Efficacy of tanshinone IIA for myocardial ischemia-reperfusion injury in rat models: a systematic review and meta-analysis (#84763)

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Efficacy of tanshinone IIA for myocardial ischemia-reperfusion injury in rat models: aystematic review and meta-analysis

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Background: Myocardial ischemia-reperfusion injury (MIRI) is severe damage to the ischemic myocardium when blood flow is restored, and it is a major complication of reperfusion therapy for myocardial infarction. Tanshinone IIA is a major constituent extracted from Salvia Miltiorrhza Bunge. Tanshinone IIA had garnered attention due to its preventative and therapeutic effects on cardiovascular diseases. In recent years, it has been used to treat MIRI in numerous animal studies. Therefore, we performed a meta-analysis on the application of tanshinone IIA in rats models with MIRI to assess the therapeutic effects of tanshinone IIA. **Methods:** A comprehensive search of PubMed, Web of Science, Embase, Cochrane Library, China National Knowledge Infrastructure database, Wanfang database, and Chinese scientific journal database was conducted to obtain studies of tanshinone IIA intervention in rats models with MIRI. We utilized SYRCLE's Risk of Bias tool to assess the quality of studies. Assessment of tanshinone IIA treatment efficacy based on superoxide dismutase (SOD), methane dicarboxylic aldehyde (MDA), and myocardial infarction area results; and subgroup analysis using ischemia duration, reperfusion duration, dosage, and route. **Results:** According to inclusion and exclusion criteria, eleven eligible studies were selected from 274 studies. In rats models with MIRI, tanshinone IIA dramatically boosted SOD levels while decreasing MDA levels and myocardial infarction area. In addition, the duration of myocardial ischemia and reperfusion duration impacted the efficacy of tanshinone IIA. At the same time, more high-quality research studies are required to determine the effectiveness and guiding criteria of tanshinone IIA. Animal studies showed that tanshinone IIA had a significant therapeutic influence on ischemia duration of less than 40 min and reperfusion duration of less than 180 min and that it was more effective when supplied through intravenous injection, intraperitoneal injection, and intragastric at doses of above 5 mg/kg. **Conclusions:** Tanshinone IIA can enhance SOD activity and decline MDA levels to ameliorate oxidative stress damage during MIRI. In addition, tanshinone IIA could decrease myocardial

infarction area, suggesting that it mitigated the damage caused by MIRI in rats and had a myocardial protective effect. These findings can further inform the MIRI treatment strategy.

Efficacy of Tanshinone IIA for Myocardial Ischemia-Reperfusion Injury in Rats Models: A Systematic Review and Meta-Analysis

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ABSTRCT

Background: Myocardial ischemia-reperfusion injury (MIRI) is severe damage to the ischemic myocardium when blood flow is restored, and it is a major complication of reperfusion therapy for myocardial infarction. Tanshinone IIA is a major constituent extracted from Salvia Miltiorrhza Bunge. Tanshinone IIA had garnered attention due to its preventative and therapeutic effects on cardiovascular diseases. In recent years, it has been used to treat MIRI in numerous animal studies. Therefore, we performed a meta-analysis on the application of tanshinone IIA in rats models with MIRI to assess the therapeutic effects of tanshinone IIA.

Methods: A comprehensive search of PubMed, Web of Science, Embase, Cochrane Library, China National Knowledge Infrastructure database, Wanfang database, and Chinese scientific journal database was conducted to obtain studies of tanshinone IIA intervention in rats models with MIRI. We utilized SYRCLE's Risk of Bias tool to assess the quality of studies. Assessment of tanshinone IIA treatment efficacy based on superoxide dismutase (SOD), methane dicarboxylic aldehyde (MDA), and myocardial infarction area results; and subgroup analysis using ischemia duration, reperfusion duration, dosage, and route.

Results: According to inclusion and exclusion criteria, eleven eligible studies were selected from 274 studies. In rats models with MIRI, tanshinone IIA dramatically boosted SOD levels while decreasing MDA levels and myocardial infarction area. In addition, the duration of myocardial ischemia and reperfusion duration impacted the efficacy of tanshinone IIA. At the same time, more

high-quality research studies are required to determine the effectiveness and guiding criteria of tanshinone IIA. Animal studies showed that tanshinone IIA had a significant therapeutic influence on ischemia duration of less than 40 min and reperfusion duration of less than 180 min and that it was more effective when supplied through intravenous injection, intraperitoneal injection, and intragastric at doses of above 5 mg/kg.

Conclusions: Tanshinone IIA can enhance SOD activity and decline MDA levels to ameliorate oxidative stress damage during MIRI. In addition, tanshinone IIA could decrease myocardial infarction area, suggesting that it mitigated the damage caused by MIRI in rats and had a myocardial protective effect. These findings can further inform the MIRI treatment strategy.

Keywords Myocardial ischemia-reperfusion injury, Tanshinone IIA, Superoxide dismutase, Methane dicarboxylic aldehyde, Myocardial infarction area, Systematic review, Meta-analysis

Introduction

According to the World Health Organization (WHO), ischemic heart disease remains the primary cause of death worldwide, accounting for 16% of all deaths (*Wang et al. 2022*). As the world's population ages, the incidence of acute myocardial infarction rises annually (*Reynolds et al. 2017*). Acute myocardial infarction therapies significantly influence the global population's economic burdens *(Pasala et al. 2022*). Prompt restoration of coronary blood supply is the most effective method for preventing myocardial cell mortality due to myocardial ischemia injury. At this stage, the coronary blood flow is restored chiefly using percutaneous coronary intervention, coronary artery bypass grafting operations, and thrombolytic technology (*Biscaglia et al. 2022*).

Myocardial ischemia-reperfusion injury (MIRI) is a secondary damage in ischemic heart disease. It refers to the sudden restoration of blood flow to the ischemic myocardium, which can lead to cardiac myocyte dysfunction, structural damage to cardiac myocyte cells, and cell death, causing deterioration of cardiac function (*Evans et al. 2020; Liu et al. 2018*). The cellular and molecular events underlying MIRI are complex and driven by multiple pathways, such as oxygen free radicals, energy metabolism disorder, calcium overload, autophagy, apoptosis mitochondrial dysfunction, microvascular injury, etc (*Chen et al. 2023; He et al. 2022*). MIRI's precise mechanism is not yet completely understood. There is no currently comprehensive treatment for MIRI.

Tanshinone IIA is a lipid-soluble diterpenoid isolated from traditional Chinese medicine Salvia Miltiorrhza Bunge (*Huang et al. 2022*). Through anti-inflammatory, lipid-regulating, antioxidant, and anti-apoptotic mechanisms, among others, it can play a preventive role in many cardiovascular disorders (*Li et al. 2018*). It is frequently used to treat people with cardiovascular disease (*Ren et al. 2019*).

MIRI could affect indications such as oxidative stress and myocardial infarction area. The myocardial infarction area can directly indicate the severity of MIRI. During ischemia, reactive oxygen species and reactive nitrogen species, such as superoxide anions, and nitric oxide, are overproduced and impair redox balance, thus leading to oxidative stress *(Chen et al. 2021*). Superoxide dismutase (SOD) and methane dicarboxylic aldehyde (MDA) are essential oxidative stress markers (*Tian et al. 2019*).

This study was undertaken by doing an exhaustive literature review on the intervention of tanshinone IIA in MIRI rats. Its therapeutic effects on rats models with MIRI were systematically evaluated in the hope of providing medical evidence for its clinical application.

