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Original Article

The role of mitochondrial reactive oxygen species in initiating mitochondrial damage and inflammation in wasp-venom-induced acute kidney injury

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Abstract: Acute kidney injury induced by stings from multiple wasps is a medical emergency and is a driving factor of acute renal dysfunction. Numerous studies have shown that mitochondrial reactive oxygen species (mtROS) play a key role in ischemia-reperfusion injury-, cisplatin-, and sepsis-induced acute kidney injury. However, the role of mtROS and its underlying mechanisms in wasp-venom-induced acute kidney injury remain inconclusive. In this study, we investigated the role and mechanisms of mtROS in mitochondrial damage and inflammation in a mouse model of acute kidney injury induced using wasp venom. Changes in mitochondrial function, transcription factor A (TFAM) expression, and DNA maintenance levels, renal function, stimulator of interferon gene (STING) expression, and inflammatory mediator levels in model mice with or without the mtROS scavenger Mito-Tempo were analyzed *in vivo*. Downregulation of mtROS levels reversed renal damage and mitochondrial dysfunction, and reduced STING expression and inflammation in the kidneys of model mice. The suppression of mtROS levels also improved the decrease in TFAM levels and mitochondrial DNA copy numbers in the kidneys of the model mice. In summary, the existing evidence in this study shows that mtROS contribute significantly to mitochondrial damage and inflammation in acute kidney injury induced by wasp venom. (DOI: 10.1293/tox.2024-0046; J Toxicol Pathol 2025; 38: 17–26)

Key words: acute kidney injury, inflammation, mitochondrial reactive oxygen species, wasp venom

Introduction

Acute kidney injury (AKI) is caused by many causes, including trauma, sepsis, and nephrotoxic drugs, and wasp venom is also considered an important causative agent of AKI in rural mountainous terrain in East, South, and Southeast Asia, including Japan, China, India, Thailand, and Indonesia[1]. According to previous studies, the mechanisms of wasp-venom-induced AKI may be related to its direct nephrotoxicity, as well as secondary to rhabdomyolysis[2]. Additionally, our own research and that of others have indicated that inflammation has a substantial impact on the pathophysiology of AKI attributed to wasp venom[1, 3, 4]. However, the precise mechanism underlying wasp-venom-induced AKI progression remains unclear. Currently, no specialized treatments for wasp-venom-induced AKI are available, and clinical interventions primarily include supportive therapy and plasma exchange[1]. Despite advances in plasma exchange technology, mortality from wasp-venom-induced AKI is still approximately 30–50%[2]. Therefore, further investigation into the role and mechanisms of wasp venom are required to aid the development of novel treatments.

Previous studies have suggested that abnormal increases in reactive oxygen species (ROS) levels are associated with AKI[5]. Increasing evidence suggests that ROS are produced mainly by the mitochondria, and mtROS account for up to 90% of intracellular ROS[6], which play a crucial role in AKI[5, 7]. In ischemia-reperfusion injury (IRI), cisplatin-, and sepsis-induced AKI, elevated mtROS levels induce oxidative stress, which accelerates the progression of kidney disease[8–10]. Oxidative stress induced by mtROS influences mitochondrial function, decreases mitochondrial membrane potential, and promotes mitochondrial DNA (mtDNA) cytosolic leakage[9, 11, 12]. The effects of mtROS on renal injury have been established in other forms of AKI; however, the detailed mechanism by which mtROS initiate wasp-venom-induced mitochondrial dysfunction and inflammation in AKI remains unclear.

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mtDNA is circular double-stranded DNA that resides in the mitochondrial matrix and contributes considerably to mitochondrial function and mtDNA damage, which is a hallmark of AKI[13, 14]. Mitochondrial transcription factor A (TFAM) is a protein encoded by nuclear DNA that binds mtDNA to compact and stabilize the genome and initiate transcription and replication of mtDNA[15]. A decrease in TFAM levels disrupts the binding of TFAM to mtDNA, leading to the release of mtDNA into the cytoplasm and destabilization[16, 17]. Previous studies have shown that mtROS can suppress TFAM expression in the kidney by inhibiting TFAM transcription and promoting its degradation, thereby affecting the stability of mtDNA[9]. Consistently, our recent study indicated that in wasp-venom-induced AKI, cyclic GMP-AMP synthase (cGAS), which is a cytoplasmic DNA recognition receptor, can identify mtDNA released into the cytoplasm and activate stimulator of interferon gene (STING) signaling[3, 18]. STING activation can further activate downstream inflammatory signaling pathways and induce an inflammatory response in wasp-venom-induced AKI[3]. In light of these findings, we speculated that mtROS may affect the stability of mtDNA and activate cGAS/STING signaling in wasp-venom-induced AKI.

In the present study, we aimed to explore the role and mechanism of action of mtROS in mitochondrial damage and inflammation during wasp-venom-induced AKI. Changes in mitochondrial function, TFAM expression, mtDNA maintenance, renal function, STING expression, and release of inflammatory mediators were analyzed in wasp-venom-induced AKI mouse models, with or without an mtROS scavenger.

