Remote ischemic conditioning in experimental hepatic ischemia-reperfusion: A systematic review and meta-analysis

CHUN TIAN[1], AIHUA WANG[2] and YONGHONG KUANG[2]

[1]Department of Anesthesiology, Yongchuan Hospital of Chongqing Medical University, Chongqing 402160, P.R. China;
[2]Department of Science and Education, Yongchuan District People's Hospital of Chongqing, Chongqing 400010, P.R. China

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Abstract. Remote ischemic conditioning (RIC), including pre-conditioning (RIPC, before the ischemic event), per-conditioning (RIPerC, during the ischemic event), and post-conditioning (RIPostC, after the ischemic event), protects the liver in animal hepatic ischemia-reperfusion injuries models. However, several questions regarding the optimal timing of intervention and administration protocols remain unanswered. Therefore, the preclinical evidence on RIC in the HIRI models was systematically reviewed and meta-analyzed in the present review to provide constructive and helpful information for future works. In the present review, 39 articles were identified by searching the PubMed, OVID, Web of Science and Embase databases spanned from database inception to July 2024. According to the preferred reporting items for systematic reviews and meta-analyses guidelines, data were extracted independently by two researchers. The primary outcomes evaluated in this study were those directly related to liver injury, such as alanine transaminase (ALT), aspartate transaminase (AST) and liver histopathology. The risk of bias was assessed using the risk of bias tool of the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE). The findings were expressed as standardized mean difference (SMD) and analyzed using random-effects models. Egger's test was used to evaluate the publication bias. RIC significantly reduced the changes in ALT, AST and liver histopathology (all P<0.00001). These effects had two peaks, with the first peak of RIPerC/RIPostC occurring earlier, regardless of models and species. RIPerC/RIPostC exerted significant effects on changes in ALT and AST [ALT SMD (95% confidence interval (CI]): RIPC -1.97 (-2.40, -1.55) vs. -2.78 (-3.77, -1.78); P=0.142; AST SMD (95%CI): RIPC -1.45 (-1.90, -0.99) vs. -2.13 (-2.91, -1.34); P=0.142], and RIPC had a greater effect on liver histopathology change [SMD (95%CI): RIPC -2.68 (-3.67, -1.69) vs. -1.58 (-2.24, -0.92); P=0.070]; however, no interactions were observed between the two groups in the meta-regression analysis. RIC is the most effective in experimental HIRI, using a 10-25-min dose. These outcomes suggest that RIC may be a promising strategy for treating HIRI; however, future studies using repeated doses in animal models with comorbidities will present novel ideas for its therapeutic application. The protocol of present study was registered with PROSPERO (CRD42023482725).

Correspondence to: Dr Aihua Wang or Dr Yonghong Kuang, Department of Science and Education, Yongchuan District People's Hospital of Chongqing, 375 Huilongdadao Road, Yongchuan, Chongqing 400010, P.R. China
E-mail: wangaihuawah86@163.com
E-mail: yhkuang0412@163.com

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Introduction

Ischemic conditioning intervention therapy originated for the management of cardiovascular diseases in the 1980s, conferring cardiac protection from a subsequent or ongoing ischemia-reperfusion injury (IRI) (1). Subsequently, organ protection in brain and liver diseases has also been investigated, but its apparent preclinical benefits have not been consistently translated into clinical practice (2,3). Remote ischemic conditioning (RIC) can activate ischemia tolerance through transient, non-fatal induction of remote or limb ischemia by simply inflating a blood pressure cuff on a leg or arm (4). This highly attractive treatment strategy is beneficial in terms of economy, safety, non-invasion and ease of promotion.

RIC, including three types of pre-conditioning (RIPC, before the ischemic event), per-conditioning (RIPerC, during the ischemic event) and post-conditioning (RIPostC, after the ischemic event) based on the timing of induction, has shown promise in treating several cardiovascular and cerebrovascular diseases (5,6). RIC protects the liver from IRI via several mechanisms such as neuro-humoral, mitochondrial autophagy and exosomal gene mechanisms (4). Although RIC has a strong short-term hepatoprotective effect against hepatic ischemia-reperfusion injuries (HIRIs) during liver-related surgeries, it is neutral in improving long-term outcomes such as hepatocyte apoptosis index, duration of hospital stay and survival rate (2). The potential explanation is interactions with liver-protecting anesthetics.

Although RIC does not cause harm in patients undergoing liver-related surgery and has elevated to human trials,

human clinical evidence is limited. Importantly, there are still several unanswered questions about the optimal timing of intervention and administration protocols (such as one compared with two limbs and the number and duration of cycles, among others). Therefore, the preclinical evidence on RIPreC, RIPerC and RIPostC in the HIRI models was systematically reviewed and meta-analyzed in the present review to provide constructive and helpful information for future works.

Materials and methods

This systematic review was prepared in accordance with the recommendations of preferred reporting items for systematic reviews and meta-analyses (PRISMA) to assess the methodological quality. Preclinical (non-human) studies were included to compare the effects of RIC on HIRI animal models. This systematic review has been registered with PROSPERO, registration number: CRD42023482725.

Search strategy. A comprehensive literature search was conducted on July 26, 2024 using the PubMed (https://pubmed.ncbi.nlm.nih.gov), OVID (https://ovidsp.ovid.com), Web of Science (https://clarivate.com) and Embase databases (https://www.embase.com) for articles published from the inception to July 2024. Search terms comprised various combinations of 'remote ischemic conditioning' or 'limb ischemic conditioning' or 'remote ischemic treatment' or 'remote ischemic adaptation' or 'remote ischemic preconditioning' or 'distant ischemic preconditioning' or 'limb ischemic preconditioning' or 'remote ischemic perconditioning' or 'limb ischemic perconditioning' or 'remote ischemic postconditioning' or 'limb ischemic postconditioning' or 'RIC' or 'RIP' or 'RIPC' or 'RPC' or 'RIPerC' or 'IperC' or 'RPostC', 'hepatic ischemia-reperfusion' or 'liver graft' or 'liver transplantation' or 'liver resection' or 'hepatectomy'. The search terms were adjusted according to different search engines. There were no language restrictions for the articles that were included. In addition, the authors manually searched the references of included studies and other existing meta-analyses to obtain more eligible studies. A specific search strategy is presented in Table SI.

Inclusion and exclusion criteria. The authors CT and AW independently reviewed and retrieved the full-text articles simultaneously. Different views were discussed among all authors, and duplicate articles in all databases were merged. The latest and most complete study was included when duplicate studies were from the same population.

The inclusion criteria are as follows: i) Any non-human species, any sex, in the models of hepatic ischemia-reperfusion; ii) interested intervention was limb RIC compared with the control group without RIC; iii) controlled studies with a separate control group; and iv) interested outcomes were postoperative liver synthetic function and liver histopathological injury.

The exclusion criteria are as follows: i) Human subjects, *in vitro* or computer studies; ii) retrospective or single-arm studies; iii) case studies, cross-over studies, studies without a separate control group, editorials, meta-analyses and reviews; iv) only the abstract of a study was available; and v) no reports of postoperative aminotransferase levels or data were from review articles.

Data extraction. The authors CT and AW independently extracted data from each article. Any disagreements were resolved by the consensus of a third reviewer (YK). The following information was extracted from the included articles: First author; year of publication; country or region of studies; animal model (species, sex, sample size, the method of ischemic induction, the duration of ischemia); parameters of RIC (body part, unilateral or bilateral, number of cycles per treatment, duration of occlusion and release per cycle) and interested outcomes. The published graphs were enlarged and measured using Grab software (2) if the information was unavailable in the text. If data were not reported or unclear, the reviewers tried to contact the respective study authors by e-mail (maximum of two attempts). Furthermore, it should be stated that it was impossible to separate the RIPerC and RIPostC groups easily; hence, these were combined to form one group.

Quality assessment. The included animal model studies were assessed using the risk of bias tool of the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE). This assessment was performed independently by CT and AW. Any disagreements were resolved by consensus. Categories for the quality investigation included sequence generation, baseline characteristics, allocation concealment, random housing, blinding for the performance bias, random outcome assessment, blinding for the detection bias, incomplete outcome data, selective outcome data and other sources of bias. Each category was classified as high, low or uncertain risk.

The methodological quality of the results was evaluated using the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) guidelines (2). Ultimately, the quality of evidence for each outcome was rated as high, moderate, low or very low.

Primary and secondary outcomes. The primary outcomes evaluated in this study were those directly related to liver injury, such as alanine transaminase (ALT), aspartate transaminase (AST) and liver histopathology. The secondary outcomes assessed were lactate dehydrogenase (LDH), tumor necrosis factor-α (TNF-α), interleukin (IL) -6, IL-10, IL-1β, apoptosis and other possible outcomes.

Statistical analysis. The analysis was performed using the Review Manager version 5.4 software (The Nordic Cochrane Center; The Cochrane Collaboration, 2020). The continuous outcomes were reported as standardized mean difference (SMD) with 95% confidence interval (CI). The dichotomous outcomes were presented as odds ratio (OR) with 95%CI, and the random-effects model was used for analysis. Subgroup meta-regression or sensitivity analyses were then performed using Stata/MP (version 17.0; StataCorp LLC), and descriptive analysis was conducted if meta-analysis was inappropriate. Publication bias was assessed using Egger's linear regression test. $P<0.05$ was considered to indicate a statistically significant difference.

