Nonsteroidal Mineralocorticoid Receptor Antagonist Finerenone Protects Against Acute Kidney Injury–Mediated Chronic Kidney Disease Role of Oxidative Stress

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Abstract—Acute kidney injury induced by ischemia/reperfusion (IR) is a frequent complication in hospitalized patients. Mineralocorticoid receptor antagonism has shown to be helpful against renal IR consequences; however, the potential benefit of novel nonsteroidal mineralocorticoid receptor antagonists such as finerenone has to be further explored. In this study, we evaluated the efficacy of finerenone to prevent the acute and chronic consequences of ischemic acute kidney injury. For the acute study (24 hours), 18 rats were divided into sham, bilateral renal ischemia of 25 minutes, and rats that received 3 doses of finerenone at 48, 24, and 1 hour before the ischemia. For the chronic study (4 months), 23 rats were divided into sham, rats that underwent 45 minutes of bilateral ischemia, and rats treated with finerenone at days 2 and 1 and 1 hour before IR. We found that after 24 hours of reperfusion, the untreated IR rats presented kidney dysfunction and tubular injury. Kidney injury molecule-1 and neutrophil gelatinase associated to lipolacin mRNA levels were increased. In contrast, the rats treated with finerenone displayed normal kidney function and significantly lesser tubular injury and kidney injury molecule-1 and neutrophil gelatinase associated to lipolacin levels. After 4 months, the IR rats developed chronic kidney disease, evidenced by kidney dysfunction, increased proteinuria and renal vascular resistance, tubular dilation, extensive tubule-interstitial fibrosis, and an increase in kidney transforming growth factor-β and collagen-I mRNA. The transition from acute kidney injury to chronic kidney disease was fully prevented by finerenone. Altogether, our data show that in the rat, finerenone is able to prevent acute kidney injury induced by IR and the chronic and progressive deterioration of kidney function and structure. (Hypertension. 2017;69:870-878. DOI: 10.1161/HYPERTENSIONAHA.116.08526.)* Online Data Supplement

Key Words: endothelin receptors ■ fibrosis ■ ischemia ■ renal circulation ■ reperfusion

Renal ischemia/reperfusion (IR) accounts for many cases of acute kidney injury (AKI).1 It is now acknowledged that AKI can lead to chronic kidney disease (CKD) development and increased mortality and cardiovascular complications.2 After an ischemic AKI episode, the renal tissue experiences several alterations. In the acute phase, the tubular epithelial cells and the endothelial cells are injured, activating an inflammatory response that may extend the degree of the initial injury. In the regeneration phase, the surviving epithelial cells redifferentiate and re-establish the normal tubular structure.3 However, depending on the severity of the AKI episode, this phase might be defective or incomplete and lead to chronic inflammation, capillary rarefaction, fibrosis development, and ultimately to CKD.4,5 In a rat model of AKI, we showed previously that a single severe episode of bilateral kidney ischemia induced CKD.6 The knowledge about the mechanisms of AKI and AKI to CKD transition has rapidly evolved, in the past decade, but there is still no pharmacological approach to prevent or delay CKD induced by AKI.1,7,8 We demonstrated previously that mineralocorticoid receptor (MR) antagonism with spironolactone is an efficient strategy to prevent both, the acute and chronic consequences of renal IR.6,9 Of note, a recent randomized study on 233 patients showed that spironolactone administration to patients with cardiac surgery 12 to 24 hours before the surgery and at days 0, 1, and 2 post-surgery did not modify the AKI incidence when compared with placebo.10

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The available MR antagonists like spironolactone or eplerenone are contraindicated in patients with compromised renal function, mainly because of the risk of hyperkalemia. Novel nonsteroidal antagonists with a better safety profile, especially on the hyperkalemic risk are an exciting alternative in this context. We therefore reported previously that the nonsteroidal MR antagonist BR-4628 is able to prevent the acute injury associated with kidney IR. The use of the nonsteroidal MRA was associated with a reduced incidence for developing hyperkalemia, renal failure, or renal impairment versus spironolactone in patients with heart failure and CKD. We therefore evaluated the efficacy of finerenone to prevent the acute and chronic deleterious consequences of an ischemic AKI episode in a preclinical model of AKI-induced CKD.