Materials and Methods

Materials and reagents

The wasp venom from *Vespa mandarinia* was extracted using a wasp venom collector (FF-3) was purchased from XieKe Biotech (Shanxi, China) following a previously described protocol[19]. The extracted wasp venom was stored at −20°C and dissolved in saline before use. Primary antibodies were purchased from the following sources: anti-TOM20 (#11802-2-AP), anti-STING (#19851-1-AP), and anti-GAPDH (#60004) (Proteintech, Wuhan, China). The anti-TFAM antibody (#ab307302) was purchased from Abcam (Cambridge, MA, USA). Mito-Tempo (MT, an mtROS scavenger) was purchased from TargetMol (Shanghai, China) and dissolved in dimethyl sulfoxide-corn (DMSO)-Corn oil (1:9) as a stock solution. The ATP detection kit (#S0026) was purchased from Beyotime Biotechnology (Shanghai, China). MitoSOX Red Mitochondrial Superoxide Indicator (MitoSOX Red) (#M36008) was purchased from Invitrogen (Carlsbad, CA, USA). TIANamp Genomic DNA Kit (#DP304) was purchased from Tiangen (Beijing, China).

Animal experiments

Animal experiments were approved by the Institutional Review Committee of Xiangyang Central Hospital (protocol code 2022-M12). Male C57BL/6 mice (8–10 weeks) were housed under standardized conditions with a controlled temperature (22°C), regulated humidity (55–65%), and 12-h light/dark periods. The animals were fed standard chow under specific pathogen-free conditions, had *ad libitum* access to tap water, and were acclimatized for 1 week. All animals (20–25 g) were divided into three groups (n=6 per group): Control, AKI, and AKI + MT. In control group, mice were subcutaneously injected with 0.9% saline at five different sites, as well as an intraperitoneal injection of DMSO-corn oil (1:9). For the AKI group, mice were subcutaneously injected with 5 mg/kg wasp venom at five different sites (1 mg/kg as 0.1 mL at each site), followed by an intraperitoneal injection of DMSO-Corn oil (1:9). A wasp venom-induced AKI model was established according to our previously published protocol[20]. The feasibility of the dosage and administration method in our model was confirmed through multiple experiments[3, 18, 19]. AKI + MT group was created to inhibit the mitochondrial ROS burst in the kidney, this group was administered an intraperitoneal injection of 10 mg/kg MT at 6- and 1-h before injecting wasp venom. Six hours after the injection of saline or wasp venom, the mice were euthanized, and blood and renal tissue samples were collected for further analysis.

Biochemistry analysis

Serum creatinine (Scr) and blood urea nitrogen (BUN) levels were quantified via an automatic blood biochemical analyzer to evaluate renal function[3].

Renal histopathology

Fresh kidney tissue samples were encased in paraffin, then hematoxylin and eosin (H&E) staining was performed on 2 μm continuous tissue slices. Renal tubule injury was evaluated based on damage to the renal tubules, as described previously, including loss of brush border, dilation of tubules, formation of casts, and necrosis of tubular[20]. Following our established protocol[21], tubular injury specimens were scored based on the percentage of tubules exhibiting tubular cell atrophy, loss of brush border, cast formation, and tubular dilation: 0, none; 1, <10%; 2, 11–25%; 3, 26–45%; 4, 46–75%; and 5, >76%.

Immunohistochemistry (IHC)

Immunohistochemical staining was performed using paraffin-embedded mouse kidney sections as per our previously published protocol[22]. Briefly, the sections embedded in paraffin were dewaxed in xylene and rehydrated with graded concentrations of ethanol. To block endogenous peroxidase activity, the sections were incubated in 3% H_2O_2 for 10 min. Antigens were retrieved by heating in citrate buffer (pH 6) for 1 h and blocking them in 10% normal goat serum for 1 h, followed by anti-TFAM (1:1,000) or anti-STING (1:500) overnight at 4°C, and then secondary antibodies were also incubated with horseradish peroxide-conjugated secondary antibodies and 3,3-diaminobenzidine substrate.

Renal mtROS measurement

mtROS was measured using MitoSOX Red, a mitochondrial fluorescent probe for the selective detection of superoxide in mitochondria, according to a previously published method[23]. In the mitochondria, superoxide oxidizes MitoSOX Red and produces strong red fluorescence. Fresh renal tissues were immediately frozen and cut into 5 mm cryostat sections. Fluorescence microscopy was used to acquire images of the sections after incubating with 5 μM MitoSox Red at 37°C for 30 min. ImageJ software was used to quantify the fluorescence intensity.

Renal ATP measurement

The ATP content in the renal tissue was measured using an ATP assay kit. Briefly, fresh kidney samples were mixed with the lysis buffer supplied by the kit. The ATP levels in the samples were assessed by mixing the supernatant with luciferase reagent, which contains luciferase and luciferin; the reaction between ATP, luciferase and luciferin produces bioluminescence. The ATP concentrations were determined according to the standard curve.

mtDNA copy number assay

The copy number of mtDNA was measured using real-time qPCR as previously described[3]. Briefly, total genomic DNA was isolated from the kidney tissues using a TIANamp Genomic DNA Kit, with the 18S rRNA gene serving as an internal reference. By amplifying the mt-ND1 gene encoded in mitochondria, the mtDNA content was determined. The relative ratio between mt-ND1 amount of the 18S rRNA was calculated to measure the mtDNA copy number. Table 1 lists the primer sequences and the 2 delta-delta threshold cycle ($2^{-\Delta\Delta}$Ct) method was used for all quantifications.

