Original Paper

Rotenone Protects Against Acetaminophen-Induced Kidney Injury by Attenuating Oxidative Stress and Inflammation

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Key Words

Acetaminophen • Rotenone • Kidney injury • Inflammation • Oxidative stress

Abstract

Background/Aims: In clinic, excessive acetaminophen (APAP) can cause kidney damage with uncertain mechanisms. Recently, accumulating evidence demonstrated a pathogenic role of mitochondrial dysfunction in the kidney injury. Thus, in this study, rotenone, a mitochondrial complex I inhibitor, was applied to the mice with APAP-induced acute kidney injury to evaluate the effect of mitochondrial complex I inhibition on APAP nephrotoxicity. **Methods:** After 3 days of rotenone pretreatment, mice were administered with APAP (300mg/kg) by intraperitoneal injection for 24 h. Then the kidney injury, inflammation, and oxidative stress were evaluated. **Results:** APAP significantly enhanced the BUN, serum creatine, and cystatin C levels in line with a moderate alteration of renal morphology. Strikingly, rotenone treatment normalized BUN, serum creatinine, and cystatin C levels, as well as the kidney morphology. Meanwhile, APAP enhanced tubular injury markers of NGAL and KIM-1 by 347- and 5-fold at mRNA levels, respectively. By Western blotting, we confirmed a 15-fold increment of NGAL in APAP-exposed kidneys. Importantly, rotenone treatment largely normalized NGAL and KIM-1 levels and attenuated inflammatory response in APAP-treated mice. Similarly, rotenone treatment enhanced the expressions of SOD1-3 compared with APAP group in line with a significant

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suppression of kidney MDA content. Finally, we observed that inhibition of mitochondrial complex III failed to protect against APAP-induced nephrotoxicity. **Conclusion:** Mitochondrial complex I inhibitor rotenone protected kidneys against APAP-induced injury possibly via the inhibition of mitochondrial oxidative stress and inflammation.

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Introduction

Acute kidney injury (AKI) is rapidly increasing worldwide [1]. Among the various forms of AKI, drug-induced AKI is a common concern in clinic [2]. Kidneys are primarily involved in filtering and concentrating various substances and chemical agents [3]. Acetaminophen (APAP), an analgesic and antipyretic agent, is better known as paracetamol in many countries. APAP is used in adults and children worldwide with a safety profile in therapeutic doses [4-6]. However, excessive APAP can cause severe hepatotoxicity, nephrotoxicity, and even death in experimental animals and humans [7]. Although APAP-induced nephrotoxicity is less common than hepatotoxicity, renal tubular damage and acute renal failure also occur even in the absence of liver injury [8, 9].

The renal tubule is densely packed with mitochondria to generate sufficient ATP via oxidative phosphorylation (OXPHOS) to power the solute transport in the kidney [10]. Besides the energy production, mitochondria also play important roles in mediating the signaling transduction, cell proliferation, cell growth, and cell death [11, 12]. The dysfunction of mitochondria could cause ATP depletion, reactive oxygen species (ROS) overproduction and subsequent inflammation [13, 14]. Mitochondria in the proximal tubule can be damaged by therapeutic drugs such as ifosfamide, cisplatin, tenofovir, and gentamicin, leading to the AKI. Recently, the mitochondrion is becoming a potential target for the treatment of AKI [15].

APAP has been reported to promote liver injury through a mitochondrial dysfunction-associated mechanism [16, 17]. In kidney, recent researches documented an involvement of mitochondrial dysfunction in the pathogenesis of CKDs [18-21]. Inhibiting the activity of the abnormal mitochondria in obstructed kidney by a mitochondrial complex I inhibitor significantly ameliorated kidney injury [22]. To define the role of mitochondrial complex I inhibitor rotenone in APAP-induced AKI, we pretreated the mice with a mitochondrial complex I inhibitor rotenone to determine (1) whether the inhibition of mitochondrial complex I can attenuate kidney damage in APAP-induced AKI, and (2) the effect mitochondrial complex I inhibition on the inflammation and oxidative stress in this experimental model.

Materials and Methods

Reagents and antibodies

Acetaminophen and rotenone were bought from Sigma (St Louis, MO). Rabbit antibodies against SOD2 and NGAL were obtained from Abcam (Cambridge, MA). Rabbit antibody against β -actin was purchased from Cell Signaling Technology (Beverly, MA). Peroxidase-conjugated AffiniPure goat anti-rabbit secondary antibodies were purchased from Bi Yuntian (Shanghai, China).