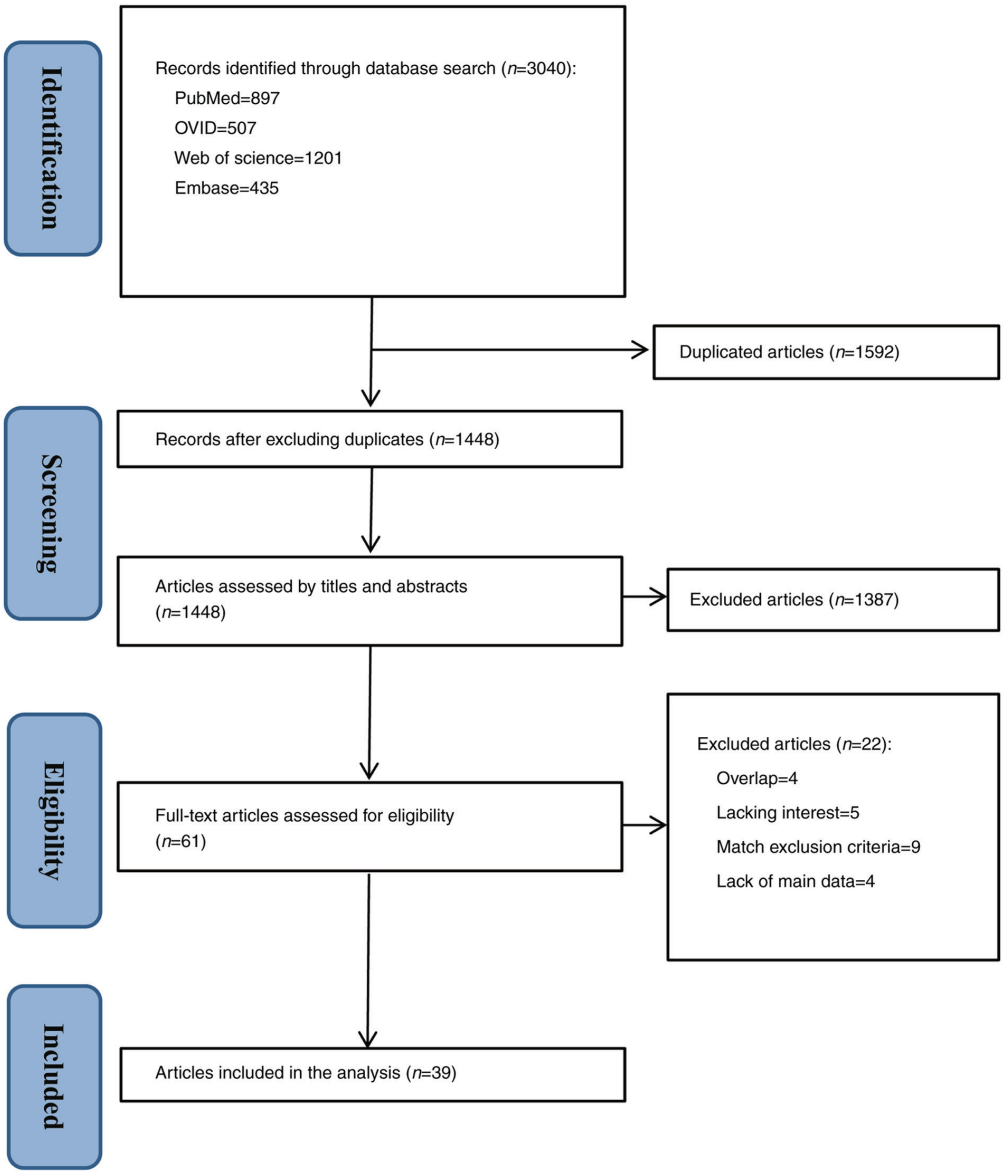


Figure 1. PRISMA flowchart of the papers selection. PRISMA, preferred reporting items for systematic reviews and meta-analyses.

Results

Study characteristics. The reviewers initially identified 3,040 relevant articles, of which 1,592 were duplicates. Excluding duplicates, a total of 1,448 studies were left for analysis. After analyzing the titles and abstracts, 1,387 articles that did not fulfil the criteria were also excluded, and the remaining 61 studies were selected for a full reading. After reviewing the full texts, 39 animal model studies met the eligibility criteria for data synthesis (Fig. 1).

All 39 included articles/studies (101 animal experiment records) were published between 2006 and 2023 and conducted in 10 countries (China, n=15; United Kingdom, n=5; Brazil, n=3; Korea, n=3; Hungary, n=3; Switzerland, n=3; Germany, n=2; Canada, n=2; Sweden, n=2; and Turkey, n=1). In total, 32 of 101 animal experiments studied Sprague Dawley rats (n=337), 12 used Wistar rats (n=178), 16 tested Lewis rats (n=144), 28 examined C57BL/6 mice (n=270), five used wild-type mice (n=82) and eight studied New Zealand white rabbits (n=50). To induce HIRI, various animal models, such as orthotopic liver transplantation (OLT), 70% liver I/R, total hepatic ischemia (THI), hemorrhagic shock-resuscitation (HSR) I/R or hindlimb I/R, were used. RIC was primarily performed by occluding the femoral vascular bundle, femoral artery or hind limbs. Table I summarizes the experimental characteristics of all the involved studies (7-45).

Quality assessment. The SYRCLE's risk of bias tool was used to evaluate the risk of bias in the included animal experiments studies (Fig. 2). In quality assessment, 15 studies (7,9,18,22,26, 27,31,32,36-40,44,45) were analyzed with the same baseline characteristics among groups, but none of them conducted random sequence generation or allocation concealment. A total of 30 studies (7-12,14,15,17-28,31,32,34,35,37-41,44) had complete outcome data and they also demonstrated a low risk of attrition and reporting biases. Furthermore, blind bias could not be evaluated in most studies because of the characteristics of animal experiments.

Table I. Characteristics of the included studies.

First author/s, year	Country	Species	Model (min)	Method of RIC	Number and duration of cycles	Time of administration	Time of assessment[a]	Interested outcomes	(Refs.)
Mukkala *et al*, 2023	Canada	M, wild-type mice	HSR-I/R (150)	Right hind limb, non-invasive	RIPC, 4x5/5 min	RIPC, 30 min pre-HSR	2 h	ALT, liver histopathology (liver necrosis area) TNF-α, IL-6, IL-10	(7)
Li *et al*, 2023	China	M, Sprague Dawley rats	OLT (75)	Hind limbs, non-invasive	RIPerC, 3x5/5 min	RIPerC, at the onset of the recipient anhepatic phase	3 h	ALT, AST, liver histopathology (Suzuki classification)	(8)
Zhou *et al*, 2021 (ex1-5)	China	M, Sprague Dawley rats	HSR-I/R (60)	Left hind limb (femoral artery), invasive	Ex1-5, RIPC, 3x5/5 min	RIPC, 30 min pre-HSR	0, 2, 6, 12, and 24 h	ALT, AST TNF-α, IL-1β	(9)
Niu *et al*, 2020 (ex1 and 2)	China	M, Sprague Dawley rats	Hindlimb I/R (240)	Hind limbs, noninvasive	Ex1, RIPC, 3x5/5 min Ex2, RIPostC, 3x1/1 min	RIPC, 30 min pre-ischemia RIPostC, at reperfusion	0 h	ALT, AST LDH, TNF-α, IL-1β, IL-10, apoptosis index	(10)
Choi *et al*, 2020	Korea	M, Sprague Dawley rats	70% liver I/R (30)	Unilateral hind limb, non-invasive	RIPC, 3x5 /5 min	RIPC, 30 min pre-ischemia	2 h	ALT, AST, liver histopathology (3-point scale) TNF-α	(11)
Koh *et al*, 2019	Korea	M, C57BL/6 mice	70% liver I/R (30)	Hind limbs, noninvasive	RIPC, 4x3 /3 min	RIPC, 24 min pre-ischemia	6 h	ALT, AST, liver histopathology (Suzuki classification) TNF-α, IL-6, IL-10	(12)
Emontzpohl *et al*, 2019 (ex1-8)	Germany	M, Lewis rats	OLT (480)	Hind limbs (Infrarenal aorta), invasive	Ex1-4, RIPC, 4x5/5 min Ex5-8, RIPostC, 4x5/5 min	RIPC, 40 min pre-ischemia RIPostC, after graft reperfusion	1, 3, 24, and 168 h	ALT, AST, liver histopathology (Suzuki classification)	(13)
Li *et al*, 2018 (ex1-3)	China	M, C57BL/6 mice	OLT (235.7±18.3)	Hind limbs (femoral vascular bundle), invasive	Ex1-3, RIPerC, 3x5/5 min	RIPerC, during the time from anesthetisation to graft reperfusion	2, 24, and 72 h	ALT, AST, liver histopathology (Suzuki classification) TNF-α, apoptosis index	(14)

Table I. Continued.

First author/s, year	Country	Species	Model (min)	Method of RIC	Number and duration of cycles	Time of administration	Time of assessment[a]	Interested outcomes	(Refs.)
Kambakamba *et al*, 2018 (ex1-12)	Switzerland	M, C57BL/6 mice	Ex1-6, Standard hepatectomy (68%) Ex7-12, Extended hepatectomy (86%)	Right hind limb (femoral vessels), invasive	RIPC, 3x5/5 min	RIPC, 30 min pre-ischemia	0, 24, 48, 72, 96, and 168 h	ALT, AST	(15)
Gomes *et al*, 2018	Brazil	M & F, weaning Wistar rats	THI (60)	Right hind limb (femoral vessels), invasive	RIPC, 3x5/5 min	RIPC, 30 min pre-THI	24 h	ALT, AST, liver histopathology (Scheuer scores)	(16)
Gao *et al*, 2018 (ex1 and 2)	China	Ex1, M, Sprague Dawley rats Ex2, M, Wistar rats	70% liver I/R (60)	Right hind limb (femoral artery), invasive	RIPostC, 1x5/5 min	RIPostC, 60 min post-ischemia	6 h	ALT, AST TNF-α, IL-1β, apoptosis index	(17)
Czigany *et al*, 2018 (ex1-8)	Germany	M, Lewis rats	OLT (480)	Hind limbs (Infrarenal aorta), invasive	Ex1-4, RIPC, 4x5/5 min Ex5-8, RIPostC, 4x5/5 min	RIPC, 40 min pre-ischemia RIPostC, after graft reperfusion	1, 3, 24, and 168 h	ALT, AST, liver histopathology (4-point scale) LDH, IL-10	(18)
Liang *et al*, 2017	China	M, Sprague Dawley rats	OLT (45)	Hind limbs, non-invasive	RIPerC, 3x5/5 min	RIPerC, at the onset of the recipient anhepatic phase	3 h	ALT, AST, liver histopathology (Suzuki classification)	(19)
He *et al*, 2017	China	M, Sprague Dawley rats	OLT (75)	Hind limbs, non-invasive	RIPerC, 3x5/5 min	RIPerC, at the onset of the recipient anhepatic phase	3 h	ALT, AST	(20)
Ruan *et al*, 2016	China	M, Sprague Dawley rats	70% liver I/R (60)	Hind limbs, non-invasive	RIPC, 3x10/10 min	RIPC, 60 min pre-ischemia	6 h	ALT, AST, liver histopathology (Suzuki classification) TNF-α, IL-6, IL-10	(21)

Table I. Continued.