Materials and methods

Protocol and registration

This article is a meta-analysis according to Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)(*Page et al. 2021*). The review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (registration number: CRD42022344447).

Search strategy

The computers were employed to search databases such as PubMed, Web of Science, Cochrane Library, Embase, China National Knowledge Infrastructure (CNKI), Wanfang database (Wanfang), and Chinese Scientific Journals database (VIP). The search period spanned from the launch of the individual databases to July 25, 2022. All searches were conducted using both subject and free words. The search phrase will include three components: intervention strategy, illness, and animal species. The only permitted languages were English and Chinese. Two authors (XB, Zhang and HH, Jiang) separately searched English and Chinese databases for all relevant articles using the following terms. The retrieval words include: “Myocardial reperfusion Injury,” “Injuries, Myocardial Reperfusion,” “Myocardial Reperfusion Injuries,” “Reperfusion Injuries,

Myocardial," "Myocardial Ischemic Reperfusion Injury," "Reperfusion Injury, Myocardial," "Injury, Myocardial Reperfusion," "Rats," "Rat," "Rattus," "Rattus norvegicus," "Rats, Norway," "Rats, Laboratory," "Laboratory Rat," "Laboratory Rats," "Rat, Laboratory," "tanshinone IIA." The detailed search strategy is provided in Table S1.

Inclusion and Exclusion Criteria

Included studies met the following criteria: (1) Rats models with MIRI, specifically by ligation of the left anterior descending coronary artery to cause ischemia and reperfusion; (2) Tanshinone IIA as an intervention with no restrictions in terms of dosage, route of administration, or duration of treatment; (3) Randomized controlled trials; (4) Superoxide dismutase (SOD), methane dicarboxylic aldehyde (MDA) and myocardial infarct area as one of the outcome measures.

The following criteria were used to exclude studies: (1) Animal species were not rats; (2) Tanshinone IIA combined with other intervention therapies; (3) The study of clinical case reports, duplicates, reviews, and unrelated studies; (4) Not English and Chinese studies, published after July 25, 2022.

Study selection and data extraction

Two researchers (XB, Zhang and LL, Zhang) independently performed the literature search, data extraction, and cross-checking. With the assistance of a third party, contentious matters were discussed and settled (CJ, Li). Using Endnote X9 software, duplicate material was filtered away to start the screening process. The second phase consisted of reading the titles and abstracts of the literature to eliminate unrelated works. Finally, the whole text of the article was reviewed and evaluated against the inclusion criteria to find those that met them.

Assessment of Risk of Bias in Individual Studies

The SYRCLE's Risk of Bias tool was employed to evaluate the risk bias of experimental animal research (*Sun et al. 2020*). Two authors (XB, Zhang and C, Chen) independently assessed the quality of the included studies using the SYRCLE's risk of bias tool, which analyzed sequence generation, baseline characteristics, allocation concealment, random housing, blinding, random outcome assessments, incomplete outcome data, selective outcome reporting, and other sources of bias. Disagreements on quality assessment were resolved by discussion and agreement. A "yes" indicated that the risk of bias in this study was low, a "no" suggested that the risk of bias was

high, and an "unclear" meant that there were insufficient details to ascertain the study's risk of bias.

Statistical analysis

The meta-analysis was carried out using the RevMan 5.4 and Stata 16.0 software. Compilation of data based on the relative risk (relative risk, RR) with a 95% confidence interval (confidence interval, CI). Standard mean difference (SMD) or mean difference (MD) and 95% CI were implemented on measurement data. When the heterogeneity is minimal ($p > 0.1$, $I^2 < 50\%$), a fixed effect model is used to combine statistics; when the heterogeneity is substantial ($p \leq 0.1$, $I^2 \geq 50\%$), a random effect model is employed. Meta-analysis applying the random-effects model will be conducted to aggregate RR. To identify the origins of heterogeneity, subgroup and sensitivity analyses were performed. Using Egger's test and funnel plots, publication bias was assessed.

Results

Study selection

Two hundred and seventy-four articles were obtained based on the specified article search strategy. Initially, 78 duplicates were eliminated through Endnote X9 software. Secondly, reviewing the titles and abstracts of the articles resulted in the elimination of 153 articles. Reading the whole article led to the elimination of 32 articles. Eventually, 11 articles were included in this meta-analysis (Fig. 1).

Study Characteristics

This meta-analysis consisted of eleven articles, nine written in Chinese (*Dai et al. 2013; Ding et al. 2020; Fu 2006; Hu et al. 2015; Jiao 2016; Ma et al. 2017; Tang et al. 2017; Yang 2010; Zhang & Zhang 2010*) and two written in English (*Li et al. 2016; Yuan et al. 2014*). There were a total of 378 rats enumerated, with 137 in the control group and 241 in the experimental group. Tanshinone IIA was applied to the experimental group, whereas the control group was given normal saline, blank, or carboxymethylcellulose sodium. The technique described in the included article for establishing the rats models with MIRI was ligation and release of the left anterior descending coronary artery. SOD was documented as an outcome measure in six investigations, MDA in five, and myocardial infarct area in six. The characteristics of the included articles are described in

Table 1.

Quality Evaluation

The SYRCLE's Risk of Bias tool was applied to assess the risk of bias in 11 articles. One study (*Tang et al., 2017*) described the randomization method, while other studies just illustrated randomization without describing the randomization method. The experimental group and the control group possessed identical baseline characteristics. Each study presented its findings precisely and comprehensively. None of the studies indicated any other bias, so they were all classified as "low risk." None of the studies provided information regarding allocation concealment, whether animals were housed randomly during the experiment, whether caregivers and/or investigators were blinded, random outcome assessment, or blinding of outcome assessment, hence they were all deemed as “unclear” (Fig. 2). Overall, the included articles were not of particularly high quality. In the included studies, some flaws in the design and implementation of animal experimental methodology need to be addressed to translate fundamental research into clinical investigations feasible.

Meta-Analysis of Primary Outcomes

Superoxide Dismutase

Six of the included studies (*Ding et al., 2020; Tang et al., 2017; Jiao et al., 2016; Hu et al., 2015; Dai et al., 2013; Fu et al., 2006*) reported the impact of tanshinone IIA on SOD levels intervention in rats models with MIRI (experimental group, n = 123; control group, n = 123). Units: U/mol. Due to the heterogeneity of the articles ($p < 0.00001$, $I^2 = 100\%$). We performed the subgroup analysis to investigate the sources of heterogeneity based on ischemia duration (15 min ≤ time < 30 min, 30 min ≤ time < 40 min or 40 min ≤ time ≤ 45 min), reperfusion duration (30 min ≤ time < 120 min, 120min ≤ time < 180min or 180min ≤ time ≤ 480min), dosage (5 $mg \cdot kg^{-1} \cdot d^{-1} \leq$ dosage < 20 $mg \cdot kg^{-1} \cdot d^{-1}$, 20$mg \cdot kg^{-1} \cdot d^{-1} \leq$ dosage < 30$mg \cdot kg^{-1} \cdot d^{-1}$ or 30$mg \cdot kg^{-1} \cdot d^{-1} \leq$ dosage ≤ 60$mg \cdot kg^{-1} \cdot d^{-1}$) and route (i.v, i.g or i.p). The results showed that heterogeneity was significantly lower in the subgroup with the ischemic duration of 40 - 45 min (Table 2) (Supplementary Fig. S1–S4), suggesting that this factor may be the cause of heterogeneity. However, it should be noted that there was still considerable heterogeneity in the remaining subgroups (Table 2) (Supplementary Fig. S1–S4), suggesting that heterogeneity might come from other factors. As

SOD could be susceptible to many reasons. We considered that variations in the testing instruments and methodologies employed in various studies may have been one of the main causes. Aside from the factors listed above, we found no other significant methodological differences between the six studies. Therefore, we employed a random-effects model to combine the data from six studies. According to this meta-analysis, the tanshinone IIA group was able to increase SOD levels significantly more than the control group (MD = 21.95, 95% CI [14.69-29.20], $p < 0.00001$) (Fig. 3).