Animals

Eight-week-old male BALB/c mice were purchased from Nanjing Medical University and were maintained under a 12 h: 12 h light/dark cycle at $22 \pm 2^\circ\text{C}$, and a relative humidity of $55 \pm 10\%$. Mice were given the diet and water ad libitum. All animal experimental procedures were approved by the Nanjing Medical University Institutional Animal Care and Use Committee.

Establishment of APAP-induced kidney injury model and rotenone treatment

After 1-week adaption, mice were randomly divided into three groups (N=7 per group): (1) Vehicle group, (2) APAP group, and (3) APAP + Rotenone group. Vehicle and APAP groups were fed with jelly diet

with vehicle and APAP + Rotenone group was pretreated with 250 ppm rotenone mixed in the jelly diet for 3 days. Then the mice were fasted for 12h but kept free access to water before the intraperitoneal injection of a single dose of APAP (300mg/kg, dissolved in saline) or equal volume of saline. All the animals were fed with the same food as before after the APAP injection. After APAP injection for 24 h, the mice were sacrificed and the blood and kidney tissues were harvested for analyses. In a separate experiment, mice were treated with vehicle or rotenone for four and a half days to evaluate the toxicity of rotenone *in vivo*.

Azoxystrobin treatment in mice with APAP challenge.

After 1-week adaption, mice were randomly divided into four groups (N=8 per group): (1) Vehicle group, (2) Azoxystrobin group, (3) APAP group, and (4) APAP + Azoxystrobin group. Vehicle and APAP groups were fed with jelly diet with vehicle. The mice in azoxystrobin and APAP + azoxystrobin groups were treated with 25mg/kg/day azoxystrobin mixed in the jelly diet. After 3 days pretreatment, mice were fasted for 12h but kept free access to water before the intraperitoneal injection of a single dose of APAP (300mg/kg, dissolved in saline). All the animals were fed with the same food and water as before after the APAP injection. After APAP injection for 24 hours, mice were sacrificed and the blood and kidney tissues were harvested for analyses.

Blood biochemistry

Serum samples were collected from blood by centrifugation at 1,500 rpm for 10 min and stored in -80°C freezer before they were analyzed. Serum creatinine (Cr) and blood urea nitrogen (BUN) were evaluated using a serum biochemical autoanalyzer (Hitachi 7600 modular chemistry analyzer, Hitachi Ltd, USA). The levels of cystatin C in the serum were determined by a mouse cystatin C ELISA kit (E-EL-M0389c, Elabscience, China).

Histopathological examination

Kidney tissues were fixed in 4% paraformaldehyde (PFA) for 16 h. Following embedding in paraffin, sections with 5µm thickness were prepared and stained with Periodic Acid Schiff (PAS) and analyzed under the light microscope. The histological damage was quantified using the following blinded scoring system. Degree of renal injury was graded from 0 to 4: 0=normal; 1=mild, involvement of less than 25% of the cortex; 2=moderate, involvement of 25 to 50% of the cortex; 3=severe, involvement of 50 to 75% of the cortex; and 4=extensive damage involving >75% of the cortex [22].

Western Blot

The kidney tissues were lysed in a RIPA lysis buffer (Bi Yuntian, Shanghai, China), and centrifuged at 12,000×g at 4°C for 15 min to isolate the proteins. The protein concentration was determined using the BCA Protein Assay Kit (Bi Yuntian, Shanghai, China). Proteins were denatured in 95°C water bath for 5 min and then separated by SDS-polyacrylamide gel electrophoresis, transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA), and blocked in blocking buffer (150 mM NaCl in 10 mM Tris, pH 7.5 containing 5% non-fat dry milk) for 1 h at room temperature. The membranes were incubated with primary antibodies against NGAL (1:1000), SOD2 (1:1000) and β-actin (1:2000) overnight at 4°C, and then washed three times with washing buffer (20 mM Tris-HCl, 137 mM NaCl, 0.2% Tween 20, pH 7.5). Then the membranes were incubated with horseradish peroxidaseconjugated secondary antibody for 1 h at room temperature, washed three times, and visualized with ECL kits (Amersham, Piscataway, NJ, USA).

