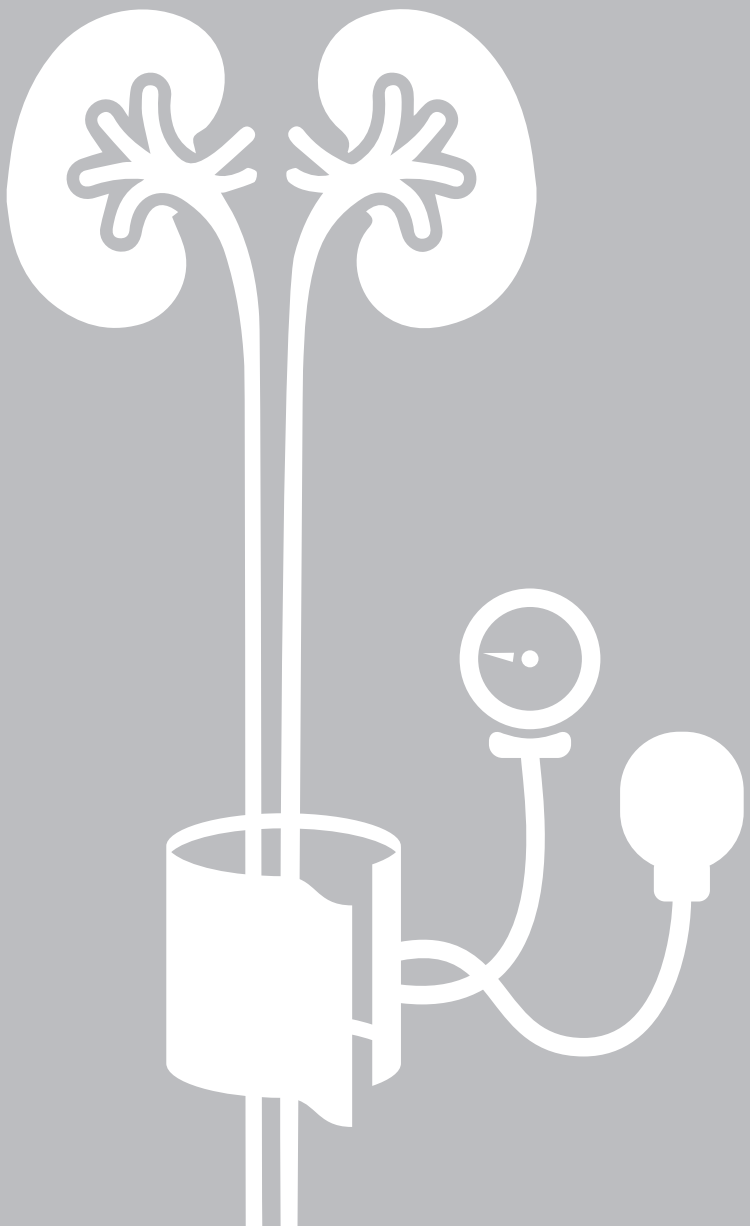
Ischemic preconditioning to reduce ischemia-reperfusion injury of the kidney



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Ischemic preconditioning to reduce ischemia-reperfusion injury of the kidney

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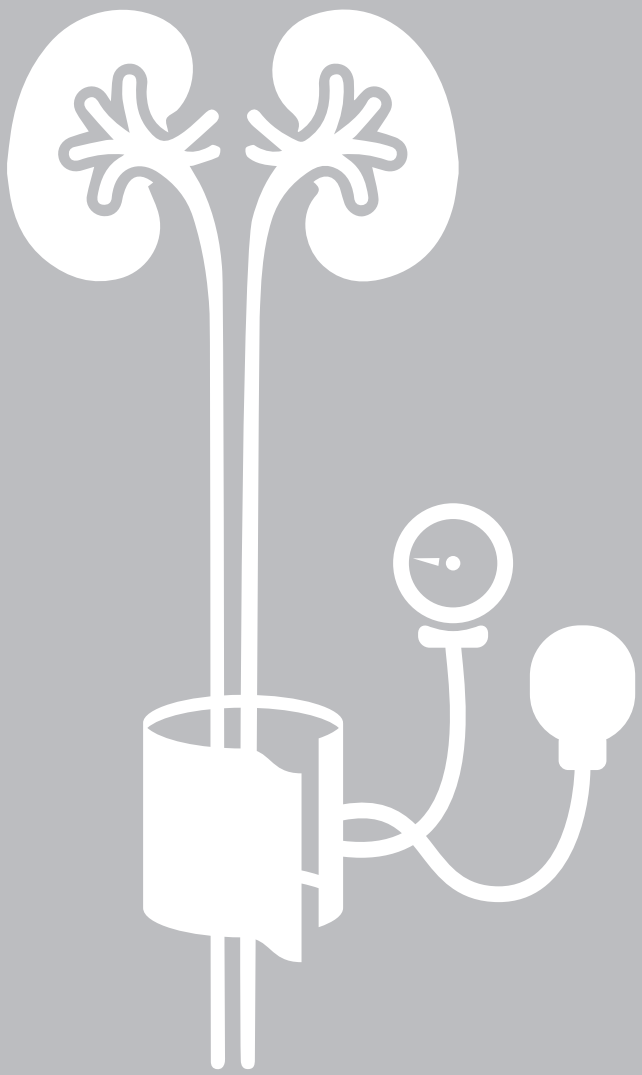
Contents



1

General introduction

ISCHEMIA REPERFUSION INJURY OF THE KIDNEY

A 69-year-old patient walked into the hospital without any complaints and was scheduled to undergo elective open abdominal aneurysm repair. The operation required 40 minutes of suprarenal clamping of the aorta, temporarily obstructing the renal blood flow and therefore causing the patient to develop renal failure. As a result, the elective intervention tragically sentenced this man to lifelong dependency on haemodialysis. This dramatic clinical presentation of the patient in the intensive care unit provides the starting point of our interest in ischemia-reperfusion injury of the kidney.

The type of renal damage described above can occur in aorta surgery, kidney transplantation, iodine contrast administration for imaging, sepsis, kidney operations, and in major operations with large hemodynamic changes. The pathophysiology of this type of renal damage is well understood: the blood flow is the most important regulatory guardian of the fundamental needs of the human (renal) cell, which depends on a strict range of parameters for its viability, such as temperature, electrolyte balance, nutrition and oxygenation. Compensating mechanisms enable the cell to cope with minor changes in these parameters, but larger disturbances can have devastating effects, such as cell death, tissue damage, loss of organ function, organ failure, and even death. The specific type of renal damage described in the case above is called ischemia-reperfusion injury (IRI) and is caused by the temporary obstruction of or lower blood flow to the organ or tissue. The degree of damage is determined by the duration of the obstruction or lower blood flow and the type of tissue involved, as tissues differ in susceptibility to IRI.

In addition to the brain and heart, the kidney is particularly vulnerable to IRI because of its high energy demand and intricate microvascular network. This high energy demand is illustrated by the fact that apart from the heart, the kidney contains the highest number of mitochondria in the body. The primary tasks of the kidney involve continuous cellular processes with large energy and oxygen consumption, including maintaining electrolyte, acid-base and osmolality balance, excreting waste products and reabsorbing vital nutrients [1]. Blood flow to the kidney is high, with approximately 20% of the cardiac output running through both kidneys. However, the anatomy of the renal vasculature leads to low oxygen concentrations in specific parts of the kidney, which are just sufficient under normal physiological circumstances. This results in a narrow range of supply and demand of oxygen and nutrient concentrations, making the kidney highly susceptible to IRI [2]. The typical period of renal ischemia that causes irreversible damage is longer than 30 minutes [3].

In order to attenuate the detrimental effects of IRI, it is essential to understand its underlying pathophysiological mechanism. In the past thirty years, our understanding of the molecular pathways in IRI has greatly increased. It is assumed that mitochondria, the energy producing organelles in cells, play a pivotal role in IRI. In the absence or severe reduction of blood circulation, the cell becomes deprived of oxygen, switching its energy cycle from the aerobic citric acid cycle (the producer of adenosine triphosphate; ATP) to anaerobic metabolism. The latter generates H^+, pyruvic acid and lactate, thereby lowering the intracellular pH. Simultaneously, ATP depletion leads to ATP-dependent ion transporters dysfunction, disrupting the intracellular ion homeostasis. Due to the acidic intracellular environment, the cell transports H^+ outside the cell in exchange for Na^+. This increase in intracellular Na^+ reverses the activity of the Na^+/Ca^{2+} exchanger, causing an influx of Ca^{2+}. The subsequent rise in intracellular and mitochondrial calcium levels induces cell swelling and eventually cell death [4].

Restoration of the circulation is essential to discontinue the harmful effects of ischemia. However, paradoxically, reperfusion is also detrimental to the ischemic tissue and greatly contributes to the total degree of IRI. For instance, in animal models of myocardial infarction, reperfusion injury accounts for 50% of the total infarct size [5]. Reperfusion injury is caused by the induction of prolonged opening of the mitochondrial permeability transition pore (mPTP), which is located in the inner mitochondrial membrane. The opening of the mPTP leads to depolarisation of the mitochondrial membrane and an increase in its permeability, causing disruption of the mitochondria and eventually cell death. The extent of the mPTP opening determines whether the cell can recover, undergo apoptosis, or undergo necrotic cell death. Two pathophysiological processes following reperfusion affect the opening of the mPTP. First, the acidic environment during the ischemic period prevents the opening of mPTP. After reperfusion, abrupt restoration of intracellular pH occurs due to the re-activation of Na^+/H^+-exchanger and Na^+/HCO_3-transporter, which result in mPTP opening. Second, abrupt restoration of oxygen levels causes this oxygen to react with enzymes and metabolites of anaerobic metabolism, forming reactive oxygen species (ROS) that cause oxidative stress, lipid peroxidation, damage to membrane of cells, and prolonged opening of the mPTP [6-9]. Because of its pivotal role in IRI, the pharmacological inhibition of mPTP-opening has been proposed as a promising strategy to reduce IRI. However, despite the identification of some substructures of the mPTP, its exact anatomical structure remains unclear [10, 11]. It is therefore not yet possible to specifically target the mPTP with pharmacological agents [12].

ISCHEMIC CONDITIONING STRATEGIES

The current strategy to reduce renal IRI is to shorten the ischemic period and, in transplantation surgery, to cool the organ. However, these strategies are often insufficient or not possible. Another method that has been shown to diminish IRI is local ischemic preconditioning (LIPC). LIPC is the application of short, harmless periods of ischemia to a certain tissue in order to reduce IRI caused by a subsequent long, harmful period of ischemia [13]. In 1986 Murry et al. [14] were the first to describe LIPC in a canine myocardial infarction model. A protocol of 4x5 minutes of ischemia and reperfusion proved highly successful in reducing myocardial infarct size upon 40 minutes of index ischemia and reperfusion (Figure 1.1). However, the same LIPC protocol was not successful in reducing IRI after three hours of index ischemia and reperfusion [14], indicating that protection can only be accomplished with a selected conditioning protocol and that its efficacy is dependent on the severity of IRI. Since this landmark study, LIPC has been shown to induce protection against IRI in many organs. Although the first study investigating the protective effects of LIPC for renal IRI, published in 1997 [15], showed no protection, later studies did show highly successful reduction of renal IRI [16] (Figure 1.1).

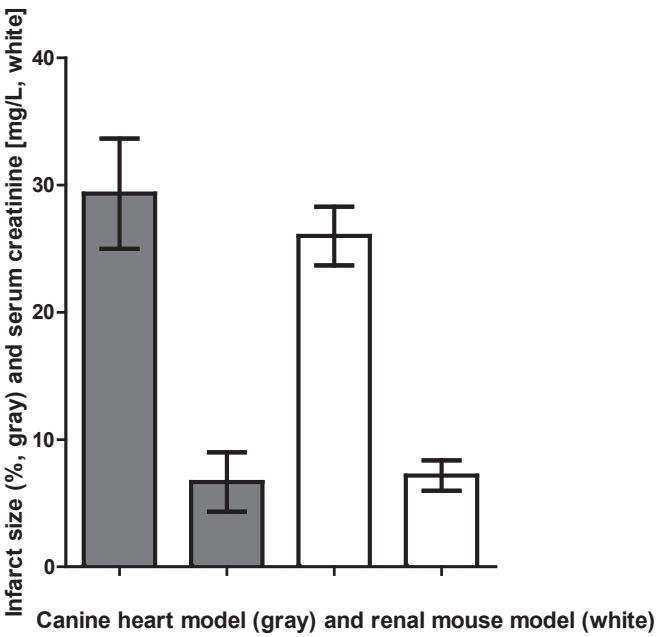


Figure 1.1. Infarct size in a canine heart model of Murry et al. in 1986 and serum creatinine concentrations in the study of Islam et al. after 48 hours, 45min IRI, in control and 4x 4min LIPC mice, figure adapted from [14, 15].

It was not until decades after the discovery of LIPC that the mechanism of action in LIPC was explored. Since then, thousands of studies have reported over 100 different molecules related to the mechanism underlying LIPC [17]. Despite this vast amount of data, the precise mechanism through which LIPC prevents IRI remains unclear. However, some pieces of the puzzle have been identified. With the mitochondrion being the key in IRI, the same organelle provides the basis for the protective mechanism of LIPC. More specifically, inhibiting mPTP opening prevents IRI [18] and LIPC inhibits the opening of mPTP [19, 20]. Since mPTP opening occurs in the first minutes of reperfusion, the protective agent of LIPC has to be active in this timeframe, making appropriate timing of the LIPC stimulus crucial [21]. In animals, protective effects of LIPC have been found in studies where the LIPC protocol was applied a few minutes or hours before IRI, the so-called early window of protection, as well as in studies with a long interval of days or even weeks between LIPC and IRI, the late window of protection.

The signalling pathways underlying LIPC can be divided into two main categories. In the first category, LIPC directly inhibits mPTP opening by activating specific signalling cascades, including the Reperfusion Injury Salvage Kinase (RISK) and Survivor Activating Factor Enhancement (SAFE) pathways (Figure 1.2) [22-24]. In the second category, LIPC indirectly inhibits mPTP opening by attenuating a number of the intracellular changes caused by ischemia and reperfusion. These intracellular changes include four distinctive mechanisms of action: 1) reduction of reactive oxygen species (ROS) formation [25, 26]; 2) opening of the ATP-dependent mitochondrial K^+ channels, which reduces the mitochondrial Ca^{2+} overload, thereby inhibiting mPTP opening [24, 27-29]; 3) reduction of the ATP consumption and/or preservation of ATP production during ischemia and reperfusion [30, 31]; and 4) delaying the restoration of the intracellular pH, thereby maintaining the acidic environment and inhibiting the opening of mPTP [32]. The precise mechanism of the two main pathways is not yet fully understood, and the direct and indirect effects of LIPC on the mPTP can act simultaneously and have a certain overlap in their working mechanism [23, 24].

Overall, there is sufficient evidence to suggest that, with a specific LIPC protocol and a determined IRI period, LIPC is effective in reducing IRI in a wide range of animal models, in different organs [33]. Although there is growing evidence of how LIPC works, it is not yet possible to predict the most effective application of LIPC for a specific species, organ or tissue; there is no overview of all animal studies performed. Such a meta-analysis could identify characteristics that determine the effectiveness of LIPC protocols and could give answers to the translational difficulties between animal studies and human trials.

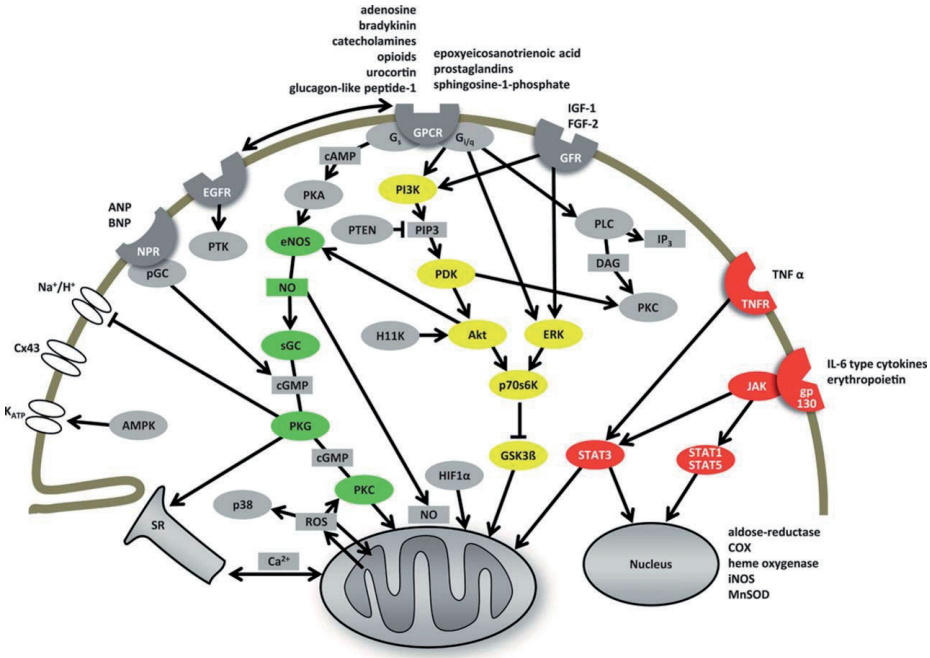


Figure 1.2. The Reperfusion Injury Salvage Kinase (RISK, in yellow) and Survivor Activating Factor Enhancement (SAFE, in red) pathways [17].
Akt indicates protein kinase B; AMPK, cyclic adenosine monophosphate–activated kinase; BNP, brain natriuretic peptide; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; COX, cyclooxygenase; Cx 43, connexin 43; DAG, diacylglycerol; EGFR, epidermal growth factor receptor; ERK, extracellular regulated kinase; FGF, fibroblast growth factor; Gs/Gi/q, stimulatory/inhibitory G protein; GPCR, G protein-coupled receptor; gp130, glycoprotein 130; GSK3β, glycogen synthase kinase 3 β; H2S, hydrogen sulfide; H11K, H11 kinase; HIF1α, hypoxiainducible factor 1α; IGF, insulin-like growth factor; iNOS, inducible NO synthase; IP3, inositoltrisphosphate; JAK, Janus kinase; KATP, ATP-dependent potassium channel; Na^+/H^+, sodium/proton-exchanger; NPR, natriuretic peptide receptor; pGC, particulate guanylate cyclase; p38, mitogen-activated protein kinase p38; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; PI3K, phosphatidylinositol [4,5)-bisphosphate 3-kinase; PKC, protein kinase C; PKG, protein kinase G; PLC, phospholipase C; PTEN, phosphatase and tensin homolog; PTK, protein tyrosin kinase; ROS, reactive oxygen species; sGC, soluble guanylate cyclase; SR, sarcoplasmic reticulum; STAT, signal transducer and activator of transcription; and TNFα, tumor necrosis factor α. The NO/PKG pathway is displayed in green, the RISK pathway in yellow, and the SAFE pathway in red.