Methods

Experimental Protocols

All the animals were kept housed at Centre d’Explorations Fonctionnelles de CRC (agreement no. A75-06-12). They were maintained in a constant temperature and humidity in light controlled room with a 12-hour light cycle. They had ad libitum access to food and water. All experiments were conducted in accordance with the institutional guidelines and the recommendations for the care and use of laboratory animals put forward by the French Ministry of Agriculture. Male Wistar rats (Janvier Laboratories, France) weighing 270 to 300 g were included in the study. Finerenone was administered at a dose of 10 mg/kg by oral gavage in vehicle (40% Kolliphor, 10% ethanol, and 50% water). In the first set of experiments (the acute study), 18 rats were divided into 3 groups: sham-operated rats (sham; n=5), rats subjected to bilateral renal ischemia of 25 minutes (IR; n=8), and rats that received 3 doses of finerenone at 48, 24, and 1 hour before the ischemia was induced (IR+Fine; n=5). The rats were studied 24 hours after reperfusion. For the second set of experiments (chronic study), 23 rats were divided into 3 groups: sham-operated rats (sham; n=7), rats with 45 minutes of bilateral ischemia (IR; n=9), and rats treated with finerenone at days 2 and 1 hour before the induction of IR (IR+Fine; n=7). Rats were studied 4 months after IR.

For the antioxidant experiment with 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPOL), 19 rats were divided into 3 groups: sham-operated rats (sham; n=6), rats that underwent 25 minutes of ischemia (IR; n=7), and rats that received TEMPOL (100 mg/kg IV) 30 minutes before the induction of the ischemia (IR+TEMPOL; n=6). In this case, all parameters were evaluated 24 hours after reperfusion.

Kidney IR Injury Model

The rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (30 mg/kg). The body temperature was monitored to be ±37°C with a rectal probe along the surgery. An abdominal incision was performed, and both renal pedicles were exposed and dissected. Kidney ischemia was achieved through the collocation nontraumatic vascular clamps over the pedicles. After 45 minutes (chronic study) or 25 minutes (acute study), the clamps were released and the reperfusion was allowed and verified by the return of oxygenated blood to the kidney. The abdominal incision was closed in 2 layers with 5-0 sutures.

Evaluation of Functional Parameters

After 24 hours of reperfusion, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg) and placed on a heating pad. The right femoral artery was catheterized with polyethylene tubing (PE-50) to monitor the mean arterial pressure with the help of an LWX214 unit. The left renal artery was dissected, and the renal blood flow was recorded by placing an ultrasound flow probe (Transonic) filled with ultrasonic coupling gel around the artery. At the end of the experiment, a blood sample was taken and the plasma creatinine and urea concentrations were determined in an automatic analyzer (Konelab 20; Thermo Fisher Scientific). The right kidney was removed and fixed in Bouin Fixative solution, and the left kidney was quickly frozen for molecular studies.

Histological Analysis

After fixation of the tissue, the kidney slices were dehydrated and embedded in paraffin. Sections of 4 μm were made and stained by hematoxylin and eosin or Sirius red stain. For each rat, 10 cortical fields were visualized and analyzed on a Leica DM4000 microscope at a magnification of ×200. In the acute study, the percentage of injured tubules was determined. A blinded researcher counted the number of tubules presenting luminal casts, cell detachment, or dilation. The percentage of injured tubules was expressed as a relation to the total number of tubules in each field. After Sirius red staining, the percentage of interstitial fibrosis was assessed. The score of tubule-interstitial fibrosis was obtained by using the following scale: 1=<25%, 2=26% to 50%, 3=51% to 75%, and 4>75% of Sirius red-positive area.