Methane Dicarboxylic Aldehyde

Five studies (*Jiao et al., 2016; Hu et al., 2015; Zhang et al., 2010; Yang et al., 2010; Fu et al., 2006*) investigated the effect of tanshinone IIA on MDA levels after intervention in rats models with MIRI (experimental group, n = 123; control group, n = 123). Unit: nmol/ml. Because of the heterogeneity of the results ($p < 0.00001$, $I^2 = 99\%$), We conducted a subgroup analysis to ascertain the causes of heterogeneity according to ischemia duration (15 min ≤ time < 30 min, 30 min ≤ time < 40 min or 40 min ≤ time ≤ 45 min), reperfusion duration (30 min ≤ time < 120 min, 120min ≤ time < 180min or 180min ≤ time ≤ 480min), dosage (5 $mg \cdot kg^{-1} \cdot d^{-1}$ ≤ dosage < 20 $mg \cdot kg^{-1} \cdot d^{-1}$, 20$mg \cdot kg^{-1} \cdot d^{-1}$ ≤ dosage < 30$mg \cdot kg^{-1} \cdot d^{-1}$ or 30$mg \cdot kg^{-1} \cdot d^{-1}$ ≤ dosage ≤ 60$mg \cdot kg^{-1} \cdot d^{-1}$) and route (i.v, i.g or i.p). The results showed a decrease in heterogeneity in the subgroup with the dosage of 30 - 60 mg/kg and route of i.v (Table 3) (Supplementary Fig. S5–S8), suggesting that these factors may be responsible for the heterogeneity. However, substantial heterogeneity persisted in the remaining subgroups (Table 3) (Supplementary Fig. S5–S8), suggesting that heterogeneity may have other causes. We made use of a random-effects model. Meta-analysis indicated that the tanshinone IIA group significantly reduced MDA levels compared to the control group (MD = -1.44, 95% CI [-2.06 to -0.82], $p < 0.00001$) (Fig. 4).

Meta-Analysis of Secondary Outcomes

Myocardial infarction area

Six of the included studies (*Ding et al., 2020; Ma et al., 2017; Li et al., 2016; Hu et al., 2015; Yuan et al., 2014; Yang et al., 2010*) examined the impact of tanshinone IIA on myocardial infarction area intervention in rats models with MIRI (experimental group, n = 154; control group, n = 170). Units: %. We executed a subgroup analysis

to determine the origins of heterogeneity based on ischemia duration (15 min ≤ time < 30 min, 30 min ≤ time < 40 min or 40 min ≤ time ≤ 45 min), reperfusion duration (30 min ≤ time < 120 min, 120min ≤ time < 180min or 180min ≤ time ≤ 480min), dosage (5 $mg \cdot kg^{-1} \cdot d^{-1}$ ≤ dosage < 20 $mg \cdot kg^{-1} \cdot d^{-1}$, 20$mg \cdot kg^{-1} \cdot d^{-1}$ ≤ dosage < 30$mg \cdot kg^{-1} \cdot d^{-1}$ or 30$mg \cdot kg^{-1} \cdot d^{-1}$ ≤ dosage ≤ 60$mg \cdot kg^{-1} \cdot d^{-1}$) and route (i.v, i.g or i.p), as the results were heterogeneous ($p < 0.00001$, $I^2 = 100\%$). The results showed that heterogeneity was reduced in the subgroup with an ischemia duration of 40 - 45 min (Table 4) (Supplementary Fig. S9–S12), implying that this factor may be the source of heterogeneity. However, there was also substantial heterogeneity in the remaining subgroups (Table 4) (Supplementary Fig. S9–S12), demonstrating that heterogeneity may have additional sources. A random-effects model was chosen. Compared to the control group, the tanshinone IIA group was able to significantly reduce myocardial infarction area (MD = -10.00, 95% CI [-13.55 to -6.45], $p < 0.00001$) (Fig. 5).

Publication bias analysis

The publication bias of the SOD, MDA, and myocardial infarction area meta-analysis was evaluated using Egger's test and funnel plots, respectively. The results indicated that funnel plots and Egger's tests for each SOD, MDA, and myocardial infarction area revealed poor symmetry in the distribution of the literature, indicating a degree of publication bias that may be attributable to factors such as the poor quality of the included articles (Fig. 6).

Sensitivity analysis

To evaluate the consistency and reliability of the meta-analysis results, sensitivity tests were carried out on the SOD, MDA, and myocardial infarction area contained in this study. The evaluated meta-analyses for each indication were eliminated one by one, and the remaining studies were combined. The results of the sensitivity research indicated that the sensitivity analysis did not significantly affect the combined effect sizes for the SOD, MDA, and myocardial infarction area. This demonstrated that the conclusions of this meta-analysis were more dependable (Fig. 7).

Discussion

This systematic review and meta-analysis researched the success factors of animal studies by collecting studies of tanshinone IIA in the treatment of MIRI rats, summarizing its efficacy and utility, and providing solid evidence for future MIRI treatments. The left anterior descending coronary artery was used as the ligation and release site in the rat models with MIRI included in this systematic review and meta-analysis to validate the validity and reasonableness of the input data, which could reduce bias. SOD was the principal oxygen radical scavenging enzyme in the organism. MDA was a crucial indicator of the severity of the free radical injury. In MIRI, the cellular oxidative stress equilibrium can be perturbed, and both SOD and MDA levels can be used as indicators of the oxidative stress equilibrium in the body (*Zhang et al. 2022*). The myocardial infarction area is a direct reflection of the severity of MIRI. Therefore, we executed a systematic review and meta-analysis to gather information regarding tanshinone IIA therapy in rat models with MIRI.