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from kidney tissue using the Trizol reagent following the manufacturer's instruction (Takara, Japan), and then the RNA was reverse transcribed using reverse transcription kit (Takara, Japan). Oligonucleotides were designed using Primer5 software and the sequences are shown in Table 1. qRT-PCR amplification was performed using the SYBR Green Master Mix (Vazyme, Nanjing, China) and the PRISM 7500 Real-Time PCR Detection System (Applied Biosystems, Foster City, CA, USA). Cycling conditions were 95°C for 10min, followed by 40 repeats of 95°C for 15s and 60°C for 1min. The mRNA expression levels were normalized to GAPDH and were calculated using delta method from threshold cycle numbers.

Kidney lipid peroxidation (MDA) assay

Kidney tissue was homogenized in PBS containing 1 mM EDTA and centrifuged at 10,500 ×g for 10 min at 4°C. Thiobarbituric acid-reactive substances (TBARS) was based on the formation of malondialdehyde (MDA) by using a commercially available TBARS Assay kit (Jianchen, Nanjing, China) following the manufacturer's instructions. MDA value was normalized to the kidney protein content as determined by BCA kit (Bi Yuntian, Shanghai, China). The amount of lipid peroxidation was expressed as nmol/mg protein.

Statistical analysis

All data were represented as means ± SD. Statistical analysis was performed using ANOVA analysis followed by a Bonferroni posttest or unpaired Student's t-test. $P < 0.05$ was considered statistically significant.

Table 1. Primers used for real-time PCR

Primer	Sequence
KIM-1_F	5'-ATGAATCAGATTCAAGTCTTC-3'
KIM1_R	5'-TCTGGTTTGTGAGTCCATGTG-3'
NGAL_F	5'-TGGCCCTGAGTGTCTCATGTG-3'
NGAL_R	5'-CTCTTGTAGCTCATAGATGGTGC-3'
IL-6_F	5'-GGCAATTCTGATTGTATG-3'
IL-6_R	5'-GACTCTGGCTTTGTCTTT-3'
IL-1β_F	5'-CAACCAACAAGTGATATTCTCCATG-3'
IL-1β_R	5'-GATCCACACTCTCCAGCTGCA-3'
MCP-1_F	5'-CTTCTGGGCTGTGTTCA-3'
MCP-1_R	5'-CCAGCCTACTCATTGGGATCA-3'
ICAM-1_F	5'-CGCTTCCGCTACCATCAC-3'
ICAM-1_R	5'-GGCGGCTCAGTATCTCCTC-3'
SOD1_F	5'-AGCCCGCGGATGAAG-3'
SOD1_R	5'-CCTTCCAGCAGTCACATTGC-3'
SOD2_F	5'-CGGCCTACGTGAACAATCTC-3'
SOD2_R	5'-GATAGCCTCAGCAACTCTCC-3'
SOD3_F	5'-TTCTTGTCTACGGCTTGCTAC-3'
SOD3_R	5'-CTCCATCCAGATCTCCAGCACT-3'
GAPDH_F	5'-GTCTTCACTACCATGGAGAAGG-3'
GAPDH_R	5'-TCATGGATGACCTTGGCCAG-3'

Results

Rotenone treatment improved renal dysfunction induced by APAP

After 24 h APAP treatment at a dose of 300mg/kg, mice in APAP alone group were significantly less active than those in the control group with no animal death. In rotenone-treated animals, such a less active status was significantly restored. The BUN and serum creatinine levels in mice were significantly increased as compared with the vehicle group (Fig. 1A & B). In contrast, the BUN and blood creatinine levels in rotenone-treated animals were strikingly normalized (Fig. 1A & B). In addition, we measured serum cystatin C which serves as a more precise marker of kidney function than serum creatinine and BUN [23, 24]. The data showed that APAP treatment increased serum cystatin C by 2.26-fold ($P < 0.01$) compared with the control animals, which was also largely normalized by the treatment of rotenone (Fig. 1C). Moreover, rotenone administration also significantly attenuated APAP-induced renal tubular injury as shown by the tubular injury scores (Fig. 1D & E). These data indicated a potent role of rotenone in protecting against APAP nephrotoxicity.

Rotenone treatment blocked the upregulation of tubular injury markers of NGAL and KIM-1

Recently, new AKI biomarkers of neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) were found to be more reliable and sensitive than BUN and creatinine in a number of experimental and clinical studies [25, 26]. Compared with the vehicle group, APAP treatment enhanced the mRNA levels of NGAL and KIM-1 by 347- and 5-fold, respectively (Fig. 2A & B). By Western blotting, we further confirmed a 15-fold upregulation of NGAL protein in the kidneys of APAP-treated mice (Fig. 2C & D). Strikingly, such increments of NGAL and KIM-1 were largely normalized by the treatment of rotenone (Fig. 2A-D). These data further confirmed a protective role of rotenone against APAP-induced acute kidney injury.

