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Abstract

Purpose: This meta-analysis evaluated changes in ferroptosis-related factors during intestinal ischemia-reperfusion injury by integrating data from animal experiments. **Methods:** Five databases were searched and 11 studies were selected from an initial pool of 105 publications. Primary indicators included Chiu's score, Fe^{2+}, and Glutathione Peroxidase 4 (GPX4), while secondary metrics included malondialdehyde (MDA), glutathione (GSH), reduced glutathione/oxidized glutathione (GSH/GSSG), solute carrier family 7 member 11 (SLC7A11), ferritin heavy chain 1 (FTH1), and Superoxide Dismutase (SOD). Sensitivity analyses were performed to assess heterogeneity and ensure the stability of the results. Funnel plots were employed to address publication bias. Statistical analyses were conducted using Review Manager 5.3. **Results:** The meta-analysis of the eleven selected studies indicated that intestinal IRI significantly increased Chiu's score (standard mean difference: 4.97, $P < 0.00001$) and oxidative stress markers such as MDA (5.41, $P < 0.00001$) while decreasing SOD levels (5.64, $P = 0.01$). Ferroptosis was significantly increased during intestinal IRI, as shown by the elevation of Fe^{2+} (4.31, $P < 0.00001$) and reductions in GPX4 (-4.43, $P < 0.00001$), GSH (-2.45, $P < 0.00001$), GSH/GSSG (-3.69, $P < 0.00001$), SLC7A11 (-3.61, $P = 0.02$), and FTH1 (-3.10, $P < 0.0001$). **Conclusion:** Intestinal IRI leads to increased Chiu's score, MDA, and Fe^{2+} levels, alongside decreased levels of GPX4, GSH, GSH/GSSG, FTH1, and SLC7A11, all of which are associated with the promotion of ferroptosis. The ischemia model employing 45 minutes of ischemia appears to yield superior outcomes regarding the evaluated markers.

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Ferroptosis in intestinal ischemia-reperfusion injury: a systematic review and meta-analysis

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Competing interests

All authors declare that they have no competing interests related to this study.

Author contributions

ZC Zhao conceptualized this article, performed data retrieval and collection, and wrote the article; YX Wu performed data retrieval and collection; LY Chang assessed the inclusion of the article using the SYRCLE risk of bias tool; Y W presents methodology and performs sensitivity analysis; DB Li assessed the inclusion of the article using the SYRCLE risk of bias tool; Y Xing re-tested the article for risk of bias; and YF Leng revised this article. Corresponding authors had full access to all data and were ultimately responsible for submitting the article for publication. All authors read and approved the final manuscript.

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Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Abstract

Purpose: This meta-analysis evaluated changes in ferroptosis-related factors during intestinal ischemia-reperfusion injury by integrating data from animal experiments. **Methods:** Five databases were searched and 11 studies were selected from an initial pool of 105 publications. Primary indicators included Chiu's score, Fe^{2+}, and Glutathione Peroxidase 4 (GPX4), while secondary metrics included malondialdehyde (MDA), glutathione (GSH), reduced glutathione/oxidized glutathione (GSH/GSSG), solute carrier family 7 member 11 (SLC7A11), ferritin heavy chain 1 (FTH1), and Superoxide Dismutase (SOD). Sensitivity analyses were performed to assess heterogeneity and ensure the stability of the results. Funnel plots were employed to address publication bias. Statistical analyses were conducted using Review Manager 5.3. **Results:** The meta-analysis of the eleven selected studies indicated that intestinal IRI significantly increased Chiu's score (standard mean difference: 4.97, $P < 0.00001$) and oxidative stress markers such as MDA (5.41, $P < 0.00001$) while decreasing SOD levels (5.64, $P = 0.01$). Ferroptosis was significantly increased during intestinal IRI, as shown by the elevation of Fe^{2+} (4.31, $P < 0.00001$) and reductions in GPX4 (-4.43, $P < 0.00001$), GSH (-2.45, $P < 0.00001$), GSH/GSSG (-3.69, $P < 0.00001$), SLC7A11 (-3.61, $P = 0.02$), and FTH1 (-3.10, $P < 0.0001$). **Conclusion:** Intestinal IRI leads to increased Chiu's score, MDA, and Fe^{2+} levels, alongside decreased levels of GPX4, GSH, GSH/GSSG, FTH1, and SLC7A11, all of which are associated with the promotion of ferroptosis. The ischemia model employing 45 minutes of ischemia appears to yield superior outcomes regarding the evaluated markers.

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Keywords: Ischemia-reperfusion injury. Ferroptosis. Meta-analysis. Gut.

1. Introduction

Intestinal ischemia-reperfusion injury (IRI) is a common complication associated with intestinal surgery, characterized by an initial interruption of blood supply to the intestine followed by subsequent reperfusion(1). The mechanisms underlying IRI are complex, involving hypoxia, the release of inflammatory mediators, and cell death. During ischemia, cells shift to anaerobic metabolism to generate ATP, which reduces ATPase activity, impairing both the reuptake of Ca^{2+} into the endoplasmic reticulum and extrusion across cell membranes. This disruption leads to calcium overload and structural damage to mitochondria(2). Reperfusion restores aerobic metabolism, facilitating Ca^{2+} influx and generating reactive oxygen species (ROS). The increase in ROS promotes the opening of the mitochondrial permeability transition pore (mPTP), initiating a cascade of events that aggravates cellular damage and induces endothelial dysfunction(3). Intestinal IRI triggers several signaling pathways, including the JAK/STAT and Toll-Like Receptor-mediated signaling pathways, which further upregulate ROS levels by activating MAPK, SIRT1, and caspase-3. This tissue damage extends beyond the intestine, potentially leading to systemic complications such as acute coronary syndrome and multiple organ dysfunction, which contribute to high mortality rates in affected patients(4). Current research underscores the importance of targeting intestinal IRI as an important strategy to mitigate mortality risk in high-risk patients.

