Quercetin alleviates acute kidney injury by inhibiting ferroptosis

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ABSTRACT

Introduction: Ferroptosis is an iron-dependent regulated necrosis and has been proven to contribute to the progress of acute kidney injury (AKI). Quercetin (QCT), a natural flavonoid which is commonly found in numerous fruits and vegetables, has extensive pharmacological effects, such as anti-oxidant, anti-inflammatory and anti-senescence effects.

Objectives: This study aims to explain whether ferroptosis is a therapeutic strategy to AKI, and to explore the effect of QCT on AKI ferroptosis.

Methods: NRK-52E cells and HK-2 cells were used for in vitro ferroptosis studies. Morphology of cells was detected by transmission electron microscopy. Lipid ROS was assayed using flow cytometry. In vivo, AKI
Acute kidney injury
Activation transcription factor 3
Macrophages

was induced by ischemia–reperfusion (I/R) or folic acid (FA). To explore the molecular mechanisms, RNA-sequence analysis was performed. Transwell was used to detect macrophage migration.

**Results:** We discovered that quercetin (QCT), a natural flavonoid, inhibited ferroptosis in renal proximal tubular epithelial cells. QCT blocked the typical morphologic changes of ferroptotic cells by reducing the levels of malondialdehyde (MDA) and lipid ROS and increasing the levels of glutathione (GSH). Moreover, QCT ameliorated AKI induced by I/R or FA. RNA-sequence analysis highlighted activation transcription factor 3 (ATF3), as it was the dominant one among all the 299 down-regulated genes by QCT. Knockdown of ATF3 could significantly increase the levels of SLC7A11, GPX4 and increased the cell viability. In addition, ferroptotic cells were found to be extremely pro-inflammatory by recruiting macrophages through CCL2, while QCT inhibited the chemotaxis of macrophages induced by ferroptosis in AKI.

**Conclusions:** Collectively, these results identify QCT as a ferroptosis inhibitor and provide new therapeutic strategies for diseases related to ferroptosis.

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In vitro experiments: the following reagents were used at the indicated concentrations: Erastin (Era) (MedChemExpress, cat number: HY-15763) (1 μM) and RSL3 (MedChemExpress, cat number: HY-100218A) (0.5 μM) for all experiments. QCT (10 μM) or Fer-1 (1 μM) (Aladdin, cat number: Q111273 or F129882, respectively) was added at the same time with Era or RSL3. The cell death inducing was brefeldin A (Bre-A) (1 μM) and subjected to the flow cytometry analysis to examine the amount of ROS within cells. Cells were analyzed with the Invitrogen™ Attune™ NxT Flow Cytometer (Invitrogen, USA) and were calculated using the FlowJo Software.

Drug treatment

Male C57BL/6j mice (20–22 g, 7–8 week) were used for all studies. Mice were housed in a room with a 12 h/12 h light/dark cycle, and habituated in the room 3 days before experiments. All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, with the approval of Center for New Drug Safety Evaluation and Research, China Pharmaceutical University.

Animal model

Renal ischemia/reperfusion model

Renal ischemia was induced in male mice according to the previous reports [24,25]. Before operation, the drug QCT or Fer-1 was administered. Mice were anesthetized to expose the both kidneys through flank incision. Then mice were kept on a homeothermic plate to induce ischemia for 30 min by clamping the renal pedicle with nontraumatic clamps. Sham groups were subjected to sham operation without induction of ischemia. Blood samples were collected at the time of euthanasia. After reperfusion for 24 h, all the mice were sacrificed. One kidney was fixed in 4% phosphate-buffered formaldehyde for histological analyses, and the other one was snap frozen for subsequent molecular analysis.

Folic acid nephropathy model

Folic acid nephropathy, a classic model of kidney tubulointerstitial injury and inflammation, was induced by a single i.p. injection of FA (200 mg/kg) in 0.3 mol/L sodium bicarbonate as reported previously [8]. All the mice were euthanized 24 h after drug treatment. Blood samples were collected at the time of euthanasia. One kidney was fixed in 4% phosphate-buffered formaldehyde for histological analyses, and the other one was snap frozen for subsequent molecular analysis.

Detection of MDA and GSH levels in cell and kidney tissue samples

Individual levels of MDA and GSH in cell and kidney tissue were measured using MDA and GSH activity assay kits respectively according to the manufacturer’s protocol. Individual contents of MDA and GSH were measured at 450 and 405 nm, respectively, with a microplate fluorometer. Total protein concentration was measured using the Bradford method (Beyotime Institute of Biotechnology, Haimen, China).

