence of RCM. (B) Histologic quantification of renal IRI is not significantly changed in any of the groups. (C) Osmotic nephrosis in RCM-treated mice was reduced in Nec-1–treated mice, but not in


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those that received Nec-1i. Osmotic nephrosis in subcapsular tubules is quantified in D. Concentrations of serum creatinine (E) and serum urea (F) are evaluated 48 hours after reperfusion (24 hours after application of RCM). n=8 per group. **P<0.01; ***P<0.001 compared with the IRI-pretreated PBS group. Sham versus IRI-treated mice: P<0.001 (not indicated in E and F). n.s., not significant.

Figure 5. Nec-1 prevents osmotic nephrosis and CIAKI. (A and B) Eight-week-old mice undergo sham surgery or CIAKI treatment as described for Figures 3 and 4, and kidney sections are stained for immunohistochemistry of RIP1 at 400-fold magnification in the presence or absence of PBS, Nec-1, or Nec-1i as indicated (A) and RIP1-positive tubules/tubular cells are counted and quantified for each group (B) (n=6 per group). (C) LDH release of primary freshly isolated renal tubules from wild-type mice (open bars) compared with tubules from RIP3-deficient mice (gray bars) after cold preparation or 60 minutes and 240 minutes of incubation at 37°C in PBS in the presence and absence of RCM and Nec-1 as indicated. Wild-type tubules that are incubated 60 minutes in hypoxia followed by 60 minutes of reoxygenation serve as positive controls. n=8 per group. *P<0.05 compared with the IRI-pretreated PBS group. n.s., not statistically significant.
Figure 6. Functional influence of RCM on peritubular capillaries is affected by Nec-1 in vivo. Eight-week-old male C57BL/6N mice receive 250 μl RCM via the tail vein 15 minutes after intraperitoneal injection of DMSO or Nec-1 with and without IRI pretreatment, as indicated. IVM is performed to detect changes in the diameters of peritubular capillaries and renal tubules. Representative images are depicted in A, and in higher magnification in B. Nec-1 prevents the return to baseline transtubular diameters after 20-25 minutes (C).
of reoxygenation served as a positive control. As expected, LDH release increased over time in the isolated tubules, but no further increase in LDH release was detected when RCM was added, despite the use of high concentration (100 μl/ml). Accordingly, and in line with the finding from TKPTS cells in Figure 1E, positivity for propidium iodide in the tubules increased over time without further increases caused by RCM (Figure 2D).

An Ischemia/Reperfusion Injury–Based Model System Allows Investigation of CIAKI

In humans, several studies have reported osmotic nephrosis as a typical morphologic feature that accompanies renal failure despite the use of high concentration (100 μl/ml) of RCM injected intravenously. We therefore treated unilaterally nephrectomized mice with high doses of indomethacin (up to 100 μg/kg) in addition to high doses of N-nitro-l-arginine methyl ester. We therefore treated unilaterally nephrectomized mice with high doses of indomethacin (up to 100 μg/kg) in addition to high doses of N-nitro-l-arginine methyl ester using concentrations up to 100 μg/kg, respectively, after 16 hours of water deprivation followed by intravenous application of 250 μl RCM. In our hands, this did not induce detectable AKI as measured by unchanged serum urea or creatinine concentrations 24 or 48 hours after injection of RCM (Supplemental Figure 8). Our subsequent approach was based on achieving the required loss of renal function through an easy-to-reproduce ischemia/reperfusion injury (IRI)–based setting. After 24 hours of reperfusion, serum urea and creatinine levels and histologic analysis of the renal damage were used to demonstrate low SDs in this system. At 24 hours after reperfusion, we analyzed various concentrations of RCM to induce CIAKI and found that 250 μl of RCM that was rapidly injected via the tail vein confers an ideal setting (Supplemental Figure 9). We subsequently used this dose to characterize the time course of CIAKI in this model for within the first 96 hours after RCM injection (Supplemental Figure 10). According to these data, we performed the following experiments with 250 μl of RCM applied via the tail vein (RCM group) and read out serum markers and histology 24 hours later (48 hours after reperfusion). These were compared with the IRI-treated mice that received 250 μl of PBS instead of contrast media (PBS group) (Figure 3, A–G). We will further refer to the difference between the RCM group and the PBS group as our model for CIAKI. In addition, we assessed the effect of volume and N-acetylcysteine application in this model and found no protective effects for these regimes (Supplemental Figure 11). As quantified in Figure 3C and to the best of our knowledge, this is the first in vivo model that reliably and closely mimics the phenotype of tubular cell osmotic nephrosis in quantifiable resolution (Supplemental Figure 12). As expected from the in vitro data, blockade of apoptosis did not influence this CIAKI model in all parameters tested (Figure 3), but also did not worsen the outcome, as would be anticipated in a purely necroptotic cell death.