Ferroptosis is a form of iron-dependent programmed cell death that is involved in the pathogenesis of intestinal IRI. This type of cell death is characterized by its unique morphological characteristics, including mitochondrial shrinkage, loss or reduction of mitochondrial cristae, and increased membrane density(5). Ferroptosis is primarily associated with two key metabolic processes: iron accumulation and the accumulation of lipid peroxides. Iron accumulation within cells promotes the generation of ROS through the Fenton reaction, directly damaging cellular structures. Moreover, iron regulates sensitivity to ferroptosis by influencing

lipoxygenase, an enzyme involved in lipid peroxidation(6). The cysteine/glutathione (GSH)/glutathione peroxidase 4 (GPX4) axis plays a central role in regulating ferroptosis. This pathway relies on the cystine-glutamate antiporter system Xc- to transport cystine into cells, where it is reduced to cysteine and then used for GSH synthesis(7). GPX4 plays an essential role in converting GSH to its oxidized form (GSSG), thereby reducing ROS production and protecting cells from lipid peroxidation damage(8). Depletion of GSH results in increased oxidative stress reduced GPX4 activity, and changes in key regulators of lipid peroxidation, such as ferritin heavy chain 1 (FTH1), heme oxygenase 1 (HO-1), solute carrier family 7 member 11 (SLC7A11), and nuclear receptor coactivator 4 (NCOA4), which collectively contribute to increased ferroptosis(6). Recent in vivo and in vitro studies have established a strong association between ferroptosis and the pathophysiological mechanisms of intestinal IRI, highlighting its potential as a therapeutic target(9). However, the understanding of ferroptosis remains limited, and extensive experimental validation is required before clinical interventions targeting ferroptosis can be applied to mitigate intestinal IRI.

This review collected the available animal studies focused on ferroptosis in the context of intestinal IRI. By conducting a systematic evaluation and meta-analysis of 11 relevant studies, this research aims to elucidate the changes in ferroptosis-related factors during intestinal IRI and to assess the impact of varying durations of intestinal ischemia on the ferroptosis process. The findings deepen the understanding of the intestinal IRI model, provide a basis for the design of future animal experiments, and potentially facilitate the advancement of clinical trials to target ferroptosis in intestinal IRI.

2. Methods

This study was registered on the Prospective Registry for Systematic Reviews platform (PROSPERO, registration ID: CRD42024569670) with all relevant

information and modifications.

2.1 Search strategy

A comprehensive literature search was conducted using five databases: PubMed, Web of Science, Cochrane Library, Embase, and Scopus. The search terms included "ischemia-reperfusion injury," "intestines," and "ferroptosis," targeting studies published up to July 20, 2024 (Table 1). This search was conducted independently by two authors, Zicen Zhao and Yuxuan Wu.

2.2 Inclusion and exclusion criteria

The inclusion criteria were: 1) All studies using intestinal ischemia-reperfusion models with superior mesenteric artery occlusion (including all species and genders). 2) Studies focused on changes in ferroptosis-related factors. The exclusion criteria were: 1) Studies involving animals with comorbidities. 2) In vitro studies and computational models. 3) Any intestinal ischemia-reperfusion model not employing superior mesenteric artery occlusion. 4) Studies with unavailable data or where data extraction was not possible. 5) Duplicate publications(10).

2.3 Data collection

Data extraction was performed independently by Zicen Zhao and Yuxuan Wu using a standardized data extraction form designed for this study. If numerical data were unavailable in the text, tables, or charts, the WebPlotDigitizer software was used to extract data from graphical representations. The following data were collected: Study details: author, year of publication, language, and country. 1) Animal characteristics: species, gender, age, weight. 2) Model parameters: ischemia-reperfusion interval (clamping and unclamping of the superior mesenteric artery) and anesthetics used. 4) Outcome measures: Chiu's score, levels of GPX4, Fe2+, MDA, SOD, GSH, GSH/GSSG, FTH1, and SLC7A11.

2.4 Data analysis

Risk of bias assessment: Two authors, Liya Chang, and Dongbin Li, independently assessed the risk of bias using the Systematic Review Center for Laboratory Animal Experiments (SYRCLE) risk of bias tool(11). In cases of disagreement, a third author, Yang Xing, was consulted to reach a consensus.

Assessment of heterogeneity: When $I^2 \leq 50\%$, heterogeneity was considered small, and a fixed-effects model was employed. In contrast, $I^2 > 50\%$ indicated moderate heterogeneity, and a random-effects model was used. For $I^2 \geq 75\%$, heterogeneity was deemed significant(12).

Data analysis: Data synthesis was performed using Review Manager 5.4 software. The standardized mean difference (SMD) and 95% confidence interval (CI) were used to analyze continuous data(12). For $I^2 > 50\%$, heterogeneity sources were explored through subgroup and sensitivity analyses. A descriptive analysis was performed if $I^2 \geq 75\%$ and heterogeneity sources could not be resolved. Funnel plots were generated if more than 10 studies were included.

3. Result

3.1 Study selection

A total of 105 articles were retrieved from the five databases. After removing 50 duplicates, 39 additional articles were excluded after reviewing titles and abstracts. The remaining 16 articles were independently evaluated in their entirety by two authors based on predetermined inclusion and exclusion criteria, resulting in the inclusion of 11 studies(13-23). The retrieval process is illustrated in Fig. 1.

3.2 Basic characteristics of the included studies

A total of 11 studies involving 202 mice were included in the meta-analysis. The main characteristics of these studies were summarized, including 1) first author; 2) year of publication; 3) language; 4) species and gender of animals; 5) age and weight of animals; 6) ischemia/reperfusion time; and 7) anesthetic used (Table 2).

3.3 Quality assessment of research

The quality of the 11 included studies was independently assessed using a 10-item systematic review scale for animal experiments based on the SYRCLE. The results are presented in Fig. 2. Most of the included studies achieved a score of 6. All studies adequately described baseline characteristics, reported random housing of animals, and provided complete outcome data without selective reporting. However, three studies did not provide detailed information about their randomization methods for the allocation sequence (17, 18, 20). Furthermore, most studies lacked sufficient information regarding the blinding of trial caregivers and researchers, the concealment of allocation sequence methods, and the random selection of animals for outcome assessment. While the overall scores indicate a moderate quality level, the quality of the studies was considered average due to these limitations.

3.4 Effectiveness of primary indicators

To evaluate the success of intestinal IRI injury modeling and the extent of ferroptosis, Chiu's score and key ferroptosis markers, such as Fe^{2+} and GPX4, were selected as the primary indicators.

3.4.1 Chiu's score

The degree of mucosal injury following intestinal IRI was assessed using Chiu's score, which was reported in nine of the included studies. Six of these studies used a 45-minute ischemic duration, while three studies used a 60-minute ischemic duration. As shown in Fig. 3A, intestinal IRI significantly increased mucosal injury scores compared to the control group (I^2 = 77%, SMD = 4.97, 95% CI = 3.36 to 6.57, P < 0.00001). When comparing the 60-minute ischemia group (I^2 = 78%, SMD = 4.86, 95% CI = 2.01 to 7.70, P = 0.0008) to the 45-minute ischemia group, a higher incidence of mucosal injury was observed in the latter (I^2 = 72%, SMD = 5.33, 95% CI = 3.23 to 7.42, P < 0.00001). Sensitivity analyses demonstrated stable outcomes for Chiu's score, confirming the reliability of the results.