Renal function, histology and immunohistochemistry

Serum creatinine and BUN were determined to monitor renal function as previously described [26,27]. Kidney sections (5 μm) were stained with hematoxylin & eosin (H&E). Tubular injury was evaluated by a pathologist in a blinded manner and was scored based on the degree of damage, as previously described [7,8]. Brush border loss, vacuolization, cell desquamation, tubule dilatation, and tubule degeneration were all scored from 0 to 3, and then all scores were added to yield the tubular injury score, which had a maximal value of 15. Images were obtained with an Olympus BX41 microscope.

Masson stain of kidney tissue

Tubular injury in kidney sections was evaluated after Masson staining by a pathologist who was blinded to the nature of the samples. Evidence of cell injury (loss of brush border or vacuolization), cell desquamation, and tubular dilation and signs of regeneration were scored on a semiquantitative zero to three scale, and results from each item were added to yield the tubular injury score, which had a maximal value of 18 [8].

Iron measurements

Cells (2 × 10^6) were rapidly homogenized in iron assay buffer with iron assay kit (sigma Aldrich, cat number: MAK025) as previously described [28]. Briefly, iron is released by the addition of an acidic buffer. Samples were tested to measure total iron (Fe^{2+} and Fe^{3+}). Released iron could react with the iron probe resulting in a colorimetric (593 nm) product, proportional to the iron present. Then the solution was centrifuged at 13,000g for 10 min at 4 °C to remove insoluble material and was measured at 593 nm with a microplate fluorometer.

Real time PCR analysis

Total RNA from tissues or cells were extracted using Trizol (Invitrogen), and reverse transcribed into cDNA using a cDNA synthesis kit (Takara). Quantitative PCR was done with a Step one plus Real-Time PCR system (Applied Biosystems, USA) with gene-specific primers. The amount of RNA was calculated by the comparative threshold cycle method. All primers were custom-made by Genscript. The primer sequences are shown in supplementary Table 1.

RNA-Seq Profiling

RNA-Seq Profiling was performed by Novogene. Briefly, the cells were treated with Era (1 μM) with or without QCT (10 μM) for 24 h in the incubator. Then the RNA was isolated using the Trizol reagent. A total amount of 3 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer’s recommendations and index codes were added to attribute sequences to each
QCT protects the kidney in both I/R and FA induced AKI

According to previous reports, ferroptosis of renal tubular cells contributes significantly to the process of AKI [7,8]. Based on the potent inhibitory effect of QCT on ferroptosis, we explored the possible role of QCT in AKI. QCT itself did not have an effect on normal mice, including kidney coefficient and the levels of blood urea nitrogen (BUN) and blood creatinine (CRE) (Fig. S2A). The experimental procedure of I/R induced AKI and administration of drugs is depicted in schematic diagram (Fig. 2A). QCT displayed a protective role by reducing kidney coefficient and the content of BUN and CRE in I/R-AKI (Fig. 2B). Tubular injury was evaluated in kidney sections by Masson staining (Fig. 2C) and HE staining (Fig. S2B), which showed that QCT significantly reduced histologic injury. To confirm these findings, we performed another AKI model using FA (Fig. 2D). Consistently, QCT protected the functional acute renal failure (Fig. 2E) and structural organ damage (Fig. 2F and Fig. S2C) in mice of FA-induced AKI model. Lipid peroxidation in kidney tissues was measured by levels of GSH and MDA. QCT increased the level of GSH and reduced MDA content in the kidney of both AKI models (Fig. 2G). Immunofluorescence staining of kidney tissue section showed QCT reduced kidney cell death with TUNEL assay kit (Fig. S2D). Together, these data support the hypothesis that QCT is a promising drug candidate for treatment of AKI by inhibiting ferroptosis of tubular epithelial cells.