Oxidative stress is an important pathogenic factor in myocardial injury. Myocardial ischemia and hypoxia can generate massive amounts of oxygen radicals, resulting in an increase in the permeability of cardiac cell membranes and even necrosis (*Petrosillo et al. 2005*). SOD eliminates free radicals and inhibits the formation of more potent hydroxyl radicals (*Fujii et al. 2022*). Elevated SOD levels can increase the body's antioxidant capacity, thereby reducing the degree of myocardial injury. For this reason, SOD was chosen as one of the primary endpoints. The results indicated that tanshinone IIA treatment of the rats models with MIRI increased serum SOD levels. Similar outcomes were observed in the subgroups of ischemia duration, dosage, and route. However, when subgroup analysis was undertaken according to reperfusion duration, the results for the 180 - 480 min category were negative (MD = 5.47, 95% CI [-0.56 to 11.50], p = 0.08). Noting that this group consisted of only one study, this conclusion was relatively unreliable and would need to be confirmed by additional research in the future. In addition, we discovered that the duration of ischemia might be a cause of heterogeneity and that subgroup analysis only partially decreased heterogeneity. We speculated that this may have been caused by measurement errors at various research institutions. MDA is the final product of lipid peroxidation by oxygen radicals, which can induce cell membrane degeneration and alter membrane fluidity and permeability, so indicating the extent of oxygen radical injury to cardiac myocytes (*Nehra et al. 2022; Tawfik et al. 2021*). SOD can be used as an indicator of the body's antioxidant capacity, while MDA can be used to measure levels

of free radicals. Consequently, MDA was chosen as one of the primary outcomes of this study. The results showed that tanshinone IIA could reduce MDA levels in the serum of rats models with MIRI, with consistent findings across subgroups of reperfusion duration, dosage, and route. However, subgroup analysis revealed that tanshinone IIA did not significantly decrease MDA levels in the subgroup with ischemia duration between 40 and 45 minutes (MD = -1.12, 95% CI [-2.27 to 0.03], $p = 0.06$). The results suggested that tanshinone IIA may be less efficient in improving MIRI in the presence of longer ischemic times. However, this subgroup only contained three studies, so the validity of this conclusion needed to be confirmed by further research. Even though subgroup analysis served to reduce subgroup heterogeneity, there were still subgroups with substantial heterogeneity. We thought that the source of heterogeneity may be some variation in the measuring techniques used across research.

The myocardial infarction area reflected the extent of MIRI and provided a visual representation of the tanshinone IIA's therapeutic effect. The results indicated that tanshinone IIA reduced the area of myocardial infarction in rats models with MIRI, with consistent findings across subgroups of ischemia duration, reperfusion duration, dosage, and route. Furthermore, we found that the duration of ischemia may be a factor contributing to heterogeneity. Nevertheless, there was still unexplained variation, which we hypothesized was due to variations in detection equipment and methodologies across studies.

This was the first systematic review of the effects of tanshinone IIA on rats models with MIRI. The included articles were subject to a thorough filtering and inspection procedure. It can be inferred that the overall quality of the included articles was not particularly excellent. Despite the rigorous evaluation of the effect of tanshinone IIA intervention on the rats models with MIRI, this study had a few drawbacks. Shortcomings of this study: Firstly, only articles written in Chinese and English were sought. The search for grey articles and conference articles was insufficient due to the difficulty of acquiring both. Secondly, certain results were highly heterogeneous, with a few included publications and outcome indicators showing some publication bias. Thirdly, this article was a meta-analysis review based on animal experiments that may have been affected by the quality of the original study design, resulting in a lower quality overall and potentially affecting the accuracy of the results. The fourth common limitation of these studies was the absence of descriptions of allocation concealment, blinding for

caregivers and/or investigators, random housing, and blinding for outcome assessment. Therefore, the included reports were classified as uncertain-risk. A larger sample size and higher-quality studies are required to further validate the results and establish the validity of the study's conclusions.

CONCLUSION

In this meta-analysis, eleven articles were considered for inclusion. More reliable preclinical evidence was acquired through the study of SOD, MDA, and myocardial infarction area. A comprehensive examination of the use of tanshinone IIA in rats models with MIRI found great efficacy in the treatment of tanshinone IIA when the ischemia duration was less than 40 min and the reperfusion duration was less than 180 min. Tanshinone IIA had a significant therapeutic effect when administered intraperitoneal injection, intragastric, and intravenous injection at doses above 5 mg/kg. Consequently, the transference of tanshinone IIA from the laboratory to the clinical treatment of MIRI is of considerable value.

Funging

This study was supported by the Shandong Natural Science Foundation Joint Fund Project (ZR2021LZ044), Jinan's "20 articles of higher education" funded projects (2020GXRC005). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

Shandong Natural Science Foundation Joint Fund Project: ZR2021LZ044.

Jinan's "20 articles of higher education" funded projects: 2020GXRC005.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

Xiao-Bin Zhang conceived and designed the experiments, performed the experiments, prepared figures and/or tables, and approved the final draft.

He-He Jiang prepared performed the experiments, prepared figures and/or tables, and approved the final draft.

Lin-Lin Zhang performed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Chen Chen performed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Meng-Zhen Xing analyzed the data, prepared figures and/or tables, and approved the final draft.

Dong-Qing Du analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

Yu-Jie Li analyzed the data, prepared figures and/or tables, and approved the final draft.

Yu-Ning Ma analyzed the data, prepared figures and/or tables, and approved the final draft.

Yu-Xia Ma conceived and designed the experiments, prepared figures and/or tables, and approved the final draft

Chun-Jing Li conceived and designed the experiments, prepared figures and/or tables, and approved the final draft

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the Supplemental Files.

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Figure 1

Figure 1 Flow diagram of the literature selection process.

