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Aprocitentan mitigates ischemia reperfusion induced kidney injury in rats by attenuating inflammation and pyroptosis through suppressing NF-κB/NLRP3/caspase-1 signaling pathway

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ABSTRACT

Introduction and aim. Renal ischemia-reperfusion (I/R) injury is a primary cause of acute kidney injury (AKI). The NF-κB/NLRP3/caspase-1 signaling system is crucial for I/R-induced kidney damage, which leads to inflammation, pyroptosis, and tissue damage. Aprocitentan has anti-inflammatory and vasoprotective properties, suggesting its potential nephroprotective advantages.

The aim of this investigation was to evaluate the protective advantages of aprocitentan against renal I/R injury in rat models. This was achieved by evaluating the impact of aprocitentan on inflammation, pyroptosis, and kidney function by altering the NF-κB/NLRP3/Caspase-1 pathway.

Material and methods. Using twenty-four adult male Sprague-Dawley rats, four separate groups were formed: sham group, I/R control group, dimethyl sulfoxide group (DMSO) and aprocitentan group (10 mg/kg). Renal ischemia/reperfusion was induced by a period of forty minutes of bilateral ischemia, which was followed by two hours of reperfusion. To evaluate the renoprotective effect of aprocitentan urea, creatinine, neutrophil gelatinase-associated lipocalin (NGAL), phosphorylated nuclear factor kappa B p65 (NF-κB p65), NOD-like receptor protein 3 (NLRP3), and cysteine-aspartic protease-1 (caspase-1), were measured, along with histopathological examination.

Results. Compared to the sham group, the I/R control group had significantly increased concentrations of urea, creatinine, NGAL, NF-κB p65, NLRP3, as well as caspase-1. These signs decreased in the aprocitentan group, leading to enhanced renal function, reduction of inflammation, and inhibition of pyroptosis. Histology showed a decrease in tubular inflammation and necrosis in the aprocitentan group.

Conclusion. Aprocitentan decreases inflammation and inhibits pyroptosis by suppressing the NF-κB/NLRP3/Caspase-1 pathway. These results illustrate its promising potential to prevent ischemic renal diseases, including acute kidney injury.

Keywords. acute kidney injury, caspase-1, endothelin receptor antagonist, ischemia-reperfusion, pyroptosis, NLRP3 inflammasome

Introduction

Renal I/R injury is a significant clinical concern and one of the main causes of AKI, leading to a high incidence of morbidity and mortality globally.[1] This disorder occurs when the kidney blood supply is temporarily interrupted (ischemia) and then resumed (reperfusion). Despite the fact that reperfusion is necessary to avoid irreversible tissue damage, it conversely worsens kidney injury, resulting in decreased kidney function and, in serious cases, end-stage kidney disease.[2]

Damage to renal I/R is a crucial subject in both therapeutic and experimental research, as it manifests frequently in clinical conditions, including kidney transplants, partial nephrectomy, septic shock, and cardiovascular procedures.[3] Renal I/R injury is particularly crucial in the transplantation of the kidney; it is a strong predictor of delayed graft function as well as long-term allograft survival.[4] While techniques for preserving organs have improved, I/R injury is still an important issue that makes it difficult for transplants to succeed.

Growing evidence indicates that pyroptosis – a pro-inflammatory type of programmed cell death regulated by the NLRP3 inflammasome and caspase-1– has a pivotal role in AKI after ischemia reperfusion injury.[5,6] During renal I/R, damage-associated molecular patterns (DAMPs) initiate NF-κB and the NLRP3 inflammasome, leading to caspase-1 activation and the liberation of mature interleukins, this includes interleukin-1 beta (IL-1β) and interleukin-18 (IL-18) that trigger an intense inflammatory cascade.[6] In experimental I/R models, treatments that inhibit NLRP3 or caspase-1 have shown lower kidney damage, suggesting that the pyroptosis pathway is a viable target.[7] Endothelin-1 has been shown to worsen renal damage by resulting in vasoconstriction and inflammation,[8] aprocitentan is a new dual endothelin receptor blocker. Therefore, we proposed that aprocitentan could reduce I/R-induced kidney damage by suppressing NLRP3/caspase-1-mediated pyroptosis and NF-κB-driven inflammation.

Aprocitentan is a dual endothelin receptor antagonist (ERA) approved for the treatment of resistant hypertension in adults. Aprocitentan blocks endothelin receptor type A (ETA) and endothelin receptor type B (ETB) receptors that decrease blood pressure and improve cardiovascular health.[9] Aprocitentan has shown the ability to reduce the levels of pro-inflammatory cytokines, especially IL-1β and tumor necrosis factor-alpha (TNF-α), thus decreasing the inflammatory response in I/R.[10]

NF-κBp65 it is an important subunit of the NF-κB transcription factor and plays an essential role in the inflammatory response associated with renal I/R. Oxidative stress and inflammatory mediators activate NF-κBp65, which moves to the nucleus and starts the transcription of pro-inflammatory genes after I/R. This procedure improves tissue damaging and increases pro-inflammatory cytokines, adhesion molecules, and enzymes, resulting in kidney damage. In the I / R animal model, reducing NF-κBp65 particularly reduces

renal damage and inflammation.[11] Moreover, NF-κBp65 controls tubular cell apoptosis and fibrosis, each of which are significant characteristics of I/R-induced kidney failure.[12]

The NLRP3 inflammasome is an important contributor to kidney damage during renal I/R. I/R promote NLRP3 activation due to cellular stress and mitochondrial malfunction as well, leading to the formation of reactive oxygen species (ROS) and Generation of pro-inflammatory cytokines (IL-1β and IL-18). Stimulation of NLRP3 inflammasomes plays a role during kidney tubular injury, inflammation, and fibrosis during I/R.[13] NLRP3 activation may worsen kidney damage by inducing pyroptosis, which causes more tissue destruction.[14] Medications that reduce kidney injury after an ischemic episode incorporate in the inhibition of the NLRP3 inflammasome. Research indicates that targeting NLRP3 may enhance kidney function by decreasing inflammation and oxidative stress, possibly providing a method to address renal I/R.[15]