The inhibitory effect of QCT on ferroptosis was depended on the repression of ATF3

Next, we embarked to decipher the molecular mechanisms that may account for the QCT's inhibition of ferroptosis. As ferroptosis is dependent upon intracellular iron and occurs due to lipid peroxide accumulation, we first determined the influence of QCT on intracellular iron levels and expression of acyl-CoA synthetase long-chain family member 4 (ACSL4), which is an essential component for lipid peroxide accumulation [30]. No obvious differences of intracellular iron and expression levels of ACSL4 mRNA were found in all groups (Fig. 3A, B). We then explored the antioxidant defenses of the cell, and results revealed a dramatic decrease of GPX4 mRNA and protein accompanied by ferroptosis, while QCT rescued the defective expression (Fig. 3C, D). To further explore the molecular mechanisms, we performed 52E cell RNA-sequence analysis. Compared with Era group, activation transcription factor 3 (ATF3) was predominately low among all the down-regulated genes, while SLC7A11, a subunit unique to system Xc⁻, was higher...
in the QCT treated group (Fig. 3E). Enrichment analysis revealed that QCT-changed genes were correlated with the glutathione metabolic pathway (Fig. 3F). PCR results also confirmed QCT could upregulate the levels of SLC7A11 and SLC3A2, and downregulate the levels of ATF3 as well as Heme Oxygenase 1 (Hmox-1) (Fig. 3G). To elucidate whether impaired expression of ATF3 was responsible for the inhibition of ferroptosis, we knocked down the expression of ATF3 with siRNA, and the knockdown efficiency was confirmed both at the mRNA and protein levels as shown in Fig. 3H. Interestingly, knockdown of ATF3 signficantly increased the expression of SLC7A11 and GPX4, while it decreased the lipid ROS levels, and resulted in an increase of cell viability (Fig. 3I). These results suggest that the ATF3 block by QCT may contribute to its inhibition on ferroptosis.

The mechanism of action of QCT was confirmed in vivo. Similar to the in vitro studies, QCT had no effect on levels of ATF3 and Hmox1 in normal mice, but significantly decreased the levels of ATF3 as well as Hmox-1 in mice subjected to the I/R-AKI model (Fig. 4A, B, Fig. S4 A, B). The mRNA and protein levels of GPX4 were not influenced by QCT in normal mice (Fig. S4 B, C). But they were diminished in the I/R group and were significantly restored after QCT treatment (Fig. 4 B, C). In accordance with the I/R-AKI model, all the results were repeatable in mice of the FA-AKI model (Fig. 4-D-F). In addition, no obvious iron accumulation was found in all groups in both models (Fig. S3 A, B). These in vivo findings fit well with in vitro evidence that QCT inhibits ferroptosis through the reduction of ATF3.
Fig. 2. QCT possessed the protective effect to the kidney in both I/R and FA induced AKI. QCT or Fer-1 was administrated 3 times/day, first at the time of model induction, and then 8 h intervals. (A) The scheme of I/R-induced AKI model and drug treatment. (B) Kidney coefficient and BUN and blood CRE levels in I/R-AKI mice. Kidney coefficient = Kidney weight/body weight x 100. (C) Representative Masson staining, and pathological scores of the kidney in I/R-AKI groups. The scale bar represents 50 μm. (D) The scheme of FA-induced AKI model and drug treatment. (E) Kidney coefficient and BUN and blood CRE levels in FA-AKI mice. Kidney coefficient = Kidney weight/body weight x 100. (F) Representative Masson staining, and pathological scores of the kidney in FA-AKI groups. The scale bar represents 50 μm. (G) MDA and GSH levels in kidney tissues in both I/R-AKI and FA-AKI groups. (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001, compared with Control (CON) group; #P < 0.05, ##P < 0.01, ###P < 0.001, compared with I/R or FA group.
Fig. 3. Inhibitory effect of QCT on ferroptosis was depended on the repression of ATF3. The cells were treated with drugs as indicated ahead for 24 h. (A) Intracellular iron levels in 52E cells. (n = 3). (B) RT-PCR results of ACSL4 in 52E cells. (n = 4). (C) RT-PCR results of GPX4 in 52E cells. (n = 4). (D) Western blot results of GPX4 in 52E cells. (n = 3). (E) Changed genes in Era + QCT treated group compared with Era group displayed in volcano plot assayed by RNA-sequence. The 52E cells were treated with Era (1 μM) with or without QCT (10 μM) for 24 h. (F) Enrichment analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway in Era + QCT group compared with Era group assayed by RNA-sequence in 52E cells. (G) RT-PCR results of SLC7A11, SLC3A2, ATF3, Hmox-1 in 52E cells. (n = 4). (H) and (I) After transfected with siRNA for 24 h, the cells were treated with Era (1 μM) for 24 h and the level of ATF3, SLC7A11, GPX4, lipid ROS levels, and cell viability in 52E cells were determined. (n = 4–6). *P < 0.05, **P < 0.01, ***P < 0.001, compared with CON group; #P < 0.05, ##P < 0.01, ###P < 0.001, compared with Era or RSL3 group.
Ferroptotic cells induced the recruitment and chemotaxis of macrophages through CCL2