3.4.2 Fe^{2+}

Iron accumulation contributes to the activation of ROS, which damages cells

and acts as a key marker of ferroptosis. Fe^{2+} levels were examined in eight studies, six of which involved a 45-minute ischemic duration and two with a 60-minute duration. Fe^{2+} levels were significantly elevated in the intestinal IRI group compared to the control group (I^2 = 83%, SMD = 4.31, 95% CI = 2.61 to 6.01, P < 0.00001). Notably, heterogeneity within the 60-minute ischemia subgroup was reduced (I^2 = 50%), suggesting that ischemic duration could account for the observed heterogeneity in Fe^{2+} levels. The incidence of elevated Fe^{2+} levels was significantly higher in the 45-minute ischemia group compared to the 60-minute group (I^2 = 82%, SMD = 6.05, 95% CI = 3.28 to 8.82, P < 0.0001) (Fig. 3B). Sensitivity analyses confirmed the stability of the outcomes for Fe^{2+}.

3.4.3 GPX4

GPX4, a critical enzyme involved in the ferroptosis pathway, was evaluated in all included studies. Of these, eight involved a 45-minute ischemic duration, and three involved a 60-minute duration. The results indicated a significant decrease in GPX4 levels following intestinal IRI (I^2 = 87%, SMD = -4.43, 95% CI = -6.01 to -2.85, P < 0.00001), highlighting the role of GPX4 in regulating ferroptosis. Among the subgroups, the 60-minute ischemia group showed less heterogeneity (I^2 = 23%), further suggesting that ischemic duration contributed to the heterogeneity in GPX4 levels. A more significant decline in GPX4 was observed in the 45-minute ischemia group compared to the 60-minute group (I^2 = 90%, SMD = -5.95, 95% CI = -8.46 to -3.44, P < 0.00001), indicating that the 45-minute ischemic model was superior to the 60-minute model in inducing ferroptosis (Fig. 3E).

3.5 Effectiveness of secondary indicators

Key indicators of oxidative stress, including MDA and SOD, as well as ferroptosis regulators such as GSH, GSH/GSSG, FTH1, and SLC7A11, were selected as secondary indicators for evaluating the impact of intestinal IRI.

3.5.1 MDA and SOD levels

A summary of the studies assessing MDA levels in comparison to SOD levels indicated that MDA was significantly elevated in the intestinal IRI group compared to the control group (I^2 = 75%, SMD = 5.41, 95% CI = 3.55 to 7.27, P < 0.00001). This suggests that intestinal IRI exacerbates oxidative stress in intestinal tissues. In the subgroup analysis, the 45-minute ischemia group showed a significantly higher incidence of elevated MDA levels compared to the 60-minute ischemia group (I^2 = 81%, SMD = 7.01, 95% CI = 3.83 to 10.19, P < 0.0001) (Fig. 3C). Three studies investigated SOD levels, and the pooled data demonstrated that intestinal IRI led to a reduction in SOD levels (I^2 = 84%, SMD = -5.64, 95% CI = -10.14 to -1.14, P = 0.01) (Fig. 3D).

3.5.2 Ferroptosis factor levels

Pooling the data from studies that assessed GSH, GSH/GSSG, FTH1, and SLC7A11 revealed significant reductions in these ferroptosis-related factors following intestinal IRI. The reductions were observed for GSH (I^2 = 58%, SMD = -2.45, 95% CI = -3.33 to -1.57, P < 0.00001) (Fig. 4A), GSH/GSSG (I^2 = 70%, SMD = -3.69, 95% CI = -5.17 to -2.21, P < 0.00001) (Fig. 4B), FTH1 (I^2 = 79%, SMD = -3.10, 95% CI = -4.61 to -1.59, P < 0.0001) (Fig. 4C), and SLC7A11 (I^2 = 83%, SMD = -3.61, 95% CI = -6.70 to -0.53, P = 0.02) (Fig. 4D). These findings suggest that intestinal IRI inhibits key negative regulators of ferroptosis. In subgroup analyses for GSH, the 45-minute ischemia group had a greater incidence of reduced GSH levels compared to the 60-minute ischemia group (I^2 = 74%, SMD = -3.14, 95% CI = -4.91 to -1.37, P = 0.0005). Similar results were observed for GSH/GSSG (I^2 = 72%, SMD = -4.55, 95% CI = -7.26 to -1.84, P = 0.001) and FTH1 (I^2 = 89%, SMD = -5.43, 95% CI = -10.67 to -0.20, P = 0.04). These findings further indicate that the ferroptosis was more effectively triggered after 45 minutes of ischemia than 60 minutes when modeling intestinal IRI in mice.

3.6 Sensitivity analysis

Sensitivity analyses were conducted to evaluate the stability of the results.

Primary indicators, including Chiu's score, Fe^{2+}, and GPX4, remained unaffected, with no changes in heterogeneity and yielding statistically significant outcomes. Secondary indicators, such as MDA, GSH, GSH/GSSG, and FTH1, also retained statistical significance after excluding specific studies. Notably, the exclusion of the study by Xinrun Wang (2023) resulted in an I^2 value of 0 for GSH, suggesting this study may have contributed to the heterogeneity observed in GSH levels. However, the results for SOD and SLC7A11 proved to be unstable, as the exclusion of certain studies rendered these outcomes no longer statistically significant.

4. Discussion

This study evaluated the changes in intestinal mucosal damage and various ferroptosis-related factors at the onset of intestinal IRI. The development of an accurate animal model significantly informs clinical studies. Our meta-analysis of the 11 included studies indicated that during intestinal IRI, the intestinal injury indices, including Chiu's score and MDA levels, increased while SOD levels decreased. Moreover, ferroptosis-related factors such as Fe^{2+} levels increased, while GPX4, GSH, GSH/GSSG ratio, FTH1, and SLC7A11 levels decreased. Notably, the intestinal IRI model demonstrated better results with 45 minutes of ischemia than with 60 minutes.