CD68 immunohistochemistry was performed as described previously. Briefly, after antigen retrieval with citrate buffer (pH 6; 15 minutes at 100°C), 4-μm-thick sections were incubated overnight at 4°C with mouse anti-rat CD68 monoclonal antibody (ab31630 antibody; Abcam, Inc.). Primary antibody binding was detected with a biotinylated horse anti-mouse IgG antibody (BA-2001; Vector Laboratories). Signal was amplified using Vectastain ABC HRP kit (PK-4002; Vector Laboratories). Finally, staining was developed with ImmPact DAB peroxidase substrate (SK-4105; Vector Laboratories).

All sections were coded and scored by blinded investigators. For each section, a mean score was calculated from at least 5 high-power fields. Detection of apoptotic tubular cells was performed on 4-μm-thick paraffin-embedded kidney sections using the ApopTag peroxidase-in situ apoptosis detection kit (S7100; Millipore), according to the manufacturer instructions.

RNA Extraction and Real-Time Polymerase Chain Reaction

Total RNA was extracted from kidney cortex with TRIZOL reagent (Life Technologies) according to the manufacturer’s instructions. The reverse transcription was performed with 2 μg of RNA and the M-MLV Reverse transcriptase Kit (Life Technologies). Transcript levels of genes were analyzed by real-time polymerase chain reaction (fluorescence detection of SYBR green) in an iCycler iQ apparatus (Bio-Rad). The mRNA levels were normalized by the amount of 18S rRNA and endogenous control. The primer sequences of the analyze genes are listed in the Table S1 in the online-only Data Supplement.

Oxidative Stress Analysis

Malondialdehyde levels were assessed through the colorimetric quantification of the malondialdehyde-thiobarbituric acid adduct by using the Lipid peroxidation (malondialdehyde) Assay kit (Abcam), following the manufacturer instructions. Plasma 8-hydroxy-guanosine (8OHdG) levels were measured in plasma according to the manufacturer instructions (589320; Cayman Chemical Company).

Immunoprecipitation and Western Blotting

For immunoprecipitation analysis, the kidney tissues from 2 rats were pooled and the proteins were extracted in dideened lysis buffer (three pools per group). Endothelin-B (ET-B) receptor was immunoprecipitated using protein A agarose beads (Thermo Scientific) and 4 μg of anti-ET-B antibody (Santa Cruz Biotechnology). As a negative control, the proteins were incubated with the protein A agarose beads and 4 μg of IgG isotype. The immunoprecipitated proteins were eluted by boiling in Laemmli buffer, blotted, and probed for anti–cysteine sulfenic acid (1:5000 dilution; Millipore) and ET-B (1:500 dilution). The experiment showing the immunoprecipitation with ET-B–specific antibody and IgG isotype control is shown in Figure S1.
To study the expression of E-cadherin and α-smooth muscle actin, snap frozen renal cortex were lysed in Complete Lysis-M (04719956001; Roche). Protein concentrations were determined with the bicinchoninic acid method, and equal amounts of protein were resuspended in Laemmli sample buffer and boiled for 5 minutes at 95°C. The proteins were separated by SDS-PAGE on Criterion TGX gels (5671085; Bio-Rad) and transferred to TransBlot Turbo nitrocellulose membranes (1704159; Bio-Rad). The membranes were blocked for 1 hour in 20 mmol/L Tris, 150 mmol/L NaCl, and 1% Tween, pH 7.5/5% nonfat dry milk and incubated overnight at 4°C with the primary antibodies, including mouse monoclonal anti α-smooth muscle actin (ab7817; Abcam; 1/500 dilution), mouse monoclonal anti E-Cadherin (ab76055; Abcam; 1/1000 dilution), and mouse monoclonal anti β-actin (A2228; Sigma; 1/7500 dilution) antibodies. Subsequently, the membranes were washed and incubated with the corresponding secondary antibody, and immunoreactivity was visualized with enhanced chemiluminescence (ECL, RPN2106; GE Healthcare). ECL was detected on a Fujifilm LAS 4000. Digital pictures were quantified by densitometric analysis with ImageJ.

Statistics

The results are represented in box plots as medians with max and min values. The statistical differences among the groups were determined by ANOVA test using the Bonferroni post hoc test for multiple comparisons in the GraphPad Prism 6 software. The $P < 0.05$ was defined as statistically significant.