After the discovery of LIPC in 1986 [14], Pryzklenk et al. conducted their landmark study in 1993: an ischemic preconditioning (IPC) stimulus applied to the circumflex coronary artery diminished IRI that was caused by a 60 minute occlusion of the left anterior descending coronary artery [35]. These coronary arteries supply different areas of the cardiac muscle, without anastomoses between the two. The protective effect of IPC on a distant tissue undergoing ischemia and reperfusion is called remote ischemic preconditioning (RIPC). Later studies reported that various remote organs and tissues can be preconditioned

to protect the heart from IRI [36]. Furthermore, other organs including the kidney can also be protected by RIPC (Figure 1.3) [59].

Thus, RIPC seems applicable to many organs or body parts and produces a universal protective signal throughout the body, and its mechanism of action appears to resemble that of local IPC. Although not as extensively investigated, available literature shows that the protective effect of RIPC is initiated by similar pathways and that a similar degree of protection is achieved [38]. Recent evidence suggests that the signal is transmitted either humorally, immunologically or neurogenically, or via a combination of these pathways [17, 39].

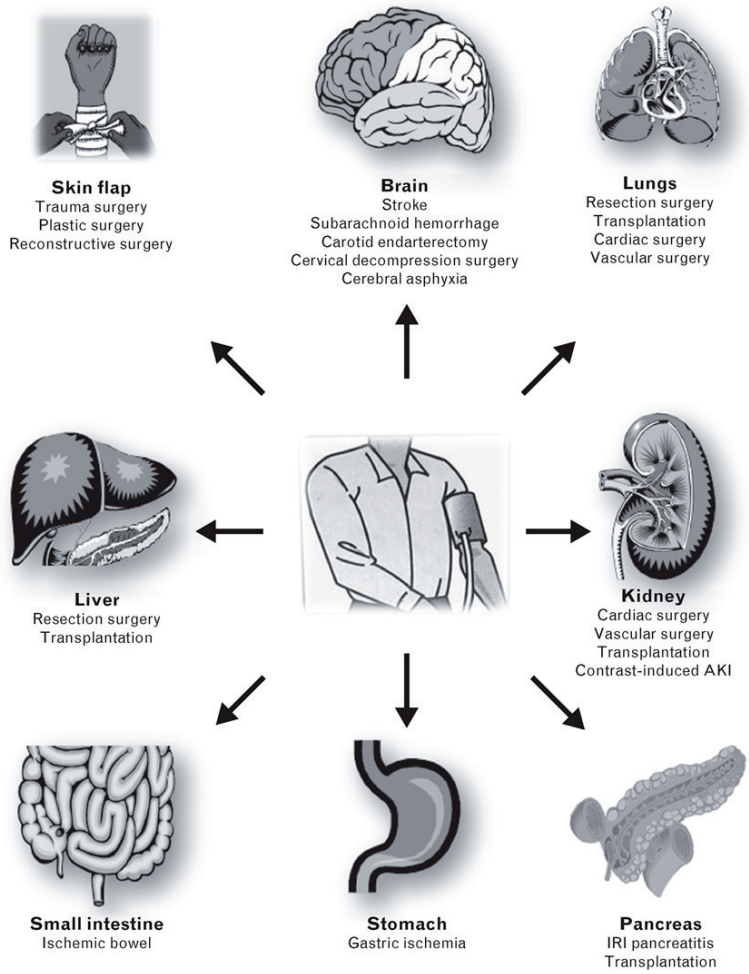


Figure 1.3. A demonstration of the noncardiac organs and tissue in which RIPC may offer protection against IRI. In each organ and tissue, there are a number of clinical settings in which RIPC has been or is currently being investigated [59].

Compared to local IPC, RIPC has major advantages in terms of translation to the clinical setting. An IPC stimulus applied directly to the kidney is often highly invasive, since it requires laparotomic or laparoscopic access to the renal vasculature, and clamping introduces risks of complications, such as damage to the vasculature, bleeding and infection. A commonly used method to apply RIPC on the other hand, is the inflation of a blood pressure cuff around an extremity, at a pressure beyond the systolic blood pressure (Figure 1.4). Using an extremity is favourable, since skeletal muscle can tolerate an interruption of blood flow for periods up to several hours without suffering irreversible damage. Remote conditioning protocols typically consist of cycles of three to five minutes of ischemia, during which damage due to compression of vessels and nerves in the extremity is negligible. Even in patients with atherosclerosis and peripheral vascular disease, it appears to be safe to occlude the vasculature by blood pressure cuff to apply RIPC [40]. Furthermore, the procedure can be performed without anaesthesia, is inexpensive, and effortless. Although clinical trials investigating the protective effect of RIPC on renal IRI initially showed positive results [41], these were not always replicated in later trials [40]. A systematic review and meta-analysis of all clinical trials is helpful to summarize all available evidence on the protective effect of RIPC on renal IRI in patients, identify possible adverse effects and improve the design of future clinical studies by analyzing the influence of decisive variables of these trials.

The currently used RIPC protocols in clinical trials are still the same protocols that were used by Murry et al. in his LIPC experiment in 1986 [14]. All trials use a similar two to four cycles of five to ten minutes protocol to precondition the target organ. With accumulating

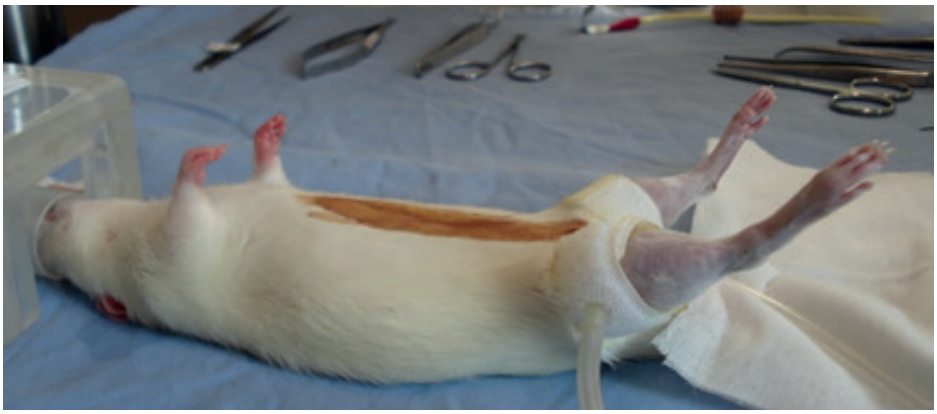


Figure 1.4. An anesthetised Sprague-Dawley rat undergoing remote ischemic conditioning by bilateral occlusion of the hind limbs with blood pressure cuffs.

disappointing results, exploring different IPC protocols could optimize the effectiveness of IPC for the human kidney. There is, for instance, evidence that repeating the RIPC stimulus over several days (RepRIPC) protects the heart from IRI [42]. A possible indication of the positive effect of RepRIPC is the improvement of the endothelial function of the vasculature [43]. Although there is no evidence for beneficial effects of RepRIPC in renal IRI models, our hypothesis is that RepRIPC could also increase the RIPC effectiveness in renal IRI models.

Apart from renal surgery and transplantation, contrast induced nephropathy is a field in which RIPC could be applied to reduce renal damage in patients. Iodine contrast administration causes acute kidney injury, known as contrast induced nephropathy (CIN). Although the precise mechanism underlying CIN remains unknown, evidence exists that contrast media have direct toxic effects on the tubular cells, resulting in altered mitochondrial function and apoptosis. Moreover, compelling evidence exists from experimental models that renal ischemia, resulting from contrast induced vasoconstriction, plays a key role in the pathogenesis of CIN [44, 45]. The outer part of the medulla has an area with high oxygen demand and is therefore vulnerable to contrast induced vasoconstriction of the vasa recta. When vasoconstriction resolves and the oxygen supply is restored, post-ischemic cells produce free oxygen radicals. The formation of free radicals contributes, at least in part, to the renal tubular cell injury [46]. This part can be explained by IRI, and can therefore possibly be diminished by RIPC. Results of RIPC significantly reducing CIN have already been shown in a small study in high-risk patients [47]. A second study in patients with medium risk of CIN showed no significant protection [48]. It remains unclear whether patients who are, according to the Dutch guidelines, at risk of CIN can benefit from RIPC.

Despite the advantages of RIPC compared to LIPC, these preconditioning strategies cannot be used in patients for whom the moment that IRI occurs is not predictable, such as renal IRI in an acute ruptured aneurysm. A solution for this problem was found in 2003 by Zhao et al. [50]; applying a brief local ischemic stimulus within minutes after index ischemia protected the tissue from IRI to the same extent as IPC. This phenomenon is referred to as ischemic postconditioning (IPostC), and was first reported to reduce renal IRI in 2007 [51]. In analogy to preconditioning, the postconditioning stimulus can be successfully applied to either the target organ (LIPostC) or to a remote organ (RIPostC). It may seem counter-intuitive to protect tissue from IRI after restoration of the blood flow, but because IRI is largely effectuated after reperfusion, a swift protective signal appears to reduce IRI.

Although the underlying mechanism is only partly understood, most molecules involved in IPC signalling have also been implicated in LIPostC and RIPostC signalling, and the inhibition of mPTP opening appears to be the key factor of the protective effect as well [32, 52, 53]. Despite the fact that strategies have been shown to influence the status of the mitochondrial permeability transition pore, it has been postulated that LIPostC prevents opening of the mPTP by delaying the normalization of the intracellular pH [54]. RIPostC on the other hand, is believed to cause the release of various signalling molecules, such as adenosine, opioids, and cytokines, which act on the mitochondrial permeability transition pore through the activation of the cyclic guanosine monophosphate, Protein Kinase G (cGMP/PKG), RISK, or SAFE pathway [55]. Therefore a combination of LIPostC and RIPostC could be more effective in reducing renal IRI, and this has not yet been tried before.

Similar to RIPC, preclinical studies on IPostC have yielded contradictory results and it is unclear which characteristics influence IPostC efficacy, and which conditioning protocols are most effective. Meta-analysis and systematic review of preclinical studies have proven useful in optimizing the design of both preclinical and clinical studies [56], but are not available for IPostC. Conducting such analyses could make it possible to analyze the influence of different variables, thereby improving future study design. For human trials on IPostC only limited data is available, herein protective effects have only been observed in subgroups or on secondary endpoints [57, 58].

RATIONALE AND AIMS OF THIS THESIS

This thesis focusses on ischemic conditioning strategies and their value in reducing renal IRI. In order to achieve successful translation of these strategies to the clinical setting, we have employed animal models, systematic reviews and meta-analyses of preclinical and clinical data, and conducted a clinical trial. The objectives of this thesis are:

The efficacy of IPC in animal models

- To evaluate the available evidence on the efficacy of local and remote IPC against experimental renal IRI, to assess treatment efficacy, and to identify variables that influence efficacy.
- To optimize the efficacy of the IPC protocol in an animal model for renal IRI.

The effectiveness of RIPC in patients

- To evaluate the efficacy of RIPC in patients undergoing major cardiac or vascular surgery in order to reduce renal IRI based on existing evidence from clinical trials.
- To investigate the efficacy of RIPC in patients undergoing procedures using iodine containing contrast agents to reduce renal IRI.

OUTLINE OF THE THESIS

Chapter 1 provides an outline of the clinical problem and an introduction to the pathophysiology of renal IRI and proposed mechanisms of local and remote IPC and IPostC. This introduction is followed by systematic reviews and meta-analyses on (R)IPC (**Chapter 2**) and (R)IPostC (**Chapter 3**) against renal IRI, providing a detailed overview of the current preclinical evidence. The next step is to fill gaps in the current literature on animal studies to optimize the efficacy of IPC in reducing renal IRI in an animal model. Optimization could be achieved by a combination of successful IPC stimuli. In the first animal study, we hypothesized that LIPC in combination with RIPC could enhance the protective effect (**Chapter 4**) and in the second animal experiment (**Chapter 5**), we studied the optimization of RIPC by repeating the RIPC stimulus over seven days. Repeating RIPC is an extensive combination of the protective effects of the early and late window of protection. In the next chapters, the efficacy of RIPC to reduce renal IRI is investigated in humans. In **Chapter 6**, a systematic review and meta-analysis describe the effects of RIPC on renal IRI in all available randomized controlled trials studying renal IRI after major cardiac or vascular surgery. **Chapter 7** and **8** address a randomized controlled trial using RIPC to reduce contrast-induced nephropathy. **Chapter 9** provides a general discussion of the thesis and future perspectives, and **Chapter 10** summarizes the main findings of this thesis.

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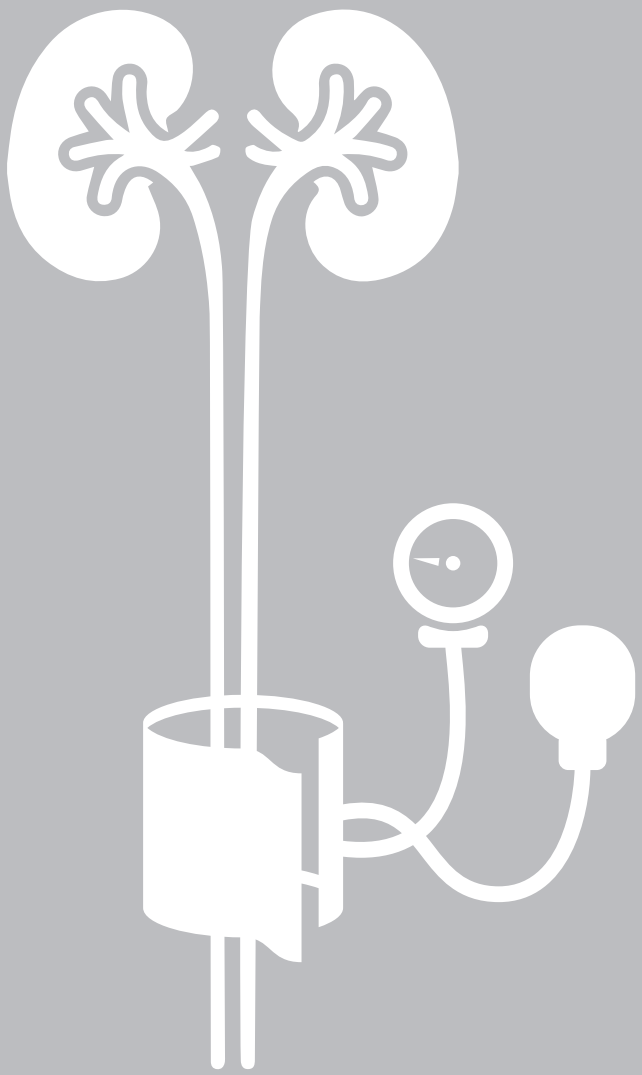

2

Ischemic preconditioning in the animal kidney, a systematic review and meta-analysis

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ABSTRACT

Ischemic preconditioning (IPC) is a potent renoprotective strategy which has not yet been translated successfully into clinical practice, in spite of promising results in animal studies.

We performed a unique systematic review and meta-analysis of animal studies to identify factors modifying IPC efficacy in renal ischemia/reperfusion injury (IRI), in order to enhance the design of future (clinical) studies.

An electronic literature search for animal studies on IPC in renal IRI yielded fifty-eight studies which met our inclusion criteria. We extracted data for serum creatinine, blood urea nitrogen and histological renal damage, as well as study quality indicators. Meta-analysis showed that IPC reduces serum creatinine (SMD 1.54 [95% CI 1.16, 1.93]), blood urea nitrogen (SMD 1.42 [95% CI 0.97, 1.87]) and histological renal damage (SMD 1.12 [95% CI 0.89, 1.35]) after IRI as compared to controls. Factors influencing IPC efficacy were the window of protection (<24 hours = early vs. ≥24 hours = late) and animal species (rat vs. mouse). No difference in efficacy between local and remote IPC was observed.

In conclusion, our findings show that IPC effectively reduces renal damage after IRI, with higher efficacy in the late window of protection. However, there is a large gap in study data concerning the optimal window of protection, and IPC efficacy may differ per animal species. Moreover, current clinical trials on RIPC may not be optimally designed, and our findings identify a need for further standardization of animal experiments.

INTRODUCTION

Ischemic preconditioning (IPC) is a potent protective strategy in which application of a brief episode of ischemia and reperfusion (I/R) results in tolerance to subsequent ischemia/reperfusion injury (IRI) [1–3]. The conditioning stimulus has been shown to be effective when applied either to the target organ itself (local IPC; LIPC [4]) or to a remote organ or tissue (remote IPC; RIPC [5]). LIPC and RIPC were both originally discovered in the dog heart, and have been successfully reproduced in a variety of animal species, using various organs, *e.g.* heart, intestine, brain, liver and kidney. There is a large variety in the IPC protocols used: the preconditioning stimulus may be one continuous ischemic period, or it may be comprised of two or more cycles of brief ischemia. Moreover, the interval between the preconditioning stimulus and the index ischemia may vary, and positive results in animals have been found for both short intervals of a few minutes or hours (the so-called early window of protection), as well as for long intervals of days or even weeks (late window of protection).

Thus, IPC poses a promising alternative to existing treatments for IRI in humans, since current strategies to reduce this important and common clinical problem are inadequate. Next to the heart, the kidney is one of the major organs of interest for clinical application of IPC. Its high energy demand and intricate microvascular network render the kidney especially sensitive to IRI, which is a major cause of kidney injury in *e.g.* renal artery stenosis and renal surgery [6, 7]. Furthermore, renal IRI is a major cause of cardiovascular morbidity and mortality, and is associated with delayed graft function after transplantation, renal damage in cardiac and aortic surgery, and shock [8–11]. In animal models, both LIPC and RIPC have been shown to be effective tools to protect the kidney (*e.g.* [12, 13]).

Where do we stand in terms of the translation of IPC to beneficial treatment for patients? LIPC has not been studied in the human kidney, but several clinical studies have been conducted in the heart: a number of studies have investigated LIPC in patients undergoing coronary artery bypass grafting (CABG) surgery, which collectively show that LIPC reduces inotrope requirements, ventricular arrhythmias, and shortens intensive care unit stay [14]. For RIPC, several clinical trials have been performed for cardiac as well as renal IRI, but their outcome is not clear-cut: many studies report protective effects of RIPC after CABG surgery, heart valve surgery, or abdominal aortic aneurysm repair, but not all findings have been positive ([15–18] and recently reviewed in [19]).