The intestine plays a central role as an immune organ and regulates various bodily functions. Under pathological conditions, blood supply is significantly reduced, resulting in IRI, which damages the intestine through mechanisms such as vasoconstriction, inflammatory responses, and cell death(24). Oxidative stress, defined as an imbalance between oxidants and antioxidants, is a key component of intestinal IRI(25). MDA, a product formed by the reaction between lipids and oxygen radicals, serves as an indicator of lipid peroxidation. In contrast, SOD is a superoxide-specific scavenger that catalyzes the decomposition of the superoxide anion into oxygen (O_2) and hydrogen peroxide (H_2O_2), thus mitigating oxidative stress(26). The oxidative stress imbalance under pathological conditions directly impacts MDA and SOD levels(27), making these biomarkers crucial for evaluating intestinal injury. Shen

et al. found that intestinal IRI led to increased Chiu's scores, elevated MDA levels, and decreased SOD levels(28), findings corroborated by our meta-analysis. Furthermore, it was demonstrated that the deviation between Chiu's score and MDA levels was greater with 45 minutes of ischemia compared to 60 minutes. This suggests that a 45-minute ischemia duration yields more consistent results for developing an animal model of intestinal IRI.

Ferroptosis, a novel form of regulated cell death, has emerged as a promising target for mitigating intestinal IRI. Ferroptosis operates through various mechanisms, with the Cyst(e)ine/GSH/GPX4 pathway being the most classical. This pathway involves the transmembrane cyst(e)ine-glutamate antiporter System Xc-, which transports cyst(e)ine into the cytosol to synthesize GSH. GPX4 plays a critical role in this process by converting GSH to GSSG, thereby reducing ROS production and protecting cells from lipid peroxidation(8). Another crucial component of ferroptosis regulation is System Xc-, an amino acid anti-transporter composed of two subunits: SLC7A11 and SLC3A2. Inhibition of System Xc- affects GSH synthesis, leading to reduced GPX4 activity, lipid ROS accumulation, and, ultimately, ferroptosis(29). Furthermore, Fe^{2+}, stored in the labile iron pool (LIP), and ferritin (composed of ferritin light chain [FTL] and FTH1), play a key role in ferroptosis. Disruption of iron metabolism can significantly upregulate FTL and FTH1, thereby inhibiting ferroptosis(30).

This study synthesized the changes in ferroptosis-related factors following intestinal IRI, showing that IRI increased Fe^{2+} levels while decreasing GPX4, GSH, GSH/GSSG, FTH1, and SLC7A11. Notably, the subgroup analysis indicated that the deviations in Fe^{2+}, GPX4, GSH, GSH/GSSG, and FTH1 from the control group were greater in the 45-minute ischemia group compared to the 60-minute ischemia group. This finding further supports the conclusion that a 45-minute ischemia duration is optimal for studying changes in ferroptosis-related factors in the intestinal IRI animal model. The insights gained regarding ferroptosis-related mechanisms during intestinal IRI can serve as a foundation for developing therapeutic interventions in clinical settings. Understanding these pathways opens possibilities for targeting ferroptosis to mitigate tissue damage in surgeries involving IRI, such as bowel

resection or transplantation.

Strengths and limitations

This study represents the first meta-analysis to assess the changes in various ferroptosis-related factors during intestinal IRI and to perform a subgroup analysis based on different ischemic durations to identify the optimal time for model development. This provides a valuable foundation for future animal experiments investigating ferroptosis in intestinal IRI. However, there are several limitations to the study. First, the overall quality of the included studies was relatively average, with some studies lacking raw data, which hindered certain aspects of the meta-analysis. Second, the amount of data obtained from animal experiments was small, suggesting that future research may require larger sample sizes to validate the findings. Lastly, the sensitivity analyses revealed certain studies with a significant impact on the heterogeneity of the results, indicating that despite similar quality assessment scores, some studies had unstable outcomes, which could affect the reliability of the meta-analysis results. Further high-quality research is essential to validate these findings and refine the model.

5. Conclusion

This meta-analysis assessed the changes in ferroptosis-related factors during intestinal IRI and demonstrated that Chiu's score, MDA, and Fe^{2+} levels significantly increased, while GPX4, GSH, GSH/GSSG, FTH1, and SLC7A11 levels decreased. The results indicated that the 45-minute ischemia duration yielded more consistent and favorable outcomes than the 60-minute one, providing strong evidence for future animal model preparation and experimental design. These findings offer valuable insights into optimizing the modeling of intestinal IRI and contribute to understanding ferroptosis in this context.

References

1. Eltzschig HK, Eckle T. Ischemia and reperfusion--from mechanism to translation. Nat Med. 2011;17(11):1391-401. DOI:10.1038/nm.2507

2. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Ischemia/Reperfusion. Compr

Physiol. 2016;7(1):113-70. DOI:10.1002/cphy.c160006

3. Tang J, Zhuang S. Histone acetylation and DNA methylation in ischemia/reperfusion injury. Clin Sci (Lond). 2019;133(4):597-609. DOI:10.1042/cs20180465

4. Li G, Wang S, Fan Z. Oxidative Stress in Intestinal Ischemia-Reperfusion. Front Med (Lausanne). 2021;8:750731. DOI:10.3389/fmed.2021.750731

5. Tang D, Chen X, Kang R, Kroemer G. Ferroptosis: molecular mechanisms and health implications. Cell Res. 2021;31(2):107-25. DOI:10.1038/s41422-020-00441-1

6. Xu S, He Y, Lin L, et al. The emerging role of ferroptosis in intestinal disease. Cell Death Dis. 2021;12(4):289. DOI:10.1038/s41419-021-03559-1

7. Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. Nat Rev Mol Cell Biol. 2021;22(4):266-82. DOI:10.1038/s41580-020-00324-8

8. Li J, Cao F, Yin HL, et al. Ferroptosis: past, present and future. Cell Death Dis. 2020;11(2):88. DOI:10.1038/s41419-020-2298-2

9. Zhou P, Zhang S, Wang M, Zhou J. The Induction Mechanism of Ferroptosis, Necroptosis, and Pyroptosis in Inflammatory Bowel Disease, Colorectal Cancer, and Intestinal Injury. Biomolecules. 2023;13(5). DOI:10.3390/biom13050820

10. Gonzalez LM, Moeser AJ, Blikslager AT. Animal models of ischemia-reperfusion-induced intestinal injury: progress and promise for translational research. Am J Physiol Gastrointest Liver Physiol. 2015;308(2):G63-75. DOI:10.1152/ajpgi.00112.2013

11. Hooijmans CR, Rovers MM, de Vries RB, et al. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 2014;14:43. DOI:10.1186/1471-2288-14-43

12. Huang BH, Guo ZW, Lv BH, et al. A role for curcumin in preventing liver fibrosis in animals: a systematic review and meta-analysis. Front Pharmacol. 2024;15:1396834. DOI:10.3389/fphar.2024.1396834