Results

Finerenone Administration Prevents the Acute Consequences of Renal IR

We first evaluated the potential benefit of finerenone administration to prevent AKI induced by IR. The rats subjected to bilateral renal IR presented renal dysfunction, evidenced by an increase in the plasma levels of creatinine and urea 24 hours after reperfusion (Figure 1A and 1B). In contrast, the increase of creatinine and urea is blunted in rats that received finerenone. The kidney mRNA levels of the tubular injury markers kidney injury molecule-1 and neutrophil gelatinase associated to lipolacin were also evaluated. Renal IR induced a marked increase of >30-fold in kidney injury molecule-1 and neutrophil gelatinase associated to lipolacin expression, which was prevented by MR antagonism with finerenone (Figure 1C and 1D). Histological analysis revealed the presence of tubular lesions (tubular dilation, casts, and cell detachment) in the rats with renal ischemia. These alterations were significantly reduced in the rats treated with finerenone (Figure 1E), as shown in the quantification of injured tubules (Figure 1F). We performed immunohistochemistry against CD68 as a macrophage infiltration marker and TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) assay for apoptosis detection after 24 hours of ischemia. As shown in Figure S2, ischemic AKI was characterized by increased macrophage infiltration, in contrast, the rats treated with finerenone tend to have a reduced macrophages infiltrate. Moreover, we observed a trend of apoptosis induction 24 hours after IR injury, which was not observed in the rats receiving finerenone. In addition, the rats that received the finerenone treatment showed increased levels of E-cadherin expression, 24 hours after ischemia, suggesting improved epithelial cell integrity (Figure S3).

Renal ischemia was associated with a greater amount of malondialdehyde levels in the kidney as an index of oxidative stress. The increase in malondialdehyde levels was prevented by finerenone administration (Figure 2A). Moreover, the plasma levels of 8OHdG were increased by 3-fold in the IR group, whereas the IR+Fine group displayed normal 8OHdG levels (Figure 2B). In addition, the mRNA levels of the antioxidant transcription factor Nrf2 and of superoxide dismutase 3 were higher only in the finerenone-treated rats (Figure S4). We reported previously that a cysteine sulfenic acid modification on ET-B receptor is a crucial step in the mechanism of...
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In this study, we confirm that ET-B receptor suffers this modification in the kidney of rats that underwent IR and we show that this modification is prevented by finerenone administration (Figure 2C).

Antioxidant Therapy Prevents Renal Dysfunction and ET-B Receptor Sulfenic Acid Modification in Renal IR

To establish whether oxidative stress production is directly linked to kidney dysfunction and ET-B receptor post-translational sulfenic modification, we studied rats that received the superoxide dismutase mimetic TEMPO as an antioxidant before the induction of the renal IR. Bilateral kidney IR induced an increase in plasma creatinine (from 21.4±0.9 µmol/L in sham to 86.2±13.4 µmol/L in IR rats), an effect prevented by TEMPO administration (47.4±8.2 µmol/L; Figure 3A). The antioxidant property of TEMPO was confirmed with lower levels of malondialdehyde (29.5±3.1 nmol/mg) in rats with IR treated with TEMPO, as compared with rats with IR without TEMPO (42.9±2.82 nmol/mg; Figure 3B). Importantly, the cysteine sulfenic acid modification that occurs in the ET-B receptor during kidney IR was fully prevented by TEMPO antioxidant treatment (Figure 3C).