Caspase-1 is a critical enzyme involved in cell death and inflammation and is an important contributor to renal I/R. In I/R, caspase-1 activation during the inflammatory response, breakdown of pro-inflammatory cytokines (IL-1β and IL-18) to their mature form, results in tissue damage. Inhibition of caspase-1 reduces renal damage and improves kidney function after I / R, suggesting that caspase-1 is a mediator of kidney inflammation and injury. It also appears to trigger renal tubular cell pyroptosis, thus worsening tissue damage following I/R.[16]

NF-κB p65 represents a transcription element that increases the synthesis of pro-inflammatory cytokines and components of the inflammasome, effectively priming cells for pyroptosis. NLRP3 is an intracellular sensor that forms the NLRP3 inflammasome complex when detecting cellular stress or damage signals; this then activates pro-caspase-1. Caspase-1 mediates the processing of pro–IL–1β and pro–IL-18 into active cytokines and cleaves gasdermin D, the executor protein that produces membrane pores that promote cell lysis and inflammatory cell death.[17] These three factors were measured as key markers of NF-κB inflammasome and pyroptosis in the kidney tissue**.**

Limited studies have directly examined therapeutic agents targeting the NF-κB/NLRP3/caspase-1 axis as a coordinated pathway, despite emerging evidence highlighting the impact of inflammation and pyroptosis on renal I/R injury. Although the protective potential of NLRP3 or caspase-1 inhibitors has been independently shown, few interventions have concurrently suppressed upstream inflammatory signaling and downstream pyroptotic mechanisms. Aprocitentan presents a unique opportunity to modulate vascular and inflammatory responses by dual blocking of endothelin A and B receptors. However, its mechanistic efficacy in the context of renal I/R injury and its impact on NF-κB/NLRP3/caspase-1 cascade of NF-B/NLRP3/caspase-1 remain unexplored. Since NLRP3 and caspase-1 are essential for renal ischemia-reperfusion injury, this study aims to investigate the less studied potential of aprocitentan, which can affect these inflammatory pathways by antagonizing endothelin receptors. Aprocitentan could provide upstream

modulation in addition to direct inflammasome inhibition, improving the possibilities for treatment beyond traditional inflammasome-targeted therapies.

Aim

The objective of this investigation is to estimate whether the dual endothelin receptor antagonist aprocitentan exhibits any possible nephroprotective advantages in a rat model of I/R. The research project relies on the mechanisms of pyroptosis and inflammation to offer a detailed understanding of their beneficial effects.

Material and methods

Animals' preparation

A total of 24 mature male Sprague-Dawley rats, weighting between 200 and 250 grams, were sourced from the Faculty of Science University of Kufa. They were kept in the University of Kufa's Faculty of Science's animal department, under standard settings. The average daily temperatures were preserved at 24 ± 2°C, and 60-65% humidity, with the rats having unrestricted access to food and tap water. The animals were kept in cages, with a 12 hour light/dark cycle.

Experimental design

Twenty-four male adult rats (Sprague-Dawley) randomly assigned to four groups using a simple randomization method (n=6 each group). The sample size of n=6 per group was chosen based on previous studies of I/R injury that detected significant differences with similar group sizes[18,19]: Sham, I/R control, I/R+DMSO, and I/R+aprocitentan. The Sham group underwent a surgical operation without renal ischemia, while the I/R control group underwent renal ischemia-reperfusion injury without any drugs or vehicle interference. The I/R+DMSO group suffered an I/R injury and received an injection of DMSO as medication solvent, while the I/R+aprocitentan group suffered I/R injury and received 10 mg/kg of aprocitentan (TargetMol Chemicals, China). Aprocitentan (or an equivalent DMSO vehicle) was injected intraperitoneally twice, 24 hours and 60 minutes before the onset of ischemia to guarantee systemic drug exposure throughout the I/R insult. The dose of 10 mg/kg was chosen after previous studies indicating effective endothelin receptor blockade at this dose in rats.[20] After the duration of the experimental period, blood samples and kidneys were collected for further assessment, and then all animals were sacrificed. The experiments carried out in this study were reviewed and approved by the Ethics Committee of the Faculty of Pharmacy of the University of Kufa, Najaf, Iraq (Approval No. 3177/2025-2-4).

Renal I/R model

The animals received ketamine (100 mg/kg) (Interchem, Holland) and xylazine hydrochloride (10 mg/kg) (Alfasan, Holland) intraperitoneally to induce anesthesia.[21] After induction of anesthesia, the limbs were secured, the area around the incision was shaved and it was then disinfected with iodine spray. Subsequently, a retroperitoneal flank incision. The renal pedicles were cautiously recognized by blunt dissection to gently separate the connective tissues and adipose tissue surrounding the left and right renal arteries and veins, anteriorly and posteriorly. The abdominal cavity was accessed via a retroperitoneal flank incision, exposing both kidneys. Bilateral renal occlusions were performed for 40 minutes using non-traumatic microvascular clamps (Minechina/Germany). The color shifted from red to dark maroon in 10 minutes, and the whole surface was an obvious indication of occlusion. Then a sterilized gauze was placed on the rat. When the ischemia phase and withdrawing the clamp was removed, the color of the kidney rapidly change from a dark maroon to dark pink, signifying successful reperfusion. After verifying reperfusion, the wound was bound and wrapped in clean gauze, both saturated with 0.9% saline to avoid dehydration. After 2 hours of reperfusion, blood was taken directly from the heart for parameter measurements, and the kidneys were harvested for evaluation of the experimental parameter assessment; afterwards, the rats were sacrificed by cardiac puncture. The kidney was sagittally divided into two parts; one was kept in 10% formaldehyde for histological purposes, and the other was placed in the freezer at -80°C in phosphate buffered saline (PBS) for the enzyme-linked immunosorbent assay (ELISA) analysis.[22] All outcomes assessments, including histopathological scoring and biomarker measurements, were performed by investigators blinded to group allocations.

Blood sample collection

At the end of the procedure, when the rats remained under anesthesia, approximately 2-4 milliliters of blood were collected directly from the cardiac chamber. The blood sample was placed in a gel tube free of anticoagulant agents and centrifuged at 3000 rpm for approximately 10 min to extract serum. Serum was next used to evaluate urea and creatinine spectrophotometrically and NGAL levels using a commercial ELISA kit (Shanghai Ideal Medical Technology Co., Ltd./China, Cat No: ADLEL-RT00241).