AKI is histologically characterized by tubular cell death and inflammation. Regulated necrosis including ferroptosis is thought to be extremely pro-inflammatory and immunogenic [1]. But how ferroptosis trigger inflammation is poorly understood. Through RNA-sequence and enrichment analysis of KEGG signaling pathways in the Era group compared with the Control group of 52E cells, we found that changed genes in the Era group were enriched in inflammatory pathways, such as “response to tumor necrosis factor”, “response to molecules of bacterial origin”, “response to lipopolysaccharide” and “response to cytokine” (Fig. 5A). Many inflammation related genes were up-regulated by Era, such as MMP9, Ptgs2, Lcn2 (Fig. 5B). Of note, macrophage chemokines, CCL2 and CCL7, were also increased in the Era group compared with the Control group (Fig. 5B). RT-PCR results confirmed the increase of chemokines induced by Era, whereas QCT treatment significantly decreased both levels of CCL2 and CCL7 (Fig. 5C). To test whether ferroptotic cells could promote macrophage migration, we added 52E cells treated with Era or DMSO in the lower chamber of the transwell and iBMDMs in the upper chamber for 24 h.
Results showed a significant increase of migrated cells by ferroptotic cells, while administration of RS102895, an inhibitor of CCR2 to the upper chamber, curtailed the migration of macrophages (Fig. 5D). In addition, Era alone without 52E cells didn’t improve the migration of macrophages (Fig. S5 A), and RS102895 had no influence on cell viability of iBMDMs (Fig. S5 B), which excluded a non-specific effect of the drugs. Collectively, these results prove that ferroptotic cells induce a pro-inflammatory state by triggering the recruitment of macrophages through CCL2, and that QCT can inhibit ferroptosis-induced inflammation in vitro.

QCT diminished the inflammation in mice of AKI models

To further confirm the pro-inflammatory effect of ferroptotic cells induced by chemotaxis of macrophages in vivo, we treated the mice with RS102895. The results showed a protective effect of RS102895 in FA-AKI, reducing kidney coefficient and blood CRE and BUN (Fig. 6A). Masson staining of kidney tissues also showed that RS102895 significantly reduced histologic injury (Fig. 6B). We further investigated the effect of QCT or Fer-1 on kidney inflammation. Infiltrated macrophages were increased in

![Image of a graph showing enrichment analysis of KEGG signaling pathway in Era group compared with Control group assayed by RNA-sequence in 52E cells.](A) Enrichment analysis of KEGG signaling pathway in Era group compared with Control group assayed by RNA-sequence in 52E cells. (B) Changed inflammation-related genes in Era group compared with Control group displayed in heatmap assayed by RNA-sequence in 52E cells. (C) RT-PCR results of CCL2, CCL7 in 52E cells after treatment for 24 h. (n = 4). (D) Left, iBMDM migration assay (visualized by crystal violet stain) using 52E cells treated with Era or DMSO for 24 h. Right, Quantitative analysis of the cell migration number. (n = 3). The scale bar represents 50 μm. ***P < 0.001, compared with CON group; ##P < 0.01, ###P < 0.001, compared with Era group.