13. Zhao X, Wang Z, Wu G, et al. Apigenin-7-glucoside-loaded nanoparticle alleviates intestinal ischemia-reperfusion by ATF3/SLC7A11-mediated ferroptosis. J Control Release. 2024;366:182-93. DOI:10.1016/j.jconrel.2023.12.038

14. Wang Y, Li B, Liu G, et al. Corilagin attenuates intestinal ischemia/reperfusion injury in mice by inhibiting ferritinophagy-mediated ferroptosis through disrupting

NCOA4-ferritin interaction. Life Sci. 2023;334:122176. DOI:10.1016/j.lfs.2023.122176

15. Zhu L, Lian W, Yao Z, et al. Integrated Analysis of Ferroptosis and Immunity-Related Genes Associated with Intestinal Ischemia/Reperfusion Injury. J Inflamm Res. 2022;15:2397-411. DOI:10.2147/jir.S351990

16. Li Y, Feng D, Wang Z, et al. Ischemia-induced ACSL4 activation contributes to ferroptosis-mediated tissue injury in intestinal ischemia/reperfusion. Cell Death Differ. 2019;26(11):2284-99. DOI:10.1038/s41418-019-0299-4

17. Li K, Wang A, Diao Y, Fan S. Oxidative medicine and cellular longevity the role and mechanism of NCOA4 in ferroptosis induced by intestinal ischemia reperfusion. Int Immunopharmacol. 2024;133:112155. DOI:10.1016/j.intimp.2024.112155

18. Wang X, Shen T, Lian J, et al. Resveratrol reduces ROS-induced ferroptosis by activating SIRT3 and compensating the GSH/GPX4 pathway. Mol Med. 2023;29(1):137. DOI:10.1186/s10020-023-00730-6

19. Ma X, Wang G, Yu W. Role of nuclear receptor coactivator 4-mediated ferritinophagy in intestinal ischemia-reperfusion injury in mice: relationship with ferroptosis. Chinese Journal of Anesthesiology. 2022;42(8):980-4. DOI:10.3760/cma.j.cn131073.20220327.00819

20. Zhang LL, Ding K, Liao SS, et al. Sestrin2 reduces ferroptosis via the Keap1/Nrf2 signaling pathway after intestinal ischemia-reperfusion. Free Radic Biol Med. 2024;214:115-28. DOI:10.1016/j.freeradbiomed.2024.02.003

21. Zhang T, Zhang L, Hao Y, et al. Effect of SLC7A11 in dexmedetomidine pretreatment induced reduction of ferroptosis caused by intestinal ischemia-reperfusion injury in mice. Chinese Journal of Clinical Pharmacology and Therapeutics. 2021;26(7):738-43. DOI:10.12092/j.issn.1009-2501.2021.07.003

22. Wang X, Leng Y, Gao H, Jia X. Role of STAT3⁄NCOA4-mediated ferritinophagy in salidroside-induced attenuation of intestinal is- chemia-reperfusion injury in mice: relationship with ferroptosis. Chinese Journal of Anesthesiology. 2023;43(11):1316-21. DOI:10.3760/cma.j.cn131073.20230722.01108

23. Deng F, Zhao BC, Yang X, et al. The gut microbiota metabolite capsiate promotes Gpx4 expression by activating TRPV1 to inhibit intestinal ischemia reperfusion-induced ferroptosis. Gut Microbes. 2021;13(1):1-21.

DOI:10.1080/19490976.2021.1902719

24. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol. 2012;298:229-317. DOI:10.1016/b978-0-12-394309-5.00006-7

25. Refaie MMM, Amin EF, Hassan MN, et al. Sacubitril/valsartan protective effect on induced intestinal ischemia/reperfusion injury via immune modulation of IL6/STAT1 pathway. J Pharm Pharmacol. 2024;76(7):788-97. DOI:10.1093/jpp/rgae031

26. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev. 2014;94(2):329-54. DOI:10.1152/physrev.00040.2012

27. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014:360438. DOI:10.1155/2014/360438

28. Shen J, Zhan Y, He Q, et al. Remifentanil Promotes PDIA3 Expression by Activating p38MAPK to Inhibit Intestinal Ischemia/Reperfusion-Induced Oxidative and Endoplasmic Reticulum Stress. Front Cell Dev Biol. 2022;10:818513. DOI:10.3389/fcell.2022.818513

29. Jiang L, Kon N, Li T, et al. Ferroptosis as a p53-mediated activity during tumour suppression. Nature. 2015;520(7545):57-62. DOI:10.1038/nature14344

30. Gammella E, Recalcati S, Rybinska I, et al. Iron-induced damage in cardiomyopathy: oxidative-dependent and independent mechanisms. Oxid Med Cell Longev. 2015;2015:230182. DOI:10.1155/2015/230182