Fig. 1. Rotenone treatment significantly attenuated APAP-induced kidney injury. The blood levels of BUN (A), creatinine (B), and cystatin C (C) were determined in mice. (D) Kidney sections were stained with PAS and evaluated by microscope ($\times 400$). (E) Tubular injury score by blinded scoring system. Data were expressed as mean $\pm$ SD, $n=7$ in each group. * $P<0.05$

compared to the vehicle group; ** $P<0.01$ compared to the vehicle group; *** $P<0.001$ compared to the vehicle group; ## $P<0.01$ compared to the APAP group.

Fig. 2. Rotenone treatment suppressed the upregulation of NGAL in APAP-treated mice. (A) mRNA expression of NGAL in kidneys was determined by qRT-PCR. (B) mRNA expression of KIM-1 in kidneys was determined by qRT-PCR. (C) Western blot of NGAL. (D) Quantitative analysis of NGAL Western blots. Data were expressed as mean $\pm$ SD, $n=7$ in each group. * $P<0.05$ compared to the vehicle group; ** $P<0.01$ compared to the vehicle group; # $P<0.05$ compared to the APAP group; ## $P<0.01$ compared to the APAP group.

Rotenone treatment attenuated APAP-induced inflammatory response in kidney

Evidence suggested that APAP-induced renal damage was partly through the inflammation [27]. Indeed, the level of inflammatory cytokines of IL-6, IL-1 β , MCP-1 and ICAM-1 were significantly upregulated in the kidneys of APAP-treated mice determined by qRT-PCR. Strikingly, the upregulation of these inflammatory cytokines was reduced by rotenone treatment (Fig. 3A-D). These results suggested that the attenuation of inflammation might be attributable to the rotenone effect on protecting against APAP nephropathy to some extent.

Rotenone treatment ameliorated APAP-induced oxidative response in the kidney

Dysfunctional mitochondria contribute to the overproduction of ROS. Here we measured three key antioxidant enzymes of SOD1, SOD2, and SOD3. As shown by the data, APAP challenge significantly reduced the mRNA expressions of SOD2 (a mitochondria-specific SOD) and SOD3 (extracellular SOD) with no effect on the SOD1 (intracellular SOD) expression (Fig. 4A-C). In animals with rotenone treatment, APAP-induced downregulation of SOD2 and SOD3 was strikingly reversed (Fig. 4B & C). Moreover, although APAP did not affect the mRNA expression of SOD1, rotenone administration further enhanced renal SOD1 expression in APAP-treated animals (Fig. 4A). By Western blotting, we further confirmed the downregulation of SOD2 at protein levels, which was partly but significantly reversed by rotenone treatment (Fig. 5A & B). In agreement with the regulation of SOD1-3, we observed that the oxidative stress marker of MDA in kidney was elevated after APAP challenge, which was significantly suppressed by rotenone administration (Fig. 5C). These findings suggested that rotenone suppressed APAP-induced oxidative stress in kidney possibly via modulating the expressions of SODs, and such an antioxidant action might finally ameliorate the inflammation and kidney injury.

Fig. 3. Rotenone treatment suppressed the inflammation in the kidneys of mice treated with APAP. (A) mRNA levels of IL-6 were determined by qRT-PCR. (B) mRNA levels of IL-1 β were determined by qRT-PCR. (C) mRNA levels of MCP-1 were determined by qRT-PCR. (D) mRNA levels of ICAM-1 were

determined by qRT-PCR. Data were expressed as mean $\pm$ SD, n=7 in each group. * P<0.05 compared to the vehicle group; ** P<0.01 compared to the vehicle group; *** P<0.001 compared to the vehicle group; # P<0.05 compared to the APAP group; ## P<0.01 compared to the APAP group.

Fig. 4. Rotenone treatment enhanced the mRNA expressions of SOD1-3 in the kidneys of mice challenged with APAP. (A) mRNA levels of SOD1 were determined by qRT-PCR. (B) mRNA levels of SOD2 were determined by qRT-PCR. (C) mRNA levels of SOD3 were determined by qRT-PCR.

Data were expressed as mean $\pm$ SD, $n=7$ in each group. ^{**} $P<0.01$ compared to the vehicle group; ^{***} $P<0.001$ compared to the vehicle group; [#] $P<0.05$ compared to the APAP group; ^{##} $P<0.01$ compared to the APAP group.