Tissue sample preparation

Samples of kidney tissue were taken after the blood samples were collected. For histopathological evaluation, one piece of tissue was embedded in 10% formaldehyde, while the other was placed in a freezer at -80°C before homogenizing using a high-frequency ultrasonic liquid processor within a 1:10 W/V solution of buffered phosphate buffered saline with 1% Triton X-100 and a cocktail of protease inhibitors (Roche Germany).[23] The homogenate went through a centrifuge at 5000 rpm for 10 min at 4°C. The

obtained supernatants were used to assess NF-κB p65, NLRP3, and Caspase-1 using available ELISA kits (Shanghai Ideal Medical Technology Co., Ltd./China, Cat No: ADL-EL-RT01064, Cat No: ADL-EL-RT01063, Cat No: ADL-EL-RT00752).

Histopathological examination

Kidney tissues have been extracted and preserved in 10% neutral buffered formalin. The samples were placed in paraffin, which is cut to 5 µm pieces. Tissue slices were stained with hematoxylin and eosin (H&E) for histological purposes. The kidney tissues were examined for histopathological changes, including tubular necrosis, inflammation, and severity of damage. Injury scores were given according to the level of damage, and tubular necrosis and inflammation were documented in each section. Histological changes were determined by the percentage of damaged kidney tubules. Tissue damage was determined semi-quantitatively as follows: 0=no damage (intact tubules); 1=<25% damage; 2 = 25 50% damage; 3=50–75% damage; and 4 => 75% damage.[24]

Statistical analysis

GraphPad Prism version 8.1 was employed to conduct statistical analysis. Data for normally distributed variables were presented as mean ± SD. The Shapiro-Wilk test was used to determine the normality of the data distribution. A one-way analysis of variance (ANOVA) was used to assess differences between groups, followed by Tukey's multiple comparison test. To compare histopathological changes between groups, nonparametric Kruskal-Wallis and Dunn's post-hoc tests were used. A p-value of <0.05 was considered statistically significant.

Results

Effect of aprocitentan on urea and creatinine

In contrast to the sham group, the serum urea and creatinine in the I/R control group were considerably higher ($p<0.0001$). The I/R control and vehicle (DMSO) groups did not differ significantly ($p>0.05$). Compared to rats treated with aprocitentan with the I/R control group, rats blood urea levels were considerably lower ($p<0.0001$) (Fig. 1).

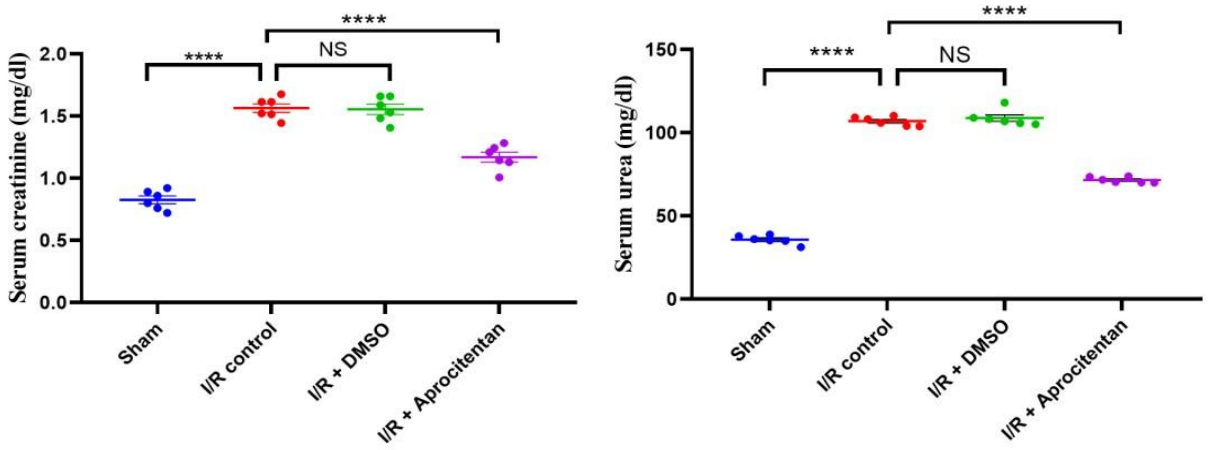

Fig. 1. Means of serum urea and creatinine concentration in the various groups (data resemble mean ±SD; n=6 biological replicates, **** – p<0.0001, and ns – p>0.05 nonsignificant vs. I/R control group, one-way ANOVA, Tukey's test)

Effect of aprocitentan on the level of NGAL

Unlike the sham group, the I/R control group's NGAL concentrations of the I/R control group were considerably higher (p<0.0001). There was no apparent difference between the vehicle and the I/R control groups (p>0.05). NGAL levels exhibited a significant decline in rats receiving aprocitentan compared to the I/R control group (p<0.0001) (Fig. 2).

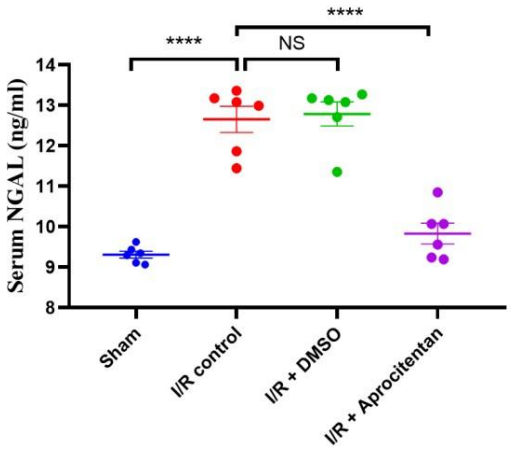

Fig. 2. Means of serum NGAL concentration in the various groups (data resemble mean±SD; n=6 biological replicates, **** – p<0.0001, and ns – p>0.05 nonsignificant vs. I/R control group, one-way ANOVA, Tukey's test)

Effect of aprocitentan on the level of the inflammatory biomarker NF-κBp65 level

The NF-Bp65 concentration of the tissue homogenate NF-κBp65 concentration was significantly higher than that of the sham group ($p<0.0001$). When comparing the DMSO group with the I/R control group, there was no noticeable difference in the NF-κBp65 level ($p>0.05$). Rats given aprocitentan had significantly lower NF-κBp65 levels of NF-Bp65 relative to the I/R control group ($p<0.0001$) (Fig. 3).