First author/s, year	Country	Species	Model (min)	Method of RIC	Number and duration of cycles	Time of administration	Time of assessment[a]	Interested outcomes	(Refs.)
Park *et al*, 2016 (ex1 and 2)	Korea	M, Sprague Dawley rats	70% liver I/R (30)	Right hind limb (femoral artery), invasive	Ex1, RIPC, 3x5/5 min Ex2, RIPerC, 3x5/5 min	RIPC, 30 min pre-ischemia RIPerC, post-ischemia	24 h	ALT, AST	(22)
Limani *et al*, 2016	Switzerland	M, C57BL/6 mice	70% liver I/R (60)	Hind limbs (femoral vascular bundle), invasive	RIPC, 3x5/5 min	RIPC, 30 min pre-ischemia	6 h	ALT, AST, liver histopathology (liver necrosis area)	(23)
Li *et al*, 2016 (ex1-3)	China	M, C57BL/6 mice	OLT (120)	Hind limbs (femoral vascular bundle), invasive	Ex1-3, RIPC, 4x6/6 min	RIPC, 48 min pre-ischemia	2, 24, and 72 h	ALT, liver histopathology (Suzuki classification) TNF-α, apoptosis index	(24)
Jia *et al*, 2015 (ex1-3)	China	M, Sprague Dawley rats	OLT (75)	Hind limbs, non-invasive	Ex1, RIPerC1, 3x1/1 min Ex2, RIPerC2, 3x5/5 min Ex3, RIPerC3, 3x10/10 min	RIPerC, at the onset of the recipient anhepatic phase	3 h	ALT, AST, liver histopathology (Suzuki classification)	(25)
Guimarães *et al*, 2015 (ex1 and 2)	Brazil	M, Sprague Dawley rats	70% liver I/R (45)	Right hind limb (femoral vascular bundle), invasive	Ex1 and 2, RIPC, 6x4/4 min	RIPC, 48 min pre-ischemia	1 and 3 h	ALT, liver histopathology (Suzuki classification) IL-6, IL-10	(26)
Czigany *et al*, 2015	Hungary	M, Wistar rats	70% liver I/R (60)	Left hind limb (femoral artery), invasive	RIPerC, 4x5/5 min	RIPerC, 20 min post-ischemia	24 h	ALT, AST, liver histopathology (liver necrosis area)	(27)
Wang *et al*, 2014 (ex1-4)	China	M, wild-type mice	70% liver I/R (45)	Right hind limb (femoral vascular bundle), invasive	Ex1-4, RIPC, 6x4/4 min	RIPC, 48 min pre-ischemia	2, 6, 12, and 24 h	ALT, AST, liver histopathology (Suzuki classification)	(28)
Uysal *et al*, 2014	Turkey	M, Wistar albino rats	70% liver I/R (30)	Left hind limb, non-invasive	RIPC, 3x10/10 min	RIPC, 60 min pre-ischemia	4 h	ALT, AST, liver histopathology (3-point scale)	(29)
Oberkofler *et al*, 2014 (ex1-3)	Switzerland	M & F, C57B1/6 mice	70% liver I/R (60)	Hind limbs (femoral vascular bundle), invasive	Ex1-3, RIPC, 4x5/5 min	RIPC, 40 min pre-ischemia	3, 6, and 24 h	ALT, AST, liver histopathology (liver necrosis area)	(30)

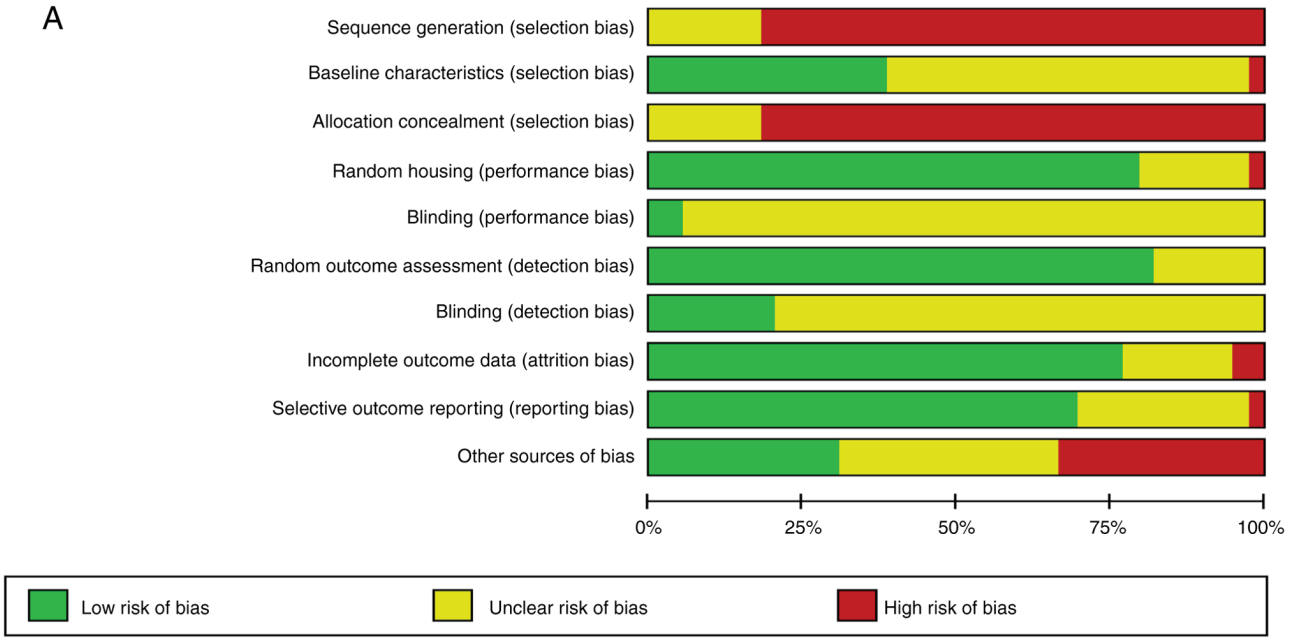
Table I. Continued.

First author/s, year	Country	Species	Model (min)	Method of RIC	Number and duration of cycles	Time of administration	Time of assessment[a]	Interested outcomes	(Refs.)
Garab *et al*, 2014 (ex1-3)	Hungary	M, Sprague Dawley rats	70% liver I/R (60)	Right hind limb (femoral artery), invasive	Ex1-3, RIPC, 2x10/10 min	RIPC, 40 min pre-ischemia	1, 2, and 3 h	ALT, AST LDH, TNF-α	(31)
Costa *et al*, 2014	Brazil	M, Wistar rats	70% liver I/R (60)	Left hind limb, non-invasive	RIPerC, 4x5/5 min	RIPerC, 20 min post-ischemia	2 h	ALT, AST	(32)
Wang *et al*, 2013 (ex1-4)	China	M, Sprague Dawley rats	OLT (60)	Hind limbs, non-invasive	Ex1-4, RIPC, 4x5/5 min	RIPC, 40 min pre-ischemia	2, 6, 12, and 24 h	ALT	(33)
Czigány *et al*, 2013 (ex1-3)	Hungary	M, Wistar rats	70% liver I/R (60)	Hind limbs (Infrarenal aorta), invasive	Ex1-3, RIPerC, 4x5/5 min	RIPerC, 20 min post-ischemia	1, 6, and 24 h	ALT, AST, liver histopathology (Suzuki classification) TNF-α	(34)
Tapuria *et al*, 2012	United Kingdom	M, Sprague Dawley rats	70% liver I/R (45)	Unilateral hind limb, non-invasive	RIPC, 4x5/5 min	RIPC, 40 min pre-ischemia	24 h	ALT, AST	(35)
Kanoria *et al*, 2012	United Kingdom	M, New Zealand white rabbits	THI (25)	Right hind limb, non-invasive	RIPC, 3x10/10 min	RIPC, 65 min pre-THI	2 h	ALT LDH	(36)
Cao *et al*, 2012 (ex1-4)	China	M, New Zealand white rabbits	70% liver I/R (25)	Hind limbs (femoral vascular bundle), invasive	Ex1-4, RIPC, 2x5/10 min	RIPC, 24 h pre-ischemia	0.5, 1, 2, and 3 h	ALT	(37)
Björnsson *et al*, 2012 (ex1-3)	Sweden	M, Sprague Dawley rats	70% liver I/R (60)	Right hind limb, non-invasive	Ex1-3, RIPC, 1x10/10 min	RIPC, 20 min pre-ischemia	0, 1, and 4 h	ALT, AST	(38)
Abu-Amara *et al*, 2011	United Kingdom	M, C57BL/6 mice	70% liver I/R (40)	Right hind limb, non-invasive	RIPC, 6x4/4 min	RIPC, 48 min pre-ischemia	2 h	ALT, AST, liver histopathology (4-point scale)	(39)
Wang (1) *et al*, 2010 (ex1-4)	Canada	F, C57BL/6 mice	70% liver I/R (60)	Right hind limb, non-invasive	Ex1-4, RIPC, 1x10/10 min	RIPC, 20 min pre-ischemia	0.5, 1, 2, and 3 h	ALT TNF-α	(40)
Wang (2) *et al*, 2010	China	M, Wistar rats	Hindlimb I/R (240)	Hind limbs, non-invasive	RIPC, 4x5/5 min	RIPC, 40 min pre-ischemia	4 h	ALT, AST	(41)
Tapuria *et al*, 2009	United Kingdom	M, Sprague Dawley rats	70% liver I/R (45)	Right hind limb, non-invasive	RIPC, 4x5/5 min	RIPC, 40 min pre-ischemia	3 h	ALT, liver histopathology (Suzuki classification)	(42)

Table I. Continued.