CIAKI Is Attenuated by Necrostatin-1, an Inhibitor of the Kinase Domain of Receptor-Interacting Protein Kinase-1

Because other forms of PCD have recently emerged, and renal cells preferentially appear to undergo programmed necrosis rather than apoptosis, we investigated the influence of the RIP1 inhibitor necrostatin-1 (Nec-1) in the in vivo CIAKI model. Importantly for the design, and in line with previously published data, application of Nec-1 24 hours after reperfusion without RCM application did not alter the amount of IRI damage (Figure 4, A and B) and had no influence on serum concentrations of urea and creatinine measured 48 hours after reperfusion; thus, effects of Nec-1 at the time 24 hours after ischemia/reperfusion (the time of RCM administration) can be attributed to its effects on CIAKI. We found an almost complete prevention of RCM-induced osmotic nephrosis in the Nec-1–treated mice, but not in mice treated with an inactive derivate of Nec-1 called Nec-11 (Figure 4C). CIAKI-induced affection of subcapsular tubules was attenuated but not completely prevented by Nec-1 (Figure 4, D and E). Furthermore, CIAKI was markedly attenuated by Nec-1 as demonstrated by the prevention of the RCM-induced increase in serum concentrations of creatinine and urea (Figure 4, E and F). We conclude that the RIP1 kinase blocker, Nec-1, protects from CIAKI in our model.

CIAKI-Induced Loss of RIP1 Positivity Is Attenuated by Nec-1

To further investigate RIP1 biology in CIAKI, we performed immunohistochemistry in sections from sham-treated and CIAKI-treated mice. RIP1 expression did not significantly change upon renal IRI. In CIAKI, however, positivity for RIP1 disappeared, and this effect was prevented by Nec-1, but not by

and affects the dilation phase (injection to 10 minutes) in peritubular capillaries (D). Upon IRI pretreatment, application of Nec-1 leads to significantly increased transtubular diameters (E) and prevents the increase in peritubular capillary diameters that is seen in the control mice (F). Note the artificial yellow appearance of the heat-exposed area in the intravital microscope. This area is not included in the quantification of tubular diameters (n>200 per value) and peritubular capillaries (n>200 per value). t-tests reveal statistical significance as depicted by the following: *P<0.05; **P<0.01; ***P<0.001.
Nec-1i (Figure 5, A and B). Because RIP1 has been well described to activate RIP3 to form the so-called necroptosome and mediate necroptosis\(^1\) but our \textit{in vitro} results suggested that RCM do not induce cell death, we hypothesized that in CIAKI, the kinase domain of RIP1 might exert novel non-cell death function. To exclude the involvement of RIP3 in this context, we investigated RIP3-deficient freshly isolated tubules and found no significant alterations in LDH release (Figure 5C), pointing to an effect that is RIP3 independent. To gain further information about the functional pathophysiology of CIAKI \textit{in vivo}, we utilized intravital microscopy (IVM).