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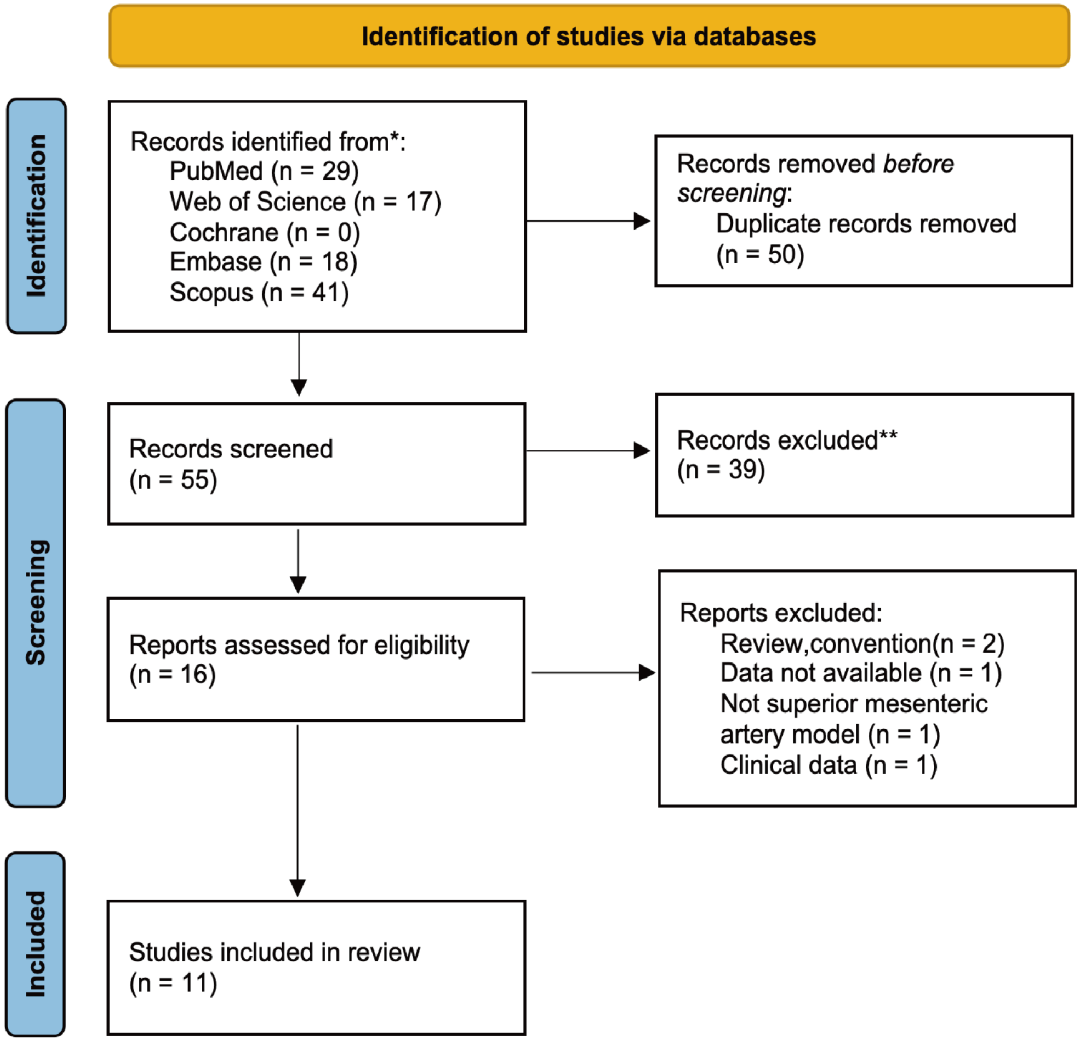

Fig. 1.

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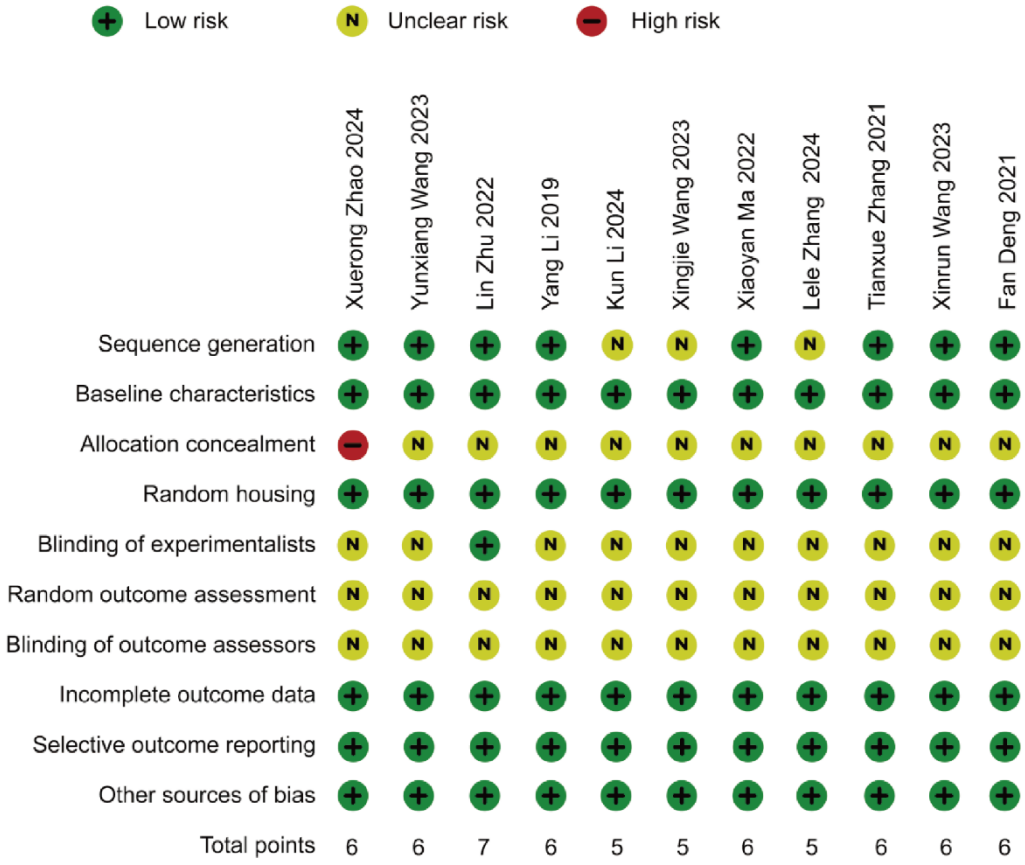

Fig. 2.

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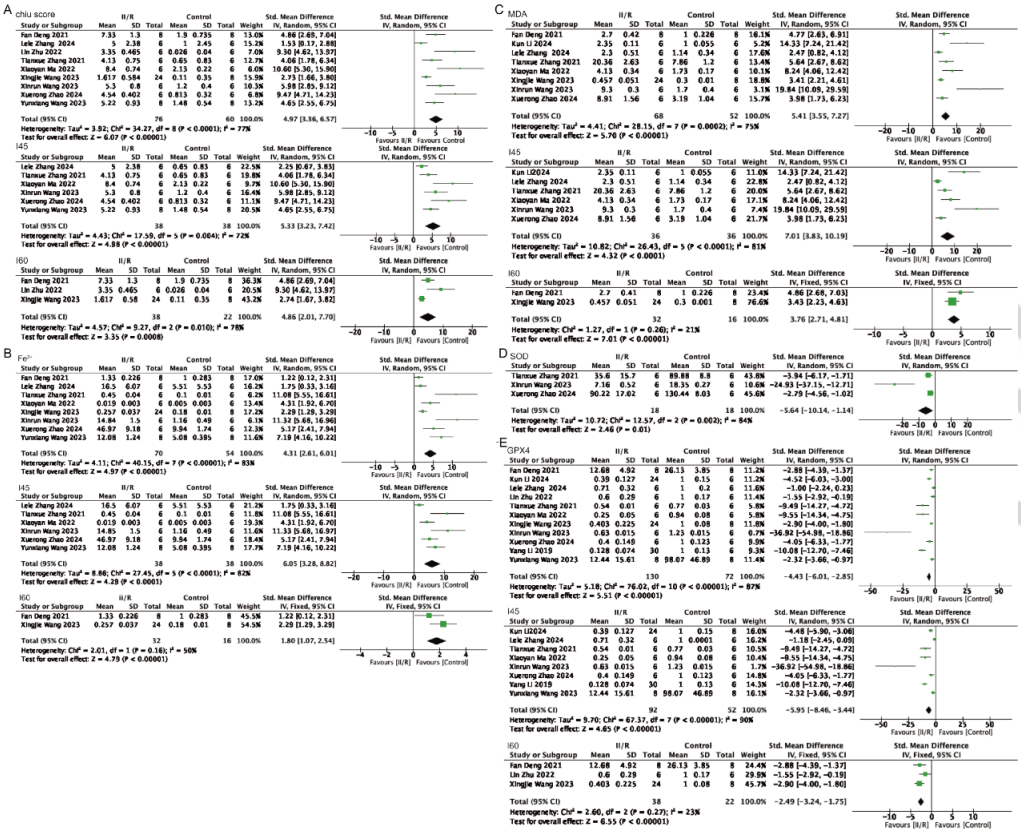