First author/s, year	Country	Species	Model (min)	Method of RIC	Number and duration of cycles	Time of administration	Time of assessment[a]	Interested outcomes	(Refs.)
Lai *et al*, 2006	China	F, Wistar rats	70% liver I/R (45)	Right hind limb (femoral artery), invasive	RIPC, 4x10/10 min	RIPC, before liver ischemia	4 h	ALT	(43)
Kanoria *et al*, 2006 (ex1-3)	United Kingdom	M, New Zealand white rabbits	THI (25)	Right hind limb, non-invasive	Ex1-3, RIPC, 3x10/10 min	RIPC, 65 min pre-THI	0.5, 1, and 2 h	ALT, AST LDH	(44)
Gustafsson *et al*, 2006 (ex1 and 2)	Sweden	F, Wistar rats	THI (60)	Right hind limb (femoral artery), invasive	Ex1 and 2, RIPC, 1x10/15 min	RIPC, 25 min pre-THI	0 and 1 h	ALT	(45)

[a]Time of post-reperfusion. RIC, remote ischemic conditioning; RIPC, remote ischemic pre-conditioning; RIPerC, remote ischemic per-conditioning; RIPostC, remote ischemic post-conditioning; Ex, experiment; OLT, orthotopic liver transplantation; I/R, ischemia-reperfusion; 70% liver I/R, complete ischemia of the median lobe and left lateral lobe (LLL) was achieved by clamping of the portal veins, hepatic arteries, and biliary branches using atraumatic microvascular clip; Hindlimb I/R, liver injury caused by the I/R injury of the hindlimb; HSR-I/R, hemorrhagic shock-resuscitation (HSR) following trauma contributes to organ dysfunction by causing I/R; THI, total hepatic ischemia; F, female; M, male; ALT, alanine transaminase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; TNF, tumor necrosis factor; IL, interleukin.



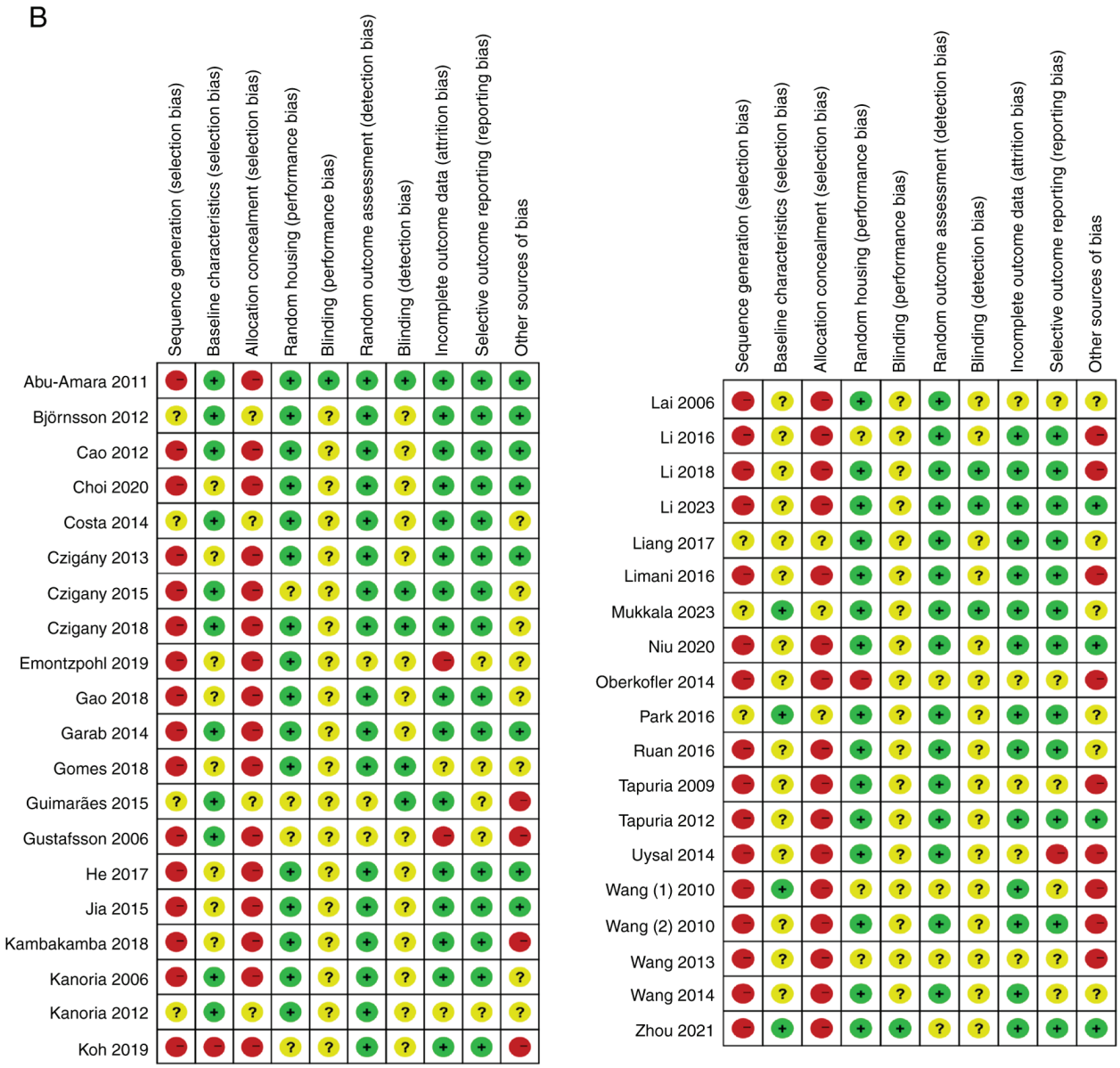


Figure 2. Risk of bias of the included studies. (A) Risk of bias graph. (B) Risk of bias summary.

Table II. Treatment effects of different factors based on primary outcomes.