Western blotting (WB)

Radioimmunoprecipitation assay (RIPA) buffer containing a cocktail of protein inhibitors was used to lyse the murine renal tissue, followed by centrifugation. The supernatant was then recovered. Thereafter, we separated the proteins on sodium dodecyl sulfate-polyacrylamide gels and electrophoretically transferred them onto polyvinylidene fluoride (PVDF) membranes. Primary antibodies were incubated at 4°C overnight after membranes were blocked for 1 h. The primary antibodies used were anti-TFAM (1:1,000), anti-TOM20 (1:2,000), anti-STING (1:500), and anti-GAPDH (1:5,000). After washing with phosphate-buffered solution, using horseradish peroxidase-conjugated secondary antibodies, PVDF membranes were incubated for 1 h at 37°C. An enhanced chemiluminescence kit was used to visualize the protein bands on PVDF films.

Analysis of the real-time polymerase chain reaction (PCR)

As our described previously[24], total RNA was extracted from the mouse kidney tissue, followed by real-time PCR. RNA was reverse transcribed to cDNA using SanSkrit TM III RT Enzyme Mix (TSINGKE). The instrument's software recorded the Ct values, and fold changes in mRNA expression were determined using the $2^{-\Delta\Delta}$Ct method. Using GAPDH as a reference gene, the results were standardized against mRNA expression levels in mouse kidney tissues. Table 1 lists the primers used in this study.

Statistical analysis

Quantitative data are presented as mean ± standard deviation, and GraphPad Prism (San Diego, CA, USA) was used for all statistical analyses. Except for the biochemistry analysis, for which a one-way analysis of variance was employed, all other experimental results were analyzed using t-tests to assess statistically significant differences between the groups. Differences were considered statistically significant at $p<0.05$.

Results

mtROS aggravated renal dysfunction in wasp-venom-induced AKI

To evaluate the effects of mtROS on wasp-venom-induced AKI, an AKI model was established by injecting C57BL/6 mice with wasp venom. Additionally, a separate group of mice was intraperitoneally injected with MT to curb excess ROS before venom injection. MT is a mitochondria-specific antioxidant[25], containing hydrophobic tetramethylpiperidine and lipophilic triphenylphosphonium cations, which are responsible for incorporation into mitochondria and scavenging mitochondrial superoxides, respectively[26]. To better understand the chemical structure of MT is shown in Fig. 1A. Compared to the control group, wasp-venom-induced AKI mice exhibited higher serum creatinine (Scr) and blood urea nitrogen (BUN) levels as well as elevated levels

Table 1. Mice Primers Used for Qpt-PCR Analysis

Genes	Forward Primer	Reverse Primer
STING	CCTCAGTTGGATGTTTGGCC	AGATCAACCGCAAGTACCCAA
18S rRNA	TTCGGAACTGAGGCCATGATT	TTTCGCTCTGGTCCGTCTTG
mtND1	GGCCCATTCGCGTTATTCTT	TCGTAACGGAAGCGTGGATA
TNF-α	GGCGGTGCCTATGTCTCA	CCTCCACTTGGTGGTTTGT
IL-6	GTTGCCTTCTTGGGACTGAT	ATTAAGCCTCCGACTTGTGA
KIM-1	AGACTGGAATGGCACTGTGA	GGCAACCACGCTTAGAGATG
GAPDH	AGTATGACTCCACTCACGGC	CACCAGTAGACTCCACGACA

STING: stimulator of interferon gene.