Finerenone Administration Prevents the AKI to CKD Transition

We next studied whether finerenone prevented the chronic consequences of ischemic AKI. Four months after the induction of the ischemia, the nontreated rats developed renal dysfunction, evidenced by increased plasma creatinine (Figure 4A) and urea (Figure 4B) levels. This increase was not observed in the rats receiving finerenone treatment (Figure 4A and 4B). Proteinuria is another marker of CKD. The untreated rats presented increased urinary protein levels (121.6±21.6 mg/24 h; n=9). In contrast, proteinuria was significantly less pronounced in rats receiving finerenone (57.6±18.9 mg/24 h; P=0.001). The renal hemodynamics were also modified 4 months after the ischemic injury as evidenced by an increase in the renal vascular resistance in the nontreated rats (Figure 4D), which was mainly a consequence of reduced renal blood flow (9.2±0.5 in IR versus 6.9±0.5 mL/min in sham; P=0.09; Figure 4E). These modifications were not observed in the rats that received finerenone. No changes in the mean arterial pressure were observed with finerenone at this dose (Figure 4F).

As expected, rats that underwent sham surgery presented normal tubular and glomerular architecture (Figure 5A). In contrast, rats that were subjected to a single IR episode developed tubular dilation, presence of tubular casts, and glomerular sclerosis after 4 months (Figure 5B). Finerenone administration before the IR episode was able to fully prevent the development of these alterations (Figure 5B). This is further evidenced in the injury score quantification. Moreover, we analyzed the mRNA levels of neutrophil gelatinase associated lipocalin and kidney injury molecule-1, both markers of tubular injury. CKD development was associated with a marked increase in the kidney mRNA levels of both markers. This elevation was not observed in the rats that received finerenone treatment (Figure 5C).

Figure 2. Effect of finerenone on oxidative stress induced by ischemia/reperfusion (IR). A, Malondialdehyde (MDA) levels were quantified in kidney lysates as indicators of lipid peroxidation. The data were normalized against the protein concentration of the tissue lysate. B, The plasma levels of 8-hydroxy-2-deoxyguanosine (8OHdG) were quantified. C, The endothelin-B (ET-B) receptor was immunoprecipitated from whole kidneys, and a Western blot against cysteine sulfenic acid was performed. One-way ANOVA was performed. *P<0.01, **P<0.001, ***P<0.00001.

Figure 3. Effect of antioxidant therapy on renal dysfunction and ET-B receptor sulfenic acid modification in renal IR. A, Plasma creatinine levels were quantified. B, Malondialdehyde (MDA) levels were quantified in kidney lysates as indicators of lipid peroxidation. C, The endothelin-B (ET-B) receptor was immunoprecipitated from whole kidneys, and a Western blot against cysteine sulfenic acid was performed. One-way ANOVA was performed. *P<0.01, **P<0.001, ***P<0.00001.
In contrast, collagen deposition is limited in rats treated with finerenone (Figure 5D). The quantification of the fibrotic area was scored and is shown in Figure 5E. The histological findings were supported by increased kidney mRNA levels of the profibrotic cytokine transforming growth factor-β and of collagen-I, in the IR group; finerenone administration prevented the increase of these fibrotic markers (Figure S5). In addition, we performed Western blot analysis for α-smooth muscle actin as a marker of fibrotic cells and for E-cadherin as an indicator of epithelial cell integrity. As shown below, after 4 months of AKI, the rats displayed increased kidney levels of α-smooth muscle actin and decreased E-cadherin expression. The effect on both proteins was not observed in the finerenone-treated rats (Figure 5F).

Discussion

In the past years, evidence supporting the potential of MR antagonism, as a therapeutic approach to prevent acute and chronic consequences of renal IR, has been accumulating. Most of the studies reporting protective effect of MR antagonism in AKI and its benefit against CKD progression have been performed with steroidal MR antagonists.6,14,15 In addition, MR blockade with spironolactone or eplerenone has shown to be beneficial in different animal models of chronic kidney injury such as hypertensive nephropathy,16 glomerulonephritis,17 adriamycin-induced nephropathy,18 lupus nephritis,19 Cyclosporine A nephrotoxicity,20,21 nephron reduction,22 unilateral ureteral obstruction,23 and diabetic nephropathy.24 Moreover, clinical studies showed that MR antagonism has a positive effect in reducing proteinuria levels in diabetic and nondiabetic patients with CKD.25–27