	ALT			AST			Liver histopathology (scores)		
Overall effect/factors	No. of experiments (animals)	SMD (95% CI)	P-value	No. of experiments (animals)	SMD (95% CI)	P-value	No. of experiments (animals)	SMD (95% CI)	P-value
Overall effect	89 (1,087)	-2.15 (-2.54, -1.75)	<0.00001	67 (797)	-1.66 (-2.06, -1.27)	<0.00001	28 (350)	-2.10 (-2.69, -1.52)	<0.00001
Time of administration									
RIPC	66 (811)	-1.97 (-2.40, -1.55)	<0.00001	44 (521)	-1.45 (-1.90, -0.99)	<0.00001	15 (178)	-2.68 (-3.67, -1.69)	<0.00001
RIPerC and RIPostC	23 (276)	-2.78 (-3.77, -1.78)	<0.00001	23 (276)	-2.13 (-2.91, -1.34)	<0.00001	13 (172)	-1.58 (-2.24, -0.92)	<0.00001
Species									
All rats	54 (605)	-1.99 (-2.51, -1.46)	<0.00001	44 (493)	-1.44 (-1.92, -0.96)	<0.0001	19 (218)	-2.23 (-3.06, -1.41)	<0.00001
Sprague Dawley rats	32 (337)	-1.82 (-2.51, -1.13)	<0.00001	25 (277)	-1.34 (-2.08, -0.61)	0.0003	10 (104)	-3.02 (-4.86, -1.18)	0.001
Wistar rats	12 (178)	-2.94 (-4.23, -1.65)	<0.00001	9 (126)	-1.93 (-3.04, -0.81)	0.0007	5 (78)	-1.97 (-2.54, -1.39)	<0.00001
Lewis rats	10 (90)	-1.48 (-2.37, -0.58)	0.001	10 (90)	-1.24 (-1.79, -0.69)	<0.0001	4 (36)	-1.66 (-2.99, -0.34)	0.01
All mice	27 (332)	-2.08 (-2.79, -1.36)	<0.00001	20 (262)	-2.34 (-3.16, -1.52)	<0.0001	9 (132)	-1.89 (-2.69, -1.09)	<0.00001
C57BL/6 mice	22 (270)	-1.85 (-2.56, -1.13)	<0.00001	15 (200)	-2.24 (-3.17, -1.31)	<0.00001	8 (122)	-1.77 (-2.59, -0.95)	<0.0001
Wild-type mice	5 (62)	-3.21 (-6.00, -0.43)	0.02	5 (62)	-2.91 (-4.94, -0.87)	0.005	1 (10)	-3.14 (-5.30, -0.98)	0.004
New Zealand white rabbits	8 (150)	-3.11 (-3.85, -2.38)	<0.00001	3 (42)	-1.15 (-2.31, 0.01)	0.05			
HIRI model (min)									
All OLT	26 (260)	-2.26 (-3.04, -1.47)	<0.00001	19 (206)	-1.84 (-2.62, -1.05)	<0.00001	15 (172)	-2.05 (-2.85, -1.24)	<0.00001
OLT (45)	1 (12)	-4.70 (-7.26, -2.14)	0.0003	1 (12)	-4.00 (-6.26, -1.74)	0.0005	1 (12)	-8.71 (-13.12, -4.31)	0.0001
OLT (60)	4 (24)	-4.06 (-6.21, -1.92)	0.0002						
OLT (75)	5 (44)	-0.84 (-3.11, 1.43)	0.47	5 (44)	-1.02 (-3.58, 1.54)	0.43	4 (34)	-1.78 (-3.66, 0.10)	0.06
OLT (120)	3 (30)	-3.49 (-4.84, -2.13)	<0.00001				3 (30)	-4.18 (-5.73, -2.63)	<0.00001
OLT (235.7±18.3)	3 (60)	-3.46 (-6.52, -0.40)	0.03	3 (60)	-3.73 (-5.89, -1.57)	0.0007	3 (60)	-0.90 (-1.45, -0.35)	0.001
OLT (480)	10 (90)	-1.48 (-2.37, -0.58)	0.001	10 (90)	-1.24 (-1.79, -0.69)	<0.0001	4 (36)	-1.66 (-2.99, -0.34)	0.01
All 70% liver I/R	41 (539)	-2.11 (-2.69, -1.54)	<0.00001	29 (365)	-1.82 (-2.51, -1.13)	<0.00001	12 (162)	-2.29 (-3.25, -1.32)	<0.00001
70% liver I/R (25)	4 (96)	-2.90 (-3.50, -2.29)	<0.00001						
70% liver I/R (30)	5 (61)	-1.71 (-3.53, 0.12)	0.07	5 (61)	-1.51 (-2.94, -0.08)	0.04	3 (44)	-2.52 (-3.91, -1.13)	0.0004
70% liver I/R (40)	1 (12)	-2.40 (-4.02, -0.77)	0.004	1 (12)	-2.10 (-3.63, -0.57)	0.007	1 (12)	-1.06 (-2.31, 0.18)	0.09
70% liver I/R (45)	9 (100)	-1.96 (-3.21, -0.71)	0.002	5 (52)	-2.02 (-3.85, -0.20)	0.03	4 (45)	-2.90 (-6.76, 0.95)	0.14
70% liver I/R (60)	22 (270)	-2.13 (-2.99, -1.27)	<0.00001	18 (240)	-1.91 (-2.88, -0.95)	<0.0001	4 (60)	-2.32 (-3.02, -1.62)	<0.00001
All THI	7 (110)	-2.86 (-3.96, -1.75)	<0.00001	4 (58)	-1.06 (-1.84, -0.27)	0.008	1 (16)	-1.27 (-2.37, -0.16)	0.02
THI (25)	4 (54)	-3.91 (-5.92, -1.91)	0.0001	3 (42)	-1.15 (-2.31, 0.01)	0.05			
THI (60)	3 (56)	-2.04 (-3.20, -0.89)	0.0005	1 (16)	-0.96 (-2.01, 0.09)	0.07	1 (16)	-1.27 (-2.37, -0.16)	0.02
Hindlimb I/R (240)	3 (36)	-3.49 (-4.69, -2.29)	<0.00001	3 (36)	-1.99 (-2.89, -1.09)	<0.0001			
All HSR-I/R	6 (72)	-1.70 (-3.17, -0.22)	0.02	6 (72)	-1.38 (-2.54, -0.21)	0.02			

Table II. Continued.

Overall effect/factors	ALT			AST			Liver histopathology (scores)		
	No. of experiments (animals)	SMD (95% CI)	P-value	No. of experiments (animals)	SMD (95% CI)	P-value	No. of experiments (animals)	SMD (95% CI)	P-value
HSR-I/R (60)	5 (50)	-1.00 (-2.02, 0.01)	0.05	5 (50)	-0.99 (-2.08, 0.10)	0.08			
HSR-I/R (150)	1 (22)	-5.38 (-7.32, -3.44)	<0.00001	1 (22)	-2.93 (-4.20, -1.66)	<0.00001			
Operation of RIC									
Non-invasive	33 (382)	-2.21 (-2.99, -1.42)	<0.00001	23 (294)	-1.51 (-2.30, -0.72)	0.0002	11 (126)	-2.42 (-3.39, -1.45)	<0.00001
Invasive	56 (705)	-2.11 (-2.56, -1.66)	<0.00001	44 (503)	-1.71 (-2.16, -1.26)	<0.00001	17 (224)	-1.93 (-2.66, -1.19)	<0.00001
Number of limbs occluded									
One limb	47 (567)	-1.88 (-2.44, -1.33)	<0.00001	36 (427)	-1.49 (-2.05, -0.92)	<0.00001	8 (98)	-2.33 (-3.88, -0.78)	0.003
Two limbs	42 (520)	-2.44 (-2.99, -1.89)	<0.00001	31 (370)	-1.87 (-2.41, -1.34)	<0.00001	20 (252)	-2.03 (-2.63, -1.44)	<0.00001
Number of cycles									
1 cycle	11 (152)	-1.93 (-3.32, -0.54)	0.006	5 (72)	-2.69 (-6.50, 1.11)	0.17			
2 cycles	7 (132)	-2.58 (-3.07, -2.09)	<0.00001	3 (36)	-1.25 (-2.18, -0.33)	0.008			
3 cycles	33 (385)	-1.91 (-2.58, -1.24)	<0.00001	32 (373)	-1.71 (-2.29, -1.12)	<0.00001	12 (158)	-1.76 (-2.53, -0.99)	<0.00001
4 cycles	31 (342)	-2.32 (-2.97, -1.67)	<0.00001	22 (264)	-1.53 (-2.04, -1.02)	<0.00001	12 (146)	-2.41 (-3.06, -1.76)	<0.00001
6 cycles	7 (76)	-2.73 (-4.54, -0.92)	0.003	5 (52)	-2.62 (-4.58, -0.67)	0.009	4 (46)	-2.04 (-5.00, 0.93)	0.18
Duration of each cycle									
1 min	2 (20)	-1.23 (-5.25, 2.79)	0.55	2 (20)	-0.54 (-2.72, 1.64)	0.62	1 (8)	-2.44 (-4.76, -0.12)	0.04
3 min	1 (20)	-1.48 (-2.50, -0.47)	0.004	1 (20)	-1.85 (-2.93, -0.76)	0.0008	1 (20)	-2.11 (-3.25, -0.97)	0.0003
4 min	7 (76)	-2.73 (-4.54, -0.92)	0.003	5 (52)	-2.62 (-4.58, -0.67)	0.009	4 (46)	-2.04 (-5.00, 0.93)	0.18
5 min	56 (667)	-2.30 (-2.80, -1.80)	<0.00001	47 (545)	-1.95 (-2.42, -1.48)	<0.00001	16 (212)	-2.01 (-2.65, -1.37)	<0.00001
6 min	3 (50)	-3.49 (-4.84, -2.13)	<0.00001				3 (30)	-4.18 (-5.73, -2.63)	<0.00001
10 min	20 (264)	-1.58 (-2.43, -0.73)	0.0003	12 (160)	-0.55 (-1.51, 0.42)	0.26	3 (34)	-1.30 (-2.92, 0.33)	0.12
Total duration of limb ischemia									
≤5 min	4 (44)	-4.31 (-8.39, -0.22)	0.04				1 (8)	-2.44 (-4.76, -0.12)	0.04
10-15 min	38 (521)	-1.84 (-2.40, -1.29)	<0.00001	28 (345)	-1.89 (-2.61, -1.17)	<0.00001	9 (136)	-1.93 (-2.82, -1.04)	<0.0001
20-25 min	39 (422)	-2.39 (-2.99, -1.78)	<0.00001	26 (296)	-1.61 (-2.14, -1.09)	<0.00001	15 (172)	-2.42 (-3.38, -1.46)	<0.00001
30 min	7 (88)	-2.02 (-4.06, 0.01)	0.05	6 (76)	-0.69 (-1.87, 0.48)	0.25	3 (34)	-1.30 (-2.92, 0.33)	0.12
40 min	1 (12)	-1.77 (-3.19, -0.35)	0.01						
Time of assessment[a]									
0 h	5 (70)	-1.68 (-3.31, -0.04)	0.04	4 (50)	-0.58 (-1.71, 0.55)	0.32			
0.5 h	3 (48)	-1.90 (-3.45, -0.34)	0.02	1 (14)	-0.32 (-1.38, 0.73)	0.55			
1 h	10 (142)	-2.09 (-3.80, -0.38)	0.02	6 (76)	-0.26 (-1.64, 1.13)	0.72	2 (28)	-0.22 (-4.48, 4.04)	0.92

Table II. Continued.