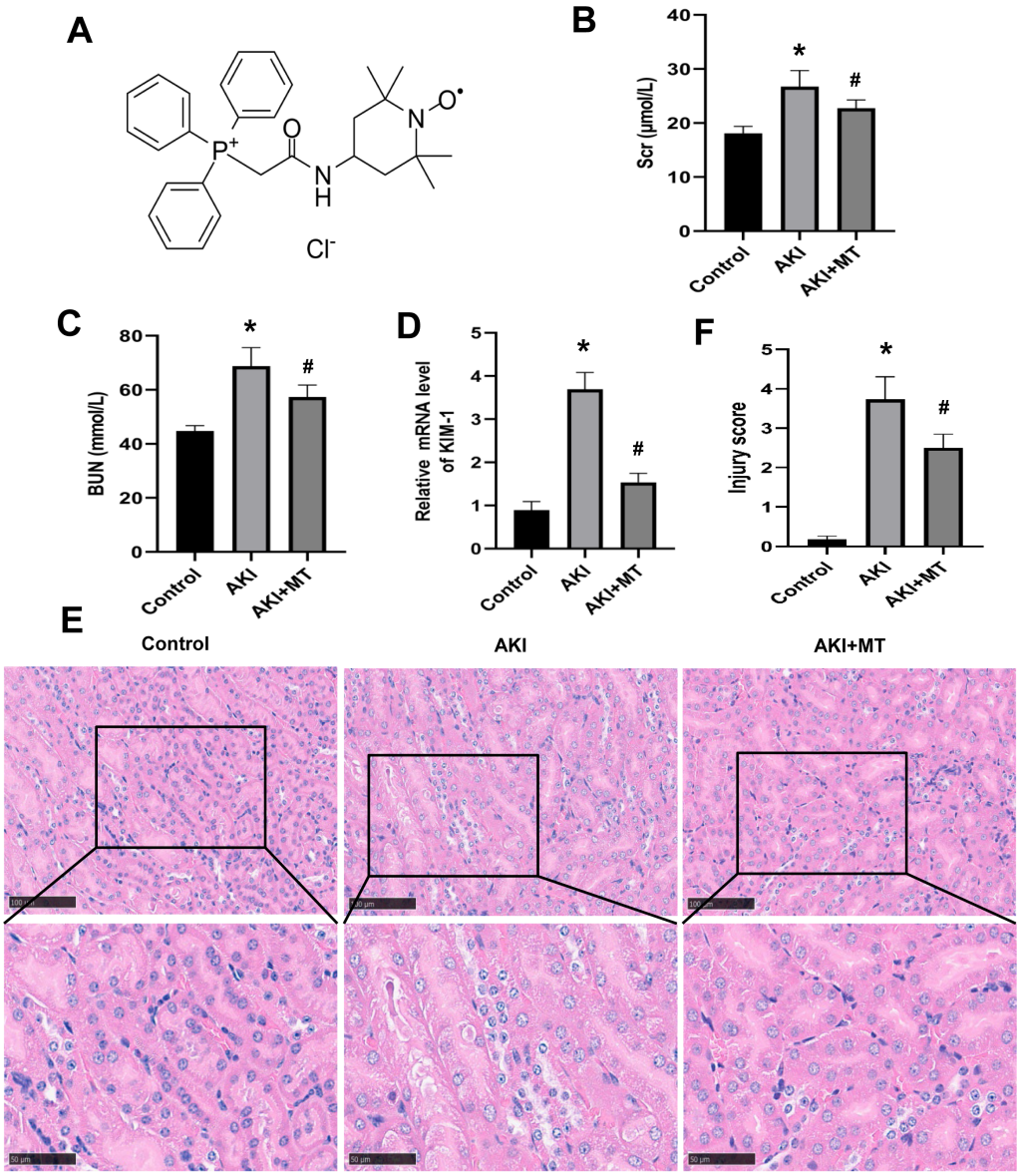

Fig. 1. Mitochondrial reactive oxygen species (mtROS) aggravated renal dysfunction in wasp-venom-induced acute kidney injury (AKI) in a mouse model. (A) Chemical structure of MT. (B, C) Renal function across groups was assessed by measuring serum creatinine (Scr) and blood urea nitrogen (BUN) (n=6). (D) KIM-1 mRNA expression was measured through real-time polymerase chain reaction (PCR) (n=6). (E) Representative images of hematoxylin-eosin staining in the kidneys of mice (scale bars=50 μm). (F) Quantitative analysis of tubular injury score in the kidneys of mice in different groups (n=6). *p<0.05 vs. CON group; *p<0.05 vs. control group; # p<0.05 vs. AKI group.

of kidney injury molecule 1 (KIM-1). Conversely, mtROS inhibition using MT pretreatment significantly improved renal dysfunction, as demonstrated by decreased levels of Scr, BUN, and KIM-1 (Fig. 1B–1D). Histological staining of kidney sections and quantitative analysis revealed that mice injected with wasp venom exhibited histopathological alterations, including cast formation, tubular cell loss, and brush border damage. Renal structural damage was attenuated by the MT injection (Fig. 1E, 1F). These results indicate that mtROS can induce kidney damage in wasp-venom-induced AKI.

mtROS promoted renal mtDNA instability and mitochondrial damage in wasp-venom-induced AKI

Mitochondrial damage was evaluated by measuring the changes in mtROS production, ATP levels (a major product of mitochondrial metabolism), TOM20 protein levels (a mitochondrial outer membrane protein), and mtDNA copy number. The MitoSOX Red fluorescence assay showed that mtROS levels were significantly increased in the renal cortex of the AKI group compared to the control group (Fig. 2A, 2B). In addition, ATP levels, mtDNA copy numbers, and TOM20 levels were decreased in the AKI group (Fig. 2C–2E). Conversely, pretreatment with the