Thus, even though the protective effect of LIPC and RIPC on renal IRI has been shown in many animal studies, translation of IPC to the clinic has, as yet, not been successful. The variety of IPC protocols used in clinical trials may be one of the reasons for this ambiguity, *i.e.* in some studies, the stimulus could have been suboptimal or incorrectly applied. There is no consensus on how many ischemic stimuli should be applied, and what the duration of the ischemic and intermediate reperfusion periods should be. It isunclear whether the early or late window of protection is most effective. Furthermore, it is unknown which patient-related factors such as age, gender or co-morbidities play a role.

Meta-analysis and systematic review of preclinical (animal) studies have previously been used to optimize experimental animal models and to improve the design of clinical trials [20–22]. In the case of IPC, meta-analysis on animal study data may provide valuable indicators to optimize the IPC protocol, as well as the window of protection in humans. It has been shown that proper analysis of animal experiments can also improve the decision making in whether or not to start a clinical trial. In addition, this approach can be used to perform a quality assessment of the current literature, including measures to avoid bias (*e.g.* randomization, concealment of allocation and blinded outcome assessment).

As such, meta-analysis of existing literature on animal models may improve future animal research in the field, thereby contributing to the Refinement and Reduction of animal experiments, as proposed by the Animal Research: Reporting *in vivo* Experiments [23] and Gold Standard Publication Checklist [24] guidelines.

This report presents innovative methods in reviewing animal studies, *i.e.* a systematic review and meta-analysis of the efficacy of IPC in experimental models of renal IRI. We set out to 1) provide a complete and systematic overview of all literature available on the effects of IPC (both local and remote) on renal IRI; 2) report summary estimates of efficacy based on meta-analysis; 3) identify factors modifying the efficacy of IPC in renal IRI, to inform the design of future clinical trials; and 4) provide insight in the quality of literature in the field of IPC and renal IRI in animal models.

ANALYSIS

Literature search strategy, inclusion and exclusion criteria

The present review was based on published results of animal studies on the effects of IPC on renal IRI, which were identified via a systematic computerized search in PubMed and Embase. The inclusion criteria and method of analysis were specified in advance

and documented in a protocol. The databases were searched for published articles up to October 19th 2011. The full search strategies for PubMed and EMBASE are included in Appendix S1, and involved the following search components: ''animal'' [25, 26], ''kidney'', ''ischemia reperfusion injury'' and ''preconditioning''. Studies were included in the systematic review if they fulfilled all of the following criteria: 1) the study assessed the effect of remote or local IPC on renal IRI; 2) the study was performed in animals *in vivo*; 3) the study was an original full paper which presented unique data. Studies were excluded if 1) the renal IRI model involved cold storage of the kidney or 2) the study was performed only in genetically modified animals. Study selection was performed independently by two reviewers (TM and KW) on the basis of title and abstract. In case of doubt, the whole publication was evaluated. Differences were clarified by discussion with a third investigator (MW). No language restrictions were applied. If necessary, papers in languages other than English were translated by scientists (native speakers for that particular language) within the Radboud University Nijmegen Medical Centre.

Study characteristics and data extraction

The following study characteristics and data items were extracted from the studies included: animal species, strain, sex, number of animals in treatment and control groups, measures of randomization, measures of blinding, number of animals excluded for statistical analysis, reason for exclusion of animals, reported outcome measures and results. Bibliographic details such as author, journal, and year of publication were also registered. Three outcome measures were assessed: serum creatinine, BUN and histological renal damage. For histology, data could be extracted if the authors used the Jablonski [27] score for renal damage, or an adapted version of this scoring system.

Data were extracted if raw data or group averages, standard deviation (SD) or standard error (SE), and number of animals per group (n) were reported, or could be recalculated. For 30 articles, relevant outcome measures or study details were not reported. We therefore contacted these authors via e-mail and received response from eight authors, six of which provided additional data. For two papers, authors reported using six to eight animals per group and we included these data using n=6 animals [28, 29]. If the number of animals was stated less specific (*e.g.* >3 animals or 4–8 animals), and the exact numbers could not be retrieved by contacting the authors, data were not included. If SE was reported, this was converted to SD for meta-analysis. If a study contained separate groups for each gender, or several preconditioning protocols, these groups were analyzed as if they were separate studies. If two or more identical groups existed, the data were

pooled for these groups. If outcomes were measured at several time points, we chose the time point at which efficacy was greatest. In eight out of 11 cases, this was 24 hours post ischemia, which was also the most common time of measurement overall (see Table S2.1). When data were presented only graphically, we contacted authors to obtain the numerical values. If these were not available, data were measured using digital image analysis software (ImageJ; http://rsbweb.nih.gov/ij/).

Assessment of methodological quality

We designed a 16-point rating system to assess the methodological quality of the included publications (see Table S2.2 and legend for details). Concerning the number of excluded animals, we assumed that there had been no exclusion if the number of animals per group mentioned in the materials and methods section was identical to the number stated in the figure legends or results section.

Data synthesis and statistical analyses

Data were analyzed using Review Manager Version 5.1 (Copenhagen, The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). Meta-analysis was performed for the outcome measures serum creatinine, BUN and histology score, by computing the standardized mean difference (SMD; the mean of the experimental group minus the mean of the control group divided by the pooled SD of the two groups). To account for anticipated heterogeneity, we used random effect models in which some heterogeneity is allowed. Subgroup analyses were predefined and performed for all outcome measures, and were used to assess the influence of variables on IPC efficacy, as well as to explore possible causes for heterogeneity. The five subgrouping variables were: timing of index ischemia (late or early window of protection), preconditioning protocol (fractionated or continuous), site of preconditioning (LIPC, RIPC or both), animal species (rat or mouse) and gender (male, female or both). Differences between subgroups were determined by calculating the difference between the respective SMDs (ΔSMD) and confidence interval (CI) of the difference. Furthermore, subgroup interaction analysis was performed in an attempt to further explain the expected study heterogeneity: we compared smaller sets of experiments by combining two subgrouping variables, *e.g.* early-RIPC *vs.* early-LIPC. Unless indicated otherwise, data are presented as SMD and 95% CIs.

For each outcome measure, we assessed the possibility of publication bias by visually evaluating the possible asymmetry in funnel plots. Finally, we investigated whether study methodology influenced the results of our meta-analysis. Pre-specified sensitivity analysis

was performed to assess whether the chosen cut-off point for early *vs.* late window of protection influenced the outcome of this subgroup analysis.

RESULTS

Study selection and characteristics

The electronic search strategy retrieved 253 records from PubMed and 270 articles from EMBASE, 310 of which were unique. Seventy-seven papers met our inclusion criteria. On the basis of predefined criteria, 19 reports were excluded and the remaining 58 articles were retrieved in full (see Figure 2.1).

The characteristics of the included studies are summarized in Table S2.1. There was a large variation in study characteristics. In 76% of the 58 included studies, the delay between the preconditioning stimulus and the index ischemia was 24 hours, which we considered to be the early window of protection. Eleven studies (19%) assessed protection in the late window of protection (timing of index ischemia ≥24 hours after IPC), and three studies (5%) reported data for both late and early window(s) of protection. For the early window of protection, the delay between IPC and index ischemia was four to 40 minutes (average 967 minutes). For the late window of protection, this was 24 hours up to 12 weeks (average 17 ± 23 days). In 28 of the 58 studies (48%), the ischemic preconditioning protocol consisted of one continuous stimulus. Twenty-two studies (38%) used only fractionated protocol(s), *i.e.* two to five cycles of brief ischemia and reperfusion, whereas eight studies employed both fractionated and continuous stimuli. The majority of studies focussed on the protective effects of LIPC on renal IRI. However, five studies assessed the effects of RIPC, using hind limb, intestine, liver or subphrenic aortic occlusion as remote stimuli. In four studies, both LIPC and RIPC of one kidney to its contralateral counterpart were performed (either intentionally, or as a result of a bilateral preconditioning stimulus and a unilateral index ischemia). Out of all 58 included studies, 14 were conducted in mice (24%), 34 in rat (59%), and ten in other species, namely rabbit (7%), dog (5%) and pig (5%). Eight out of 58 studies (14%) were performed in female animals, 37 in males (64%), and four studies used animals of both genders (7%). Nine studies did not report the gender of the animals.

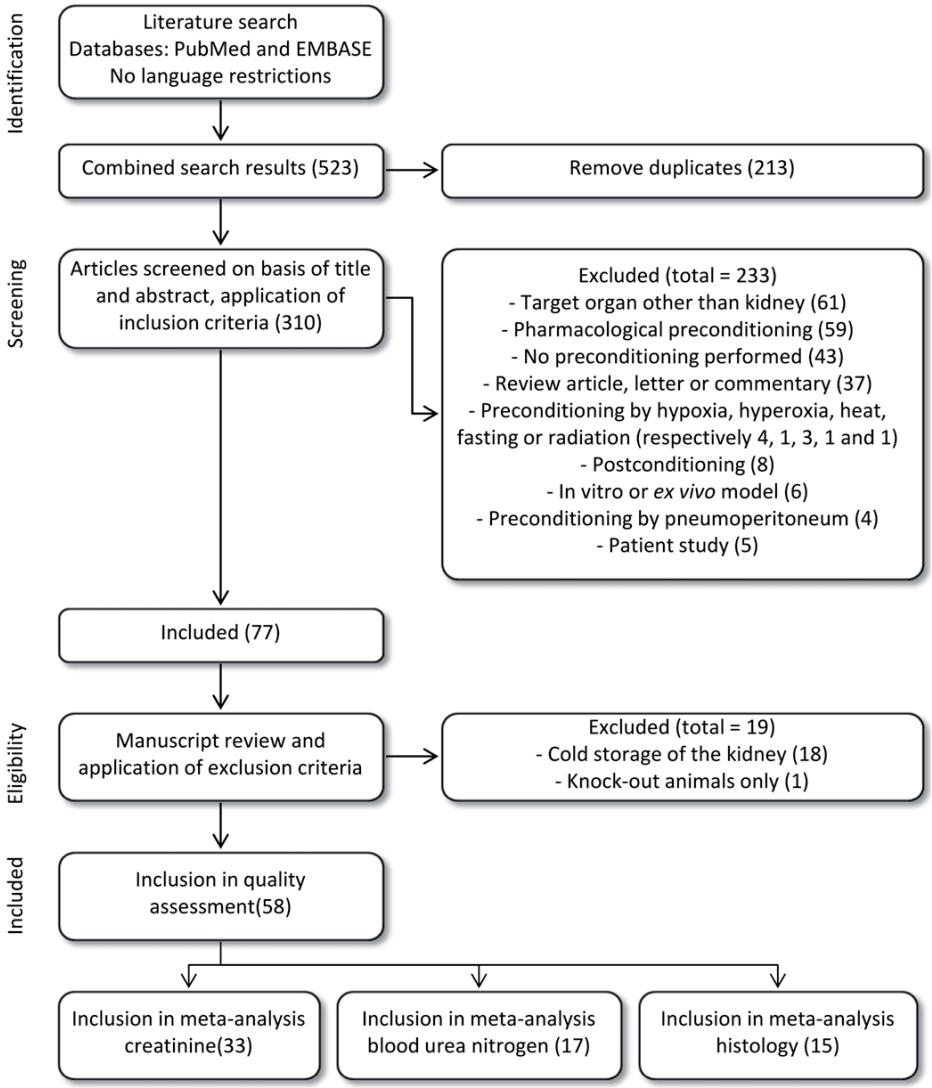

Figure 2.1. Flow chart of study selection.
The number of studies in each phase is indicated between brackets.

Methodological quality of studies

The results of the quality assessment of the 58 studies included in this systematic review are shown in Table S2.2 and Figure 2.2. On average, studies reported nine out of 16 characteristics (59 ± 10%). The lowest score was five out of 15 items (33%), and the highest scoring studies reported 12 items out of 14 (80%). In the quality assessment of

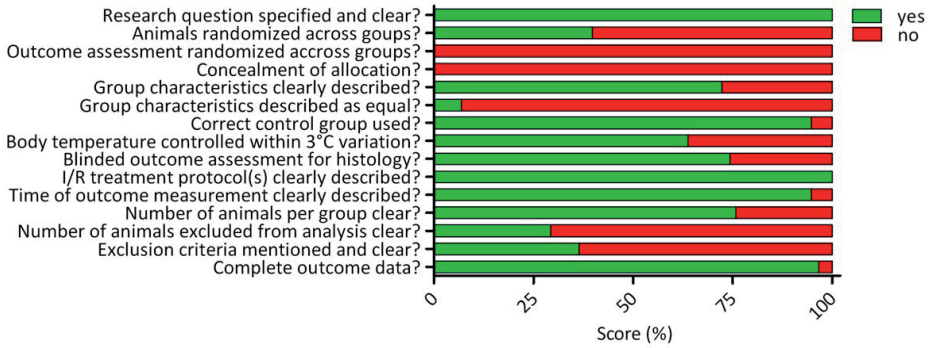

Figure 2.2. Quality assessment score, averaged per item.
Many studies scored poorly on key characteristics of scientific practice, and measures to avoid bias, such as characteristics of the subject population, randomization, blinding and exclusion criteria.

clinical trials, randomization, blinding, and description of withdrawals are key quality measures. However, only 40% of the animal studies included in this systematic review reported randomization of the animals across treatment groups. Out of the 39 studies in which renal histology was an outcome measure, 74% reported blinding of the outcome assessment. No study reported blinding for any other outcome measure. Only 29% of the studies reported the number of animals excluded, 64% of which did not state the reason for exclusion. Although the strong influence of body temperature on renal damage has been shown in both large and small animal models, 36% of the studies did not report whether the body temperature of the animals was controlled.

Meta-analysis of outcome measures

Results for the outcome measure serum creatinine are summarized in Table S2.3 and Figure 2.3. Thirty-two articles studied the effect of one or more IPC protocols on serum creatinine after renal IRI. The analysis contained 62 experiments, including data for 512 control animals which underwent renal IRI only, and 492 animals that underwent IPC + renal IRI. In 36 experiments, the SMD and 95% CI indicated that IPC significantly reduced the IRI-induced rise in serum creatinine. One study reported a negative effect of IPC on serum creatinine [30]. Overall analysis showed that IPC reduced serum creatinine after IRI (1.54 [1.16, 1.93], $p<0.00001$). Overall study heterogeneity was high (83%).

Subgroup analysis showed a beneficial effect of IPC for all subgroups, except for female (notably, this subgroup contained only two experiments and was therefore excluded from further statistical analysis). We also found a subgroup effect of the variable 'window of protection' (Table S2.2, filled squares). The ΔSMD and CI of the difference for early *vs.*

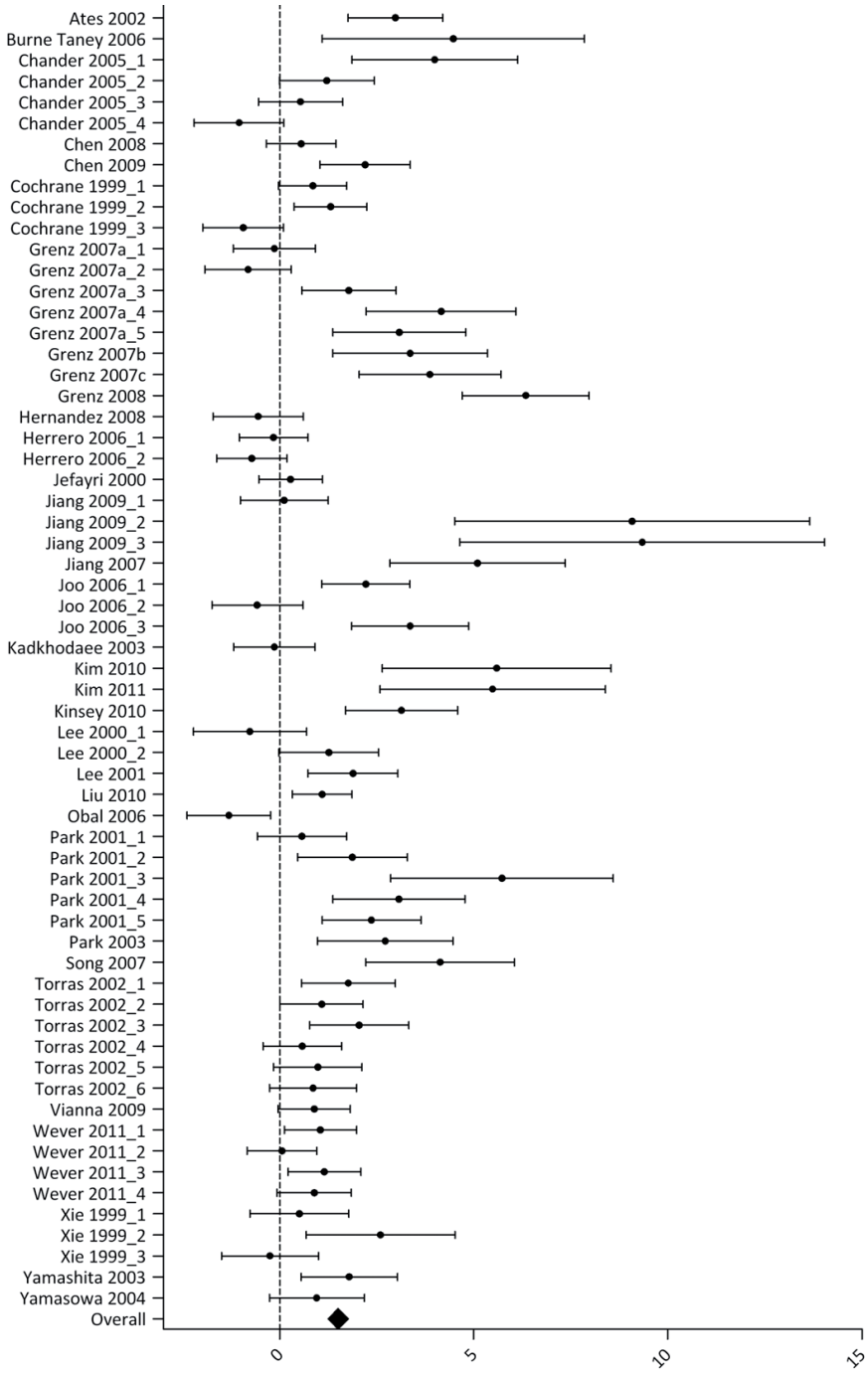

Figure 2.3. Effect of IPC on serum creatinine after renal IRI.
Left side favours control (renal IRI only), right side favours IPC. An overall beneficial effect of IPC on serum creatinine was observed (1.54 [1.16, 1.93]). Data presented as SMD and 95% CI.

late was 2.43 [1.29, 3.57], indicating that the late window of protection of IPC was more effective in reducing serum creatinine than the early window. Subgroup analysis indicated a higher IPC efficacy in studies conducted in mouse *vs.* rat (Table S2.2, triangles; ΔSMD 1.7 [1.5,1.90]). For other species (dog, pig, rabbit) subgroups were too small to perform reliable subgroup analysis. No difference in IPC efficacy was observed for continuous fractionated; ΔSMD 0.46 [20.30, 1.22]), or males only groups of mixed gender (ΔSMD 0.38 [20.60, 1.36]). For site of preconditioning, no differences were found when comparing the subgroups LIPC *vs.* RIPC (ΔSMD 0.06 [20.98, 1.10]), LIPC *vs.* LIPC +RIPC (ΔSMD 1.01 [20.44, 2.46]) or RIPC *vs.* LIPC+RIPC (ΔSMD 0.95 [20.73, 2.63]).