Fig. 5. Ferroptotic cells induced the recruitment and chemotaxis of macrophages through CCL2. (A) Enrichment analysis of KEGG signaling pathway in Era group compared with Control group assayed by RNA-sequence in 52E cells. (B) Changed inflammation-related genes in Era group compared with Control group displayed in heatmap assayed by RNA-sequence in 52E cells. (C) RT-PCR results of CCL2, CCL7 in 52E cells after treatment for 24 h. (n = 4). (D) Left, iBMDM migration assay (visualized by crystal violet stain) using 52E cells treated with Era or DMSO for 24 h. Right, Quantitative analysis of the cell migration number. (n = 3). The scale bar represents 50 μm. ***P < 0.001, compared with CON group; ##P < 0.01, ###P < 0.001, compared with Era group.
Fig. 6. QCT diminished the inflammation in murine AKI models. (A) Kidney coefficient and BUN and blood CRE levels in FA-AKI mice. (n = 6). (B) Representative Masson staining, and pathological scores of the kidneys in FA-AKI groups. The scale bar represents 50 μm. (n = 6). (C) Representative F4/80 staining photographs, and mean F4/80 positive cells in I/R-AKI mice counted in 5 fields. Black arrows indicate positive F4/80 staining cells; statistic results of F4/80+ cells/field are listed on the right. The scale bar represents 50 μm. (n = 6). (D) RT-PCR results of IL-1β, IL-6, TNF-α in I/R-AKI mice. (n = 6). (E) Representative F4/80 staining photographs, and mean F4/80 positive cells in FA-AKI mice counted in 5 fields. Black arrows indicate positive F4/80 staining cells; statistic results of F4/80+ cells/field are listed on the right. The scale bar represents 50 μm. (n = 6). (F) RT-PCR results of IL-1β, IL-6 in FA-AKI mice. (n = 6). *P < 0.05, ***P < 0.001, compared with CON group; #P < 0.05, ##P < 0.01, ###P < 0.001, compared with I/R or FA group.
mice of the I/R-AKI model, and were then reduced after QCT or Fer-1 administration (Fig. 6C). In addition, IL-1β, IL-6 and TNF-α levels in kidney were reduced by QCT or Fer-1 (Fig. 6D). Consistently, upsurge of infiltrated macrophages and pro-inflammatory cytokines were also mitigated by QCT in FA-AKI (Fig. 6E, F). All these results suggest that ferroptotic cells trigger inflammation in kidney by promoting pro-inflammatory cytokine release and the recruitment of macrophages, and inhibition of ferroptosis by QCT can alleviate the inflammation-mediated kidney injury.

Discussion

AKI is caused by factors such as kidney I/R, sepsis or nephrotoxins and is characterized by impaired kidney filtration function. The damage of the kidney tubular epithelial cells causes loss of kidney function and increases morbidity and mortality. Unfortunately, AKI still remains a headache condition which lacks specific tools for treatment until now. Understanding the pathophysiology of one disease is always the cornerstone for exploration of novel diagnostic and therapeutic strategies. Emerging evidence supports the concept that ferroptosis, among all the types of cell death, plays a crucial role in the pathophysiology of AKI, highlighting ferroptosis as a promising target for treatment of AKI.

Ferroptosis is a regulated form of cell death driven by loss of activity of the lipid repair enzyme GPX4 and subsequent accumulation of lipid-based ROS. Researchers found that ferroptosis inhibitor Fer-1 prevented AKI [7,8]. N-acetylcysteine, a lipid oxidation reducer, shows a protective role in FA and star fruit-induced AKI [31,32]. Era could inhibit cystine uptake by the cystine/glutamate antiporter (system xc−), and act on voltage dependent anion channels 2 and 3 (VDAC2/3), destroying the antioxidant defenses and promoting ROS production by the cell and ultimately leading to ferroptosis [3,29]. RSL3, a glutathione (GSH) peroxidase (GPX) 4 inhibitor, is another popularly used inducer of ferroptosis [3]. We found that QCT, an anti-oxidant, significantly inhibited both Era and RSL3-induced-ferroptosis, increased the cell viability and decreased cellular lipid ROS. As expected, QCT also showed as a therapeutic drug candidate for both I/R-AKI and FA-AKI, protecting from functional acute renal failure and structural organ damage. Meanwhile, lipid peroxidation in kidney tissues was blocked by QCT, consistent with previous studies that showed that QCT possessed the effect of alleviating intracellular ROS generation [33,34]. Interestingly, QCT is also a main component of "Huangkui capsule", which was proven to have wide renal protective effect and is widely used for diabetic nephropathy and other kidney diseases in China [35,36]. But the mechanisms behind "Huangkui capsule" remain poorly understood. The inhibitory effect of QCT on ferroptosis of kidney tubular epithelial cells shown here might provide the possible mechanism for "Huangkui capsule". However, QCT has poor bioavailability and solubility, which limits its application [37–39]. Also, unlike in vitro studies of QCT, the in vivo studies are more complex, considering the possible effect of its metabolites. The absorption and pharmacokinetics of QCT should be further studied. QCT in this manuscript was orally administrated 25 mg/kg for three times/day based on several exploration tests, while oral administration of QCT at 10 mg/kg daily was unable to protect against isoproterenol cardiotoxicity as reported [40]. Optimization of the structure of QCT to get a new compound with higher bioavailability and solubility is needed in further studies.