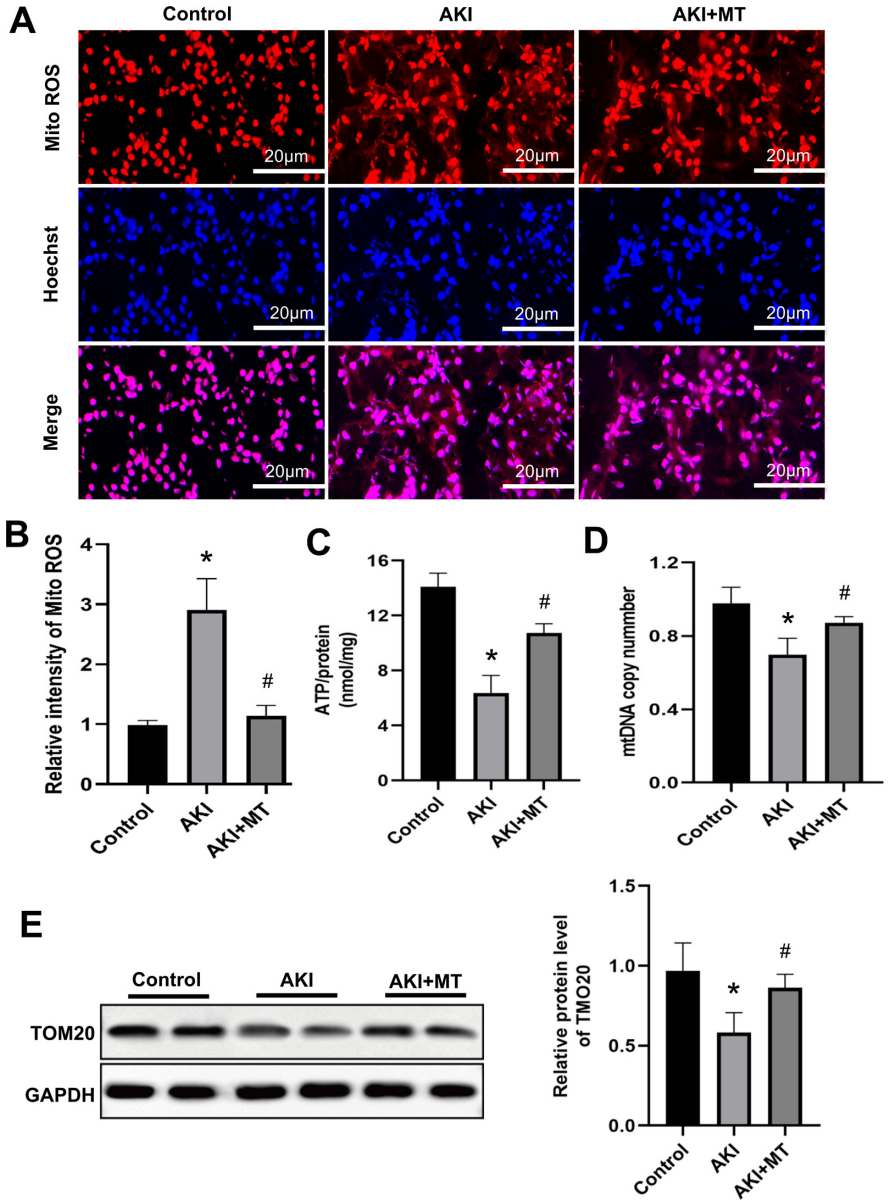

Fig. 2. Mitochondrial reactive oxygen species (mtROS) promoted mitochondrial damage in wasp-venom-induced acute kidney injury (AKI) in a mouse model. (A) Representative micrographs of MitoSOX in the renal cortex (scale bars=20 μm). (B) Quantitative analysis of the relative intensity of mtROS in the kidneys of mice in different groups (n=6). (C) The level of ATP was quantified by an ATP assay kit (n=6). (D) mtDNA copy number was measured by real-time polymerase chain reaction (PCR) (n=6). (E) Western blotting and quantitative data analysis of TOM20 in mice kidneys in different groups (n=6). *p<0.05 vs. control group; #p<0.05 vs. AKI group.

mtROS scavenger restored ATP production, TOM20 levels, and mtDNA copy number in the kidneys of the AKI group (Fig. 2C–2E). The decrease in the mtDNA copy number in the AKI group reflects the degree of mtDNA damage and instability. To investigate the potential mechanisms underlying mtDNA instability, we measured the expression of TFAM, which is essential for mtDNA replication, stability, and transcription[27], using IHC and WB analyses. The results showed that TFAM levels were significantly decreased in the kidneys of AKI model mice (Fig. 3A, 3B). However, pretreatment with MT significantly reversed this phenomenon in mouse kidneys (Fig. 3A, 3B). These results indicate that mtROS play a role in mitochondrial damage and mtDNA instability in wasp-venom-induced AKI.

mtROS promoted STING activation in wasp-venom-induced AKI

According to our recent reports, STING plays a crucial role in wasp-venom-induced AKI by regulating inflammatory response, apoptosis, and necroptosis[3]. Next, we investigated whether increased mtROS levels were associated with STING activation. As demonstrated in Fig. 4A, STING was mainly expressed in the renal tubules. IHC staining of kidney sections showed that mice with wasp venom-induced