Fig. 3.

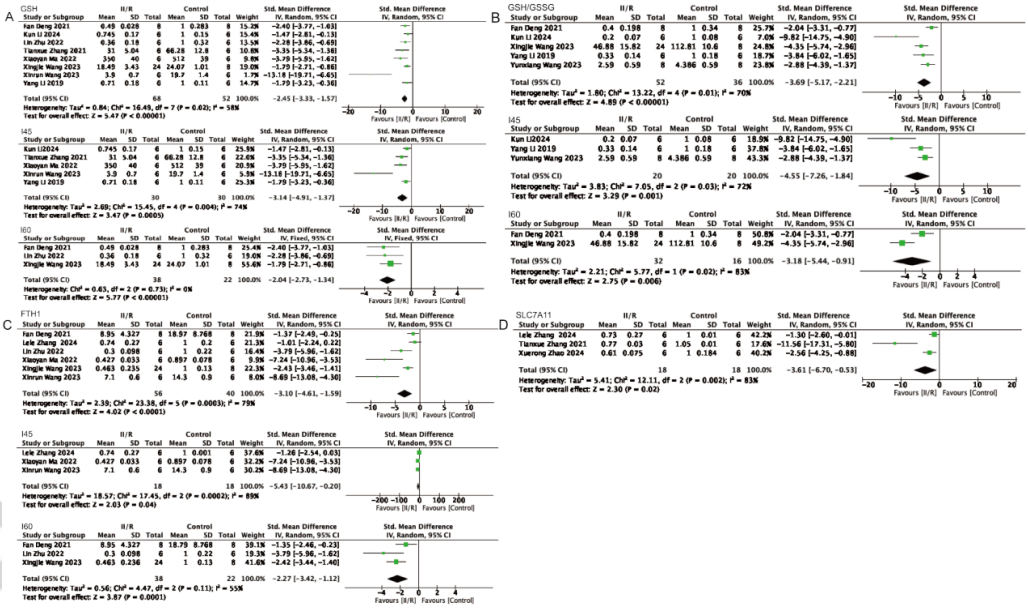

Fig. 4.

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Table 1: Search strategy

		Search	Results
WOS	#1	Reperfusion Injury (Topic) OR Reperfusion Injuries (Topic) OR Injury, Ischemia-Reperfusion (Topic) OR Injury, Ischemia Reperfusion (Topic) OR Ischemia-Reperfusion Injuries (Topic) OR Injury, Reperfusion (Topic) OR Ischemia-Reperfusion Injury (Topic) OR Ischemia Reperfusion Injury (Topic) OR Reperfusion Damage (Topic) OR Damage, Reperfusion (Topic) OR Reperfusion Damages (Topic) and Preprint Citation Index (Exclude – Database)	149782
	#2	Intestines (Topic) OR Intestine (Topic) and Preprint Citation Index (Exclude – Database)	451774
	#3	Ferroptosis (Topic) OR Oxytosis (Topic) and Preprint Citation Index (Exclude – Database)	16128
	#4	#1 AND #2 AND #3 and Preprint Citation Index (Exclude – Database)	17
Pubmed		("reperfusion injury"[MeSH Terms] OR ("reperfusion"[All Fields] AND "injury"[All Fields]) OR "reperfusion injury"[All Fields] OR ("reperfusion injury"[MeSH Terms] OR ("reperfusion"[All Fields] AND "injury"[All Fields]) OR "reperfusion injury"[All Fields] OR ("reperfusion"[All Fields] AND "injuries"[All Fields]) OR "reperfusion injuries"[All Fields]) OR ("reperfusion injury"[MeSH Terms] OR ("reperfusion"[All Fields] AND "injury"[All Fields]) OR "reperfusion injury"[All Fields] OR ("injury"[All Fields] AND "ischemia"[All Fields] AND "reperfusion"[All Fields]) OR "injury ischemia reperfusion"[All Fields]) OR ("reperfusion injury"[MeSH Terms] OR ("reperfusion"[All Fields] AND "injury"[All Fields]) OR "reperfusion injury"[All Fields] OR ("injury"[All Fields] AND "ischemia"[All Fields] AND "reperfusion"[All Fields]) OR "injury ischemia reperfusion"[All Fields]) OR ("reperfusion injury"[MeSH Terms] OR ("reperfusion"[All Fields] AND "injury"[All Fields]) OR "reperfusion injury"[All Fields] OR ("ischemia"[All Fields] AND "reperfusion"[All Fields] AND "injuries"[All Fields]) OR "ischemia reperfusion injuries"[All Fields]) OR ("reperfusion injury"[MeSH Terms] OR ("reperfusion"[All Fields] AND "injury"[All Fields]) OR "reperfusion injury"[All Fields] OR ("injury"[All Fields] AND "reperfusion"[All Fields]) OR "injury reperfusion"[All Fields]) OR ("ischaemia reperfusion injury"[All Fields] OR "reperfusion injury"[MeSH Terms] OR ("reperfusion"[All Fields] AND "injury"[All Fields]) OR "reperfusion injury"[All Fields] OR ("ischemia"[All Fields] AND "reperfusion"[All Fields] AND "injury"[All Fields]) OR "ischemia reperfusion injury"[All Fields]) OR ("ischaemia reperfusion injury"[All Fields] OR "reperfusion injury"[MeSH Terms] OR ("reperfusion"[All Fields] AND "injury"[All Fields]) OR "reperfusion injury"[All Fields] OR ("ischemia"[All Fields] AND "reperfusion"[All Fields] AND "injury"[All Fields]) OR "ischemia reperfusion injury"[All Fields]) OR ("reperfusion injury"[MeSH Terms] OR ("reperfusion"[All Fields] AND "injury"[All Fields]) OR "reperfusion injury"[All Fields] OR ("reperfusion"[All Fields] AND "damage"[All Fields]) OR "reperfusion damage"[All Fields]) OR ("reperfusion injury"[MeSH Terms] OR ("reperfusion"[All Fields] AND "injury"[All Fields]) OR "reperfusion injury"[All Fields] OR ("damage"[All Fields] AND "reperfusion"[All Fields]) OR "damage reperfusion"[All Fields]) OR ("reperfusion injury"[MeSH Terms] OR ("reperfusion"[All Fields] AND "injury"[All Fields]) OR "reperfusion injury"[All Fields] OR ("reperfusion"[All Fields] AND "damages"[All Fields]) OR "reperfusion damages"[All Fields])) AND ("intestinalization"[All Fields] OR "intestinalized"[All Fields] OR "intestinally"[All Fields] OR "intestinals"[All Fields] OR "intestine s"[All Fields] OR "intestines"[MeSH Terms] OR "intestines"[All Fields] OR "intestinal"[All Fields] OR "intestine"[All Fields] OR ("intestinalization"[All Fields] OR "intestinalized"[All Fields] OR "intestinally"[All Fields] OR "intestinals"[All Fields] OR "intestine s"[All Fields] OR "intestines"[MeSH Terms] OR "intestines"[All Fields] OR "intestinal"[All Fields] OR "intestine"[All Fields])) AND ("ferroptosis"[MeSH Terms] OR "ferroptosis"[All Fields] OR ("ferroptosis"[MeSH Terms] OR "ferroptosis"[All Fields] OR "oxytosis"[All Fields]))	29
Cochrane	#1	(Injury, Reperfusion):ti,ab,kw OR (Ischemia-Reperfusion Injury):ti,ab,kw OR (Ischemia Reperfusion Injury):ti,ab,kw	3710
	#2	(Reperfusion Injury):ti,ab,kw OR (Reperfusion Injuries):ti,ab,kw OR (Injury, Ischemia-Reperfusion):ti,ab,kw OR (Injury, Ischemia Reperfusion):ti,ab,kw OR (Ischemia-Reperfusion Injuries):ti,ab,kw	3710
	#3	(Reperfusion Damage):ti,ab,kw OR (Damage, Reperfusion):ti,ab,kw OR (Reperfusion Damages):ti,ab,kw	929
	#4	#1 OR #2 OR #3	3953
	#5	(Intestines):ti,ab,kw OR (Intestine):ti,ab,kw	33100
	#6	(Ferroptosis):ti,ab,kw OR (Oxytosis):ti,ab,kw	27
	#7	#4 AND #5 AND #6	0
Embase	#4	#1 AND #2 AND #3	18