Ferroptosis is dependent upon intracellular iron, accumulation of lipid ROS, and loss of activity of the lipid repair enzyme GPX4. In the present study, no increasing iron content was found in cells developing ferroptosis or kidney tissues of AKI mice. While disruption of GPX4 was repaired by QCT, and lipid ROS was abrogated. According to the RNA-sequence analysis, we identified ATF3 as a core factor that contributed to the ferroptosis. ATF3 belongs to a member of the ATF/cyclic AMP response element-binding (ATF/CREB) family of transcription factors, considered as a stress response-inducing gene. And ATF3 was proven to be involved in varied types of cell regulation, including stress response, cell cycle regulation, apoptosis and immune regulation [41]. ATF3 has also been reported to change in various diseases such as atherosclerosis [42], cardiac hypertrophy [43], breast cancer [44], colorectal cancer [44] and other tumors. Recently, ATF4, another member of ATF/CREB family of transcription factors, was found to promote angiogenesis and neuronal cell death through ferroptosis in an Xc-dependendent manner [45]. Wolfram reported that high levels of ATF3 correlated with low glutathione during sepsis [46]. Recently, ATF3 was proven to promote ferroptosis [47]. We also found that ATF3 was increased in AKI, and that knockdown of ATF3 increased the cell viability of the renal proximal tubular epithelium and the expression of GPX4. Our data revealed that QCT could markedly inhibit ATF3 and suggested that QCT may inhibit ferroptosis through the repression of ATF3. In addition, a strong repression by QCT of Hmox-1 was also found. Hmox-1, the enzyme that is responsible for heme degradation, is upregulated in proximal tubule cells in response to oxidant stress. But its role in ferroptosis is controversial, some reported Hmox-1 mitigated ferroptosis [48], while others found Hmox-1 accelerated ferroptosis [49]. Based on our results, the effect of QCT on Hmox-1 was more likely to be accompanied by the inhibition on ferroptosis.

Besides tubular cell death, inflammation is also an important characteristic of AKI, histopathologically correlated with renal dysfunction [50]. It is known that dying cells release inflammatory factors which amplify tissue injury. Ferroptosis, together with other types of regulated necrosis, is thought to be immunogenic and extremely pro-inflammatory, but how ferroptosis causes inflammation remains poorly characterized. Through the RNA-sequence analysis, we found that molecular inflammatory pathways were activated in ferroptotic cells. Many inflammation related genes, especially macrophage chemokines CCL2 and CCL7, were triggered by ferroptosis. Ferroptotic cells did induce the recruitment of macrophages according to our experiments. Many references showed an important role of macrophages in AKI, stimulating and amplifying inflammatory responses [51,52]. QCT and Fer-1, inhibitors of ferroptosis, significantly reduced the infiltration of macrophages and levels of inflammatory cytokines in kidney. Our findings here clearly link cell death-immune crosstalk to AKI development and define important cellular and molecular mediators which may serve as effective targets in AKI.

Conclusion

In conclusion, this study provides evidences that QCT possess a protective role on AKI via inhibiting ferroptosis. Our studies suggest that (i) QCT is a potent ferroptosis inhibitor and ameliorates AKI; (ii) QCT reduces ATF3 expression and further influences the downstream signaling pathway of ferroptosis; (iii) Ferroptosis could induce the recruitment of macrophages, triggering inflammation. We view this work as a first step toward developing treatments for AKI targeting ferroptosis, and provide the basis for the development of new therapeutic strategies for diseases related to ferroptosis.

Compliance with Ethics Requirements

All Institutional and National Guidelines for the care and use of animals (fisheries) were followed.
All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, with the approval of Center for New Drug Safety Evaluation and Research, China Pharmaceutical University. The ethical committee number for the study is B20170722-1.

Declaration of Competing Interest

The authors have declared no conflict of interest.

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Author contributions

X.G., Y.W., and X.L. designed research; Y.W., F.Q., Q.C., Y.L., C.Y., R.B., H.Y., X.C. and X.G. performed research; Y.W., Q.C., X.G. and X.L. analyzed data; X.G., Y.W., and L.Y. wrote the paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2020.07.007.

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