Despite the promising data obtained in rodents, only few clinical trials have evaluated the potential therapeutic benefit of spironolactone, mainly because of the concern of hyperkalemia as a consequence of MR antagonism in the distal tubule. This risk is even higher in patients with CKD; paradoxically, these types of patients are the ones that could benefit most from this treatment. The development of nonsteroidal MR antagonists, such as finerenone, which display a safer profile, has brought new hope for MR antagonism as a therapeutic tool to prevent or treat kidney and cardiovascular diseases. However, few studies have evaluated whether these novel MR antagonists retain their beneficial effects on different kidney diseases as those observed with previous existing steroidal MR antagonists. BR-4628 prevents kidney failure, inflammation, and DNA damage in a model of deoxycorticosterone acetate–salt induced hypertension.28 Moreover, BR-4628 was beneficial in experimental glomerulonephritis and renal IR.12,29 Finerenone offered kidney and heart protection in deoxycorticosterone acetate–salt cardiorenal injury.30 Furthermore, finerenone administration protected against cardiac injury in myocardial infarction or pressure overload–induced cardiac hypertrophy models.11,12 In patients with chronic heart failure and concomitant CKD, finerenone was at least as effective as spironolactone in decreasing NT-pro-BNP (N-terminal pro-B-type natriuretic peptide) and urinary albumin, but it was associated with significantly lower increases in serum potassium, significantly lower incidences of hyperkalemia and lower incidence of worsening renal function.33 In addition, in patients with diabetic nephropathy (T2DM and albuminuria >30 mg/g),
addition of finerenone to the standard of care (angiotensin-converting enzyme inhibitor/angiotensin-receptor blocker) resulted in dose-dependent, significant reductions in albuminuria.34 Hyperkalemia leading to discontinuation was not observed in the placebo and finerenone 10-mg groups; the incidence was 3.2% in the 15-mg group and ≤2.2% in all other groups. There were no differences in the incidence of estimated glomerular filtration rate decrease of ≥30% between the placebo and finerenone groups. In the present study, we show that MR antagonism with the new third-generation MR antagonist finerenone has a potent effect against ischemic AKI. The acute functional and structural alterations observed as a consequence of IR were efficiently prevented by finerenone. Oxidative stress is an important component of IR injury. Because IR affects the oxidative phosphorylation, there is an increased production of reactive oxygen species (ROS) during reperfusion. The contribution of ROS in the development of kidney injury is highlighted by the capacity of several antioxidants to attenuate renal IR.35–37 The excessive ROS generation can induce protein, DNA, and lipid damage. In particular, a cysteine sulfenic acid modification that occurs in ET-B receptor is crucial for the decreased NO synthesis38 and therefore the maintenance of vasoconstriction. We showed previously that MR antagonism with the nonsteroidal MR antagonist BR-4628 is able to prevent the occurrence of this inactivating post-translational ET-B receptor modification during kidney IR.12 Here, we show that finerenone administration can also prevent the increase in intrarenal oxidative stress and the ET-B sulfenic acid modification after an ischemic episode. However, whether this modification occurs as a direct consequence of ROS generation during kidney IR was unknown. Therefore, we investigated the ET-B post-translational modification in rats receiving an antioxidant treatment and found that TEMPOL administration prevented the increased oxidative stress after AKI, acute kidney dysfunction, and the sulfenic acid modification of the ET-B receptor. This supports the idea that during kidney IR, the increased ROS generation affects the ET-B receptor and the normal signaling for NO production in endothelial cells. MRAs may act through this pathway as evidenced by loss of effectiveness when a selective ET-B receptor antagonist is coadministered with an MRA.12 In this sense, we showed that specific MR gene deletion in smooth muscle cells protects against kidney IR, through affecting Rac1-mediated ROS generation.39 Therefore, during kidney IR, excessive ROS generation in smooth muscle cells might affect the proximal ET-B receptor in endothelial cells, an effect that can be prevented by MR antagonism.

MR blockade by finerenone is effective not only for preventing the acute consequences of ischemic AKI but also to limit the progression of AKI to CKD. Four months after IR, rats displayed characteristics of CKD such as proteinuria, kidney dysfunction, increased renal vascular resistance, and structural alterations. Short-term prophylactic MR antagonism (2 days) with finerenone fully prevented the progression from AKI to CKD.