Results for the outcome measure BUN are summarized in Table S2.4 and Figure 2.4. Seventeen articles studied the effect of one or more IPC protocols on BUN after renal IRI. In 20 out of 29 experiments, the IRI-induced rise in BUN was significantly reduced in animals undergoing IPC, when compared to a control group that underwent IRI only (overall effect size 1.42 [0.97, 1.87]; p<0.00001). Overall study heterogeneity was high (76%).

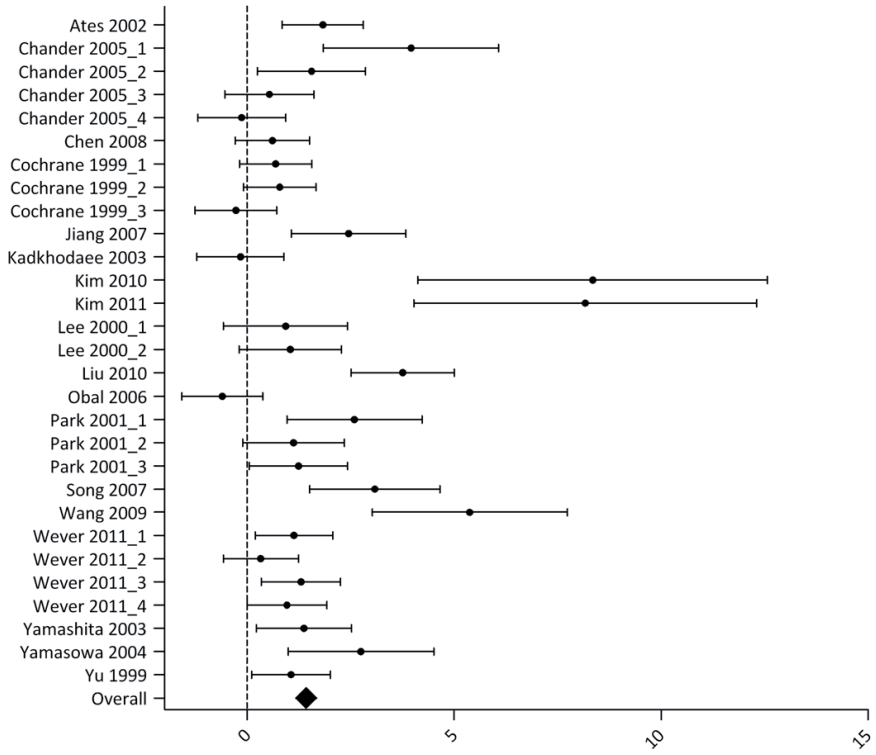

Figure 2.4. Effect of IPC on BUN after renal IRI.
Left side favours control (renal IRI only), right side favours IPC. An overall beneficial effect of IPC on BUN was observed (1.42 [0.97, 1.87]). Data presented as SMD and 95% CI.

Subgroup analysis showed that the beneficial effect of IPC on BUN was present in all subgroups. Between-subgroup analysis revealed a higher IPC efficacy in mouse *vs.* rat (Table S2.3, triangles; ΔSMD 2.12 [0.48, 3.76). No effect was found for the window of protection (early *vs.* late; ΔSMD 1.25 [20.05, 2.55]) or the IPC protocol (continuous *vs.* fractionated; ΔSMD 0.96 [20.03, 1.95]). Furthermore, the site of preconditioning did not influence IPC efficacy: LIPC *vs.* RIPC, LIPC *vs.* LIPC +RIPC and RIPC *vs.* LIPC+RIPC, respectively ΔSMD 0.2 [20.69, 1.09]), ΔSMD 0 [21.03, 1.03] and ΔSMD 0.2 [20.82, 1.22]. Subgroup analysis could not be performed for the variable 'gender', because of insufficient data.

Results for the outcome measure renal histology are summarized in Table S2.5 and Figure 2.5. Twenty-six experiments from 15 studies reported the effect of IPC on the Jablonski score for renal histology. Eight studies using a histology score not comparable with Jablonski's were excluded from analysis. Data included contained 205 control and 191 IPC-treated animals. Overall, IPC had a significant renal protective effect of 1.12 [0.89, 1.35]. Overall study heterogeneity was 63%. Subgroup analysis showed that the beneficial effect of IPC on histology was present in all subgroups. Between-subgroup analysis could only be performed for the variables window of protection, IPC protocol, gender and animal species, because of insufficient numbers of experiments in the other subgroups. No significant differences between subgroups were found (early *vs.* late, ΔSMD 1.8 [20.07, 3.67]; continuous *vs.* fractionated, ΔSMD 0.3 [20.50, 1.10]; males *vs.* mixed gender, ΔSMD 0.25 [20.58, 1.08]; rat *vs.* mice, ΔSMD 0.55 [20.14, 1.24]).

Subgroup interaction analysis

In an attempt to further explain the expected study heterogeneity, subgroup interaction analysis was performed for all subgroup interactions which contained three or more experiments. Study heterogeneity was not notably reduced by combining subgroup variables and remained on average 80 ± 6% for serum creatinine, 62 ± 23% for BUN and 47 ± 30% for renal histology. The analyses revealed no significant differences in the interaction between subgroups, and did therefore not alter the results of the subgroups analysis. Interestingly, for serum creatinine, the subgroup interactions early-RIPC and continuous-RIPC did not show an overall effect of IPC, whereas early-LIPC and continuous-LIPC did show the protective effect. This may indicate that the positive effect of an early window of protection, or the benefits of a continuous IPC protocol are less pronounced for RIPC than for LIPC. However, because of the small number of experiments in these subgroups interactions (six and three experiments, respectively), these results must be interpreted with care.

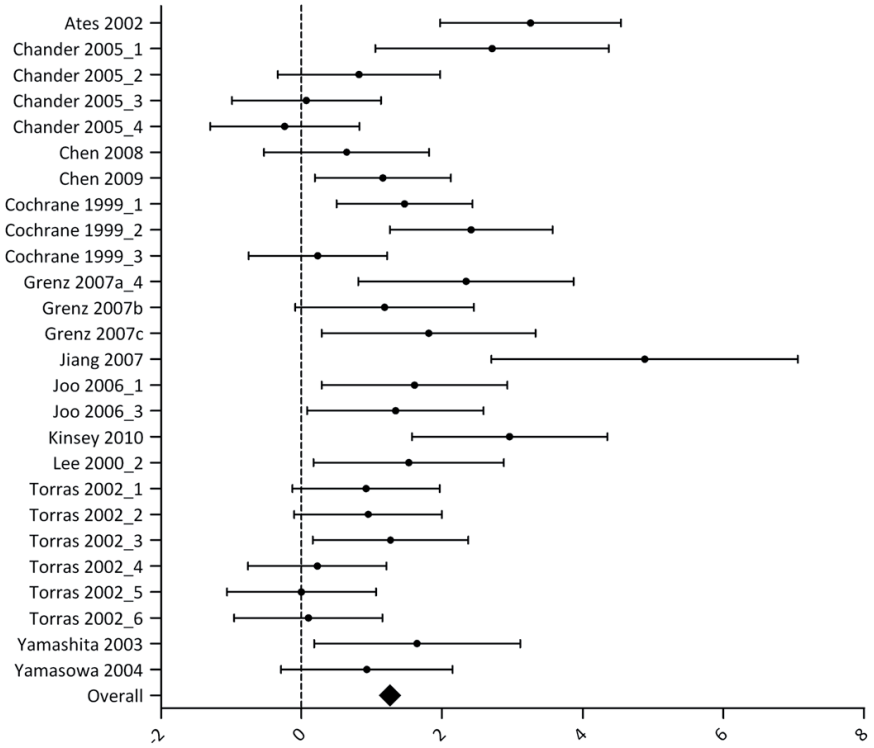

Figure 2.5. Effect of IPC on renal histology after renal IRI.
Left side favours control (renal IRI only), right side favours IPC. An overall beneficial effect of IPC on renal histology was observed (1.22 [0.89, 1.35]). Data presented as SMD and 95% CI.

Publication bias

The presence of publication bias was assessed for all outcome measures. Visual analysis of funnel plots revealed that small, negative studies appeared to be underrepresented (data not shown). This was especially true for serum creatinine and BUN, and to a lesser extent for renal histology data.

Sensitivity analysis

To assess the robustness of our findings, we undertook a sensitivity analysis by redefining the cut off-point for the early window of protection at a later time point (<48 hours) or an earlier time point (<6 hours). This did not significantly alter the outcome of any of the outcome measures (data not shown).

DISCUSSION

Here we report a unique systematic review and meta-analysis of current literature reporting experimental animal models of IPC in renal IRI. Three important outcome measures were assessed, namely serum creatinine, BUN and histological renal damage quantified by Jablonski score. For all three, protective effects of IPC were observed, *i.e.* IPC reduced serum creatinine (1.54 [1.16, 1.93]), BUN (1.42 [0.97, 1.87]) and histological damage (1.12 [0.89, 1.35]) after IRI, when compared to control animals undergoing renal IRI only. Importantly, in the clinical setting, serum creatinine currently remains the gold standard to assess renal function. In rodents however, questions have been raised regarding the reliability of creatinine for measuring renal function, since the impact of tubular creatinine excretion on creatinine clearance is even larger in mice than in humans [31]. We therefore put forward that other outcome measures, such as BUN and/or renal histology may also be of great value when translating animal study results to the human setting. Furthermore, other renal damage markers such as Kidney Injury Molecule-1 (KIM-1) and Neutrophil Gelatinase-Associated Lipocalin (NGAL) are gaining ground in clinical practice [32]. Reporting these markers in both animal and human studies may provide further information for the translation of animal study data to the human setting.

We performed subgroup analysis to investigate several predefined factors which we hypothesized to modify the efficacy of IPC in renal IRI, namely: window of protection (early or late), IPC protocol (continuous or fractionated), site of preconditioning (RIPC, LIPC or both), species (mouse or rat) and gender (male, female or mixed). The protective effects of IPC were persistent in all subgroups, for all outcome measures, except for female (only two experiments). Based on the latter observation, we propose the need for future studies should in females, since it has been shown that there is a significant difference between males and females for cardiac IPC efficacy (*e.g.* [33]).

For serum creatinine, the window of protection influenced the efficacy of IPC: IPC was more effective when conducted >24 hours before index ischemia (late window of protection), as compared to an early window of protection (<24 hours before index ischemia). We observed the same trend towards higher efficacy in the late window of protection for BUN and renal histology. The cut-off point of <24 hours for the early window could be redefined at <6 hours or <48 hours without significantly influencing these results, since the vast majority of experiments (93%) investigated a time window of either <40 minutes, or >4 days. The remaining 7% of the experiments concerned a time window of six to 24 hours between IPC and IRI. Thus, there is a large gap in these data which makes it difficult to assess the optimal window of protection for IPC. Nevertheless,

our data strongly indicate that the late window of protection might be more effective to reduce renal IRI as compared to the early window. This finding is particularly interesting since almost all clinical trials currently registered at Clinicaltrials.gov investigating the effects of LIPC and RIPC use only the early window of protection. To our knowledge data on the efficacy of combined activation of the early and late window in humans is lacking.

The second variable which influenced IPC efficacy was animal species: for serum creatinine and BUN data, IPC was more effective when performed in mice *vs.* rats. This suggests that mouse models of renal IPC may be more sensitive when compared to rat, and are thus the preferable models for future animal studies. Furthermore, this finding implicates that IPC efficacy is species specific, and therefore the protective effect may be greater, or less pronounced in large animals and humans. This illustrates the difficulty in directly translating results from animal studies to the human setting, and further studies in large animals and humans are necessary to clarify this issue.

No significant differences were observed for the variables IPC protocol (continuous *vs.* fractionated) or site of preconditioning (LIPC, RIPC or both). The latter finding is interesting, since the use of LIPC in clinical practice is limited because of the risk of damage to major vascular structures, and the fact that even brief ischemia may damage the target organ in vulnerable patients. RIPC therefore has more potential for clinical application, since the IPC stimulus can be applied to *e.g.* a limb, which is easy to handle and relatively resistant to IRI. Our finding that RIPC and LIPC are equally effective indicates that RIPC has an at least equal potential for translation to the clinic, although it must be noted that only two studies used the limb as remote organ. Subgroup analysis of the serum creatinine levels in animals undergoing simultaneous LIPC of one kidney and RIPC of the contralateral kidney show a trend towards higher efficacy (Table S2.2, filled circles), indicating that a combination of LIPC and RIPC may have an additive effect. However, this result must be interpreted with care, because of the low number of experiments included.

Methodological quality of studies

Our assessment reveals that there is much to gain in terms of the methodological quality of animals studies in this field. Key characteristics of scientific practice, and measures to avoid bias, such as characteristics of the subject population, randomization, blinding and exclusion criteria, were infrequently reported. A number of recent systematic reviews show that this is the case in many fields of animal research. For scientific and ethical reasons, it is urgent that the standards routinely applied in human research become standard of practice in animal research as well. While it is possible that some

authors merely failed to report these details, there is reason for concern, since it is unclear whether there is a significant difference between the reported study quality and the actual study quality. For this reason, better reporting of animal studies is crucial. Regrettably, there appears to be an inverse correlation between the impact factor of the journal in which the study is published, and the required detail of the materials and methods description [24]. The high heterogeneity of the data presented in this systematic review may be explained in part by the differences in study quality, as well as the lack of consensus and general standards of practice in animal studies. It has proven difficult to obtain missing data by contacting authors directly, which further emphasizes the importance of adequately reporting animal studies. However, in spite of insufficient reporting, systematic review and meta-analysis of current publications aid in making possible bias transparent, and can provide us with valuable new insights, which will support the translation of animal data to the clinical setting.

Strengths and limitations

The major strength of our study is that, as far as we are aware, we are the first performing a systematic review and meta-analysis on renal protection by IPC in animal studies. We were able to include a large number of studies per outcome measure, which enabled us to investigate the effect of several subgroup variables.

Some potential limitations should be discussed. Firstly, the extracted data are highly heterogeneous, which is most likely due to a large variety in experimental designs used and the variation in study quality. The fact that our subgroup interaction analysis did not notably reduce heterogeneity supports this notion. Although we have tried to account for this heterogeneity by using a random effects model and performing subgroup and sensitivity analysis, pooling of the results may not be appropriate for all subgroups. Therefore, differences between (small) subgroups should be interpreted with caution and be used to generate new hypotheses, rather than for drawing final conclusions. However, all studies provide information on the association between IPC and IRI in the animal kidney, and are thus valuable for this systematic review.

Secondly, the included studies may be subject to publication bias. Visual analysis of funnel plots revealed that only small, negative studies appeared to be underrepresented in current literature on IPC in renal IRI. Asymmetry was most notable in serum creatinine and BUN data. This may indicate that publication bias is present, which could cause overestimation of the effect sizes. Importantly, funnel plot asymmetry can result from non-publication of negative results, but may also be caused by other factors, such as

true study heterogeneity, or differences in study quality [34]. Our finding that the study quality is rather heterogeneous may therefore explain part of the funnel plot asymmetry.

Clinical implications

Both LIPC and RIPC (and also the combination of the two), appear to have the potential to reduce IRI, and since RIPC by brief limb ischemia has the advantage of being safe and easy to perform, the latter has the greatest potential for clinical practice. In contrast to the variety of IPC protocols used in animal studies, current clinical trials on RIPC in renal IRI are using similar preconditioning protocols, namely fractionated IPC stimuli, and a short delay between IPC and index ischemia (early window of protection). The current review indicates that even though this approach might be effective, efficacy could be even higher in the late window of protection. Future studies should be designed to investigate the optimal window of protection in patients, taking into account the possible difference between acute and delayed ischemic preconditioning. Whether a combination of the two is additive or even synergistic requires further testing in animal and human models as well.

It is important to realize that, to date, no studies (animal or human) have investigated the effect of co-medication and comorbidities such as diabetes, hypertension or obesity, on IPC in renal IRI. For the heart, it has been shown that medication and co-morbidities influence IPC efficacy (reviewed *e.g.* in [35]). Similarly, differences in IPC efficacy between genders may indicate that the optimal IPC stimulus is different in males *vs.* females. We propose that future clinical studies should be designed to optimize IPC efficacy for certain patient groups, and that animal studies in this area can inform the design of such clinical trials. Furthermore, a better mechanistic insight is needed in the cause of the observed interspecies difference. These data will give us a clue whether translation to humans is feasible.

CONCLUSION

The currently applied approach of systematic review and metaanalysis indicates that, in animal studies, IPC has an overall protective effect on the kidney, since it reduces serum creatinine, blood urea nitrogen (BUN) and renal damage as assessed by histology after IRI. We found that IPC is more effective in reducing serum creatinine when the IPC stimulus is applied >24 hours before index ischemia (late window of protection), a trend which was also observed for BUN and renal histology data.

Furthermore, serum creatinine and BUN data showed an effect of animal species on IPC efficacy: IPC was more effective when performed in mice *vs.* rats. No significant differences were observed for the variables site of preconditioning (local, remote or both) or IPC protocol (continuous *vs.* fractionated). Our review indicates that current clinical trials on RIPC may not be optimally designed, and further optimization may be necessary for successful translation to the clinical setting.