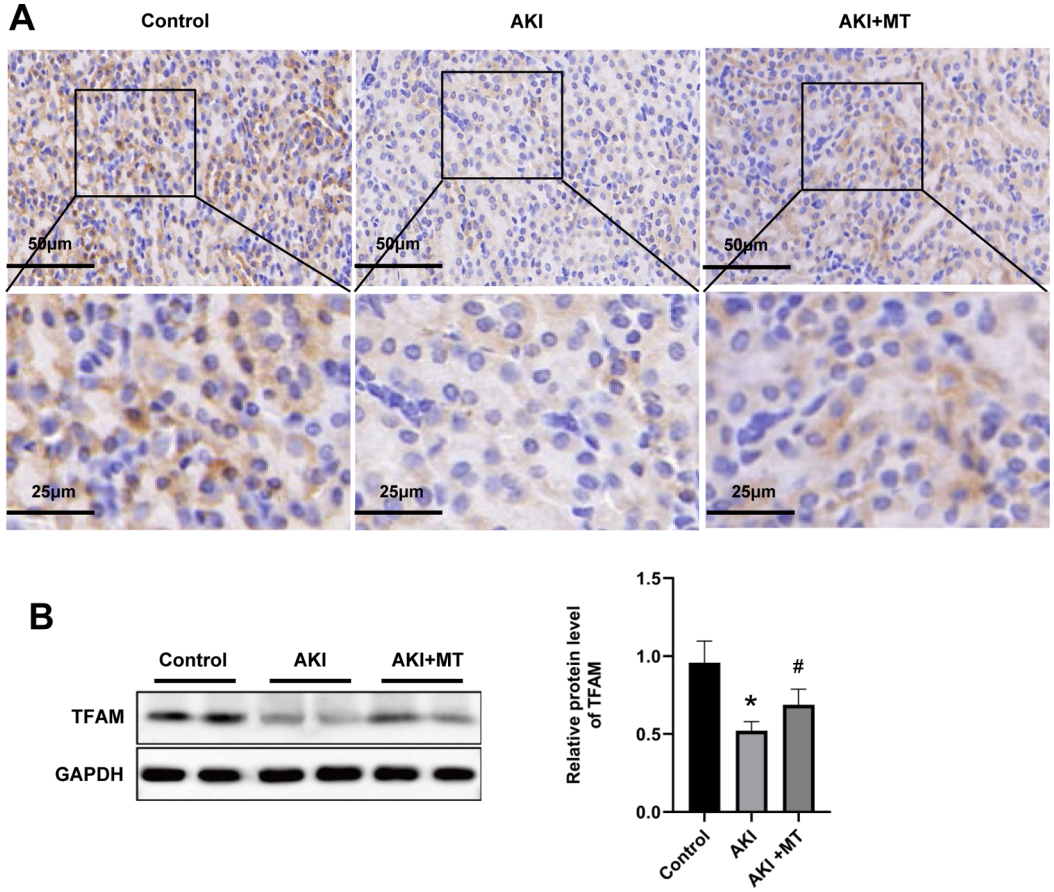

Fig. 3. Mitochondrial reactive oxygen species (mtROS) promoted mtDNA instability in wasp-venom-induced acute kidney injury (AKI) in a mouse model. (A) Representative sections of the kidney for TFAM by immunohistochemistry (scale bar=50 μm) (n=6). (B) Western blotting and quantitative data analysis of TFAM in mice kidneys in different groups (n=6). *p<0.05 vs. control group; #p<0.05 vs. AKI group.

AKI displayed higher STING levels than those in the control group. As shown in Fig. 4B, 4C, from the PCR and WB analysis results, we observed that STING protein and mRNA levels were higher than those in the control group. Consistent with our expected results, STING activation was significantly inhibited in mouse kidney sections pretreated with MT (Fig. 4A–4C). These findings suggest that mtROS promotes the activation of STING in wasp-venom-induced AKI.

mtROS exacerbated renal inflammation in wasp-venom-induced AKI

Inflammation plays an essential role in wasp-venom-induced AKI[1, 21] Overexpression of inflammatory mediators due to STING activation in wasp-venom-induced AKI animal models has been reported by our research group[3]. The expression of inflammatory mediators IL-6 and TNF-α mRNA levels were significantly raised in the AKI group (Fig. 5A, 5B). However, the levels of IL-6 and TNF-α significantly decreased in the MT-treated group compared with those in the AKI group (Fig. 5A, 5B). These results indicate that mtROS promote renal inflammation in wasp-venom-induced AKI.

Discussion

In this study, we discovered that wasp venom could cause renal dysfunction and inflammation due to the bursting of mtROS, which is the main source of oxidative stress in the kidney. In wasp-venom-induced AKI, the burst of mtROS is accompanied by the downregulation of TFAM and decreased expression of mitochondrial function-related parameters, such as TOM20, ATP, and mtDNA copy numbers. Furthermore, STING activation was observed in the wasp-venom-induced AKI, accompanied by an increase in inflammatory mediators and tubular damage marker KIM-1. All these events may collaborate toward kidney dysfunction and pathological damage. Conversely, the expression of TFAM, TOM20, ATP, mtDNA copy number, STING, and KIM-1 were markedly restored by scavenging mtROS. These findings indicated that mtROS are major contributors to renal mitochondrial dysfunction, which further mediates inflammation and contributes to AKI.

Previous studies have shown that oxidative stress plays a major role in several types of AKI, including IRI-, cisplatin-, and sepsis-induced AKI[9, 12, 28]. Our recent study revealed that oxidative damage is also an important factor in