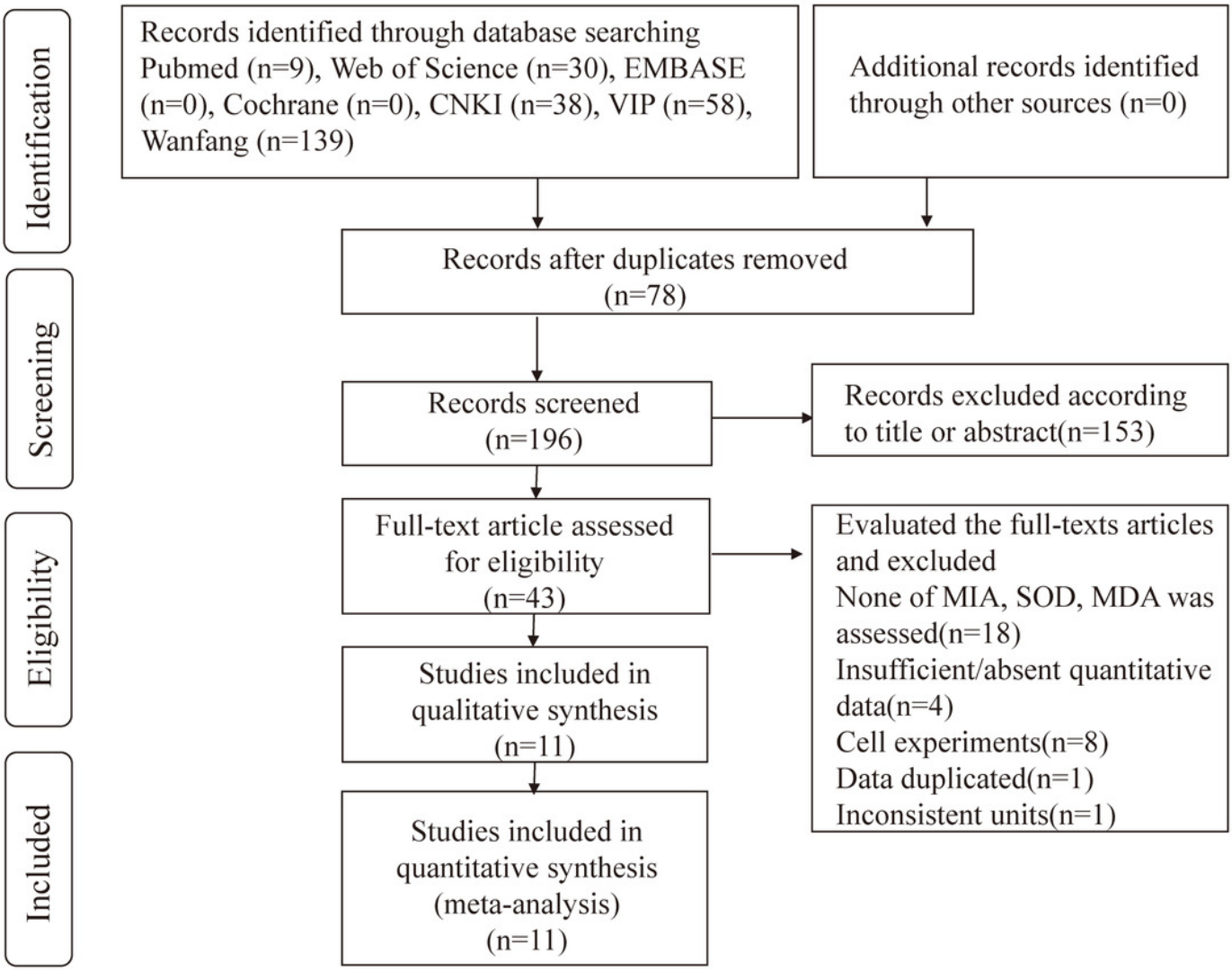

Figure 2

Figure 2 Evaluation of literature quality outcomes derived from SYRCLE's Risk of Bias utilizing the Cochrane tool.

(A) Risk of bias summary: the review authors' assessments of each risk of bias item for each included study. (B) Risk of bias graph: review authors' judgments about each risk of bias item displayed as a percentage for all included studies.

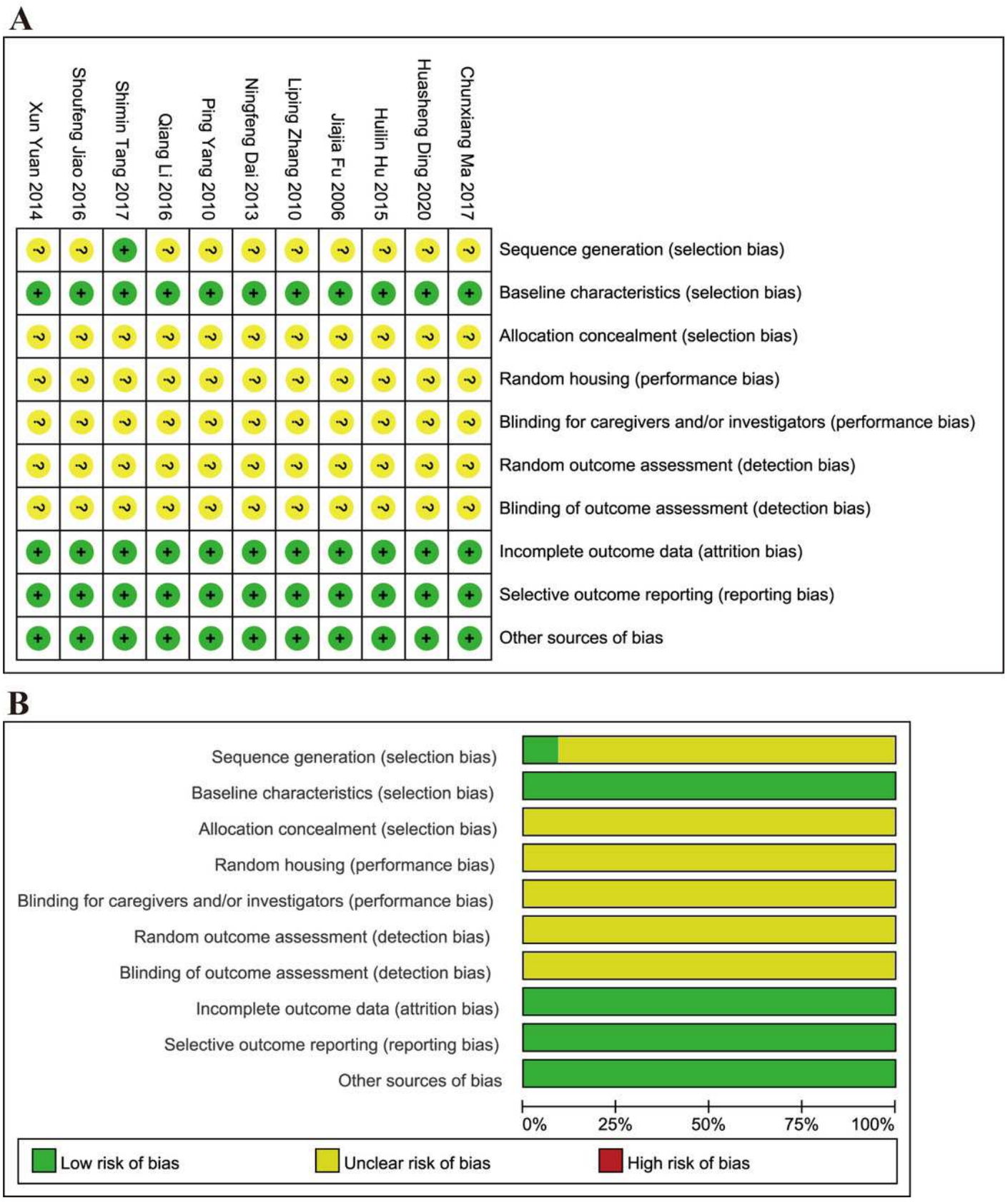
A
Xun Yuan 2014
Shoufeng Jiao 2016
Shimin Tang 2017
Qiang Li 2016
Ping Yang 2010
Ningfeng Dai 2013
Liping Zhang 2010
Jiajia Fu 2006
Huilin Hu 2015
Huasheng Ding 2020
Chunxiang Ma 2017
Sequence generation (selection bias)
Baseline characteristics (selection bias)
Allocation concealment (selection bias)
Random housing (performance bias)
Blinding for caregivers and/or investigators (performance bias)
Random outcome assessment (detection bias)
Blinding of outcome assessment (detection bias)
Incomplete outcome data (attrition bias)
Selective outcome reporting (reporting bias)
Other sources of bias
B
Sequence generation (selection bias)
Baseline characteristics (selection bias)
Allocation concealment (selection bias)
Random housing (performance bias)
Blinding for caregivers and/or investigators (performance bias)
Random outcome assessment (detection bias)
Blinding of outcome assessment (detection bias)
Incomplete outcome data (attrition bias)
Selective outcome reporting (reporting bias)
Other sources of bias
0%
25%
50%
75%
100%
Low risk of bias
Unclear risk of bias
High risk of bias

Figure 3

Figure 3 Forest plot of SOD.