	ALT			AST			Liver histopathology (scores)		
Overall effect/factors	No. of experiments (animals)	SMD (95% CI)	P-value	No. of experiments (animals)	SMD (95% CI)	P-value	No. of experiments (animals)	SMD (95% CI)	P-value
2 h	14 (182)	-2.58 (-3.70, -1.45)	<0.00001	9 (120)	-2.16 (-3.17, -1.15)	<0.0001	5 (62)	-2.46 (-4.14, -0.78)	0.004
3 h	14 (156)	-2.00 (-3.03, -0.96)	0.0002	10 (98)	-1.27 (-2.47, -0.07)	0.04	7 (70)	-3.58 (-5.67, -1.50)	0.0007
4 h	4 (54)	-1.66 (-3.68, 0.36)	0.11	3 (42)	-1.96 (-4.36, 0.44)	0.11	1 (14)	-1.95 (-3.30, -0.60)	0.005
6 h	10 (122)	-3.12 (-4.19, -2.06)	<0.00001	9 (116)	-3.17 (-4.46, -1.89)	<0.00001	3 (48)	-2.22 (-2.98, -1.46)	<0.00001
12 h	3 (26)	-2.90 (-4.72, -1.08)	0.002	2 (20)	-4.70 (-8.21, -1.20)	0.009			
24 h	16 (179)	-2.26 (-3.00, -1.53)	<0.00001	14 (163)	-1.63 (-2.25, -1.02)	<0.00001	8 (98)	-1.62 (-2.30, -0.94)	<0.00001
48 h	2 (20)	-1.54 (-7.15, 4.06)	0.59	2 (20)	-5.29 (-15.37, 4.79)	0.30			
72 h	4 (50)	-2.64 (-5.25, -0.03)	0.05	3 (40)	-3.48 (-7.54, 0.58)	0.09	2 (30)	-2.87 (-6.29, 0.56)	0.10
168 h	4 (38)	-0.16 (-0.81, 0.50)	0.64	4 (38)	-0.33 (-1.00, 0.35)	0.34		-2.10 (-2.69, -1.52)	

[a]Time of post-reperfusion. ALT, alanine transaminase; AST, aspartate aminotransferase; CI, confidence interval; RIPC, remote ischemic pre-conditioning; RIPerC, remote ischemic per-conditioning; RIPostC, remote ischemic post-conditioning; OLT, orthotopic liver transplantation; I/R, ischemia-reperfusion; 70% liver I/R, complete ischemia of the median lobe and left lateral lobe (LLL) was achieved by clamping of the portal veins, hepatic arteries, and biliary branches using atraumatic microvascular clip; Hindlimb I/R, liver injury caused by the I/R injury of the hindlimb; HSR-I/R, hemorrhagic shock-resuscitation (HSR) following trauma contributes to organ dysfunction by causing I/R; THI, total hepatic ischemia; SMD, standardized mean difference.

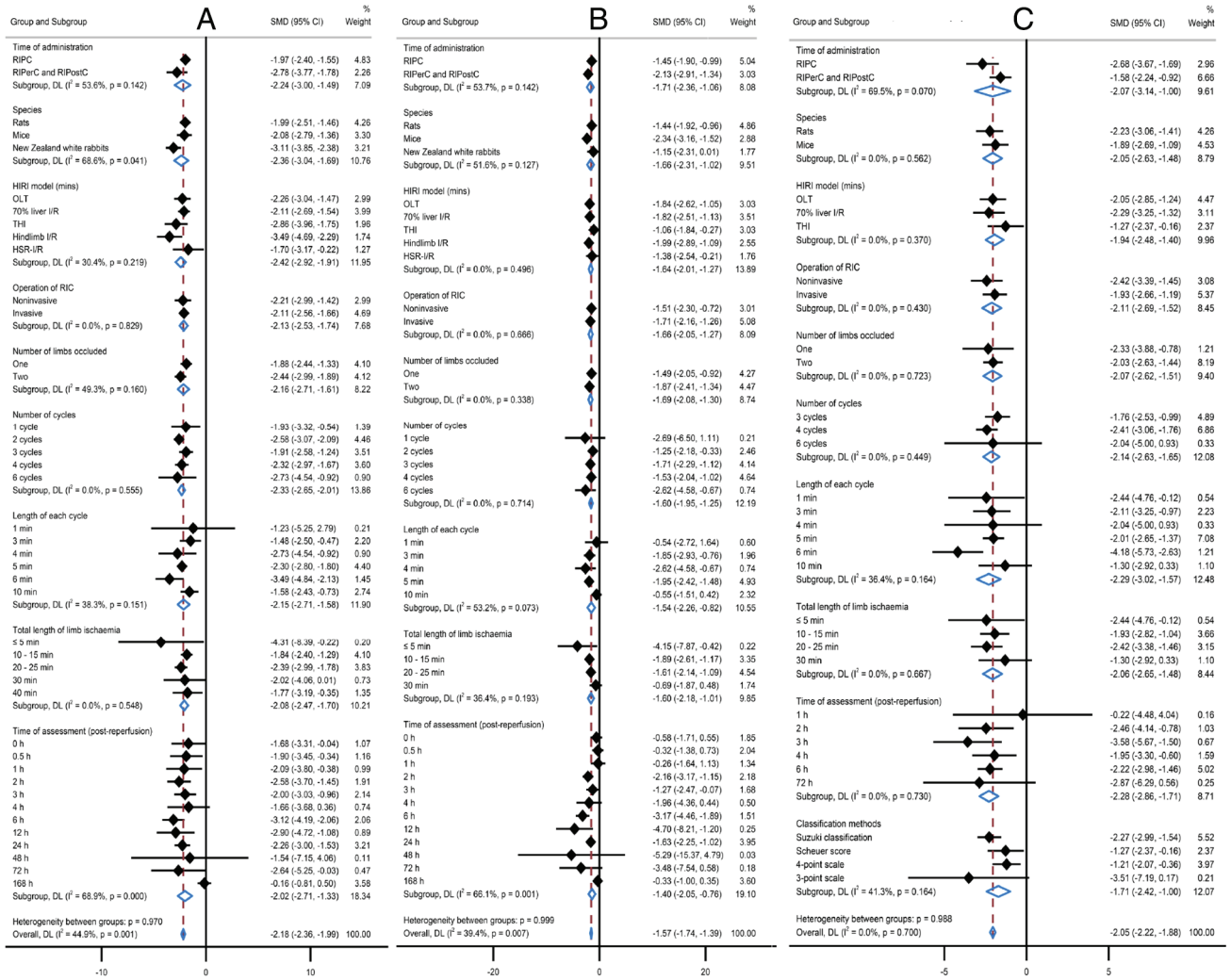


Figure 3. Subgroup analyses of all RIC studies. Changes in (A) ALT, (B) AST and (C) liver histopathology. RIC, remote ischemic conditioning; ALT, alanine transaminase; AST, aspartate transaminase.

Effects of all RIC studies. RIC was significantly effective against all the primary outcomes (ALT, AST and liver histopathology; all P<0.00001), although there were significant statistical heterogeneities: I^2=79% in the ALT change, 76% in the AST change, and 71% in the liver histopathology change (Table II; Figs. S1-S3). Notably, RIPerC/RIPostC exhibited greater effects on ALT and AST changes [ALT SMD (95%CI): RIPC -1.97 (-2.40, -1.55) vs. -2.78 (-3.77, -1.78); P=0.142; AST SMD (95%CI): RIPC -1.45 (-1.90, -0.99) vs. -2.13 (-2.91, -1.34); P=0.142], and RIPC exerted a greater effect on liver histopathology change [SMD (95%CI): RIPC -2.68 (-3.67, -1.69) vs. -1.58 (-2.24, -0.92); P=0.070]; however, there was no interactions between the two groups in the meta-regression analysis. RIC was the greatest magnitude effective if the duration of each cycle was 4-6 min or the total duration of limb ischemia was 10-25 min. ALT and AST changes significantly interacted with the assessment times, with two peaks occurring 2-3 or 6-12 h post-reperfusion lasting up to 72 h. The efficacy of RIC on ALT change was greatest in the New Zealand white rabbits model with species interaction (P=0.041), but this animal model was much fewer (n=150). In addition, there were no interactions between liver histopathology and species, the HIRI model, the operation of RIC, the number of limbs occluded, the number of cycles, the duration of each cycle, the total duration of limb ischemia, or classification methods of liver histopathology score in the meta-regression analysis (all P>0.05). The details are shown in Table II and Fig. 3.

RIC was also significantly effective against several secondary outcomes such as LDH, TNF-α and apoptosis index (all P<0.00001) and there were also significant statistical heterogeneities (I^2=75%, I^2=76%, I^2=51%, respectively; Table SII; Fig. S4). Due to the limitations of the sample size and data integrity, only a meta-regression analysis was performed by dividing the groups of RIPC and RIPerC/RIPostC, and there were no interactions between the two groups (P=0.91, P=0.16, P=0.75, respectively; Fig. S5). In addition, only four studies (five experiments; 78 animals) (7,12,21,26) evaluated IL-6 change, and five studies (13 experiments; 156 animals) (10,12,18,21,26) evaluated IL-10 change, showing that RIC was ineffective against IL-6 and IL-10 changes [SMD (95%CI): -1.47 (-3.72, 0.79); P=0.20; SMD (95% CI): -0.34 (-1.39, 0.71); P=0.52].

Effects of RIPC studies. The effects of RIPC protocol variables were assessed against the primary outcomes changes using meta-regression (Fig. 4). The effects of RIPC on ALT and AST changes were significant if the duration of each cycle was