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	#3	'ferroptosis'/exp OR ferroptosis OR oxytosis:ti,ab,kw	15781
	#2	'intestines'/exp OR intestines OR intestine:ti,ab,kw	682094
	#1	reperfusion injury'/exp OR 'reperfusion injury' OR (('reperfusion'/exp OR reperfusion) AND ('injury'/exp OR injury)) OR 'reperfusion injuries':ti,ab,kw OR 'injury, ischemia-reperfusion':ti,ab,kw OR 'injury, ischemia reperfusion':ti,ab,kw OR 'ischemia-reperfusion injuries':ti,ab,kw OR 'injury, reperfusion':ti,ab,kw OR 'ischemia-reperfusion injury':ti,ab,kw OR 'ischemia reperfusion injury':ti,ab,kw OR 'reperfusion damage':ti,ab,kw OR 'damage, reperfusion':ti,ab,kw OR 'reperfusion damages':ti,ab,kw	110248
Scopus		((TITLE-ABS-KEY (reperfusion AND injury) OR TITLE-ABS-KEY (reperfusion AND injuries) OR TITLE-ABS-KEY (injury, AND ischemia-reperfusion) OR TITLE-ABS-KEY (injury, AND ischemia AND reperfusion) OR TITLE-ABS-KEY (ischemia-reperfusion AND injuries) OR TITLE-ABS-KEY (injury, AND reperfusion) OR TITLE-ABS-KEY (ischemia-reperfusion AND injury) OR TITLE-ABS-KEY (ischemia AND reperfusion AND injury) OR TITLE-ABS-KEY (reperfusion AND damage) OR TITLE-ABS-KEY (damage, AND reperfusion) OR TITLE-ABS-KEY (reperfusion AND damages))) AND ((TITLE-ABS-KEY (intestines) OR TITLE-ABS-KEY (intestine))) AND ((TITLE-ABS-KEY (ferroptosis) OR TITLE-ABS-KEY (oxytosis)))	41

Table 2. Characteristics of included studies. w: week(s): U: unclear: min: minute(s).

Name	Year	Country	Language	Species/Gender	Age/Weight	I/R	Anesthetic
Xuerong Zhao	2	China	English	C57BL6/Male	8-12w/22-25g	45min/90min	3-4% isoflurane
Yunxiang Wang	2	China	English	C57BL6J/Male	6w/U	45min/60min	Tribromoethanol (400 mg/kg)
Lin Zhu	2	China	English	C57BL6J/Male	7-8w/20-25g	60min/240min	N
Yang Li	2	China	English	C57BL6/Male	8w/U	30,45,60 min/15,30,60, 120,240min	Pentobarbital (50 mg/kg)

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Kun Li	2	China	English	C57BL6J/Male	8w/18-22g	45min/0, 30,60,120,240 min	Pentobarbital (50 mg/kg)
Xingjie Wang	2	China	English	C57BL6/Male	6-8w/18-22g	60min/15,30,60min	5% isoflurane
Xiaoyan Ma	2	China	Chinese	C57BL6/Male	8-10w/21-25g	45min/120min	5% pentasorbital sodium (0.4ml/100g)
Lele Zhang	2	China	English	C57BL6/Male	6-8w/U	45min/120min	pentobarbital (35–40 mg/kg)
Tianxue Zhang	2	China	Chinese	C57BL6J/Male	8w/22-25g	45min/30 min	1% pentasorbital sodium (50mg/kg)
Xinrun Wang	2	China	Chinese	C57BL6/Male	6-8w/20-25g	45min/30 min	3% pentasorbital sodium (30mg/kg)
Fan Deng	2	China	English	C57BL6/Male	6-8w/U	60min/120min	isoflurane