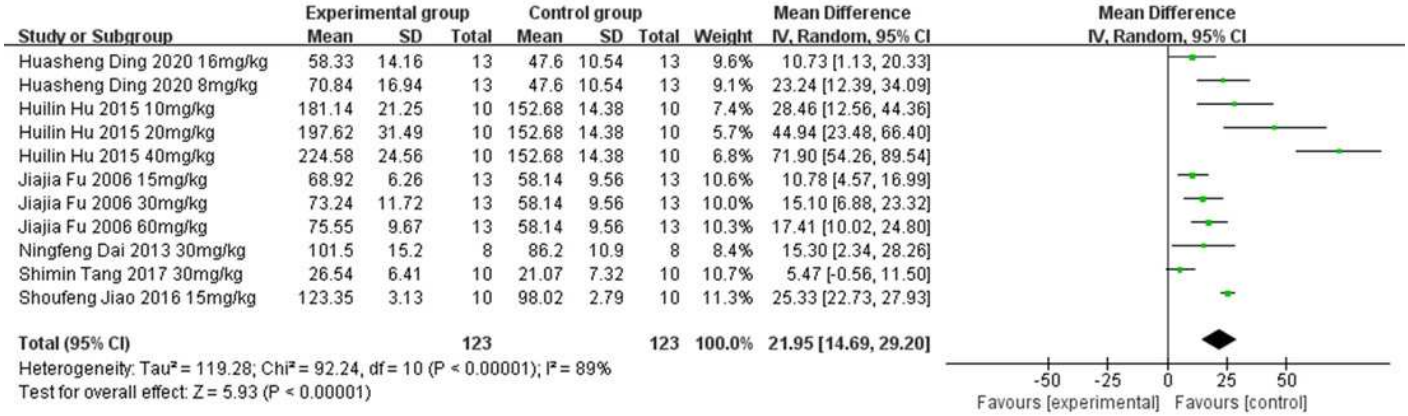

Figure 4

Figure 4 Forest plot of MDA.

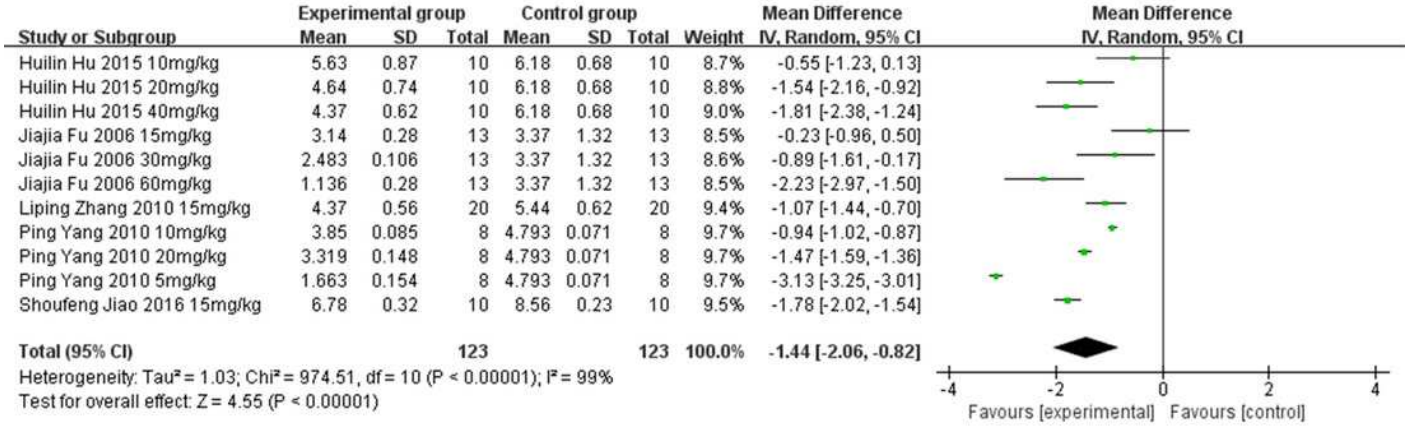

Figure 5

Figure 5 Forest plot of myocardial infarction area.

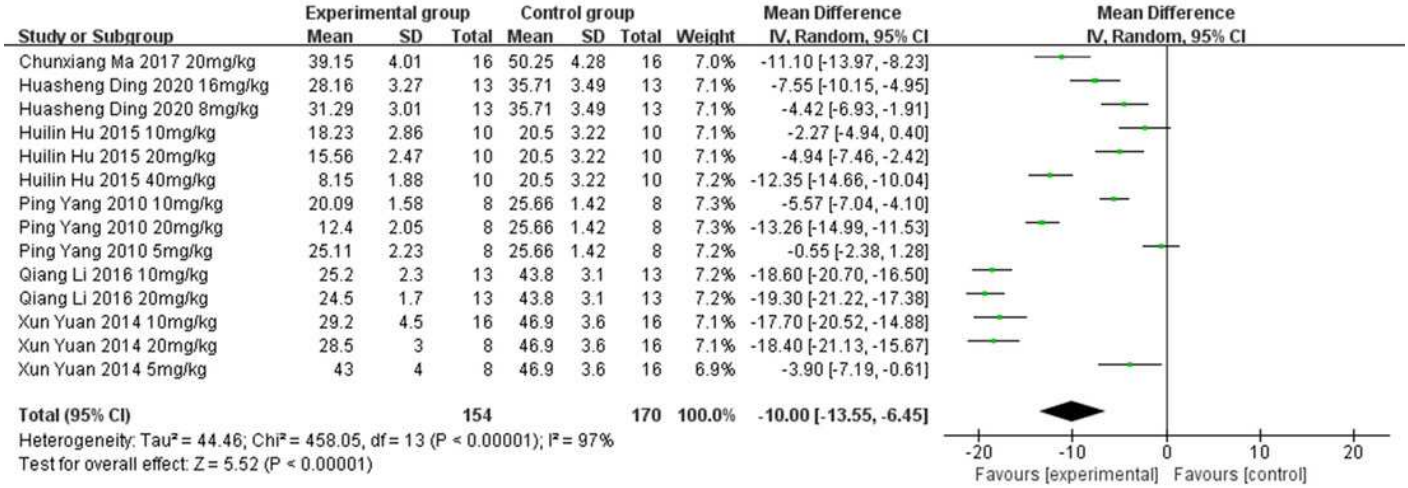

Figure 6

Figure 6 Egger's tests and funnel plots for evaluating publication bias.

(A, B) Egger's test and funnel plot for publication bias in SOD. (C, D) Egger's test and funnel plot for publication bias in MDA. (E, F) Egger's test and funnel plot for publication bias in myocardial infarction area.