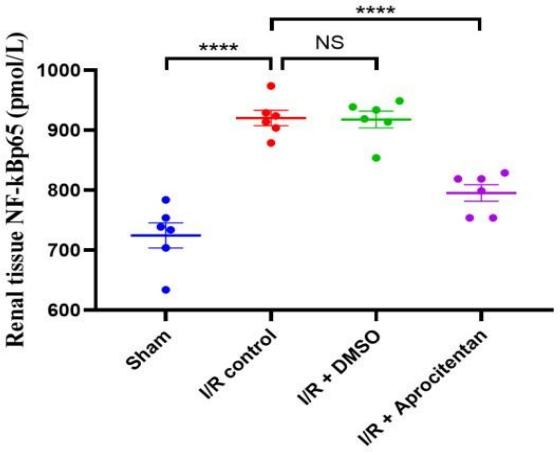

Fig. 3. Means of tissue NF-κBp65 concentration in the various groups (data resemble mean±SD; n=6 biological replicates, **** – $p<0.0001$, and ns – $p>0.05$ nonsignificant vs. control group, one way ANOVA, Tukey test)

Effect of aprocitentan on the level of NLRP3

The from the I/R control group of tissue homogenate NLRP3 concentration was significantly greater ($p<0.0001$) than that of the sham group. When comparing the DMSO group with the I/R control group, there was no noticeable variation in the level of NLRP3 level ($p>0.05$). Rats who received aprocitentan demonstrated substantial reductions in NLRP3 levels compared to the I/R control group ($p<0.0001$) (Fig. 4).

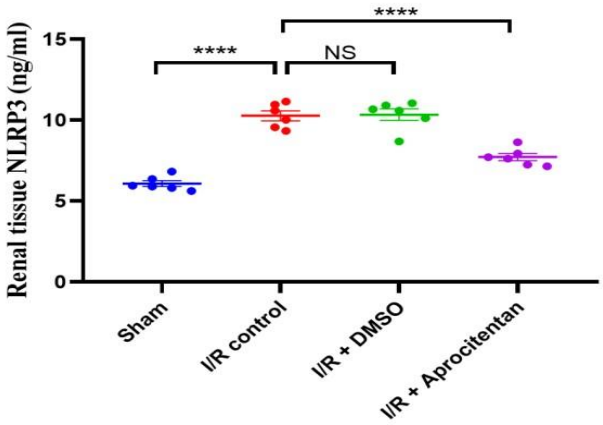

Fig. 4. Means of tissue NLRP3 concentration in the various groups (data resemble mean±SD; n=6 biological replicates, **** – p<0.0001, and ns – p>0.05 nonsignificant vs. I/R control group, one-way ANOVA, Tukey's test)

Effect of aprocitentan on the level of the caspase-1 pyroptosis biomarker

The concentration of tissue homogenate caspase-1 from the I / R control group was significantly higher than that of the sham group (p<0.0001). When comparing the DMSO group with the I/R control group, there was no noticeable difference in the level of caspase-1 level (p>0.05). Rats treated with the aprocitentan group showed a significant decline in the caspase-1 levels compared to the I/R control group (p<0.0001) (Fig. 5).

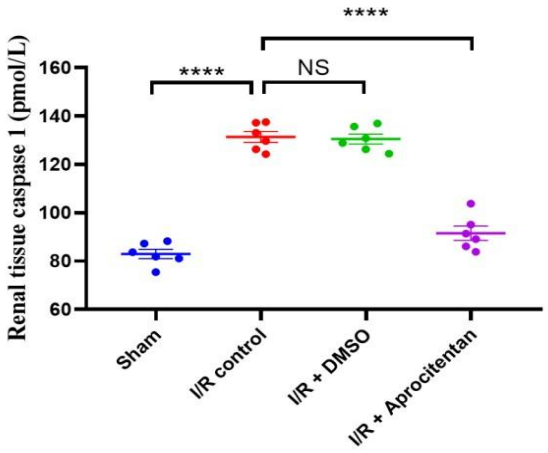

Fig. 5. Means of tissue caspase 1 concentration in the various groups (data resemble mean±SD; n=6 biological replicates, **** – p<0.0001, and ns – p>0.05 nonsignificant vs. I/R control group, one-way ANOVA, Tukey's test)

Histopathological findings

The I/R control and DMSO groups showed a significant histological change (neuronal tubular damage) in comparison with the normal tissue of the sham group (p<0.001). Further comparison that according to the histopathology and scoring system, the score of renal tubular damage was significantly lower in the aprocitentan group in contrast to the I/R control and vehicle groups (*p<0.05) (Fig. 6-10).

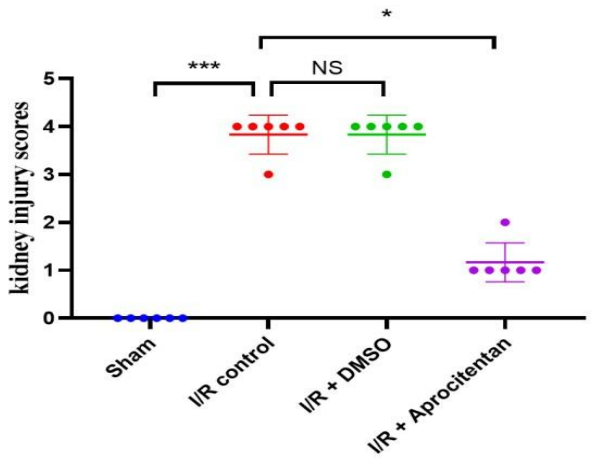

Fig. 6. Scores of kidney injury in the various groups (data resemble mean ± SD; n=6 biological replicates, *** – $p<0.001$, * – $p<0.05$, and ns – $p>0.05$ nonsignificant vs. I/R group, nonparametric Kruskal Wallis and Dunn's post hoc tests)