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Table S2.1. Study characteristics

Author	Species	Gender	Cycles (fractionation)		Prec isch	Int rep	Delay (IPC window)		Index ischemia	Time of measurement	LIPC/ RIPC	Outcome measures
Ateş, 2002 [36]	Rt	m	1	(N)	10	-	10	(E)	45	45min/24h	RIPC	Cr, BUN, H
Aufricht, 2002 [37]	Rt	m	1	(N)	45	-	18h	(E)	45	15	L+RIPC†	Other
Ayupova, 2009 [38]	Rt	m	1	(N)	30	-	14d	(L)	30	24h	LIPC	Cr‡
Burne-Taney, 2006 [39]	M	?	1	(N)	30	-	6d	(L)	30	24h	LIPC	Cr
Cao, 2010 [40]	Rt	m	3	(Y)	2	5	5	(E)	45	24h	LIPC	Cr‡
Chander, 2005 [41]	Rt	m	3	(Y)	2/3/4/5	5	5	(E)	45	24h	LIPC	Cr, BUN, H
Chen, 2008 [42]	Rt	m	4	(Y)	8	5	5	(E)	45	24h	LIPC	Cr, BUN, H
Chen, 2009 [43]	Rt	m	3	(Y)	2	5	5	(E)	45	24h	LIPC	Cr, H
Cochrane, 1999 [44]	Rt	m	1	(N)	2	-	5	(E)	45	24h	LIPC	Cr, BUN, H
			3	(Y)	2	5	5	(E)		24h	LIPC	
			3	(Y)	5	5	5	(E)		24h	LIPC	
Grenz, 2007a [45]	M	m/f*	1	(N)	4	4	4	(E)	10–60	24h	LIPC	Cr, H
			2–5	(Y)	4	4	4	(E)		24h	LIPC	
Grenz, 2007b [46]	M	m/f*	4	(Y)	4	4	4	(E)	30	24h	LIPC	Cr, H
Grenz, 2007c [47]	M	m/f*	4	(Y)	4	4	4	(E)	45	24h	LIPC	Cr, H
Grenz, 2008 [48]	M	m/f*	4	(Y)	4	4	4	(E)	45	24h	LIPC	Cr
Guye, 2010 [49]	Rb	m	3	(Y)	3	3	5	(E)	45	3h	LIPC	H‡
Hernandez, 2008 [50]	P	f	1	(N)	5	-	5	(E)	60	1/2/6/9/14d	LIPC	Cr
Herrero, 2006 [51]	Rt	m/f*	1	(N)	15	-	10	(E)	60	24h	LIPC	Cr
			2	(Y)	15	10	10	(E)	60	24h	LIPC	
Hyodo, 2009 [52]	Rt	f	1	(N)	5	-	5	(E)	45	0/6/12/24h	LIPC	Other

Table S2.1 continues on next page.

Table S2.1. ***Continued***

Author	Species	Gender	Cycles (fractionation)		Prec isch	Int rep	Delay (IPC window)		Index ischemia	Time of measurement	LIPC/ RIPC	Outcome measures
Islam, 1997 [53]	Rt	f	4	(Y)	4	11	30	(E)	20/40	90min/9d	LIPC	Other
Jang, 2008 [54]	M	m	1	(N)	30	-	8d	(L)	30	1/4/8d	LIPC	Cr‡
Jefayri, 2000 [55]	Rt	f	4	(Y)	4	11	5	(E)	45	0/2/6h	LIPC	Cr
Jiang, 2007 [56]	Rt	m	1	(N)	10/20/30	-	8d	(L)	40	2/35/70h	LIPC	Cr, BUN, H
Jiang, 2009 [57]	Rt	m	1	(N)	20	-	4d	(L)	40	24h	LIPC	Cr
Joo, 2006 [58]	M	?	4	(Y)	5	5	15	(E)	30	24h	LIPC	Cr, H
			4	(Y)	5	5	6h	(E)		24h	LIPC	
			4	(Y)	5	5	24h	(L)		24h	LIPC	
Kadkhodaee, 2004 [59]	Rt	M	3	(Y)	5	5	5	(E)	30	70	LIPC	Cr, BUN
Kim, 2010 [60]	M	M	1	(N)	30	-	8d	(L)	30	4/24h	LIPC	Cr, BUN
Kim, 2011 [61]	M	M	1	(N)	30	-	8d	(L)	30	4/24h	LIPC	Cr, BUN
Kinsey, 2010 [62]	M	M	1	(N)	24	-	7d	(L)	28	24h	LIPC	Cr, H
Kosieradzki, 2003 [63]	D	f*	1	(N)	10	-	10	(E)	45	0.5/1/2/3/4h	LIPC	Other
			1	(N)	10	-	24h	(L)				
Lazaris, 2009 [64]	Rt	M	1	(N)	15	-	15	(E)	45	45	RIPC	other
Lee, 2000 [65]	Rt	M	4	(Y)	8	5	5	(E)	45	24h	LIPC	Cr, BUN, H
Lee, 2001 [66]	Rt	M	4	(Y)	8	5	5	(E)	45	24h	LIPC	Cr
Li, 2005 [67]	D	?	1	(N)	10	-	10	(E)	60	continuous	LIPC	Other
			2-3	(Y)	10	10	10	(E)				
Liu, 2010 [68]	Rb	M	1	(N)	15	-	10	(E)	60	90	LIPC	Cr, BUN

Table S2.1 continues on next page.

Table S2.1. ***Continued***

Author	Species	Gender	Cycles (fractionation)		Prec isch	Int rep	Delay (IPC window)		Index ischemia	Time of measurement	LIPC/ RIPC	Outcome measures
Mahfoudh-Boussaid, 2007 [69]	Rt	M	2	(Y)	5	5	5	(E)	60	2h	LIPC	Other
Obal, 2006 [70]	Rt	M	3	(Y)	2	5	10	(E)	45	1/2/3d	LIPC	Cr, BUN
Ogawa, 2000 [71]	Rt	M	1 3	(N) (Y)	5 5	- 5	5 5	(E) (E)	30	?	LIPC	Other
Ogawa, 2001 [72]	Rt	M	1	(N)	4	-	30	(E)	60	?	L+RIPC†	Other
Ogawa, 2002 [73]	Rt	M	2	(Y)	3	5	5	(E)	30	?	LIPC	Other
Orvieto, 2007 [74]	P	F	1 4	(N) (Y)	10 4	- 11	15 11	(E) (E)	90	1/3/8/15d	LIPC	Cr
Park, 2001 [75]	M	M	1	(N)	5/15/30	-	8/15d	(L)	30/35	1/2d	L+RIPC†	Cr, BUN
Park, 2003 [76]	M	M	1	(N)	15/25/20/30	-	1–12wk	(L)	30	1d/1/3/4/6/12wk	LIPC	Cr
Patshan, 2006 [77]	M	M	1	(N)	25	-	7d	(L)	25	10min/3/6/24h/7d	LIPC	Other
Salehipour, 2007 [78]	D	m/f	1	(N)	5	-	10	(E)	40	2d	LIPC	Other
Sola, 2003 [79]	Rt	M	1	(N)	10	-	10	(E)	45	continuous	LIPC	Other
Song, 2007 [80]	Rt	M	3	(Y)	8	5	5	(E)	45	2h/1d	RIPC	Cr, BUN
Sugino, 2001 [81]	Rt	M	2	(Y)	3	5	5	(E)	50	continuous	LIPC	Other
Timsit, 2008 [82]	Rt	?	3	(Y)	5	5	5	(E)	60	0/1/3/11/15d	L+RIPC†	Cr‡
Toosy, 1999 [83]	Rt	F	4	(Y)	4	11	5	(E)	40	0/2/9d	LIPC	Other
Torras, 2002 [84]	Rt	M	1	(N)	5/10/15/20	-	10/20/40	(E)	40	0/1/2/3/7	LIPC	Cr, H

Table S2.1 continues on next page.

Table S2.1. ***Continued***

Author	Species	Gender	Cycles (fractionation)		Prec isch	Int rep	Delay (IPC window)		Index ischemia	Time of measurement	LIPC/ RIPC	Outcome measures
Treska, 2006 [85]	P	M	2	(Y)	5	10	10	(E)	30	0/10/20/40/60	LIPC	Other
Vianna, 2009 [86]	Rt	M	3	(Y)	5	5	5	(E)	45	1	LIPC	Cr
Wang, 2009 [87]	Rb	m/f	4	(Y)	5	5	5	(E)	240	<4h	RIPC	BUN
Wever, 2011 [88]	Rt	M	1	(N)	12	-	12	(E)	25	48h	RIPC	Cr, BUN, H
			3	(Y)	4	4	4	(E)				
Wu, 2009 [89]	Rt	F	4	(Y)	4	11	10	(E)	45	6h/24h	LIPC	Other
Xie, 1999 [90]	Rb	m/f	1	(N)	10	-	10	(E)	60	24h	LIPC	Cr
			2-3	(Y)	10	10	10	(E)				
Yamashita, 2003 [91]	Rt	M	3	(Y)	2	5	5	(E)	45	24h	LIPC	Cr, BUN, H
Yamasowa, 2005 [92]	M	m/f	3	(Y)	2	5	5	(E)	45	24h	LIPC	Cr, BUN, H
Yu, 1999 [93]	Rt	M	2	(Y)	15,30	5	5	(E)	45	24h	LIPC	BUN
			4	(Y)	5	5	5	(E)				
			4	(Y)	5	5	24h	(L)				

All durations and time points are given in minutes, unless indicated otherwise. *data retrieved from authors; †remote organ was the contralateral kidney; ‡not extracted because of missing data. Prec Isc = preconditioning ischemia, Int Rep = intermediate reperfusions, Delay = delay between IPC and index ischemia, Rt = rat, Rb = rabbit, M = mouse, D = dog, P = pig, SD = Sprague-Dawley, ? = unknown, m = male, f = female, Y = yes, N = no, E = early, L = late, LIPC = local ischemic preconditioning, RIPC = remote ischemic preconditioning, Cr = serum creatinine, BUN = blood urea nitrogen, H = renal histology assessed by Jablonski score.

Table S2.2. Methodological quality

	Ateş, 2002 [36]	Aufricht, 2002 [37]	Ayupova, 2009 [38]	Burne-Taney, 2006 [39]	Cao, 2010 [40]	Chander, 2005 [41]	Chen, 2008 [42]	Chen, 2009 [43]	Cochrane, 1999 [44]	Grenz, 2007a [45]	Grenz, 2007b [46]	Grenz, 2007c [47]	Grenz, 2008 [48]	Guye, 2010 [49]	Hernandez, 2008 [50]	Herrero, 2006 [51]	Hyodo, 2009 [52]	Islam, 1997 [53]	Jang, 2008 [54]	Jefayri, 2000 [55]	Jiang, 2007 [56]	Jiang, 2009 [57]	Joo, 2006 [58]	Kadkhodaee, 2004 [59]	Kim, 2010 [60]	Kim, 2011 [61]	Kinsey, 2010 [62]	Kosieradzki, 2003 [63]	Lazaris, 2009 [64]
Research question specified and clear?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Animals randomized across groups?	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+	-	-	-
Outcome assessment randomized across groups?	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Concealment of allocation?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Group characteristics clearly described?*	+	+[†]	+[†]	-	+[†]	+[†]	+	+[†]	+	-	-	-	-	-	-	-	+[†]	+	+[†]	+[†]	+[†]	+[†]	-	+[†]	+	+[†]	+	+	+
Group characteristics described as equal?	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Correct control group used?	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Body temperature controlled within 3°C variation?	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Blinded outcome assessment for histology?	+	NA	NA	+	+	+	+	-	+	+	+	+	+	-	+	NA	NA	+	+	+	+	+	+	+	-	NA	NA	NA	NA
I/R treatment protocol(s) clearly described?‡	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Time of outcome measurement clearly described?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Number of animals per group clear?	+	-	-	-	+	+	+	+	+	-	-	-	+	+	+	-	-	+	-	+	+	+	+	-	+	+	+	+	+
Number of animals excluded from analysis clear?	-	-	-	-	+	+	-	-	-	+	-	+	-	+	+	-	+	-	-	-	+	-	-	-	-	-	+	-	-
Exclusion criteria mentioned and clear?	NA	NA	NA	NA	NA	+	-	-	-	NA	-	NA	-	-	+	NA	NA	-	NA	+	-	-	+	NA	-	NA	+	+	NA
Complete outcome data?	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+[§]	+	+	+	+	+	+	+	+	+	+	+	+	+
Total score	7	8	6	7	10	11	9	8	10	9	8	9	7	10	8	5	9	10	7	11	10	10	9	8	7	9	7	10	7
Maximal possible score	14	13	13	14	14	15	15	15	15	14	15	14	15	15	15	13	13	15	14	15	15	15	15	14	15	13	14	14	13
Quality (%)	50	62	46	50	71	73	60	53	67	64	53	64	47	67	53	38	69	67	50	73	67	67	60	57	47	69	50	71	54

Table S2.2 continues on next page.

Table S2.2. ***Continued***

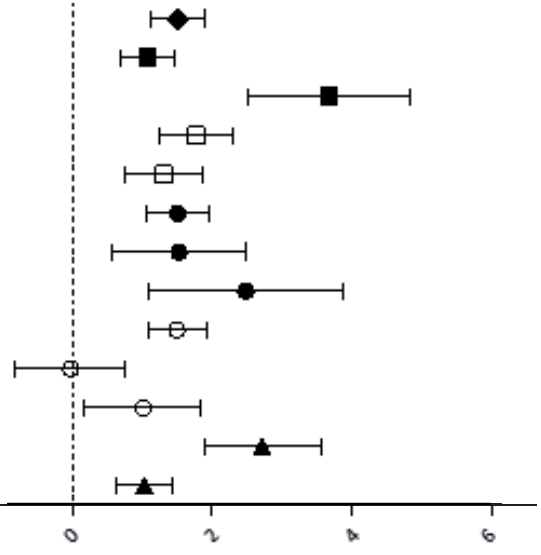
	Lee, 2000 [65]	Lee, 2001 [66]	Li, 2005 [67]	Liu, 2010 [68]	Mahfoudh-Boussaid, 2007 [69]	Obal, 2006 [70]	Ogawa, 2000 [71]	Ogawa, 2001 [72]	Ogawa, 2002 [73]	Orvieto, 2007 [74]	Park, 2001 [75]	Park, 2003 [76]	Patshan, 2006 [77]	Salehipour, 2007 [78]	Sola, 2003 [79]	Song, 2007 [80]	Sugino, 2001 [81]	Timsit, 2008 [82]	Toosy, 1999 [83]	Torras, 2002 [84]	Treska, 2006 [85]	Vianna, 2009 [86]	Wang, 2009 [87]	Wever, 2011[88]	Wu, 2009 [89]	Xie, 1999 [90]	Yamashita, 2003 [91]	Yamasowa, 2005 [92]	Yu, 1999 [93]	Overall score (%)
Research question specified and clear?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Animals randomized across groups?	+	-	+	+	+	+	-	-	-	+	-	-	-	+	-	+	-	-	+	-	-	+	+	+	-	+	-	-	+	40
Outcome assessment randomized across groups?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Concealment of allocation?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Group characteristics clearly described?*	+	+†	+†	+†	+†	+†	+†	+†	+†	-	+†	+†	-	-	+†	+†	+†	+†	+†	+†	+†	-	-	+†	+†	+†	+	-	+	76
Group characteristics described as equal?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
Correct control group used?	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	95
Body temperature controlled within 3°C variation?	+	+	-	+	+	+	-	-	+	+	+	+	-	-	+	+	-	-	+	+	-	+	-	+	-	-	-	-	+	64
Blinded outcome assessment for histology?	+	NA	NA	+	NA	+	NA	NA	NA	NA	-	NA	NA	-	NA	+	+	+	-	+	+	+	NA	+	NA	-	+	+	+	74
I/R treatment protocol(s) clearly described?‡	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Time of outcome measurement clearly described?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	95
Number of animals per group clear?	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	76
Number of animals excluded from analysis clear?	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	+	-	-	+	29
Exclusion criteria mentioned and clear?	-	NA	+	+	NA	+	-	NA	NA	+	-	-	-	NA	-	-	NA	NA	+	-	-	-	NA	NA	-	NA	-	+	NA	36
Complete outcome data?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	97
Total score	10	8	8	11	9	12	7	7	8	9	8	7	5	7	7	11	9	9	10	9	8	9	7	11	7	7	8	9	10	8
Maximal possible score	15	13	14	15	13	15	14	13	13	14	15	14	14	14	14	15	14	14	15	15	15	15	13	14	14	14	15	15	14	14
Quality (%)	67	62	57	73	69	80	50	54	62	64	53	50	36	50	50	73	64	64	67	60	53	60	54	79	50	50	53	60	71	59

1 = yes, 0 = no, NA = not applicable; *required are: species, strain, sex, and weight or age; †weight or age missing; ‡required are: number and duration of preconditioning ischemic period(s), number and duration of preconditioning reperfusion period(s), timing and duration of index ischemia; §no, but explained.

2

Table S2.3. Subgroup analysis serum creatinine

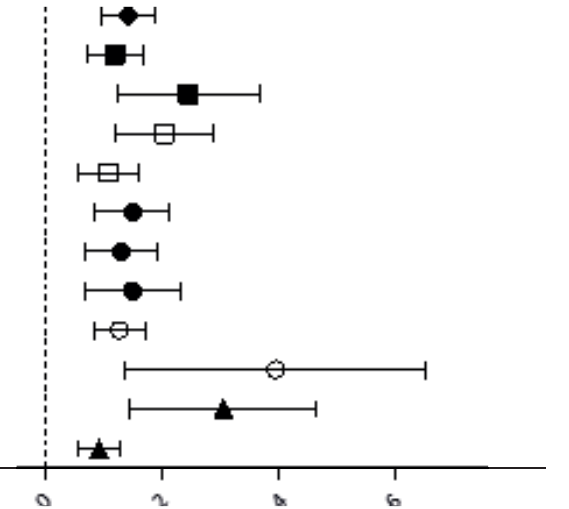
Subgroup	n experiments	n studies	I^2	n IRI only	n IRI + IPC	SMD and 95% confidence interval
Overall	62	33	83%	512	492	1.54 [1.16, 1.93]
Early	47	25	81%	413	384	1.10 [0.72, 1.48]
Late	15	9	80%	99	108	3.53 [2.45, 4.60]
Continuous	32	18	80%	257	250	1.77 [1.25, 2.29]
Fractionated	30	20	85%	255	242	1.31 [0.74, 1.87]
LIPC	51	29	83%	421	390	1.47 [1.03, 1.90]
RIPC	6	3	79%	60	60	1.53 [0.57, 2.48]
LIPC + RIPC	5	1	76%	31	42	2.48 [1.09, 3.87]
Male	42	22	79%	345	339	1.51 [1.09, 1.93]
Female	2	2	24%	17	18	-0.03 [-0.83, 0.76]
Male + female	11	4	82%	87	81	1.13 [0.25, 2.02]
Mouse	22	12	84%	173	170	2.72 [1.88, 3.55]
Rat	35	18	78%	303	286	1.02 [0.61, 1.44]



IRI = ischemia-reperfusion injury, IPC = ischemic preconditioning, SMD = standardized mean difference, LIPC = local ischemic preconditioning, RIPC = remote ischemic preconditioning.