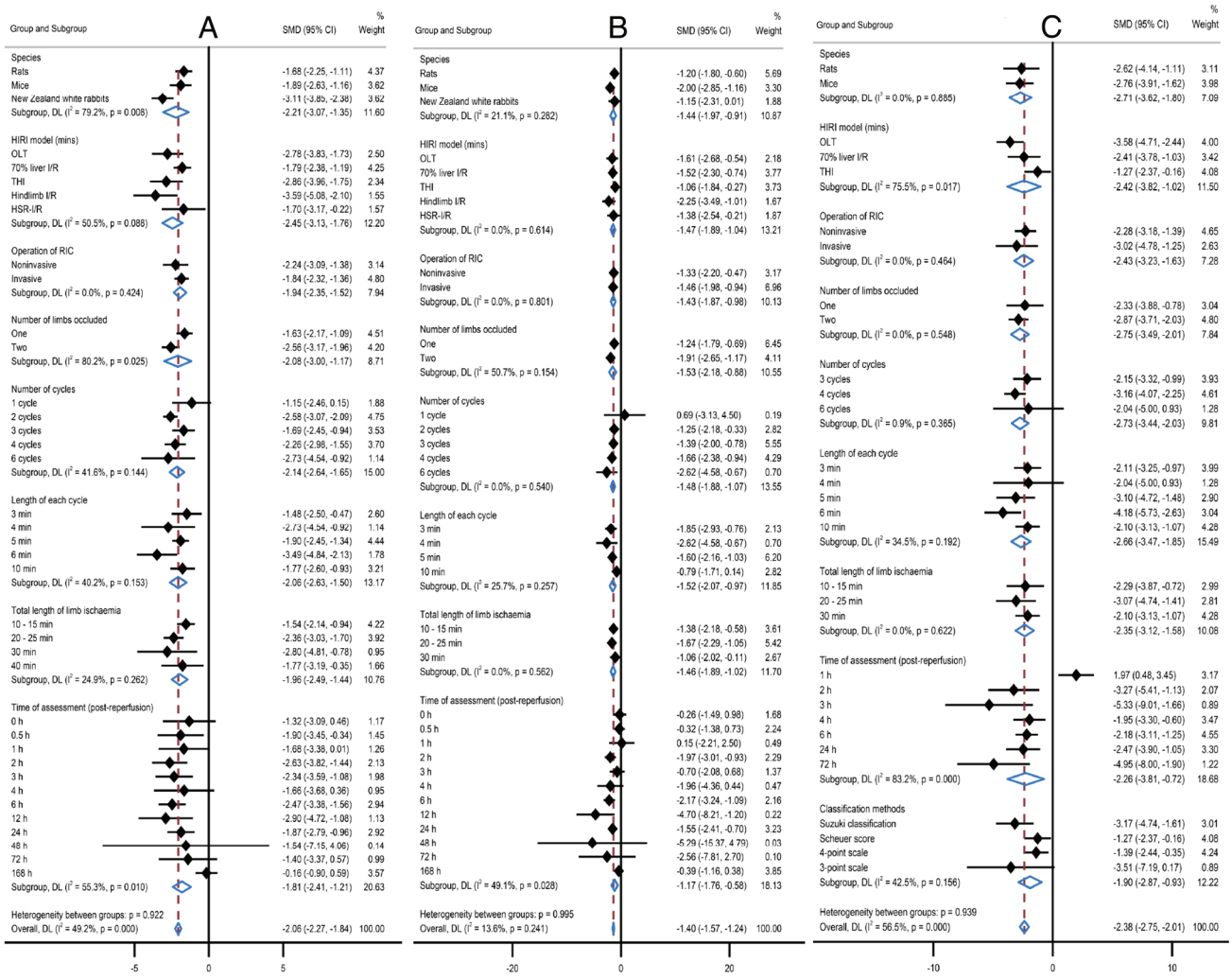


Figure 4. Subgroup analyses of all RIPC studies. Changes in (A) ALT, (B) AST and (C) liver histopathology. RIPC, pre-conditioning; ALT, alanine transaminase; AST, aspartate transaminase.

6 min or the total duration of limb ischemia was 10-25 min, with two peaks occurring at 2-3 or 6-12 h following reperfusion and lasting up to 72 h. RIPC was most effective against ALT change in New Zealand white rabbits and had no interactions in rats or mice (P=0.008). Using one or two limbs of RIPC reduced ALT but using two limbs was significantly improved than one limb [SMD (95%CI): -2.56 (-3.17, -1.96) vs. -1.16 (-2.17, -1.09); P=0.025]. In addition, the effect of RIPC on the liver histopathological change was improved in the OLT model (P=0.017), and there were no interactions between RIPC and the liver histopathological score classification method (P=0.156).

Effects of RIPerC/RIPostC studies. The effects of RIPerC/RIPostC protocol variables were assessed against the primary outcomes changes using meta-regression (Fig. 5). Similar to the RIPC protocol, the protective effects against ALT and AST changes revealed two peaks, but the first peak occurred earlier (1-3 h post-reperfusion). The efficacies of RIPerC/RIPostC on ALT and AST interacted with the RIC cycles, the most significant at one cycle of RIC (P=0.002; P<0.001), but this animal model is much fewer (n=24). Using one or two limbs of RIPerC/RIPostC reduced ALT and AST, but using one limb was a significant improvement on two limbs [ALT SMD (95%CI): -6.04 (-9.44, -2.65) vs. -2.25 (-3.25, -1.24), P=0.036; AST SMD (95%CI): -5.41 (-8.90, -1.93) vs. -1.83 (-2.60, -1.05), P=0.049]. Notably, the protection was ineffective if the duration of each cycle was 10 min or the total duration of the limb occlusion was 30 min.

Publication bias. Notably, Begg's funnel plot (Fig. 6) shows the asymmetric patterns in included studies, suggesting a possible publication bias in this meta-analysis (All Begg's statistics P<0.05). Further analyzing the sources of statistical heterogeneity revealed the presence of interactions with respect to country. The protective effects of RIC on ALT and AST changes were highest in Brazil and the impact on liver histopathology was highest in Korea (Fig. S6).

Discussion

HIRI is a complex pathophysiological process, which is essentially a series of inflammatory cascade reactions triggered by the release of a large number of inflammatory mediators following the activation of hepatic Kupffer cells, hepatic sinusoidal endothelial cells and hepatic stellate cells, which may lead to the postoperative liver dysfunction and partial

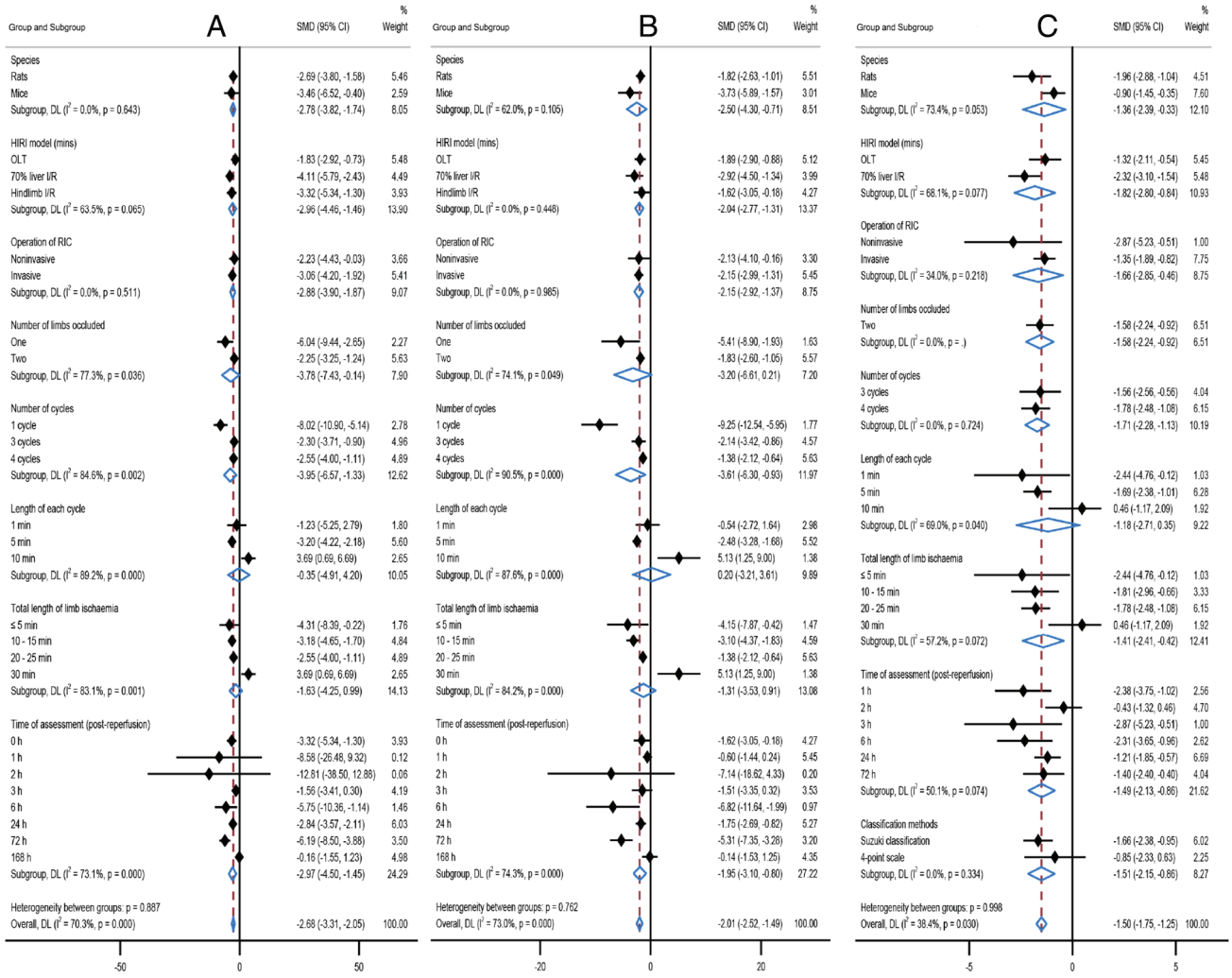


Figure 5. Subgroup analyses of all RIPerC/RIPostC studies. Changes in (A) ALT, (B) AST and (C) liver histopathology. RIPerC, per-conditioning; RIPostC, post-conditioning; ALT, alanine transaminase; AST, aspartate transaminase.

residual liver cell death, or even an increase of perioperative mortality (46,47). Various strategies such as pharmacological modifiers, physiological scavengers and physical processes have been investigated to avoid the adverse effects of HIRI following liver transplantation and liver resection (4,48). RIC is one of these novel strategies, which can induce nonlethal stress to the remote organs through transient ischemia, resulting in local and systemic tolerance to IRI. There is too little clinical evidence; however, the role of RIC in animal HIRI models has been extensively studied. To the best of the authors' knowledge, there have been no systematic reviews and meta-analyses related to this topic.