Fig. 5. Effect of rotenone treatment on APAP-induced oxidative stress in the kidneys. (A) Western blot of SOD2. (B) Quantitative analysis of SOD2 Western blots. (C) Oxidative stress marker MDA in the kidneys was measured using a commercial kit. Data were expressed as mean $\pm$ SD, $n=7$ in each group. *

$P<0.05$ compared to the vehicle group; ^{***} $P<0.001$ compared to the vehicle group; [#] $P<0.05$ compared to the APAP group.

Rotenone treatment at current dose did not cause renal toxicity

As we know, excessive inhibition of the mitochondrial activity could be toxic to highly aerobic organs like kidney [28, 29]. In order to test the nephrotoxicity of rotenone at a relatively lower dose (250 ppm in food), we treated mice with vehicle or rotenone for four and a half days. As shown by the data, compared to the vehicle group, rotenone alone had no significant effects on serum creatinine and BUN levels (Fig. 6A & B). Moreover, rotenone did not significantly increase the KIM-1 and NGAL (Fig. 6C-E). At the same time, rotenone also did not cause inflammatory response as shown by the unaltered renal expressions of IL-1 β and TNF- α (Fig. 6F & G). These data indicated that rotenone at 250ppm in food did not cause obvious nephrotoxicity in mice.

Effect of azoxystrobin treatment on APAP-induced nephrotoxicity in mice

Furthermore, we used azoxystrobin (AZOX), an inhibitor of mitochondrial complex III, to treat the mice with APAP challenge. The dose of azoxystrobin was chosen according to the previous study in mouse model [30]. The results showed that AZOX failed to protect against APAP-induced increments of serum BUN and creatinine (Fig. 7A & B). Consistently, AZOX also did not affect the upregulation of tubular injury markers of NGAL and KIM-1 (Fig. 7C-F) compared to the APAP group. Meanwhile, APAP-induced inflammatory response in kidney was also unaltered by AZOX (Fig. 8A & B). Finally, we also found that three key antioxidant enzymes of SOD1-3 in the kidneys were not influenced by AZOX compared to APAP alone group (Fig. 8C-E). These data suggested that inhibition of mitochondrial complex III with AZOX was unable to prevent the renal injury induced by APAP.

Fig. 6. Evaluation of the renal toxicity of rotenone in mice. (A) Blood BUN. (B) Serum creatinine. (C) mRNA levels of KIM-1 were determined by qRT-PCR. (D) Western blot of NGAL. (E) Quantitative analysis of NGAL Western blots. (F) mRNA levels of IL-1 β were determined by qRT-PCR. (G) mRNA levels of TNF- α were determined by qRT-PCR. Data were expressed as mean $\pm$ SD, n=8 in each group. ns (no significance) compared to the vehicle group.

Fig. 7. Effect of azoxystrobin treatment on APAP-induced nephrotoxicity in mice. (A) Blood BUN. (B) Serum creatinine. (C) mRNA levels of NGAL were determined by qRT-PCR. (D) mRNA levels of KIM-1 were determined by qRT-PCR. (E) Western blot of NGAL. (F) Quantitative analysis of NGAL Western blots. Data were expressed as mean $\pm$ SD, n=8 in each group. * P<0.05 compared

to the vehicle group; ** P<0.01 compared to the vehicle group; *** P<0.001 compared to the vehicle group; ns (no significance) compared to the APAP group.

Fig. 8. Effect of azoxystrobin treatment on the expressions of inflammatory cytokines and SOD1-3 in APAP-treated mice. (A) mRNA levels of IL-1 β were determined by qRT-PCR. (B) mRNA levels of ICAM-1 were determined by qRT-PCR. (C) mRNA levels of SOD1 were determined by qRT-PCR. (D) mRNA levels of SOD2 were determined by qRT-PCR. (E) mRNA levels of SOD3 were determined by qRT-PCR. Data were expressed as mean $\pm$ SD, n=8 in each group. * P<0.05 compared to the vehicle group; ** P<0.01 compared to the vehicle group; ns (no significance) compared to the APAP group.