Table S2.4. Subgroup analysis blood urea nitrogen

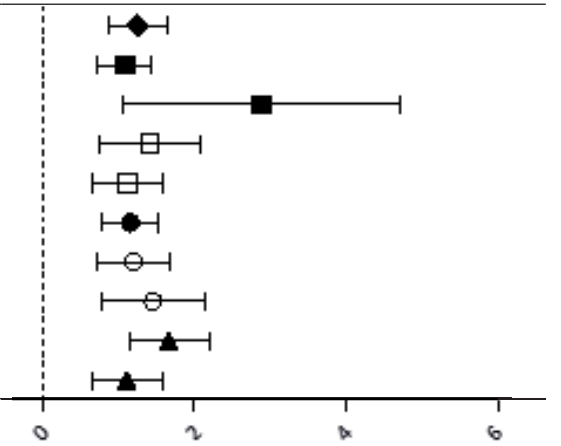
Subgroup	n experiments	n studies	I^2	n IRI only	n IRI + IPC	SMD and 95% confidence interval
Overall	29	17	76%	242	241	1.42 [0.97, 1.87]
Early	22	12	75%	197	185	1.20 [0.72, 1.68]
Late	7	5	75%	45	56	2.45 [1.24, 3.66]
Continuous	11	8	79%	94	103	2.04 [1.19, 2.89]
Fractionated	18	12	71%	148	138	1.08 [0.57, 1.59]
LIPC	20	14	81%	167	155	1.50 [0.86, 2.14]
RIPC	6	3	54%	60	60	1.30 [0.69, 1.92]
LIPC + RIPC	3	1	12%	15	26	1.50 [0.69, 2.31]
Male	27	15	74%	228	227	1.27 [0.83, 1.71]
Mixed	2	2	67%	14	14	3.94 [1.38, 6.51]
Mouse	6	4	77%	33	44	3.05 [1.45, 4.65]
Rat	21	12	60%	186	174	0.93 [0.55, 1.30]



IRI = ischemia-reperfusion injury, IPC = ischemic preconditioning, SMD = standardized mean difference, LIPC = local ischemic preconditioning, RIPC = remote ischemic preconditioning.

Table S2.5. Subgroup analysis histology

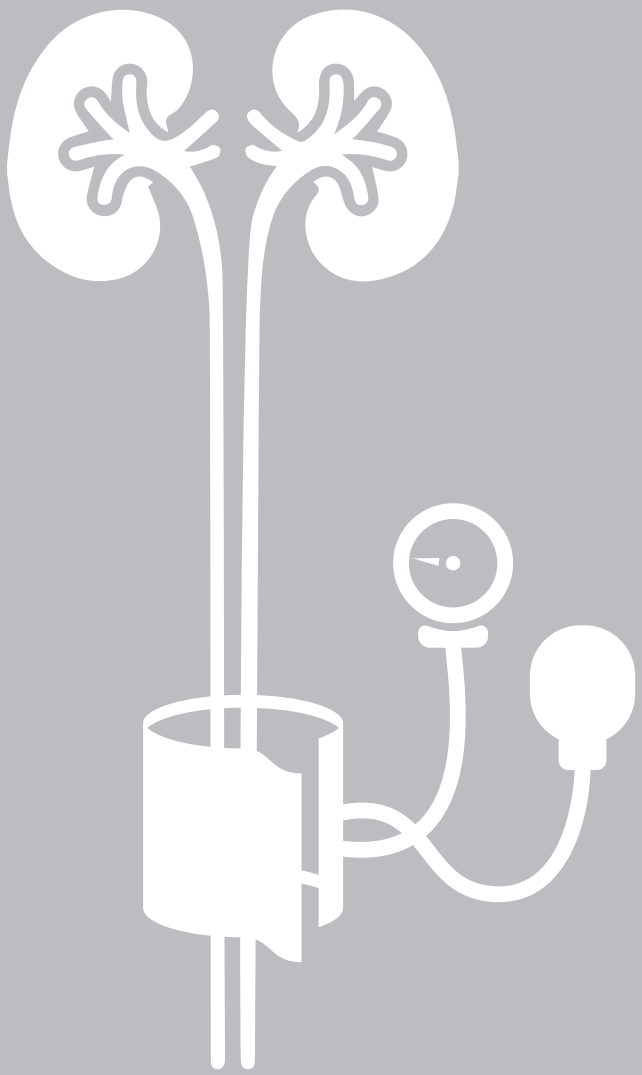
Subgroup	n experiments	n studies	I^2	n IRI only	n IRI + IPC	SMD and 95% confidence interval
Overall	26	15	63%	205	191	1.12 [0.89, 1.35]
Early	23	13	55%	180	168	1.01 [0.77, 1.25]
Late	3	3	76%	25	23	2.50 [1.64, 3.35]
Continuous	12	6	73%	104	95	1.20 [0.87, 1.54]
Fractionated	14	10	51%	101	96	1.03 [0.71, 1.35]
LIPC	25	14	58%	193	179	1.04 [0.81, 1.28]
Male	20	10	70%	166	156	1.04 [0.79, 1.30]
Mixed	4	4	0%	25	23	1.46 [0.78, 2.14]
Mouse	7	6	5%	49	44	1.66 [1.15, 2.17]
Rat	19	9	68%	156	147	0.97 [0.71, 1.23]



IRI = ischemia-reperfusion injury, IPC = ischemic preconditioning, SMD = standardized mean difference, LIPC = local ischemic preconditioning, RIPC = remote ischemic preconditioning.

Appendix S2.1. Full search strategy for PubMed and EMBASE

PubMed	Kidney	"kidney"[MeSH Terms] OR "acute kidney injury"[MeSH Terms] OR "kidney"[Tiab] OR "kidneys"[Tiab] OR "renal"[Tiab] OR "kidney transplantation"[MeSH Terms] OR "nephrology"[MeSH Terms] OR "nephrology"[Tiab]
	Preconditioning	"ischemic preconditioning"[MeSH Terms] OR "IPC"[tiab] OR "RIPC"[tiab] OR "brief ischemia"[tiab] OR "brief ischaemia"[tiab] OR "preconditioning"[tiab] OR "pre conditioning"[tiab] OR "pre-conditioning"[tiab] OR "transient ischaemia"[tiab] OR "transient ischemia"[tiab] OR "intermittent ischaemia"[tiab]OR "intermittent ischemia"[tiab] OR "continuous ischemia"[tiab] OR "continuous ischaemia"[tiab]
	Ischemia reperfusion injury	"warm ischemia"[Mesh Terms] OR "warm Ischemia"[Tiab] OR "warm Ischaemia"[Tiab] OR "cold ischemia"[Mesh Terms] OR "cold ischemia"[Tiab] OR"cold ischaemia"[Tiab] OR "primary graft dysfunction"[Mesh Terms] OR "primary graft dysfunction"[Tiab] OR "I/R"[Tiab] OR "IRI"[Tiab] OR "ischemic reperfusion"[Tiab] OR "ischaemic reperfusion"[Tiab] OR“ischemia reperfusion"[Tiab] OR“ischaemia reperfusion"[Tiab] OR “kidney ischemia"[Tiab] OR “kidney ischaemia"[Tiab] OR "renal ischaemia"[tiab] OR "renal ischemia"[tiab] OR "reperfusion injury"[Mesh Terms] OR "reperfusion injury"[tiab] OR "reperfusion injuries"[tiab] OR "ischemia reperfusion"[tiab] OR "ischaemia reperfusion"[tiab] OR "renal injury"[tiab] OR "renal injuries"[tiab]
	Animals	Laboratory animal search filter [94]
Embase	Kidney	exp kidney/ OR exp acute kidney failure/ OR exp kidney transplantation/ OR exp kidney allograft rejection/ OR (renal OR kidney OR kidneys OR nephrology).ti,ab.
	Preconditioning	exp ischemic preconditioning/ OR (IPC OR RIPC OR brief ischemia OR brief ischaemia OR preconditioning OR pre conditioning OR pre-conditioning OR transient ischaemia OR transient ischemia OR intermittent ischaemia OR intermittent ischemia OR continuous ischemia OR continuous ischaemia).ti,ab.
	Ischemia reperfusion injury	exp reperfusion injury/ OR exp cold ischemia/ OR exp primary graft dysfunction/ OR (warm ischemia OR warm ischaemia OR cold ischemia OR cold ischaemia OR reperfusion injury OR primary graft dysfunction OR I/R OR IRI OR ischemic reperfusion OR ischaemic reperfusion OR kidney ischemia OR kidney ischaemia OR renal ischaemia OR renal ischemia OR reperfusion injury OR reperfusion injuries OR ischemia reperfusion OR ischaemia reperfusion OR renal injury OR renal injuries).ti,ab. OR (cold ischemia OR cold ischemia time OR cold ischemia times OR cold ischemic time OR cold ischemic times OR cold ischaemia OR cold ischaemia time OR cold ischaemia times OR cold ischaemic time OR cold ischaemic times).ti,ab. OR (warm ischemia OR warm ischaemia).ti,ab.
	Animals	Laboratory animal search filter [95]



3

Preclinical evidence for the efficacy of ischemic postconditioning against renal ischemia-reperfusion injury, a systematic review and meta-analysis

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ABSTRACT

Background

Renal ischemia-reperfusion injury (IRI) is a major cause of kidney damage after *e.g.* renal surgery and transplantation. Ischemic postconditioning (IPostC) is a promising treatment strategy for renal IRI, but early clinical trials have not yet replicated the promising results found in animal studies.

Method

We present a systematic review, quality assessment and meta-analysis of the preclinical evidence for renal IPostC, and identify factors which modify its efficacy.

Results

We identified 39 publications studying >250 control animals undergoing renal IRI only and >290 animals undergoing renal IRI and IPostC. Healthy, male rats undergoing warm ischemia were used in the vast majority of studies. Four studies applied remote IPostC, all others used local IPostC. Meta-analysis showed that both local and remote IPostC ameliorated renal damage after IRI for the outcome measures serum creatinine, blood urea nitrogen and renal histology. Subgroup analysis indicated that IPostC efficacy increased with the duration of index ischemia. Measures to reduce bias were insufficiently reported.

Conclusion

High efficacy of IPostC is observed in animal models, but factors pertaining to the internal and external validity of these studies may hamper the translation of IPostC to the clinical setting. The external validity of future animal studies should be increased by including females, comorbid animals, and transplantation models, in order to better inform clinical trial design. The severity of renal damage should be taken into account in the design and analysis of future clinical trials.

INTRODUCTION

Renal ischemia and reperfusion injury (IRI) is a major cause of acute kidney injury (AKI) after *e.g.* renal surgery, coronary artery bypass grafting and abdominal aortic aneurysm repair, which results in increased morbidity and mortality [1]. Renal IRI is also considered an important cause of delayed graft function after renal transplantation and is associated with prolonged hospital stay and acute rejection [2, 3].

Ischemic postconditioning (IPostC) is a protective strategy in which (repeated) brief, intermittent periods of ischemia and reperfusion are applied in the early phase of reperfusion after a prolonged ischemic episode. Since its discovery in 2003 in the dog heart [4], IPostC has been shown to attenuate IRI in various organs and a variety of animal species, and is effective when applied to either the target organ, or a remote organ or tissue [5, 6]. Thus, IPostC poses a promising treatment strategy for IRI in patients.

Following the promising results obtained in animal studies, the feasibility and efficacy of renal IPostC in patients has been investigated in two clinical trials [7, 8]. Although application of local IPostC seemed feasible and safe in patients undergoing donation-after-circulatory-death kidney transplantation, it had no effect on delayed graft function incidence or renal function in a paired kidney analysis [7]. Remote IPostC (RIPostC) appeared to hasten the early recovery of graft function in patients undergoing living donor kidney transplantation, but did not affect graft function >24 hours post-operatively [8]. In addition, clinical trials investigating the effect of IPostC on the myocardium have also yielded conflicting results (reviewed in [9, 10]). Thus, the question arises why the replication of the promising results found in animals has been limited in patients, and how the translation of IPostC from animal studies to patients may be improved.

Previously, meta-analysis and systematic review of preclinical studies have proven useful in optimizing the design of both preclinical and clinical studies [11–13]. Although an overview of experimental studies in this field exists [14], a systematic review of the preclinical evidence for renal IPostC is lacking. It remains unclear if and how factors pertaining to the IPostC protocol (*e.g.* timing and duration) and the animals under investigation (*e.g.* sex, comorbidities) influence IPostC efficacy. As a result, the IPostC stimulus could have been suboptimal or incorrectly applied in clinical trials, or unsuitable for the patient population. We therefore conducted a systematic review and meta-analysis of evidence on the protective effect of IPostC in animal models of renal IRI. This approach allowed us to analyze the influence of variables such as IPostC timing, IPostC duration, sex and comorbidity on treatment efficacy. We also assessed the extent to

which the preclinical data might be at risk of bias, either through publication bias, or through factors relating to experimental design.

MATERIALS AND METHODS

For an extended version, see S3.1 Text. The review methodology was predefined and documented in a protocol [15], published online on February 12th 2015. The review question was: what is the effect of local or remote IPostC on renal function in animal models of renal IRI?

Amendments to the review protocol

After study selection, we found that the timing and duration of the IPostC protocol depended strongly on the site of postconditioning. We therefore decided to perform separate meta-analyses of studies using local, remote, and local+remote postconditioning, to avoid collinearity.

For serum creatinine and blood urea nitrogen (BUN), all data could be expressed in the same unit of measurement, but differences in baseline measurements between studies were observed. We therefore performed meta-analysis of the normalised mean difference (NMD) instead of the standardized mean difference (SMD). For renal histology, we expressed all scores as a percentage on the grading scale used, and performed meta-analysis of the mean difference (MD), instead of the SMD. This allowed us to include studies reporting the histology score as a percentage on the grading scale used.

Study identification

A systematic, computerized search in the databases Medline (via PubMed) and EMBASE (Table S3.1) was performed on February 4th 2015, using the search components 'kidney', 'ischemic postconditioning' and an animal search filter for either PubMed [16] or EMBASE [17]. To identify additional relevant studies, the reference lists of included studies and relevant reviews were hand searched. No language restrictions were applied.

Selection of studies

After removal of duplicates, all references were screened for inclusion based on their title and abstract. The following inclusion criteria were applied: the study 1) is an original article

presenting unique data with a control group, 2) is performed *in vivo* in animals with or without comorbidities, but without genetic modifications, 3) reports on renal ischemia-reperfusion injury and outcome measures related to kidney injury or function, and 4) examined the effect of remote and/or local ischemic postconditioning. Subsequently, the full-text manuscripts of eligible studies were reviewed for inclusion. Studies involving co-medication other than anaesthetics or analgesics, or a co-intervention other than collateral nephrectomy were excluded. Studies performed in a renal transplantation model were excluded from the present dataset, but labelled for future reference. In both phases, references were independently assessed for inclusion by two reviewers (KW and SJ).

Study characteristics and data extraction

Study characteristics were extracted by one reviewer (SJ) and checked for inconsistencies by a second reviewer (TM). We selected the following outcome measures for analysis: serum creatinine, BUN and renal histology scores (Jablonski [18] or comparable). Data was collected as mean and standard deviation (SD). For serum creatinine and BUN, all data was recalculated to the same unit of measurement (respectively umol/L and mmol/L). For renal histology, scores were expressed as a percentage on the grading scale used. If an outcome was measured at several time-points, data was extracted for the time-point of greatest efficacy. If a study reported data from several experimental groups, it was extracted as separate comparisons and the number of animals in the control group was corrected (number of animals divided by number of comparisons).

Risk of bias and study quality

Two reviewers (SJ and TM) independently assessed the risk of bias and study quality of each included study. In case of discrepancies, consensus was reached by discussion with a third reviewer (KW). Risk of bias was assessed using SYRCLE's Risk of Bias tool [19]. Reporting bias (item #9) was not assessed, since none of the studies reported the use of a study protocol predefining primary and secondary outcomes. When assessing selection bias, groups within a study were considered similar at baseline if sex and baseline serum creatinine did not significantlydiffer between groups (or, if baseline creatinine was unavailable, body weight). To assess whether studies were free of other risks of bias, addition of animals to groups during the experiment and a possible conflict of interest were taken into account. We also assessed reporting of the following study quality items: any randomization, any blinding, regulation of body temperature within 3°C variation and sample size calculation.

Data analysis

Data was analyzed using Stata/SE (StataCorp, Texas, USA). For the outcome measures serum creatinine and BUN, meta-analysis was performed on the NMD, which allows us to correct for baseline kidney injury by relating the magnitude of the effect of treatment to a baseline measured in untreated animals [20]. For histology, the MD was used. A random effects model was used to account for expected between-study heterogeneity. To assess heterogeneity, the I^2 and adjusted R^2 statistics were determined. To examine potential sources of heterogeneity, predefined subgroup analyses were performed on subgroups containing data from at least three studies. For the duration of IPostC ischemia, studies were categorized using increments of 0.7 log, which resulted in categories of 26–125, 126–630 and 631–3162 seconds of ischemia. For the duration of index ischemia, studies were categorized using increments of 15 minutes, resulting in categories of 16–30, 31–45, 46–60, 61–75 (no studies) and 76–90 minutes. Differences between subgroups were determined by calculating the difference in NMD and MD respectively and the 95% confidence intervals (CI) of the difference. Results are reported as a NMD or MD [95% CI], unless stated otherwise. For each outcome measure, the significance level for subgroup analyses was adjusted for the number of analyses using the Bonferroni-Holm method [21].

Publication bias was assessed for each outcome measure by visual evaluation of funnel plots, Duval and Tweedie's trim and fill analysis and by performing Egger's test for small study effects. Sensitivity analyses were carried out for creatinine and BUN using a fixed time point of outcome assessment (24 h). For histology, a sensitivity analysis was performed using only Jablonski histology scores.