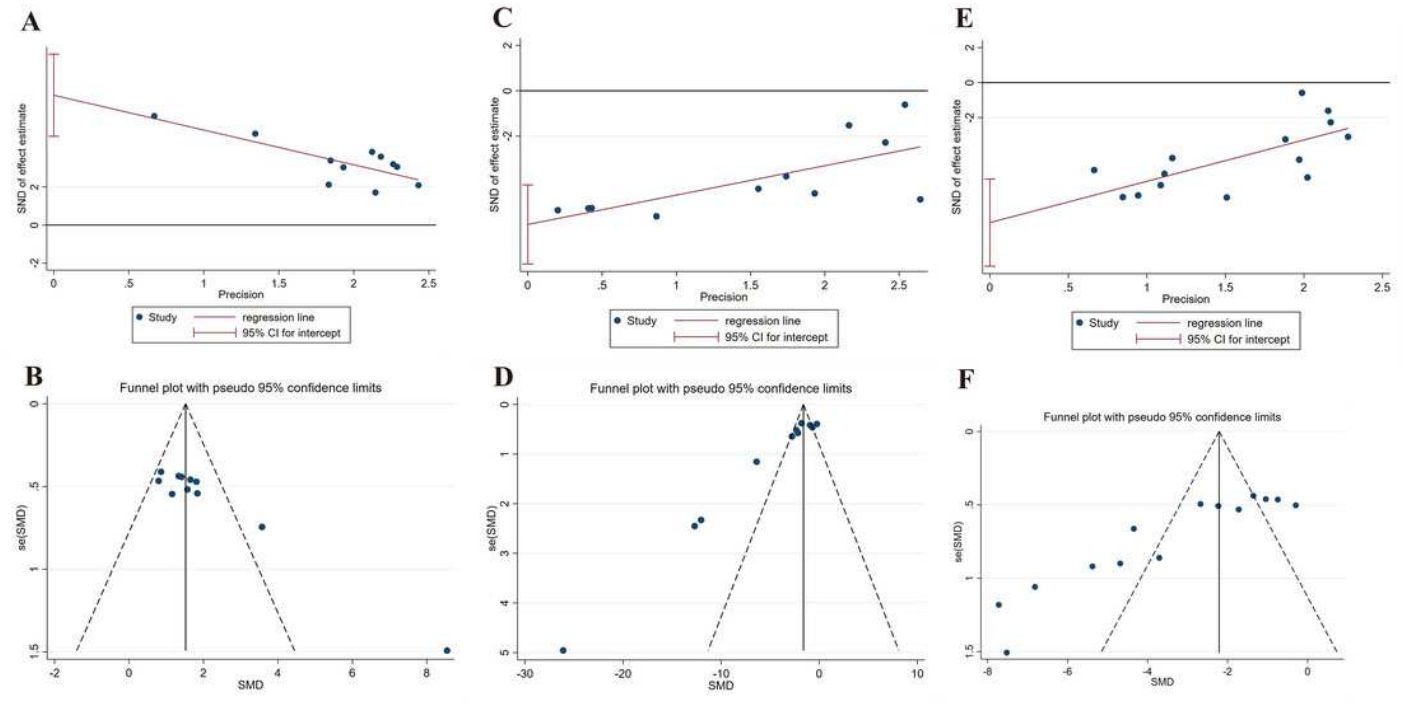

Figure 7

Figure 7 Sensitivity analysis chart for SOD, MDA, and myocardial infarction area.

(A)Sensitivity analysis chart for SOD. (B)Sensitivity analysis chart for MDA. (C)Sensitivity analysis chart for myocardial infarction area.

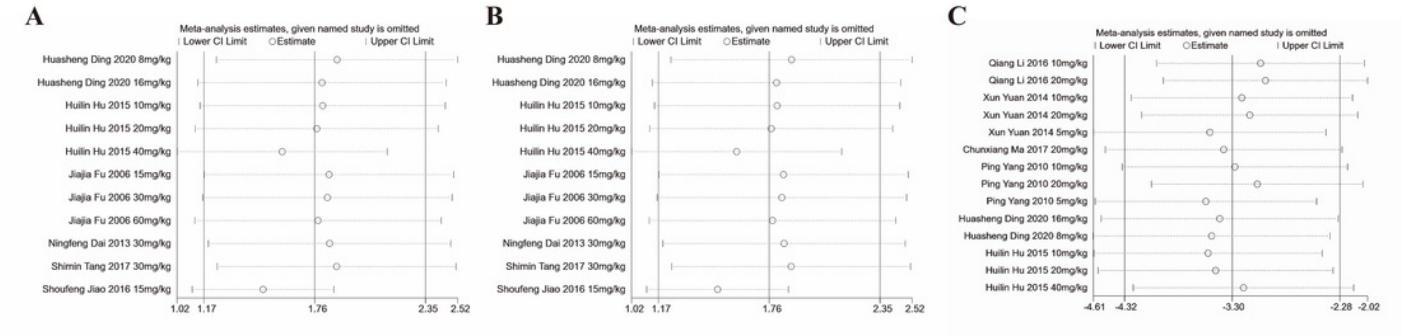

Table 1(on next page)

Table1 Summary of characteristics of the included studies

Notes: Abbreviations: TSAⅡA, Tanshinone IIA; TSAⅡA-L, Tanshinone IIA low-dose group; TSAⅡA-M, Tanshinone IIA medium-dose group; TSAⅡA-H, Tanshinone IIA high-dose group; Control, receiving either normal saline, blank, or carboxymethylcellulose sodium group; i.p, intraperitoneal injection; i.g, intragastric; i.v, intravenous injection; NS, normal saline; MIA, myocardial infarction area; SOD, superoxide dismutase; MDA, methane dicarboxylic aldehyde; CMC-Na, Carboxymethylcellulose sodium; ?, not recorded.

Table1 Summary of characteristics of the included studies

Study	Species	Sex	Age	Weight	Ischemia duration	Reperfusion duration	Groups(n)	Dose	Route	Treatment time	Outcome measure
Ding et al. (2020)	SD	Male	?	210~270g	40min	120min	Control (n=13)	NS 4ml·kg^{-1}·d^{-1}	i.p	Prior to ischemia	MIA, SOD
							TSAⅡA-L (n=13)	8mg·kg^{-1}·d^{-1}	i.p		
							TSAⅡA-H (n=13)	16mg·kg^{-1}·d^{-1}	i.p		
Ma et al. (2017)	SD	Male	8~12W	180~220g	30min	180min	Control (n=16)	NS	i.g	Prior to ischemia	MIA
							TSAⅡA (n=16)	20mg·kg^{-1}·d^{-1}	i.g		
Tang et al. (2017)	SD	Male	8W	260~280g	30min	480min	Control (n=10)	NS	i.g	Prior to ischemia	SOD
							TSAⅡA (n=10)	30mg·kg^{-1}·d^{-1}	i.p		
Jiao (2016)	SD	Male	7~8W	250~280g	30min	120min	Control (n=10)	not intervene	-	Prior to ischemia	MDA, SOD
							TSAⅡA (n=10)	15mg·kg^{-1}·d^{-1}	i.g		
Li et al. (2016)	SD	Male	?	210~250g	30min	120min	Control (n=13)	not intervene	-	Prior to ischemia	MIA
							TSAⅡA-L (n=13)	10mg·kg^{-1}·d^{-1}	i.v		
							TSAⅡA-H (n=13)	20mg·kg^{-1}·d^{-1}	i.v		
Hu et al. (2015)	Wistar	Male	20~32W	260~300g	20min	60min	Control (n=10)	not intervene	-	Prior to ischemia	MIA, SOD, MDA
							TSAⅡA-L (n=10)	10mg·kg^{-1}·d^{-1}	i.p		
							TSAⅡA-M (n=10)	20mg·kg^{-1}·d^{-1}	i.p		
							TSAⅡA-H (n=10)	40mg·kg^{-1}·d^{-1}	i.p		
Yuan et al. (2014)	SD	Male	?	250~300g	30min	120min	Control (n=16)	not intervene	-	Prior to ischemia	MIA
							TSAⅡA-L (n=8)	5mg·kg^{-1}·d^{-1}	i.v		
							TSAⅡA-M (n=16)	10mg·kg^{-1}·d^{-1}	i.v		
							TSAⅡA-H (n=8)	20mg·kg^{-1}·d^{-1}	i.v		
Dai et al. (2013)	SD	Male	?	240~320g	30min	120min	Control (n=8)	not intervene	-	Prior to ischemia	SOD
							TSAⅡA-L (n=8)	30mg·kg^{-1}·d^{-1}	i.v		
Zhang & Zhang (2010)	Wistar	?	?	?	30min	120min	Control (n=20)	NS	i.g	Prior to ischemia	MDA
							TSAⅡA (n=20)	15mg·kg^{-1}·d^{-1}	i.g		
Yang (2010)	Wistar	Male	?	250~300g	15min	30min	Control (n=8)	NS	i.g	Prior to ischemia	MIA, MDA
							TSAⅡA-L (n=8)	5mg·kg^{-1}·d^{-1}	i.g		
							TSAⅡA-M (n=8)	10mg·kg^{-1}·d^{-1}	i.g		
							TSAⅡA-H (n=8)	20mg·kg^{-1}·d^{-1}	i.g		
Fu (2006)	SD	Male	?	280~300g	45min	120min	Control (n=13)	0.5% CMC-Na	i.g	Prior to ischemia	SOD, MDA
							TSAⅡA-L (n=13)	15mg·kg^{-1}·d^{-1}	i.g		
							TSAⅡA-M (n=13)	30mg·kg^{-1}·d^{-1}	i.g		
							TSAⅡA-H (n=13)	60mg·kg^{-1}·d^{-1}	i.g		