Discussion

Drug-induced AKI is common in clinic [2, 31]. APAP is a widely used antipyretic analgesic. Appropriate amount of APAP helps to relieve pain and fever, but over dose of APAP can cause AKI [7]. It is known that severe AKI could need renal replacement therapies with high mortality [31]. This situation raised urgent requests to better understand the pathogenic mechanisms of AKI and find more effective strategies on AKI therapy. Recent studies suggested that both oxidative stress and inflammation contributed to APAP-induced renal injury [27, 32, 33]. However, the more detailed mechanisms are still elusive.

Mitochondria are complex intracellular organelles with important physiological functions. Renal tubules are densely packed with mitochondria. Mitochondrial dysfunction is thought to be a key mechanism in the pathogenesis of AKI [10]. Under the physiological conditions, excessive inhibition the mitochondrial activity could be toxic to cells and organs. However, under the pathological conditions, mitochondrial damage could result in the oxidative stress and inflammation. Thus, a suitable inhibition of the activity of the injured mitochondria could restrict the release of ROS and other toxic products to attenuate the organ injury. In agreement with this concept, a number of literatures documented that the inhibition of mitochondrial complex I activity under pathological conditions protected against the organ damage [22, 34-38]. In the present study, we also found that mitochondrial complex I inhibitor rotenone protected against APAP-induced acute kidney injury.

APAP-induced nephrotoxicity is frequently associated with the elevation of blood urea nitrogen and serum creatinine and acute tubular injury [39]. In addition to these traditional serum biochemical markers of renal injury, kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) are considered as more sensitive and reliable biomarkers [25, 26]. Previous studies have shown that the elevation of KIM-1 and NGAL is prior to the obvious renal tubule damage and the increments of serum creatinine (SCr) and blood urea nitrogen (BUN) levels [40]. Our results showed that the BUN, serum Creatinine, cystatin C, KIM-1 and NGAL were all significantly increased in the animals treated with APAP, indicating an acute kidney injury. Strikingly, administration of mitochondrial complex I inhibitor rotenone largely normalized these injury markers, suggesting an important role of mitochondrial complex I activity-associated mechanism in APAP-induced kidney injury.

The abnormal mitochondrion is positioned to be a critical player in AKI [41]. Mitochondrial damage leads to the excessive production of ROS, which could directly cause the cellular injury [42, 43]. In the present study, we tested the efficacy of mitochondrial complex I inhibitor rotenone on the oxidative stress in kidneys of mice exposed to APAP and found that rotenone treatment strikingly ameliorated APAP-induced enhancement of oxidative stress maker MDA in the kidneys. Along with this phenomenon, rotenone restored the reduction of SOD2 and SOD3 and increased SOD1, suggesting that the antioxidant activity of rotenone might be through the modulation of SODs to some extent.

Inflammation plays a detrimental role in many diseases including AKI [44]. Many studies suggested that pro-inflammatory cytokines have an important role in the pathogenesis of APAP-induced liver toxicity [45, 46]. In this APAP nephrotoxicity model, inhibition of mitochondrial complex I by rotenone led to a marked amelioration of inflammation as shown by the significant suppression of proinflammatory cytokines IL-6, IL-1 β , MCP-1 and ICAM-1. These results suggested that mitochondrial dysfunction may play an important role in mediating the inflammatory response in APAP-induced kidney injury.

Finally, azoxystrobin, an inhibitor of mitochondrial complex III, failed to protect against APAP-induced kidney injury. As we know, complex I and III are the important sources of O₂⁻ production in mitochondria [47-49]. Superoxide is released into the matrix from complex I, while complex III-generated superoxide is released into both the matrix and intermembranous space [50, 51]. In the absence of ADP, electrons derived from succinate (FADH₂-linked complex II substrate) can reversely flow to complex I to generate more O₂⁻. For this reason, complex I is considered as the major ROS-generating site in mitochondria

under the physiology and pathology [50, 52]. Therefore, inhibition of the complex I, rather than other mitochondrial complexes, such as complex III, can more significantly inhibit the production of ROS, thereby protecting against APAP-induced renal injury.

Conclusion

Our study demonstrated that inhibiting the activity of dysfunctional mitochondria by a mitochondrial complex I inhibitor significantly attenuated APAP-induced AKI in line with the blockade of oxidative stress and inflammation. These results not only indicated a mitochondria-associated mechanism in mediating APAP-induced renal injury but also suggested that targeting mitochondria might be a promising strategy for treating APAP-induced nephrotoxicity. However, we could not entirely rule out the possibility of an off-target action of rotenone in protecting against APAP-induced nephrotoxicity, which needs more evidence to be figured out in the future.

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Disclosure Statement

The authors declare that there are no conflicts of interest.

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