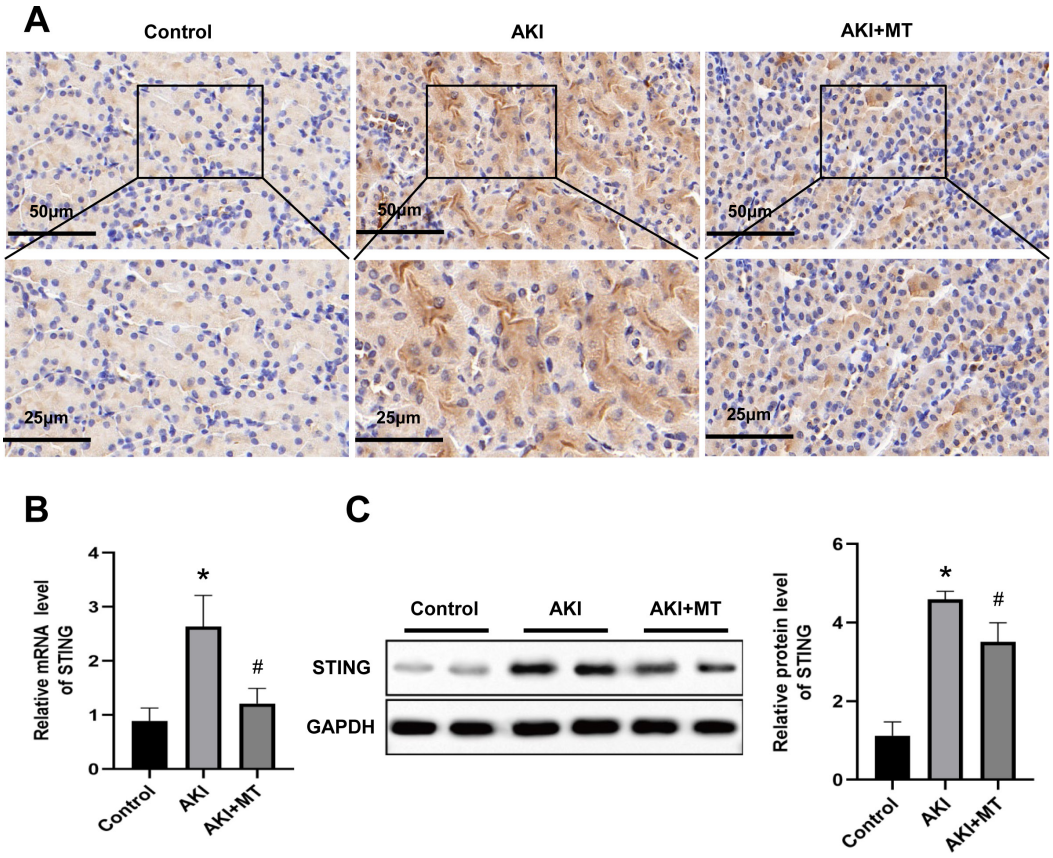

Fig. 4. Mitochondrial reactive oxygen species (mtROS) promoted the activation of stimulator of interferon gene (STING) in wasp-venom-induced acute kidney injury (AKI) in a mouse model. (A) A representative micrograph showing STING immunohistochemistry (IHC) staining in the kidneys of the mouse model (scale bar=50 μm) (n=6). (B, C) The mRNA and protein levels of STING were measured in mice kidneys in different groups (n=6). *p<0.05 vs. control group; #p<0.05 vs. AKI group.

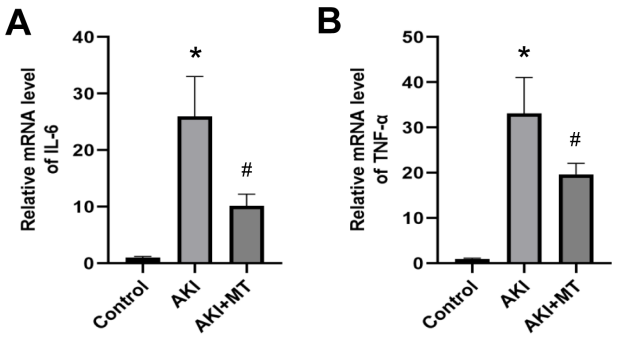

Fig. 5. Mitochondrial reactive oxygen species (mtROS) exacerbated renal inflammation in wasp-venom-induced acute kidney injury (AKI) in a mouse model. (A, B) The mRNA expression of inflammatory mediators, including IL-6 and TNF-α was measured by real-time polymerase chain reaction (PCR) (n=6). *p<0.05 vs. control group; #p<0.05 vs. AKI group.

wasp-venom-induced AKI[29]. Several studies have described mitochondria as the major source of excessive ROS[6, 30] and excessive production of mtROS can induce an imbalance between mtROS production and removal, which induces oxidative stress. Notably, downregulating mtROS levels attenuated mitochondrial dysfunction, inflammation and kidney damage in IRI-, cisplatin-, and sepsis-induced AKI model mice[9, 12, 31]. Interestingly, in the present study, an abnormal increase in mtROS levels was observed in the kidneys of the wasp-venom-induced AKI group using the MitoSOX Red mitochondrial superoxide indicator. Previous studies have indicated that wasp-venom-induced AKI is due to the direct effects of wasp venom or secondary rhabdomyolysis[1]. Other studies have shown that both phospholipase A2 (an important component of wasp venom) and myoglobin (the main harmful product of rhabdomyolysis) induce mtROS production[32, 33]. Thus, we speculate that the burst of mtROS observed in the present study may be attributed to the direct effect of phospholipase A2 and/or the indirect effect of myoglobin. The excessive production of mtROS in the kidneys of mice with wasp venom suggests the occurrence of mtROS-mediated oxidative stress. However, the role and underlying mechanisms of mtROS in mitochondrial function in wasp-venom- induced AKI remains unclear.