**Nec-1 Induces Tubular Dilation and Affects the Kinetics of the Dilation of Peritubular Capillaries after RCM Application**

The applicability of IVM to investigate AKI has recently been established by others.\(^2\)\(^3\) We utilized IVM to detect the initial events in tubules and peritubular capillaries upon RCM application in mice with \textit{in vivo} detection of renal tubules to a depth of 150 \(\mu\)m below the kidney capsule. Transtubular dilation after intravenous application of 250 \(\mu\)l RCM injection appeared throughout the first 20 minutes before diameters returned to baseline levels. Upon a single intraperitoneal application of a single dose of Nec-1 15 minutes before RCM, the return to baseline levels was prevented within the observation period. In line with this finding, we detected significantly wider tubules in the Nec-1 group after IRI pretreatment (Figure 6, A, B, C, and E). In addition, and in line with a previous report,\(^4\) RCM resulted in a significant increase in the diameter of the peritubular capillaries in the first 10 minutes (6.9 \(\mu\)m versus 7.9 \(\mu\)m, vasodilative phase) followed by a gradual decline between 10 and 25 minutes (7.9 \(\mu\)m versus 5.8 \(\mu\)m, vasoconstrictive phase). As expected upon IRI pretreatment, peritubular capillaries remained in a dilated state, but diameters remained stable and significantly different to the wild-type mice in the Nec-1 group (Figure 6, A, B, D, and E). The latter effect was also recently demonstrated for the vasa recta.\(^4\) According to the laminar flow equation of Hagen-Poiseuille, the increased blood flow might reflect the functional decrease in kidney function in CIAKI, without causing cell death, an effect that is not observed upon Nec-1 treatment. In addition, the peritubular dilation was completely abolished by intraperitoneal application of Nec-1 15 minutes before application of RCM, suggesting a functional role for RIP1 in this setting, even after IRI pretreatment. In combination with our \textit{in vitro} results, these data suggested that RCM might not kill tubular cells, but rather lead to a RIP1-associated functional renal failure that involves vasodilation of the peritubular vessels, the mechanism of which remains unclear.

**DISCUSSION**

We previously reported that application of Nec-1 protects from renal IRI when it is applied before reperfusion.\(^2\)\(^5\) Mechanistically, the effect of Nec-1 on CIAKI presented in this article is completely different, and must carefully be separated from interference with necroptosis. Most importantly, addition of Nec-1 24 hours after reperfusion does not influence the course of IRI, which is a prerequisite for the present investigation in which we address three considerations that might be of interest in the context of CIAKI. First, we question the functional relevance of apoptosis, and PCD in general, in the pathogenesis of CIAKI. Second, we introduce a new and easy-to-reproduce CIAKI mouse model. Third, we provide evidence for a non-cell death function for the kinase domain of RIP1 in regulation of vascular tone and CIAKI and its inhibitor Nec-1.

Apoptosis in tubular cells has been reported after various iodide RCM \textit{in vitro} such as 200 mg/ml of Iodixanol,\(^1\)\(^4\) 320 mg/ml of Iodixanol,\(^1\)\(^3\) and 320 mg/ml of Ioversol.\(^1\)\(^5\) These agents have several limitations. First, these concentrations to our understanding are artificially high and do not mimic the clinical situation. Second, unlike RCM used in this study, those RCM are not in everyday clinical use. Third, the detection of apoptosis was performed using nonspecific terminal deoxynucleotidyl transferase–mediated digoxigenin-deoxyuridine nick-end labeling assays.\(^3\)\(^9\)\(^4\)\(^6\)\(^4\)\(^7\) Some reports also investigated caspase-3 activity, a very good marker for apoptosis, but the activity increased only by 1.4-fold.\(^1\)\(^5\) In typical apoptotic settings, caspase-3 activity increases by \(\geq 10\)-fold.\(^4\)\(^8\)\(^4\)\(^9\)

Here we demonstrate that apoptosis only occurs \textit{in vitro} using artificially high RCM concentrations (Supplemental Figure 4), which is generally in line with some of the previously mentioned reports.\(^1\)\(^3\)\(^1\)\(^5\) \textit{In vivo}, we cannot find any protection from CIAKI that is based on blockade of caspases (Figure 3) and do not find any significant amount of cell death that might provide a concept to explain AKI.