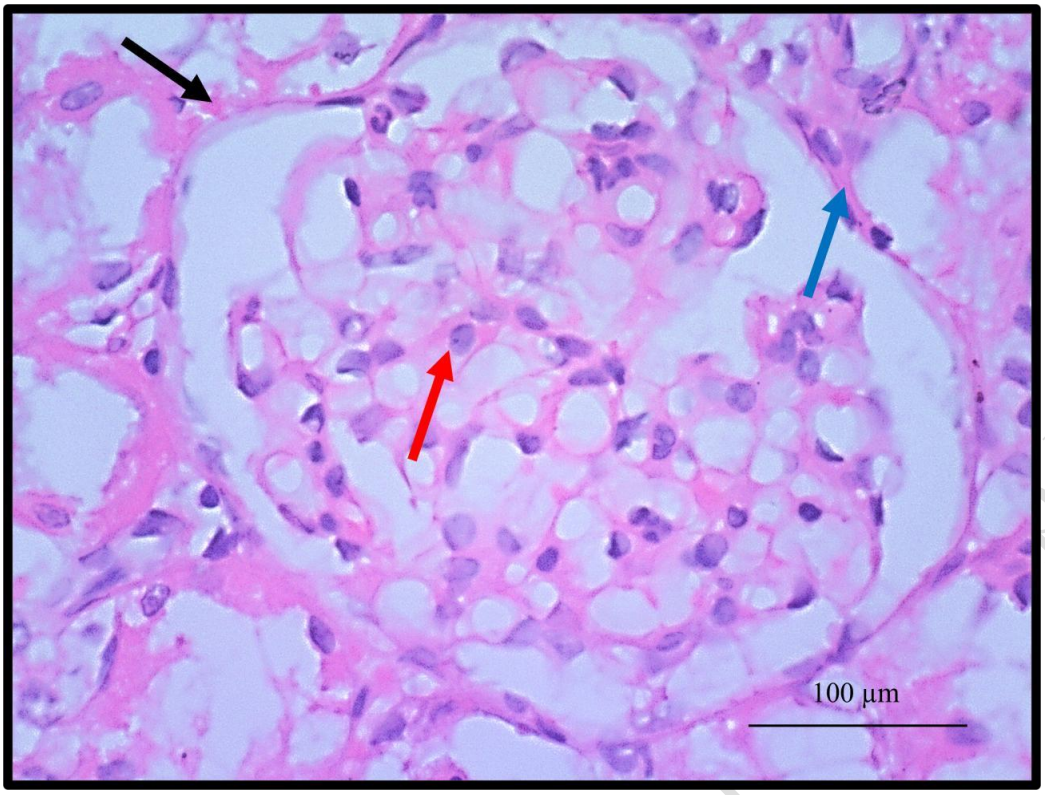

Fig. 7. Histological section in kidney of rat in sham group (the section shows normal renal tissue texture including normal glomeruli (black arrow), normal glomerular tuft (red arrow) and normal glomerular capsule (blue arrow), the proximal renal tubules show normal epithelial cells that line the tubules with normal lumen (green arrows), the tissue is stained by H&E stain and the section was taken with a digital camera attached to a light microscope at 40×magnifier scale)

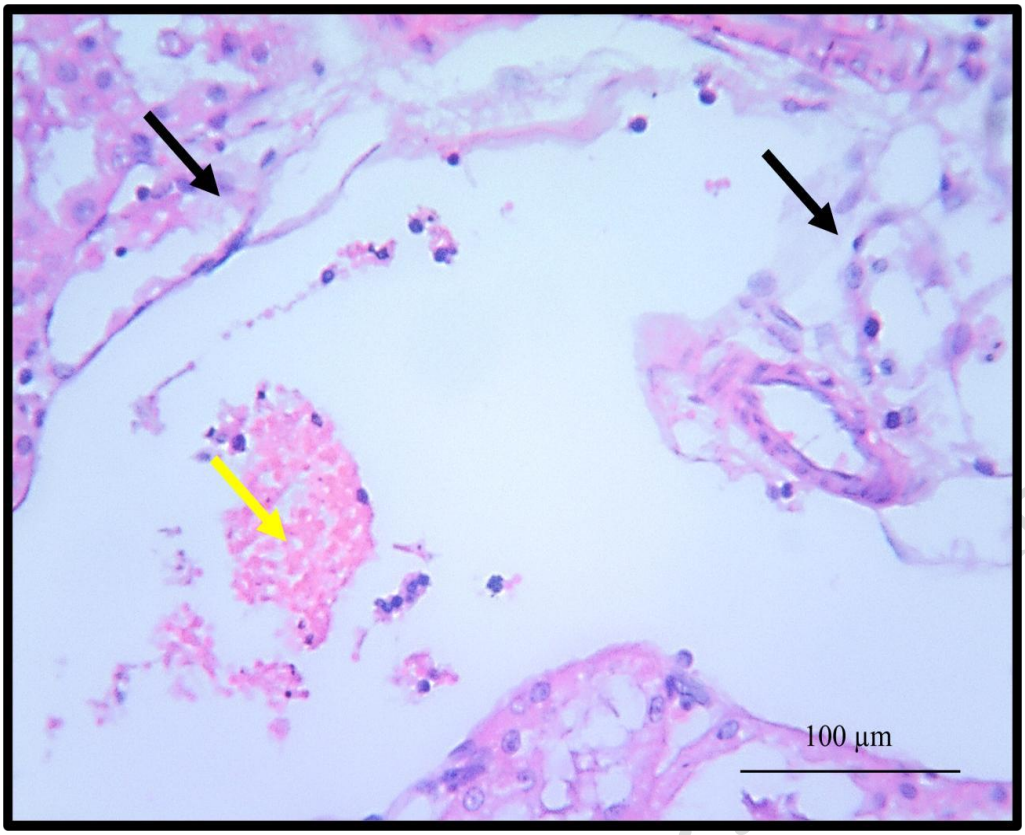

Fig. 8. Kidney histopathological section in rats of I/R control group (rats underwent clamping of the renal artery using the dorsal approach to cause ischemia for 40 minutes and reperfusion for 2 hours), the section shows a clear necrotic lesion in the renal tissue (coagulative necrosis, black arrows) with glomerular atrophy can be seen in the section of renal tissue (red arrows), the glomerular tuft shows severe necrotic change for epithelial cells of the tuft (blue arrow), blood vessels congestion as severe pathological changes can be seen in the section (yellow arrows), the tissue is stained by H&E stain and the section was taken with a digital camera attached to a light microscope at 40×magnifier scale.