Notes:

Abbreviations: TSAⅡA, Tanshinone IIA; TSAⅡA-L, Tanshinone IIA low-dose group; TSAⅡA-M, Tanshinone IIA medium-dose group; TSAⅡA-H, Tanshinone IIA high-dose group; Control, receiving either normal saline, blank, or carboxymethylcellulose sodium group; i.p, intraperitoneal injection; i.g, intragastric; i.v, intravenous injection; NS, normal saline; MIA, myocardial infarction area; SOD, superoxide dismutase; MDA, methane dicarboxylic aldehyde; CMC-Na, Carboxymethylcellulose sodium; ?, not recorded.

Table 2(on next page)

Table 2 Subgroup analysis of SOD based on ischemia duration, reperfusion duration, dosage, and route.

Table 2 **Subgroup analysis of SOD based on ischemia duration, reperfusion duration, dosage, and route.**

Criteria for grouping	Subgroup	n	Mean difference (MD)	I^2 (%)	Z	P
Ischemia duration	15 min ≤ Time < 30 min	3	48.25 (21.41, 75.08)	85	3.52	0.004
	30 min ≤ Time < 40 min	3	15.55 (0.57, 30.54)	95	2.03	0.04
	40 min ≤ Time ≤ 45 min	5	14.69 (10.60, 18.79)	21	7.03	<0.00001
Reperfusion duration	30 min ≤ Time < 120 min	3	48.26 (21.46, 75.06)	85	3.53	0.0004
	120 min ≤ Time < 180 min	7	17.12 (11.22, 23.03)	79	5.69	<0.00001
	180 min ≤ Time ≤ 480 min	1	5.47 (-0.56, 11.50)	—	1.78	0.08
Dosage	5 $mg \cdot kg^{-1} \cdot d^{-1}$ ≤ Dosage < 20 $mg \cdot kg^{-1} \cdot d^{-1}$	5	19.20 (11.05, 27.36)	84	4.62	<0.00001
	20 $mg \cdot kg^{-1} \cdot d^{-1}$ ≤ Dosage < 30 $mg \cdot kg^{-1} \cdot d^{-1}$	1	44.94 (23.48, 66.40)	—	4.11	<0.0001
	30 $mg \cdot kg^{-1} \cdot d^{-1}$ ≤ Dosage ≤ 60 $mg \cdot kg^{-1} \cdot d^{-1}$	5	23.11 (8.75, 37.46)	92	3.15	0.002
Route	i.v	1	15.30 (2.34, 28.26)	—	2.31	0.02
	i.g	4	17.51 (9.60, 25.41)	87	4.34	<0.0001
	i.p	6	29.30 (12.62, 45.98)	92	3.44	0.0006

Table 3(on next page)

Table 3 Subgroup analysis of MDA based on ischemia duration, reperfusion duration, dosage, and route.

Table 3 Subgroup analysis of MDA based on ischemia duration, reperfusion duration, dosage, and route.

Criteria for grouping	Subgroup	n	Mean difference (MD)	I^2 (%)	Z	P
Ischemia duration	15 min ≤ Time < 30 min	6	-1.59 (-2.48, -0.70)	99	3.49	0.0005
	30 min ≤ Time < 40 min	2	-1.44 (-2.13, -0.74)	90	4.06	<0.0001
	40 min ≤ Time ≤ 45 min	3	-1.12 (-2.27, 0.03)	87	1.90	0.06
Reperfusion duration	30 min ≤ Time < 120 min	6	-1.59 (-2.48, -0.70)	99	3.49	0.0005
	120 min ≤ Time < 180 min	5	-1.26 (-1.84, -0.69)	86	4.29	<0.0001
Dosage	5 $mg \cdot kg^{-1} \cdot d^{-1}$ ≤ Dosage < 20 $mg \cdot kg^{-1} \cdot d^{-1}$	6	-1.11 (-1.46, -0.75)	95	6.13	<0.00001
	20 $mg \cdot kg^{-1} \cdot d^{-1}$ ≤ Dosage < 30 $mg \cdot kg^{-1} \cdot d^{-1}$	2	-2.37 (-3.92, -0.81)	96	2.98	0.003
	30 $mg \cdot kg^{-1} \cdot d^{-1}$ ≤ Dosage ≤ 60 $mg \cdot kg^{-1} \cdot d^{-1}$	3	-1.65 (-2.38, -0.92)	72	4.44	<0.00001
Route	i.v	3	-1.32 (-2.04, -0.59)	75	3.57	0.0004
	i.g	8	-1.48 (-2.22, -0.75)	99	3.96	<0.0001

Table 4(on next page)

Table 4 Subgroup analysis of myocardial infarction area based on ischemia duration, reperfusion duration, dosage, and route.

Table 4 Subgroup analysis of myocardial infarction area based on ischemia duration, reperfusion duration, dosage, and route.

Criteria for grouping	Subgroup	n	Mean difference (MD)	I^2 (%)	Z	P
Ischemia duration	15 min ≤ Time < 30 min	6	-6.51 (-10.75, -2.26)	96	3.00	0.003
	30 min ≤ Time < 40 min	6	-14.93 (-19.18, -10.68)	94	6.89	<0.00001
	40min ≤ Time ≤ 45 min	2	-5.96 (-9.03, -2.90)	65	3.81	0.0001
Reperfusion duration	30 min ≤ Time < 120 min	6	-6.51 (-10.75, -2.26)	96	3.00	0.003
	120 min ≤ Time < 180 min	7	-12.88 (-18.04, -7.73)	97	4.90	<0.00001
	180 min ≤ Time ≤ 480 min	1	-11.10 (-13.97, -8.23)	—	7.57	<0.00001
Dosage	5 $mg \cdot kg^{-1} \cdot d^{-1}$ ≤ Dosage < 20 $mg \cdot kg^{-1} \cdot d^{-1}$	8	-7.57 (-12.27, -2.86)	97	3.15	0.002
	20 $mg \cdot kg^{-1} \cdot d^{-1}$ ≤ Dosage < 30 $mg \cdot kg^{-1} \cdot d^{-1}$	5	-13.42 (-18.33, -8.51)	96	5.36	<0.00001
	30 $mg \cdot kg^{-1} \cdot d^{-1}$ ≤ Dosage ≤ 60 $mg \cdot kg^{-1} \cdot d^{-1}$	1	-12.35 (-14.66, -10.04)	—	10.47	<0.00001
Route	i.v	5	-15.69 (-20.34, -11.05)	94	6.62	<0.00001
	i.g	4	-7.59 (-13.23, -1.94)	97	2.63	0.008
	i.p	5	-6.33 (-9.85, -2.82)	90	3.54	0.0004