A preclinical model for CIAKI should meet three major criteria. First, it must not be a lethal model to be comparable to clinically relevant CIAKI. Second, intravenous application of commonly used contrast media should lead to a measurable increase in serum urea and creatinine concentrations. Third, typical histologic changes that are regularly seen in renal biopsies, like osmotic nephrosis, should be easily detected by standard histology. We undertook several approaches that had been suggested in the literature, all of which did not meet the above-mentioned three criteria in our hands using mice (Supplemental Figure 8). From several ischemia/reperfusion studies, it is well known that kidney function was comparable in medium-sized groups 24 and 48 hours after reperfusion with low SD in mice.\(^2\)\(^5\)\(^5\)\(^0\)\(^5\)\(^2\) Therefore, we chose the time point at 24 hours after reperfusion for the induction of CIAKI. In this regard, our model is restricted to the investigation of those compounds and knockout models that do not influence IRI when applied 24 hours after reperfusion. For any agent that is investigated in this model, it needs to be assured that IRI by 48 hours is not significantly different from the PBS control at 48 hours. For instance, blockade of Fas ligand by the mAb MFL3 did exert protection from IRI when applied 24 hours after reperfusion (data not shown) with the consequence that
our model does not allow evaluation of the effectiveness of the blockade of this death receptor in CIAKI and RIP3-deficient mice could not be investigated due to the protection from IRI compared with wild-type mice (data not shown). Nec-1, however, did not influence IRI when applied later than 30 minutes after the onset of reperfusion and certainly not when applied 24 hours from the onset of reperfusion.25

Mechanistically, our study suggests a functional role of the RIP1 kinase domain that is not associated with cell death in CIAKI based on three lines of evidence. First, Nec-1 blocks CIAKI in our model. Second, the inactive Nec-1 derivate Nec-1125-28 does not affect this model, ruling out off-target effects of this compound. Third, the RIP1 expression pattern, which is unaffected by IRI (as reported earlier25), substantially changes after applications of RCM (Figure 5, A and B). RIP1 was described to be involved in both apoptosis and necroptosis, but also in activating the NF-κB pathway after TNFR1 ligation by TNFα.53 We are only beginning to understand non-cell death functions of RIP1 that can be prevented by Nec-1.54 It is of interest in this regard that the histologic pattern of osmotic nephrosis is not restricted to CIAKI, but has also been described in severe sepsis.55–57 RIP1 is also critically involved in sepsis as recently demonstrated in a model of TNFα-induced shock,58 although in this model, we could not detect osmotic nephrosis due to the early death of the mice within 24 hours after TNFα application.32 Currently, we cannot exclude a common underlying role for RIP1 in the pathogenesis of osmotic nephrosis in both CIAKI and sepsis because RIP1-deficient mice die perinatally.59 Organ-specific floxed RIP1-deficient mice will be required to further investigate the role of RIP-1 in both models. Unlike in the sepsis model, Nec-1 prevented all signs of CIAKI almost completely. Besides hydration and application of N-acetylcysteine, both of which did not influence our model (Supplemental Figure 11), and the recently suggested application of atorvastatin,60 blocking the RIP1 kinase activity may be considered a new strategy to prevent CIAKI.

In summary, we conclude that living renal cells are put into a nonfunctioning state by RCM and that this functional inhibition, rather than cell death, is prevented by Nec-1, suggesting a role for the kinase domain of RIP1 in this process. With respect to the fact that probably no other kinase inhibitor was examined in this much detail for its selectivity,26–28 we consider it justified to conclude that the RIP1 kinase domain essentially mediates the mentioned effects without the need for its default partner RIP3. These results should focus our efforts on the inhibition of the RIP1 kinase domain in this scenario rather than on the prevention of cell death. Finally, the possibility that Nec-1 and presumably other RIP1 kinase inhibitors can strongly alter peritubular perfusion might be of relevance beyond CIAKI.

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Disclosures

None.

References


Concise Methods

Complete methods are available in the Supplemental Material.

This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2012121169/-/DCSupplemental.
CORRECTION


In Figure 3A of this article, the two lower left panels showing ×400 magnifications of the PBS and sham radiocontrast media (RCM) groups were accidentally transposed. This occurred because they both had the same finding of the two conditions (*i.e.*, absence of osmotic nephrosis changes). The correct Figure 3A is printed below.

**Figure 3.** Blockade of apoptosis does not protect from RCM-induced osmotic nephrosis or AKI (CIAKI). (A) Eight-week-old male C57Bl/6 mice undergo sham surgery or bilateral renal pedicle clamping 24 hours before intraperitoneal injection of either PBS or the pan-caspase inhibitor zVAD followed by intravenous injection of RCM.