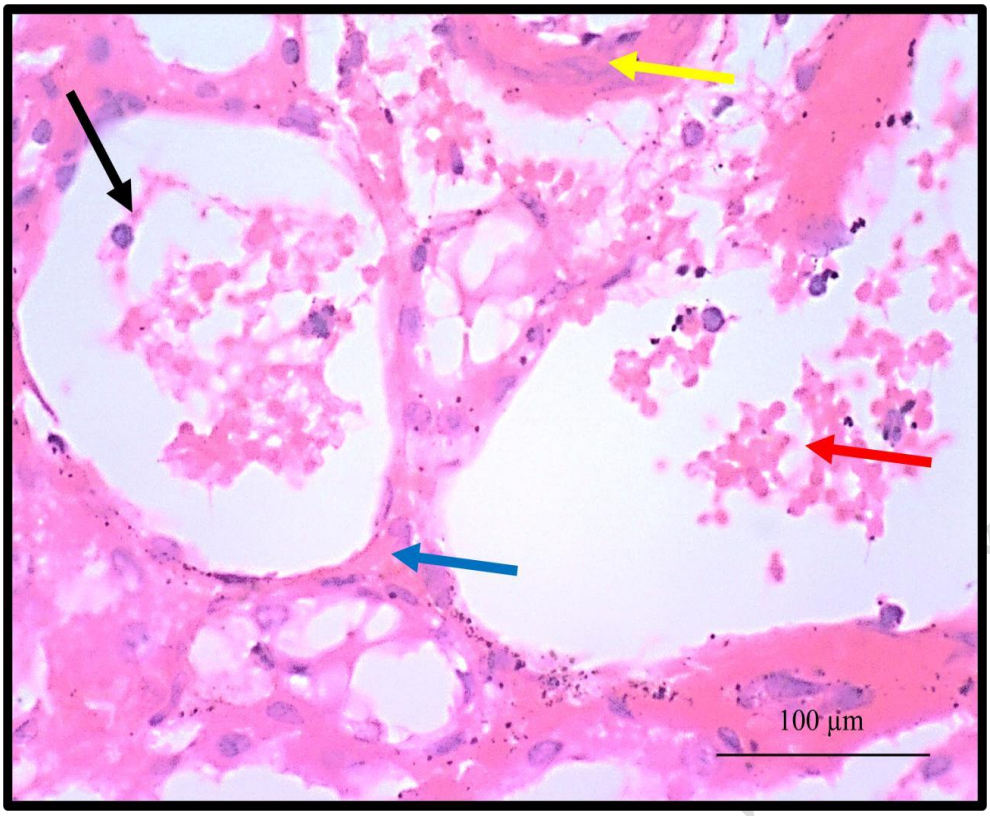

Fig. 9. Kidney histopathological section in rats of DMSO group (intraperitoneal injection with aprocitentan DMSO solvent 24 hours prior to ischemia and then one hour before ischemia, then underwent the surgical procedure, followed by 40 minutes of bilateral renal ischemia then 2 hours of reperfusion), renal tissue shows severe atrophied lesion of renal glomeruli and glomerular tuft (black arrows) with severe hypertrophic changes in the glomerular capsule (blue arrow), blood vessels show clear wall damage congestion (red arrow), the proximal renal tubules show narrowing in the lumen with hypertrophy of lining cells (yellow arrows), tissue is stained by H&E stain and the section was taken with a digital camera attached to a light microscope at 40×magnifier scale

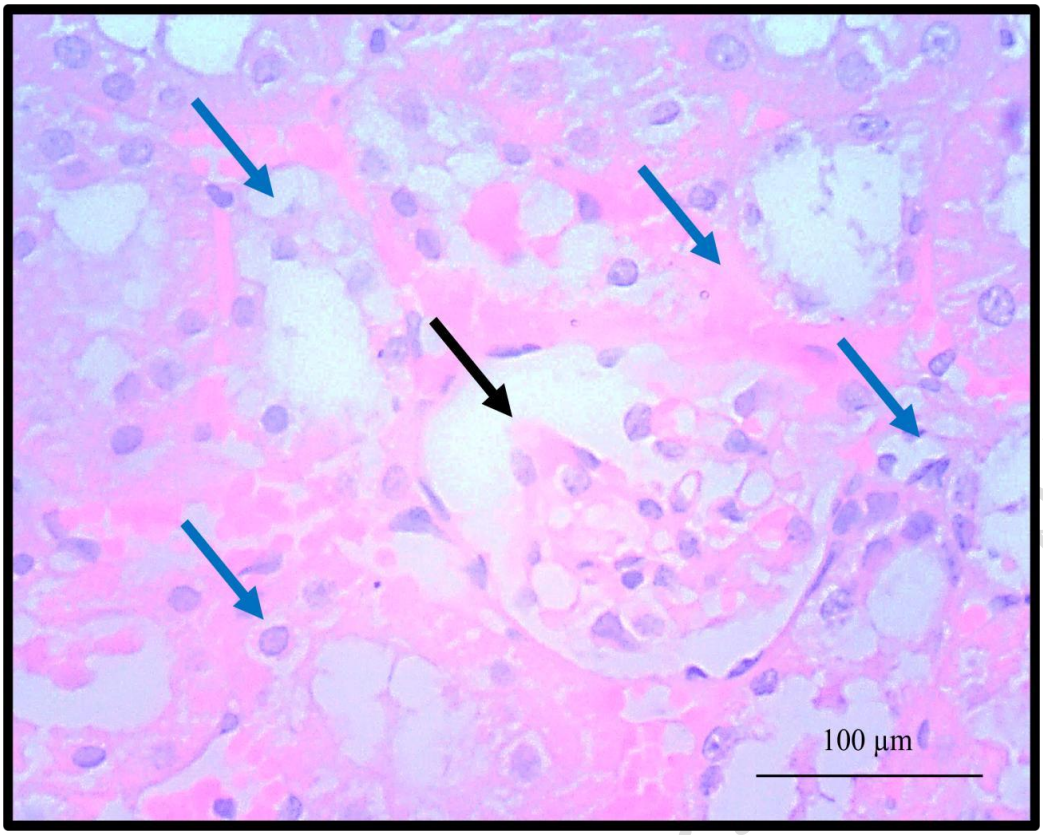

Fig. 10. The histopathological section of the kidney in rat of the treated group with aprocitentan (intraperitoneal injection with aprocitentan (10 mg/kg) both 24 and 1 hour before induction of ischemia), the section shows normal glomerular texture including normal capsule and tuft (black arrow) and some glomeruli show mild atrophied lesion (red arrow), the renal proximal tubules show mild hypertrophy and hydropic degeneration and cytoplasmic vacuolation in tubular epithelial cells (blue arrows), the tissue is stained with H&E stain and the section was taken with a digital camera attached to a light microscope at 40×magnifier scale