RESULTS

Study identification and selection

A flow chart of the study selection process is shown in Figure 3.1. The computerized search retrieved 213 references from PubMed and 272 from EMBASE. Four additional references were added after hand searching reference lists of included studies and relevant reviews. After duplicate removal, 300 references were screened based on title and abstract and 51 studies continued to the eligibility phase. Two letters to the editor [22, 23] were included since they presented unique data and sufficient methodological detail. One study investigating IPostC in a canine model of renal transplantation with cold ischemia was excluded, because of the differences in pathophysiology compared to

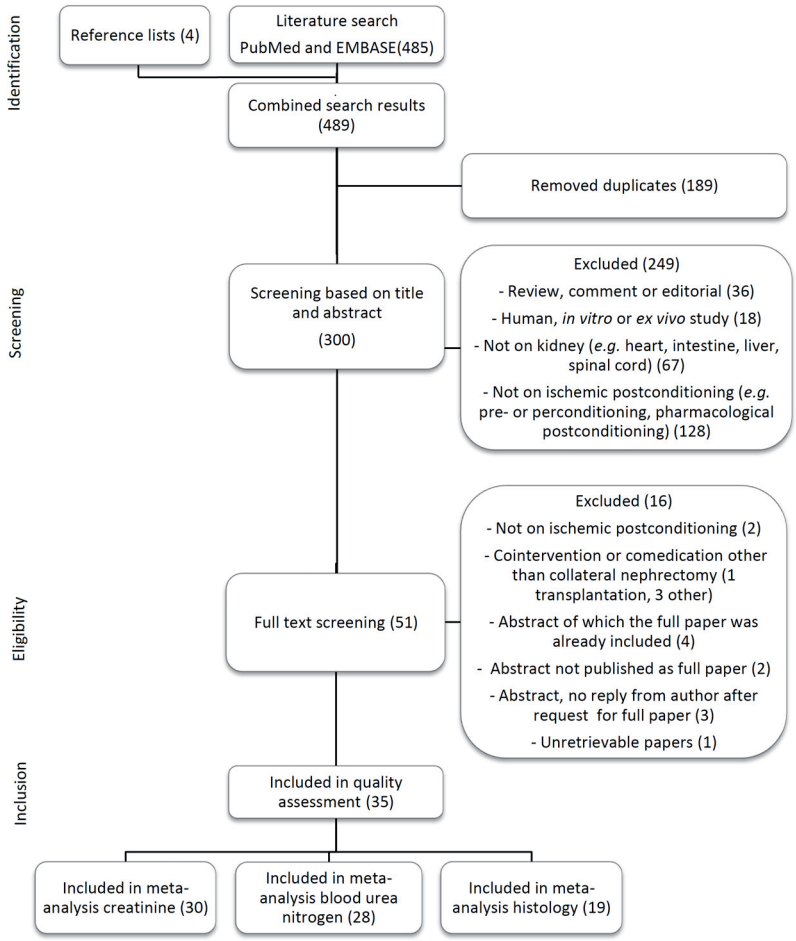

Figure 3.1. Flow chart of study selection.
The number of studies in each phase are shown between brackets.

warm ischemia. Finally, 35 studies were included in the risk of bias assessment, all but one of which reported on one or more of the selected outcome measures.

Study characteristics

The study characteristics are summarized in Table 3.1. Out of the 35 included studies, 31 were performed in rats, one in dogs and three in mice. Male animals were used in all but three studies. There were only three studies investigating the effect of RIPostC, all of which used the hind limb as remote tissue. The most commonly used durations of index ischemia

Table 3.1. Study characteristics

Study	Species/ strain	Sex	Index isch	# IPoC cycles	IPoC isch	IPoC rep	IPoC delay	Coll ntx?	Site of IPoC	Time of OM (hrs)	OM
Chen 2008[41]	R/W	m	45	6	10	10	0	Y	LIPoC	24/48/72	Cr, BUN, H(J)
Chen 2011[42]	R/SD	m	60	6	10	10	0	Y	LIPoC	4	Cr, BUN, H(D)
Chen 2014[43]	R/W	m	45	6	10	10	0	Y	LIPoC	24	Cr, BUN, H(J)
Chen 2015[44]	R/W	m	45	6	10	10	0	Y	LIPoC	24	Cr, BUN, H(J)
Eldaif 2010[45]	R/SD	m	45	4	45	45	0	Y	LIPoC	24	Cr, BUN, H(O)
Fan 2009[46]	R/SD	m	60	6	10	10	0	?	LIPoC	6	Cr, BUN, H(D)
Guo 2014[47]	R/SD	m	45	3	10	10	0	N	LIPoC	0/1/3/6/12/24/48	Cr, BUN
Ji 2012[48]	R/SD	m	45	6	10	10	0	Y	LIPoC	6/12/24/48/72	Cr, BUN, H(D)
Jiang 2010[49]	D	m	60	6	15 30 60	15 30 60	0	Y	LIPoC	72	Cr, BUN, H(J)
Jiang 2014[50]	R/SD	m	60	4	300	300	0	Y	RIPoC†	24	Cr, BUN, H(J)
Kadkhodaee 2011[22]	R/SD	m	45	4	300	300	0	Y	RIPoC†	24	Cr, BUN
Kadkhodaee 2014[51]	R/SD	m	45	4	10 300	10 300	0	Y	LIPoC RIPoC†	24	Cr, BUN, H(D)
Lemoine 201[52]	M/C57BL6	m	30	3	30	30	0	Y	LIPoC	24	Cr, BUN, H(O)
Li 2010[53]	R/SD	m	45	10	20	20	0	N	LIPoC	24	Cr, BUN, H(J)
Li 2012[54]	R/SD	f	45	10	20	20	0	Y	LIPoC	1/3/6/12/24	Cr, BUN
Liu 2007[55]	R/W	m	45	6	10	10	0	Y	LIPoC	24	Cr, BUN, H(J)
Mahfoudh-Boussaid 2012[24]	R/W	m	60	6	10	10	0	N	LIPoC	2	Cr, H(J)
Mahmoudi 2014[35]	R/SD	m/f	45	4	10	10	0	Y	LIPoC	24	Cr, BUN, H(O)
Miklós 2012[26]	R/W	m	45	4	15	15	0	N	LIPoC	2	Cr, BUN, H(J)

Table 3.1. ***Continued***

Study	Species/ strain	Sex	Index isch	# IPoC cycles	IPoC isch	IPoC rep	IPoC delay	Coll ntx?	Site of IPoC	Time of OM (hrs)	OM
Serviddio 2008[27]	R/W	m	90	3	300	180-360-720*	0	Y	LIPoC	0/0.6/24/48	Cr, BUN, H(O)
Shokeir 2012[56]	R/SD	m	45	3	300	180-360-720*	0	Y	LIPoC	2/24/48	Cr, BUN, H(O)
Shokeir 2014[57]	R/SD	m	45	3	300	180-360-720*	0	Y	LIPoC	2/24/48	Cr, BUN
Szwarc 2007[58]	M/Swiss	f	30	3	30	30	0	Y	LIPoC	0-192	Cr
Tan 2013[59]	R/SD	m	45	3	30	30	420	Y	LIPoC	1/48/168	Cr, H(D)
Tang 2008[60]	R/W	m	60	6	10	10	0	Y	LIPoC	24	Other
Tao 2012[61]	R/SD	m	45	6	10	10	0	Y	LIPoC	24	Cr, BUN, H(D)
Wang 2010 [62]	R/W	m	60	6	10	10	0	Y	LIPoC	24	Cr, BUN, H(J)
Weng 2012[28]	R/SD	m	45	6	10	10	0	Y	LIPoC	2016	Cr, BUN, H(O)
Wever 2012[23]	R/SD	m	25	6 3 9	8 300 308	8 300 308	0	Y	LIPoC RIPoC† Both†	48	Cr, BUN, H(O)
Xia 2014[25]	R/SD	m	60	6	10	10	0	Y	LIPoC	1/3/6/24	Cr, BUN, H(O)
Yun 2009 A[63]	R/SD	m	45	6	10	10	0	Y	LIPoC	1/3/6/12/24	Cr, BUN, H(J)
Yun 2009 B[64]	R/SD	m	45	6	10	10	0	Y	LIPoC	24	Cr, BUN, H(J)
Zhang 2011[65]	R/SD	m	45	3	10	10	0	N	LIPoC	6	Cr, BUN, H(D)
Zhu 2008[66]	R/W	m	60	6	10	10	0	Y	LIPoC	24	H(D)
Zhuang 2009[67]	M/C57	m	26	3	30	30	0 600	Y	LIPoC	48	Cr, BUN, H(O)

Duration of index ischemia is given in minutes, IPoC protocol timing and duration in seconds, time of outcome measurement in hours. *the duration of reperfusion elongated in each cycle; †remote tissue was the hind limb. Spec = species, Index isch = index ischemia in minutes, IPoC isch = duration of ischemic phase in IPoC protocol, IPoC rep = duration of reperfusion phase in IPoC protocol, IPoC delay = delay between end of index ischemia and start of IPoC, Col ntx = collateral nephrectomy, SD = Sprague-Dawley, W=Wistar, M= Mongrel), m = male, f = female, Y = yes, N = no, ? = unknown, LIPoC = local ischemic postconditioning, RIPoC = remote ischemic postonditioning, Cr = serum creatinine, BUN = blood urea nitrogen, H(J) = renal histology assessed by Jablonski score, H(O) = renal histology assessed by other scoring system, H(D) = descriptive reporting of renal histology.

were 45 and in. The IPostC protocol varied between studies, however, local application of six cycles of 10/10 seconds of reperfusion/ischemia was most commonly used.

Risk of bias and study quality

The results of the study quality and risk of bias assessment are shown in Figure 3.2 and Table S3.2. Randomization and blinding are essential measures to reduce bias, but are infrequently reported. Seventy-four percent of the included studies reported random allocation of the animals, however, only one study adequately specified the method of randomisation. Studies that reported blinding (46%), only did so for the outcome assessment of histology. None of the studies reported a sample size calculation. As a consequence of insufficient reporting, the risk of bias was unclear for most items of the risk of bias tool.

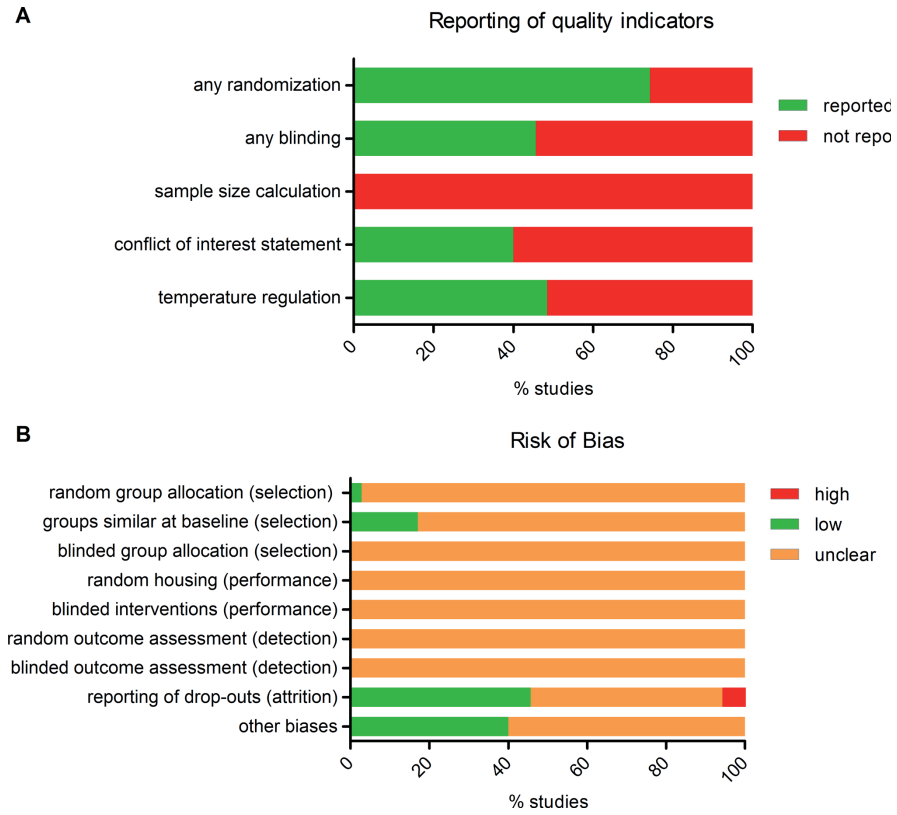

Figure 3.2. Risk of bias and study quality assessment.
Top: Reporting of five key study quality indicators was found to be poor in many cases. Bottom: Using SYRCLE's risk of bias tool, the risk of selection, performance, detection, attrition and other biases was assessed. Lack of (adequate) reporting of measures to reduce bias resulted in a high percentage of unclear risk of bias for most items.

Meta-analyses

Studies investigating local, remote, or local+remote IPostC were analyzed separately. Only the local IPostC group contained enough studies to perform subgroup analysis for any of the outcome measures. One study reporting creatinine clearance [24] was excluded from analysis because serum creatinine data could not be obtained. Data from two studies [25, 26] was excluded because serum creatinine or BUN levels were the same in the experimental group and the sham group, indicating that the experimental group did not sustain a sufficient amount of renal IRI. For renal histology, two studies were excluded due to incomplete outcome data [26, 27].

Serum creatinine

Thirty-one studies reported serum creatinine data from 39 experiments, using 258 sham animals, 247 control animals undergoing renal IRI only and 298 experimental animals undergoing both IRI and IPostC. Both the control and experimental groups contained three to 12 animals (median n=8). The IRI-induced rise in serum creatinine was reduced by both LIPostC (34 experiments; NMD 45.0 [33.4, 56.6]) and RIPostC (four experiments; NMD 49.3 [22.8, 75.7]; Figure 3.3). One study investigating the combination of LIPostC and RIPostC showed no effect (NMD 57.84 [-12.0, 127.7]).

Subgroup analysis results for the LIPostC studies are shown in S3 Table. LIPostC had a beneficial effect on creatinine in all subgroups, except for mouse, female, four cycles of LIPostC and 16–30 minutes of index ischemia. Overall heterogeneity was high (I^2 74.7%), but none of the subgroupvariables accounted for a significant proportion of the observed heterogeneity.

BUN

Twenty-eight studies reported BUN data from 36 experiments, using 226 sham animals, 222 control animals and 269 IPostC-treated animals. Both the control and experimental groups contained three to 12 animals (median n=8). The IRI-induced rise in BUN was reduced by both LIPostC (33 experiments; NMD 43.4 [30.8, 56.1]) and RIPostC (four experiments; NMD 41.0 [23.7, 58.3]; Figure 3.4). One study investigating the combination of LIPostC and RIPostC showed no effect (NMD 55.0 [-5.6, 115.6]).

Subgroup analysis results for the LIPostC studies are shown in S4 Table. The effect of species and sex on LIPostC efficacy could not be analyzed due to insufficient data. LIPostC had a beneficial effect on BUN in all subgroups, except for mouse, female, four cycles of IPostC, 631–3162 seconds of IPostC ischemia and 16–30 minutes of index ischemia.

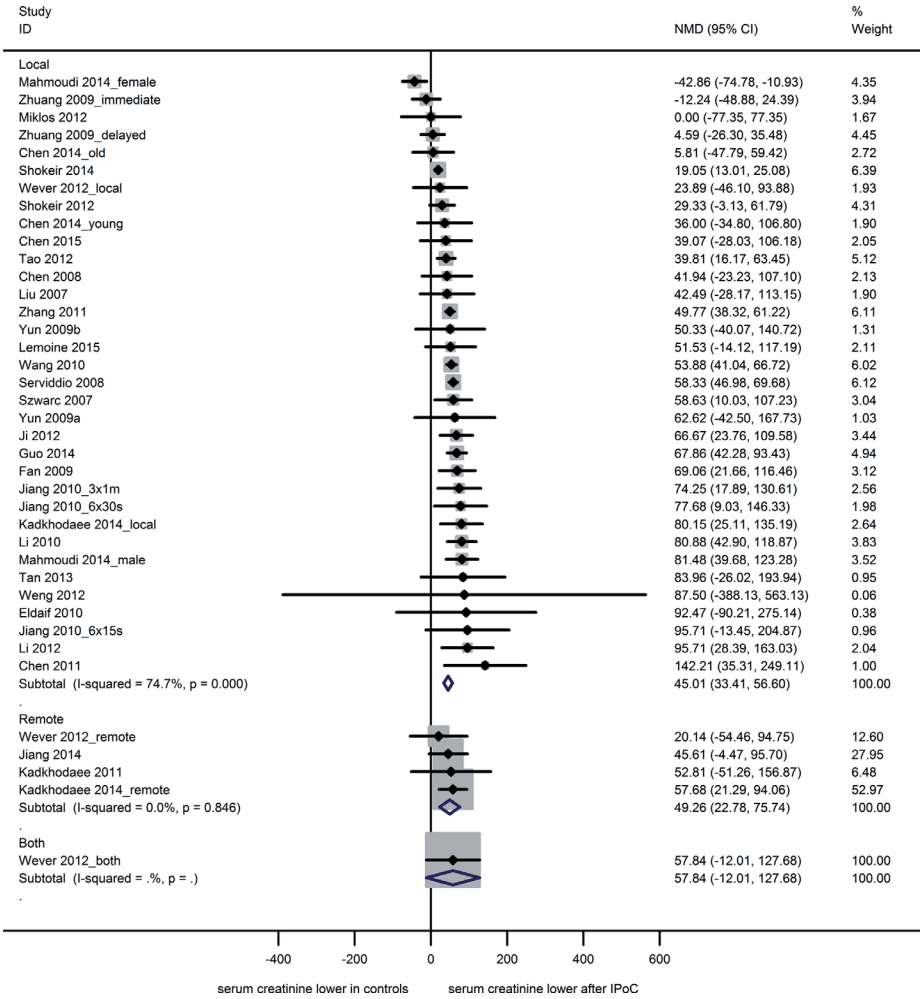

Figure 3.3. Meta-analysis creatinine.
The summary effects show a decrease in serum creatinine after local or remote IPostC. One study investigating the combination of local and remote IPostC showed no effect. Data are presented as NMD and 95% CI. Within subgroup weights from random effects analysis are shown.

Overall heterogeneity was high (I^2 71.6%). A significant proportion of heterogeneity was explained by the duration of index ischemia (adjusted R^2 44.5%; $p<0.007$), indicating that the efficacy of postconditioning increased with the duration of index ischemia. None of the other subgroup variables accounted for a significant proportion of heterogeneity.