The reviewers carefully set the primary outcomes to ensure that this systematic review and meta-analysis of preclinical studies can be applied to the clinics. Although there is still a lack of a reliable endpoint that can effectively predict the outcome of patients undergoing surgery, most ongoing HIRI clinical trials focus on the markers of liver injury (ALT and AST) as primary outcomes (2). Considering this clinical setting, the present review briefly analyzed the efficacy of RIC on HIRI in preclinical studies with ALT, AST and liver histopathology as the primary outcomes and LDH, TNF-α, and apoptosis index as secondary outcomes. The present systematic review and meta-analysis of 39 articles (101 animal experiments, 1,061 animals) confirmed that RIC (RIPC or RIPerC/RIPostC) had a powerful effect on improving ALT, AST and liver histopathology changes in the preclinical liver I/R models. In all RIPerC/RIPostC studies, ALT and AST changes appeared to be more efficacious than RIPC in both rats and mice, in 70% of liver I/R models and using one or two limbs and using invasive or non-invasive operations. However, RIPC appeared to have a more potent effect on liver histopathology change.

Significant statistical heterogeneities were present in both RIPC and RIPerC/RIPostC groups and the meta-regression subgroup analysis helped to explore the effects of these two protocol variables on the changes in primary outcomes. Significant interactions with species in RIC experiments showed that RIPC was most effective in New Zealand white rabbits. However, the RIPerC/RIPostC group lacked the New Zealand white rabbits' trial, and both groups were equally effective in rats and mice. This interspecies difference has raised concerns about treatment failure when moving into clinical trials. A recent study (2) evaluating the beneficial effects and applicability of RIPC in hepatectomy demonstrates that RIPC has some short-term liver protection against

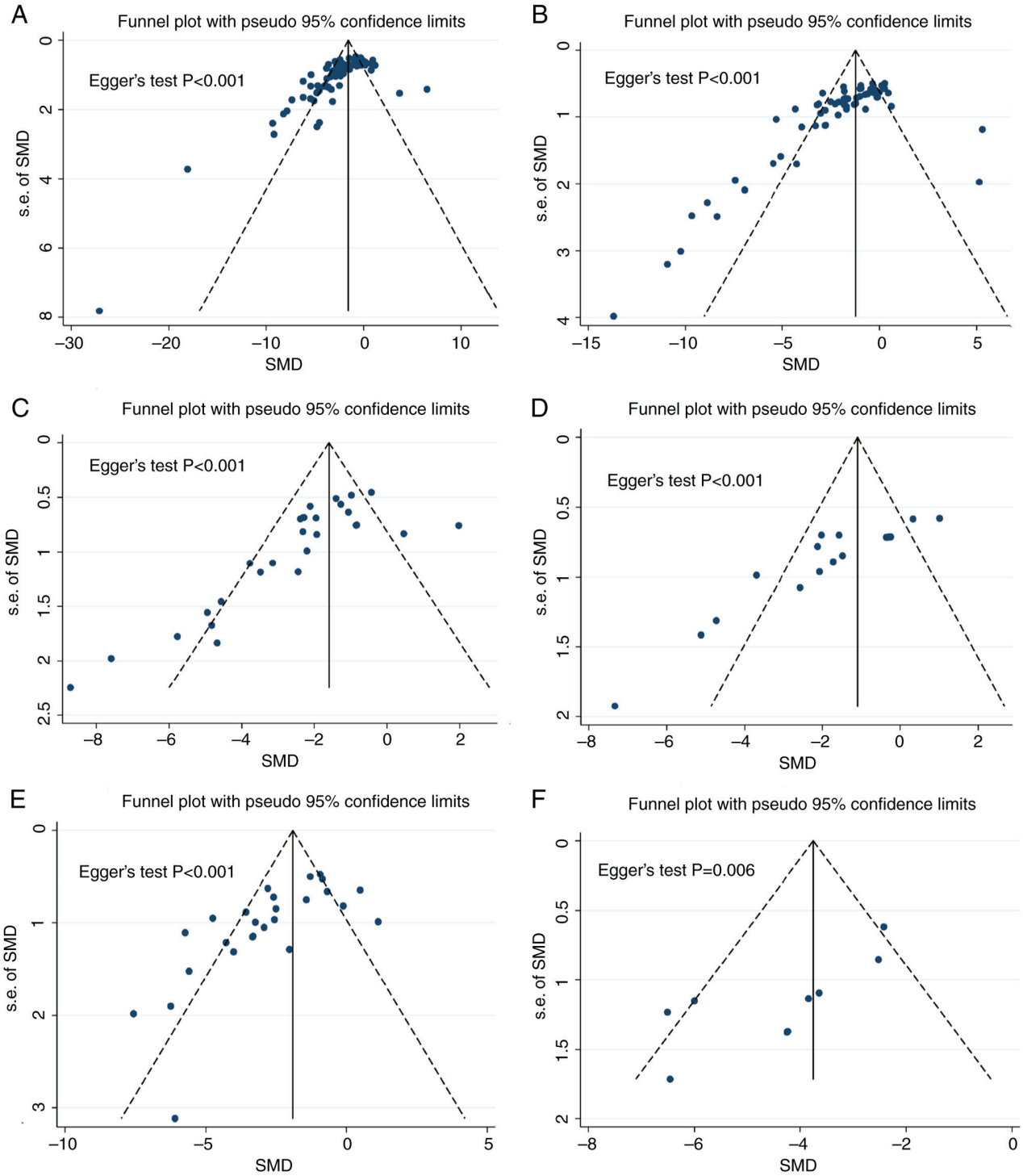


Figure 6. Egger's funnel plots of all RIC studies. Changes in (A) ALT, (B) AST and (C) liver histopathology, (D) LDH changes, (E) TNF-α changes and (F) apoptosis index. RIC, remote ischemic conditioning; ALT, alanine transaminase; AST, aspartate transaminase; LDH, lactate dehydrogenase; TNF-α, tumor necrosis factor-α.

HIRI during hepatectomy but has limited improvement in clinical outcomes.

RIPerC/RIPostC studies revealed significant interactions with the total duration of limb ischemia (defined as the dose of RIC) and RIC was ineffective when the dose was 30 min. The 40-min dose was not tested in the RIPerC/RIPostC group, but the effect began to wane in the RIPC group. However, RIPC studies showed no significant interactions with doses, suggesting the existence of a dose therapeutic window with an optimal period between 10 and 25 min. The number of limbs used for RIC may somewhat reflect the dose. However, the present review showed that RIPC was a significant improvement, compared with unilateral, regarding ALT change on both sides, whereas RIPerC/RIPostC was the opposite. Frustratingly, the reviewers gained no reasonable explanation and heterogeneity or publication bias could not be ruled out. In addition, none of the included studies involved a repeat dose regimen (RIPC+RIPerC/RIPostC), and whether this provides added benefit warrants further verification.

The mechanisms of RIC are still being explored and may involve various inflammatory mediators, receptors, gene expression and other links. Some studies have demonstrated that RIC has two protective time windows (49-51). The first protective time window (also known as the classical protective window) occurs immediately after RIC and has a strong effect. It lasts for 2-3 h and possibly relates to altering endogenous substances (such as adenosine, bradykinin and nitric oxide) produced during RIC. The second window of protection occurs several hours after RIC, and the effect is weak, lasting 72-96 h. This second window of protection may be related to the cell signaling pathway and gene regulation after releasing endogenous substances. In the present review, the two peak levels of RIC action confirmed the protective time window effect of RIC, and the first peak of RIPerC/RIPostC appeared earlier, indicating that it may take effect more quickly.

Although the present meta-analysis provided rigorous information on RIC's efficacy for HIRI, there were still some limitations, suggesting that the results of all outcomes should be interpreted with caution, needing more well-designed preclinical and clinical studies. First, the majority of articles included in this review studied healthy young male rodent models, which may not accurately reflect clinical scenarios involving comorbidities. By contrast, most patients were middle-aged and elderly with one or more comorbidities that may inhibit the effects of RIC, such as hypertension, diabetes, hepatitis B, fatty liver or cirrhosis. Second, anesthesia during RIC implementation is another concern, as propofol or sevoflurane has been reported to have liver protective effects (52,53). All current clinical studies have been conducted under propofol anesthesia or propofol combined with inhalation anesthesia, which may interfere with the effects of RIC and is also a hot topic of debate. Third, for the countries where animal experiments were conducted in this study, the sources of heterogeneity were significant. There was also a substantial risk of publication bias, with a worrying tendency to over-interpret positive results. Fourth, as shown in Table II, the number of experiments on some models and species is small, and some experiments have limited data, so it is difficult to fully consider all animal models and species differences in meta-analysis. Therefore, some species or models were simply merged, such as 'All rats' and 'All OLT' in Table II and Fig. 3. However, from the statistical data of the present review, these will not affect the main results of this study. Fifth, the optimal frequency and repeated dosing effects remain unclear. RIPC combined with RIPerC/RIPostC or a daily repetition protocol would be a promising exploration. Overall, the present review demonstrated promising preclinical evidence for RIC in HIRI, but its clinical translation requires addressing these limitations. However, it remains a comprehensive review and probably the most accurate preclinical evidence of the literatures to date.

In summary, RIC significantly alleviated HIRI in the experimental models. RIPerC/RIPostC acted more quickly and affected ALT and AST changes, whereas RIPC significantly affected liver histopathology. RIC has a dose therapeutic window and the best period is 10-25 min. However, given the significant statistical heterogeneities and risk of publication bias, future studies using repeated doses in animal models with comorbidities will generate innovative ideas for its therapeutic applications.

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Availability of data and materials

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Authors' contributions

CT was responsible for conceptualization, funding acquisition, data curation and writing the original draft. AW was responsible for validation, data curation, software, supervision, writing, reviewing and editing. YK was responsible for conceptualization, validation, writing, reviewing and editing. Data authentication is not applicable

Ethics approval and consent to participate

The present review was exempted from an ethical opinion by the Ethics Committee of Yongchuan Hospital of Chongqing Medical University as it was a systematic review of the literature.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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