Discussion

Urea and creatinine are conventional indicators of renal function that reflect the rate of glomerular filtration. Impaired filtration in AKI, including I/R injury, causes an elevation of these substances in the blood. This is in line with the I/R control rats that had considerably higher blood levels of urea and creatinine compared to the sham group rats.[25–27]

Aprocitentan treatment leads to significantly lower levels of urea and creatinine than untreated I/R control rats, which indicates preserved renal excretory function. This improvement in biochemical indicators of renal function suggests that aprocitentan maintained the glomeruli and tubular filtration systems. Although there are no comparable experiments on aprocitentan in I/R, our findings are consistent with its documented renal effects in other situations. Particularly in hypertensive rat models, dual endothelin-1 (ET-1) receptor blockage with aprocitentan lowers blood pressure without leading to renal damage.[20,28] The ability of

aprocitentan to avoid rapid increase in urea and creatinine after I/R corresponds with its history of maintaining renal function, showing that it reduced the acute loss of filtration capacity that often occurs with ischemic damage.

NGAL is an early tubular injury biomarker that increases immediately in blood and urine after renal trauma. It is considered a sensitive early sign of AKI.[29] In our I/R model, serum levels of NGAL were significantly higher in the untreated I/R group in contrast to the sham group, showed significant tubular damage, and confirm the role as an accurate early sign of ischemic AKI.[30] This elevation is consistent with previous research, which suggests that NGAL is significantly increased by ischemic kidney injury and is consistent with the degree of tubular damage.[31]

Aprocitentan treatment led to significantly lower NGAL levels compared with I/R controls. The decreased NGAL shows that aprocitentan decreased acute tubular injury in the ischemic kidneys, providing a degree of protection. The outcome is consistent with previous research on endothelin antagonists. In particular, double ERA bosentan has been found to decrease NGAL levels while improving renal function in the diabetic nephropathy model by lowering oxidative stress and inflammation.[32] Aprocitentan potentially uses comparable defensive mechanisms. Aprocitentan has been shown to relieve the effects of oxidative stress in cellular models.[33] By reducing ROS formation of ROS in the mitochondria,[33] aprocitentan could reduce the oxidative injury to tubules, which would otherwise promote elevation of NGAL. Thus, the impact in I/R suggests reduced acute tubular injury, suggesting effective renal protection, most likely by antioxidant and anti-inflammatory activities.

NF-κB p65 is an essential part of the NF-κB transcription factor complex that controls the expression of inflammatory genes. NF-κB p65 plays a critical role in the inflammatory response during renal I/R.[34] The I/R control group had substantially higher levels of renal NF-κB p65 compared to sham rats, indicating that ischemia-reperfusion stimulated this pathway. Previous investigations have shown that NF-κB p65 levels are significantly elevated in ischemic kidneys, leading to further inflammation and cell death.[12]

As an example, studies indicate that I/R increases NF-κB signaling pathways, leading to renal impairment and tissue damage.[12,35] In a similar way, another study demonstrated that NF-κB p65 levels were significantly higher in kidney damage due to I/R, which was connected to enhanced inflammatory reactions and cell death.[36]

Endothelin-1 itself can enhance inflammatory signaling; ET-1 activates the NF-κB-mediated pathway in macrophages, leading to TNF-α release.[37] Aprocitentan treatment significantly decreased NF-κB p65 in kidney tissue compared with untreated I/R rats, a sign of an anti-inflammatory effect. Aprocitentan inhibits ETA and ETB receptors, so it breaks the loop of ET-1-induced NF-κB activation.[37] This limits the transcription of pro-inflammatory genes. These findings are unique in the context of renal I/R, since previous literature has not specifically studied the influences on NF-κB in acute kidney injury. However, it is comparable to the broad anti-inflammatory effects of endothelin antagonism. Reducing NF-κB activity

might limit cytokine release and leukocyte infiltration, preserving renal tissue from inflammation. Therapies that limit NF-κB activity in I/R models may mitigate renal injury.[38] The results we see suggest that aprocitentan produces a similar benefit. Aprocitentan decreases NF-κB p65, indicating that it has the capacity to reduce I/R's early inflammatory pathways. This is essential for reducing tissue damage and preserving renal function.

NLRP3 is an intracellular pattern recognition receptor that polymerizes to form the NLRP3 inflammasome in response to danger signs (oxidative stress, ATP, mitochondrial DNA) after tissue injury.[7] The multiprotein complex activates caspase-1, leading to the production of pro-inflammatory cytokines IL-1β and IL-18.[7] In the kidneys, NLRP3 is found primarily in tubular as well as immune cells, and it is usually inactive.[3] Renal I/R activates NLRP3 by ischemic cell stress, reperfusion-induced ROS production and DAMPs.[7,12]

As a result, we found that I / R significantly increased NLRP3 levels in renal tissue (I/R control versus sham), which agrees with previous studies pointing to NLRP3 as an important modulator of inflammation and injury in I/R models.[1,39]

Rats treated with aprocitentan had considerably lower renal levels of NLRP3 compared to untreated I/R rats, indicating that the drug inhibited inflammasome activation. This is a significant finding, since it reflects that aprocitentan can modify innate inflammatory sensors beyond its hemodynamic effects.[39] To the best of our knowledge, there is no previous investigations have looked into the impact of aprocitentan on NLRP3 on renal I/R. Endothelin-1 signaling has been linked to increased production of cytokines and possibly inflammasome activity in cardiac injury models.[40]

Aprocitentan could reduce upstream triggers (including oxidative stress or NF-κB dependent signals) by blocking ET receptors, which allow NLRP3 activity.[10] Notably, aprocitentan was shown to drastically reduce mitochondrial ROS generation and malfunction in a cellular model, which indicates that its antioxidant properties may be involved in this context.[41] Aprocitentan may help preserve renal tubular cells and decrease dysfunctional inflammation after I/R by inhibiting NLRP3.[42]

Notably, aprocitentan‘s multifunctional cytoprotective activity appears in different models of organ injury. As an example, aprocitentan has been shown to reduce doxorubicin-induced heart injury by reducing mitochondrial impairment and oxidative stress.[42] These results support the idea that aprocitentan can positively alter stress response pathways (including NLRP3 activation) in many types of tissues, thus promoting organ protection.