Considering that mitochondrial function is highly related to the integrity of mtDNA[7], we speculated that excess mtROS alters mtDNA stability due to mitochondrial

function damage in AKI induced by wasp venom. Due to its unique location and structure, mtDNA is particularly susceptible to oxidative stress[9]. Previous studies have demonstrated that mtDNA resides in the mitochondrial matrix. During renal injury, mitochondrial damage causes mtDNA to leak into the cytosol[34]. In our recent study, urine mtDNA levels were elevated in patients with wasp-venom-induced AKI; however, kidney mtDNA levels were reduced in mouse models, suggesting that mtDNA leakage occurs in wasp-venom-induced AKI[3]. The results of the present study were consistent with those of other studies; the downregulation of mtDNA copy numbers was reversed by MT (an mtROS scavenger), suggesting that mtROS is significant for the stability of mtDNA[9]. MT has proven to be non-nephrotoxic and has been widely used in studying AKI[9, 12, 25, 35]. To further explore the mechanisms underlying mtDNA instability, we measured the levels of TFAM, a critical regulator of mtDNA transcription and replication, which plays a critical role in maintaining mtDNA stability[15, 36]. Our results are consistent with those of other studies[37, 38] that excessive mtROS can reduce TFAM expression, a phenomenon that was prevented by MT treatment. We speculate that the decrease in TFAM may be due to mtROS directly downregulating TFAM expression by inhibiting TFAM transcription[9]. Furthermore, the decrease in TFAM downregulates the binding capabilities of TFAM to mtDNA and subsequently results in reduced mtDNA transcription and content and dysfunctional packaging of mtDNA into nucleoids, thus promoting mtDNA instability[39]. This instability promotes mtDNA leakage and cytosolic mtDNA accumulation. The present study indicates that excessive mtROS levels lead to reduced TFAM expression and further promote mtDNA instability in wasp venom-induced AKI.

Additionally, the mtDNA copy number, we determined other reliable indicators of mitochondrial damage. In this study, increased mtROS generation was accompanied by decreased levels of TOM20 (a mitochondrial protein marker) and ATP (the main product of mitochondrial metabolism). Studies have indicated that a decrease in TOM20 levels can reduce the import of several mitochondrial proteins and affect mitochondrial morphology[40]. The decreased expression of ATP, which is primarily produced by mitochondrial oxidative phosphorylation, reflects the degree of mitochondrial function[41]. Our results indicated that during wasp-venom-induced AKI, when kidney mitochondria are injured, mitochondrial redox homeostasis decreases, leading to a vicious cycle of mtROS production and mitochondrial damage. Furthermore, severe mitochondrial damage can lead to mitochondrial dysfunction (a significant decrease in ATP levels), damage to mitochondrial integrity (reduced expression of TOM20), and ultimately affect mtDNA copy numbers. In contrast, the scavenging of mtROS significantly reversed mitochondrial damage (enhanced expression of TOM20, ATP, and mtDNA copy numbers in renal mitochondria) in AKI caused by wasp venom. These data establish that mtROS play an essential role in mitochondrial damage in wasp-venom-induced AKI.

STING relays DNA-triggered signals and activates the innate immunity[42]. Our recent study demonstrated that STING activated by mtDNA leaked into the cytoplasm, further activating downstream inflammatory signaling pathways and inducing an inflammatory response and apoptosis in wasp-venom-induced AKI[3]. In previous studies, excessive mtROS secretion has been found to cause a loss of mitochondrial integrity, releasing mtDNA into the cytoplasm, and further activating the STING pathway. Therefore, mtROS are considered upstream regulatory targets of STING signaling[43]. In this study, we found that mtROS inhibition effectively reversed alterations in STING expression. Hence, we speculated that STING activation in wasp venom is associated with excessive mtROS production, leading to mitochondrial damage that results in mtDNA leakage. Our recent study indicated that the STING pathway regulates wasp-venom-induced renal tubular inflammation *in vivo* and *in vitro*[3]. Moreover, our current study revealed that the inhibition of mtROS decreased the expression of inflammatory mediators in wasp-venom-induced AKI, indicating that excessive mtROS can promote the inflammatory response in the kidney during wasp-venom-induced AKI. Using mouse models of wasp-venom-induced AKI, we propose that excessive mtROS-mediated inflammation may be associated with a STING activation-mediated inflammatory response. An abnormal increase in mtROS can lead to leakage of mtDNA into the cytoplasm[9, 44, 45]. The overproduction of mtROS induces oxidative stress, mitochondrial dysfunction, and a decrease in TFAM levels, which further promotes mtDNA leakage into the cytosol to activate downstream STING signaling pathways and inflammatory responses. Additionally, previous studies have indicated that mtROS can be a damage-associated molecular pattern that directly induce inflammatory responses directly[46, 47]. These data indicate that mtROS strongly contributes to inflammation in wasp-venom-induced AKI.

In summary, our study demonstrated that mtROS are crucial for mitochondrial damage and inflammation in wasp-venom-induced AKI. Excessive mtROS induces mitochondrial dysfunction and subsequent inflammation in wasp-venom-induced AKI Thus, inhibition of mtROS may serve as a potential therapeutic target for wasp-venom-induced AKI.

Disclosure of Potential Conflicts of Interests: The authors report no competing interests.

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