In experimental models, the targeting of renal hemodynamics and oxygenation by RAS blockade, epigenetic changes and cell cycle arrest, has shown to have potential
for targeting the progression of AKI to CKD.\textsuperscript{6,40–42} However, the ADQI XVIII work group acknowledged that in the current clinical practice, there are no therapeutic interventions to avoid the AKI to CKD progression.\textsuperscript{7}

Altogether, our data show that in a rat model of AKI to CKD, the novel nonsteroidal MR antagonist finerenone is able to prevent acute injury induced by IR and the chronic and progressive deterioration of kidney function and structure. The underlying mechanism relies on prevention of oxidative stress and its consequence on the ET-B receptor. These preclinical data warrant further studies using nonsteroidal MR antagonists in clinical trials, to target kidney injury associated to IR.

**Perspectives**

Given the accumulating body of evidence that shows a benefit of MR antagonism against kidney injury mediated by ischemic processes, the next step would be to translate these findings into the clinical practice. The concern for hyperkalemia might be overcome with the use of novel nonsteroidal antagonists. The target population to perform a large clinical trial would be patients who will undergo cardiovascular surgery or kidney transplantation in which the likelihood of developing AKI is elevated. Whether finerenone administration might be beneficial for the prevention of oxidative injury in other organs remains to be investigated.

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Disclosures

P. Kolkhof is an employee of BAYER AG. The other authors report no conflicts.

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**Novelty and Significance**

**What Is New?**

- A novel nonsteroidal mineralocorticoid receptor antagonist prevents the acute and chronic consequences of renal ischemia/reperfusion.
- Finerenone and antioxidant treatment prevent acute kidney injury through the prevention of endothelin-B receptor sulfenylation.

**What Is Relevant?**

- This study is relevant to the treatment of acute kidney injury and to prevent its progression to chronic kidney disease. These preclinical data support the use of nonsteroidal mineralocorticoid receptor antagonists in clinical trials intended to target kidney disease.

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**Summary**

The novel nonsteroidal mineralocorticoid receptor antagonist finerenone prevents acute injury induced by ischemia/reperfusion, as well as the chronic and progressive deterioration of kidney function and structure. The underlying mechanism relies on prevention of oxidative stress and its consequence on the endothelin-B receptor.
Nonsteroidal Mineralocorticoid Receptor Antagonist Finerenone Protects Against Acute Kidney Injury–Mediated Chronic Kidney Disease: Role of Oxidative Stress

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The Non-steroidal Mineralocorticoid Receptor Antagonist Finerenone Protects Against AKI-mediated Chronic Kidney disease: Role of Oxidative Stress.

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### Supplementary table S1

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<td>CTACAAATgggAATgTCTCTgC</td>
</tr>
</tbody>
</table>
Figure S1. Representative immunoprecipitation with ETB specific antibodies and IgG isotype control.
**Figure S2.** Effect of finerenone on macrophage infiltration and apoptosis after 24h of ischemic AKI. (A) Representative CD68 immunohistochemistry images are shown for the sham, IR and IR + Fine groups. The number of CD68 positive cells was quantified in high power fields (HPF). (B) Representative TUNEL assay images are shown for the sham, IR and IR + Fine groups. The number of TUNEL positive cells was quantified in high power fields (HPF). One-way ANOVA was performed. *p<0.01.
Figure S3. The protein levels of E-cadherin were evaluated in kidneys after 24-h of ischemia induction. One-way ANOVA was performed. *p<0.01, **p<0.001.
Figure S4. The mRNA levels of (A) Nrf2 and (B) SOD3 were determined by RT-PCR in kidneys after 24-h of reperfusion. One-way ANOVA was performed. *p<0.01.
Figure S5. The mRNA levels of (A) TGF-b and (B) Collagen I were determined by RT-PCR in kidneys after 5 months of follow up. One-way ANOVA was performed. *p<0.01, **p<0.001.