Caspase-1 activity led to a cascade of inflammatory damage, which involves the production of IL-1β and IL-18, which intensify inflammation. Furthermore, it cleaves gasdermin D, which results in cell swelling and lytic death.[7] In the present study, I/R caused a substantial increase in caspase-1 in kidney tissue (I/R control versus sham), showing activation of the inflammasome/pyroptosis pathway. Previous studies have shown that caspase-1 activity in AKI can result in an increase in IL-1β/IL18 levels, worsening inflammation, and tissue destruction.[1,12]

Compared to untreated I/R rats, rats treated with aprocitentan exhibited lower levels of caspase-1. This suggests that aprocitentan successfully reduced inflammasome activity and pyroptosis in the kidney.

The direct impact on caspase-1 in I/R has not been investigated in previous studies; however, our results are in line with the protective properties of other treatments that target connected pathways. In fact, it has been suggested that treatments that block ET-1 signaling could reduce ischemic kidney injury by decreasing caspase-1 activation.[40] Dual ETA/ETB blockade of aprocitentan probably interferes with pro-inflammatory signaling sufficient to prevent the NLRP3/caspase-1 axis from full activation. This effect has important consequences: aprocitentan stops the loss of pyroptotic cells and the inflammatory cycle that would cause more damage to the kidneys by inhibiting caspase-1.

Aprocitentan-treated rats showed lower levels of NF-κB p65, NLRP3, and caspase-1, implying a reduction in the inflammasome-driven pyroptosis pathway. Thus, in the current study, inhibition of NLRP3 inflammasome and the caspase-1 action may result in less renal cells undergoing pyroptosis, which is consistent with better renal function and histology found in the aprocitentan group. Research suggests that inhibiting the NF-κB/NLRP3/caspase-1 axis could mitigate renal I/R injury.[20] Lower NF-κB p65 in the treated rats implies reduced transcription of NLRP3 and pro-IL-1β, which contributes to lower damage caused by inflammation. These findings confirm the pathological significance of inflammasome-mediated pyroptosis in AKI and the preventive potential for targeting this pathway with drugs such as aprocitentan.

Renal I/R in rats causes classic acute damage: severe tubular necrosis (loss of epithelial cells and brush boundaries, associated with cast formation), marked vascular congestion, and even glomerular structural injury. These results agree with the observations of Ghada and Tweij et al., whose work reported significant changes in the internal structure of kidneys and significant damage to tubules after I/R.[27,43] in rats treated with aprocitentan, these lesions were markedly attenuated: the glomerular architecture remained intact, milder tubular necrosis, and reduced infiltration of inflammatory cells compared to untreated I/R rats. This protective histopathological profile aligns with previous reports on endothelin blockade in AKI – for example, dual ERA tezosentan almost completely prevented acute cortical tubular necrosis (ATN) and preserved renal architecture in ischemic rat kidneys,[44] and selective ETA blocking (ambrisentan) and dual ETA/ETB blocking (bosentan) similarly decreased tubular injury, apoptosis, and inflammation in renal I/R models.[32]

The decline in NGAL level and histological injury scores in the Aprocitentan-treated group supports the hypothesis that this agent confers meaningful protection against I/R-induced AKI. NGAL reduction implies not only changes in molecular signaling, but also preserved renal function because it is a sensitive marker of tubular injury. These findings are especially relevant considering the existing clinical use as an antihypertensive agent.[45] It could be repurposed to treat perioperative AKI due to its ability to attenuate inflammation and pyroptosis, including situations such as transplantation or cardiac bypass.

This study had various limitations that need to be addressed. To begin with, the sample size was modest (n=6 each group), which limited the range to which the results can be generalized. Second, we only looked at short-term results for two hours after reperfusion; we did not assess the longer-term effects on kidney remediation or the development of chronic injury. Third, it is unclear whether other dose timing or repeated doses might increase the protective effect of aprocitentan because it was only studied at a specific dose and time point. Additionally, we evaluated pyroptosis indirectly through the measurement of NLRP3 and caspase-1 as substitute indicators instead of directly measuring the downstream executors, such as gasdermin D activation or IL-1β production. As a result, we conclude a decrease in pyroptosis from indirect signs; direct experiments should be used in the future to verify this process. Furthermore, since only male rats were used for this study, any possible sex-based variation in response was not taken into account. Finally, the use of ELISA to assess NF-κB p65 is another limitation, because it does not distinguish between active and inactive forms or indicate nuclear translocation. Future research can use immunohistochemistry (IHC), reverse transcription polymerase chain reaction (RT-PCR), or Western blotting to verify NF-κB activation and obtain more details about the mechanistic insights. Further studies are required to support our findings, and these limitations should be considered when evaluating the data.

Conclusion

This research shows that aprocitentan has significant nephroprotective properties in rat models with renal I/R. Aprocitentan efficiently reduces kidney damage by decreasing inflammation and reducing pyroptosis by inhibition of NFκB/NLRP3/Caspase-1 signaling pathway.

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Declarations

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Author contributions

Conceptualization, T.T.; Methodology, L.A.; Software, L.A.; Validation, L.A. and T.R.; Formal Analysis, T.T.; Investigation, L.A.; Resources, L.A.; Data Curation, L.A.; Writing – Original Draft Preparation, L.A.; Writing – Review & Editing, L.A.; Visualization, L.A.; Supervision, T.R.; Project Administration, T.T.

Conflicts of interest

The authors declare that they have no conflict of interest.

Data availability

All clinical and statistical data and materials are available for the benefit of science.

Ethics approval

Ethical approval for the experiments in this study was obtained from the Ethics Committee of the Faculty of Pharmacy, University of Kufa, Najaf, Iraq (Approval No. 3177/2025-2-4).

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