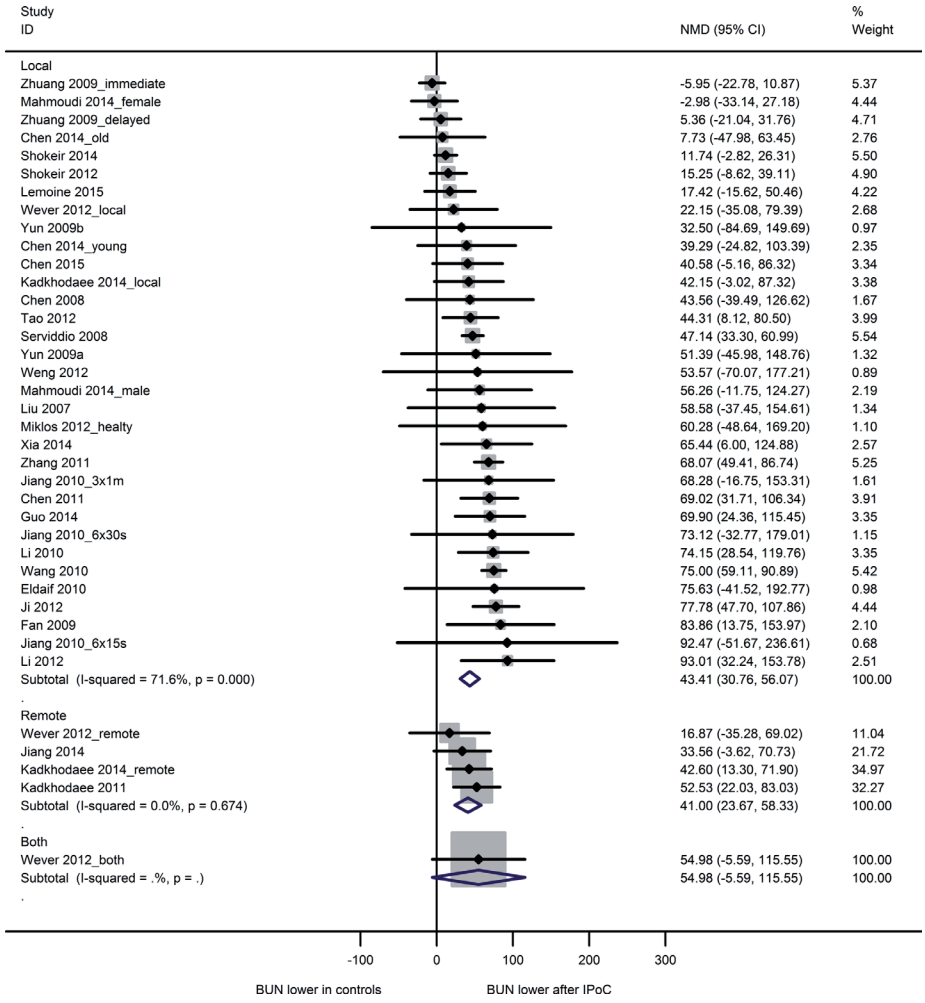

Figure 3.4. Meta-analysis blood urea nitrogen.
The summary effects show a decrease in blood urea nitrogen after local or remote IPostC. One study investigating the combination of local and remote IPostC showed no effect. Data are presented as NMD and 95% CI. Within subgroup weights from random effects analysis are shown.

Renal histology

Nineteen studies reported data on renal histology from 26 experiments, using 149 sham, 152 control and 191 IPostC-treated animals. Both the control and experimental groups contained four to ten animals (median n=8). Renal histology scores were reduced after renal IRI in animals treated with LIPostC (23 experiments; MD 27.8 [18.4, 37.2] or RIPostC (two experiments; MD 18.4 [6.4, 30.5]) or the combination of the two (one experiment; MD 1.0 [0.1, 1.93]; Figure 3.5).

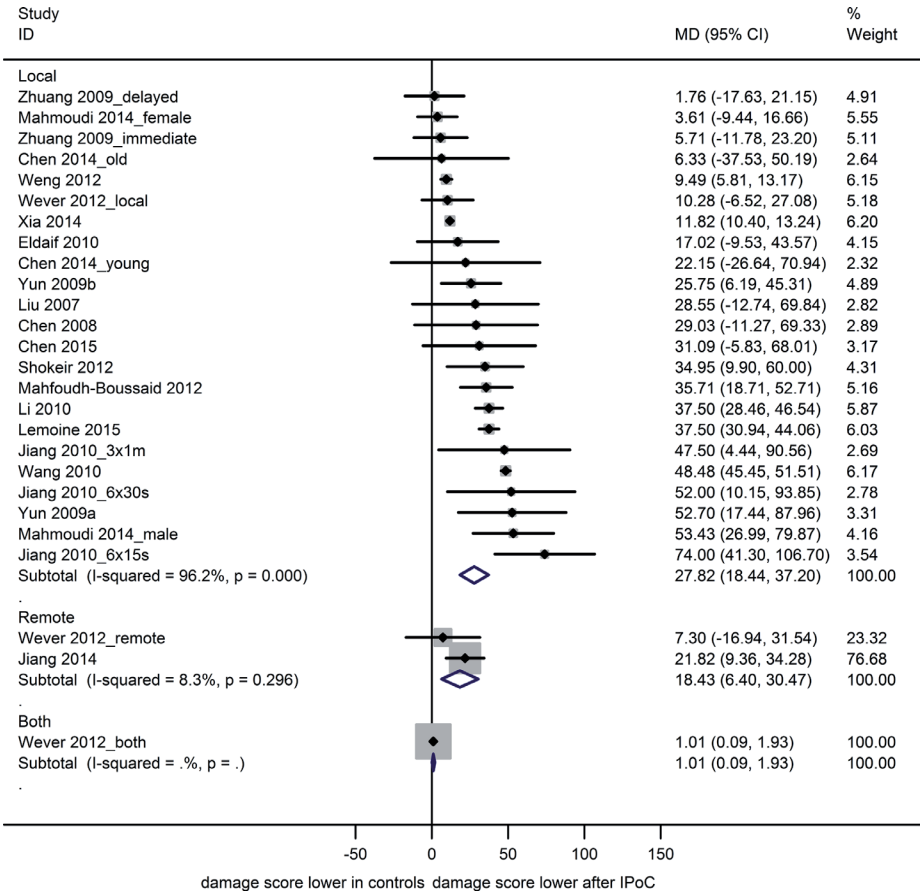

Figure 3.5. Meta-analysis renal histology.
The summary effects show a decrease renal damage score after local or remote IPostC, and the combination of the two. Data are presented as MD and 95% CI. Within subgroup weights from random effects analysis are shown.

A positive effect of LIPostC on histology scores (Table S3.5) was observed in most subgroups, similar to the results obtained for serum creatinine. The effect of species, sex and site of postconditioning on IPostC efficacy could not be analyzed due to insufficient data. Overall heterogeneity was very high (I^2 96.2%), but could not be attributed to any of the subgroup variables.

Publication bias

Publication bias could be assessed for LIPostC only, due to insufficient data for RIPostC. Possible publication bias was observed for all outcome measures when visually evaluating

funnel plots for asymmetry. Duval and Tweedie's trim and fill analysis resulted in filled data points for all outcome measures (Figure 3.6A, 3.6C and 3.6E), indicating that small, negative studies were underrepresented. However, Egger's test indicated that no small study effects were present (Figure 3.6B, 3.6D and 3.6F).

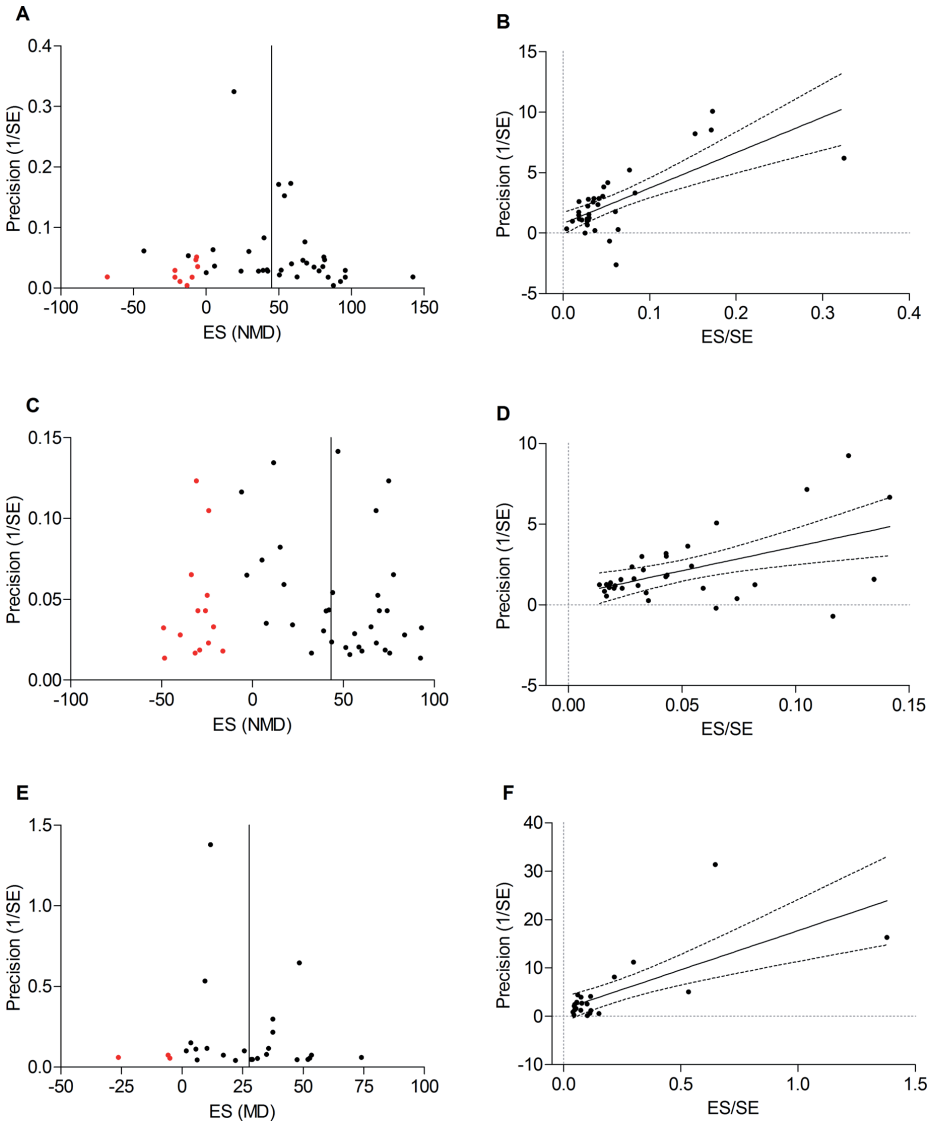

Figure 3.6. Publication bias.
Trim and fill analysis for studies on local IPostC indicates funnel plot asymmetry for respectively creatinine (A), BUN (C) and renal histology (E). The 95% confidence interval of Egger's regression line (dashed lines) does not include the origin of the graph, indicating no small study effects for creatinine (B), BUN (D) and renal histology (F).

Sensitivity analyses

Sensitivity analyses were performed to assess the robustness of our findings. For serum creatinine and BUN, a fixed time point of 24 hours for outcome assessment was chosen instead of the time point of the greatest efficacy. The analyses contained 24 studies for both creatinine and BUN. The summary effect found in the sensitivity analysis did not differ from the original analysis for either creatinine (NMD 43.3 [30.7, 55.9] *vs.* 45.0 [33.4, 56.6]) or BUN (NMD 37.3 [24.7, 50.8] *vs.* 43.4 [30.8, 56.1]). The overall heterogeneity was slightly lower in the sensitivity analyses (I^2 64.3% *vs.* 74.7% for creatinine and 69.3% *vs.* 71.6% for BUN).

For renal histology, a sensitivity analysis was performed by excluding all studies which did not use the Jablonski grading scale. The summary effect found in the sensitivity analysis on the remaining 13 studies did not differ from the original analysis (MD 40.2 [32.5, 47.8] *vs.* 27.8 [18.4, 37.2]). However, heterogeneity was considerably lower in the sensitivity analysis (I^2 41.5% *vs.* 96.2%). This was surprising, since all scales roughly scored the same features of tubular damage (*e.g.* cellular vacuolization, loss of brush border, cast formation). However, since the overall effect of IPostC was robust, we feel that our decision to pool all scoring systems is justified.

One study [28] measured serum creatinine and BUN twelve weeks after renal IRI. At this time-point, values were similar in all groups, which resulted in extremely large confidence intervals in our NMD meta-analysis. However, omitting this study had no effect on meta-analysis outcomes.

DISCUSSION

IPostC efficacy and sources of heterogeneity

This systematic review and meta-analysis provides a quantitative summary of all preclinical *in vivo* evidence on IPostC against renal IRI. Our review shows a protective effect of both LIPostC and RIPostC on renal function and histology, based on a reduction in serum creatinine, BUN and renal histology scores. The high between-study heterogeneity was partially explained by the duration of index ischemia, *i.e.* LIPostC efficacy appeared to increase as the duration of index ischemia increased. The other study characteristics under investigation did not account for significant proportions of heterogeneity, or could not be analysed due to insufficient data (especially for RIPostC). For LIPostC, the remaining heterogeneity is high, especially for renal histology. Importantly, differences in the risk of

bias between studies may represent a significant source of unexplained heterogeneity, but insufficient reporting currently prevents us from testing this hypothesis (see below).

Methodological quality

Adequate reporting of methodological details is crucial to determine the risk of bias in primary studies and to assess the quality of a body of evidence. Insufficient reporting of preclinical research methodology occurs in many fields and is often associated with an overestimation of treatment effects *e.g.* [29–31]. We show that details on key measures to reduce bias (such as randomisation and blinding) and other study quality indicators were missing from many studies included in our review. The risk of bias in most studies therefore remains unclear. Consequently, some studies may have overestimated the effect of IPostC, which may have influenced the outcome of our meta-analysis.

The number of animals per group was very low in a number of studies. This is a matter of concern, since underpowered studies have an increased risk of finding false positive results. Systematic reviews have suggested that underpowering of *in vivo* studies is common, and that this greatly contributes to translational failure [31, 32]. Since none of the included studies reported a sample size calculation, we cannot exclude the possibility of an effect of underpowering on our meta-analysis.

Study characteristics

The present review points out several apparent differences between the experimental design of current clinical trials on renal IPostC, and the preceding animal studies. Firstly, we show that 99% of the preclinical evidence was obtained from animals undergoing warm renal ischemia. In contrast, the two published clinical trials on IPostC [7, 8], as well as a third trial in progress (ISRCTN66437627), all study the effect of IPostC after renal transplantation. To our knowledge, these trials were predominantly based on results obtained in animal models of warm IRI. Only one animal study investigating IPostC after renal transplantation [33] was retrieved by our search (which was not limited to a specific model of renal IRI). This study does show a protective effect of local IPostC after transplantation, however, there are substantial differences between these models (*e.g.* warm *vs.* cold ischemia and renal denervation), and animal models using warm ischemia may not optimally predict outcomes in the clinical transplantation setting.

Secondly, we show that 90% of the animal studies investigated LIPostC, even though RIPostC is generally considered to be more applicable in clinical practice. Thus far, one

clinical trial investigated LIPostC [7], and two applied RIPostC ([8] and ISRCTN66437627). Regarding the LIPostC protocol, our meta-analysis did not identify any factors related to timing or duration which influence its efficacy. Since nearly all evidence was obtained in rats, it remains unclear whether the same timing and duration is effective in all species (including humans). Only two studies have used larger animals, whose metabolic rate is more comparable to humans. Of note, Van den Akker et al. [7] adjusted their clinical IPostC protocol to fit the metabolic rate in humans, but found no beneficial effect. Furthermore, there is not enough preclinical evidence to assess if timing and/or duration of the protocol influences the efficacy of RIPostC. We suggest that the optimal timing and duration of the postconditioning protocols should be determined separately for LIPostC and RIPostC.

Concerning the population under investigation, nearly all preclinical studies used male animals, whereas the clinical trials included both men and women. This sex bias (which is widespread in preclinical studies) is reason for concern, considering the evidence that females react differently to both IRI [34] and IPostC [35]. Secondly, we found no studies using animals with relevant comorbidities such as hypertension or diabetes mellitus, which are often present in patients undergoing renal surgery or transplantation. The absence of comorbidities in experimental animals has previously been described as a possible explanation for the translational failure of conditioning strategies [36–38].

Publication bias

Visual inspection of funnel plots, as well as trim and fill analysis, indicate a possible presence of publication bias in this field. The direction of effect did not change after trim and fill, but neutral and negative studies were underrepresented. On the other hand, Egger's test did not indicate any small-study effects, and Funnel plot asymmetry may be explained by other factors such as true heterogeneity, study quality or chance [39]. Based on this analysis we assess the risk of publication bias to be mild (histology and creatinine) to moderate (BUN). This should be kept in mind when interpreting our results, since data from a range of animal studies strongly suggested that publication bias is associated with a substantial overestimation of treatment effects [40].

Clinical implications and future perspective

This review is the first systematic overview of preclinical evidence for the efficacy of IPostC in animal models of renal IRI. It provides useful insights in the variables influencing IPostC efficacy, within the limitations inherent to combining data from different experiments.

Sensitivity analyses showed that the observed overall efficacy is robust for all outcome measures. Our finding that IPostC efficacy may increase with the duration of renal ischemia suggests that IPostC is less effective when kidney injury is mild. The severity of renal IRI in patients varies with the type of surgery they receive, their co-morbidities and additional measures which can be taken to reduce IRI. Thus, IPostC may not be equally potent in all patients, and this should be taken into account when including patients in clinical trials and analyzing clinical and preclinical results.

We also find that the body of evidence on which clinical trials are presently based is narrow, and its quality unclear. In particular, indirectness and risk of bias are reasons to interpret the preclinical findings with care. The present review points out a number of opportunities for improvement and future research, in order to increase clinical relevance of the preclinical studies and provide sufficient validity to guide clinical trial design. Preclinical studies should use both sexes, animals with relevant comorbidities, and it should be investigated whether the results obtained thus far can be replicated in transplantation models. Larger animal species may be used to better resemble the metabolic rate in humans. Importantly, to avoid effects of insufficient reporting, underpowering and publication bias in systematic reviews, it is of the utmost importance that the design, execution and reporting of animal studies is improved, for instance through the use of the GSPC and ARRIVE guidelines by authors and journals. Only then, preclinical evidence can be used to its full extent.

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SUPPORTING INFORMATION

S3.1 Text. Extended materials and methods

The review methodology was predefined and documented in a systematic review protocol [15], published online on February 12th 2015. The review question was: what is the effect of local or remote IPostC on renal function in animal models of renal IRI?

2.1 Amendments to the review protocol

After study selection, we found that the timing and duration of the IPostC protocol depended strongly on the site of postconditioning. We therefore decided to perform separate meta-analyses of studies using local, remote, and local+remote postconditioning, to avoid collinearity.

For serum creatinine and blood urea nitrogen (BUN), all data could be expressed in the same unit of measurement, but differences in baseline measurements between studies were observed. We therefore performed meta-analysis of the normalised mean difference (NMD) instead of the standardized mean difference (SMD). For renal histology, we expressed all scores as a percentage on the grading scale used, and performed meta-analysis of the mean difference (MD), instead of the SMD. This allowed us to include studies reporting the histology score as a percentage on the grading scale used.

2.2 Study identification

A systematic, computerized search in the databases Medline (via PubMed) and EMBASE (Supplemental Table S3.1) was performed on February 4th 2015, using the search components 'kidney', 'ischemic postconditioning' and an animal search filter for either PubMed [16] or EMBASE [17]. To identify additional relevant studies, the reference lists of included studies and relevant reviews were hand searched. No language restrictions were applied. Studies in a language other than English were translated using Google Translate. In case of uncertainties, a native speaker of the language was consulted.

2.3 Selection of studies

After removal of duplicates, selection of studies was performed using Early Review Organizing Software (Institute of Clinical and Health Policy, Buenos Aires, Argentina). All references were first screened for inclusion based on their title and abstract. The following inclusion criteria were applied: the study 1) is an original article presenting unique data with a control group, 2) is performed *in vivo* in animals with or without comorbidities,

but without genetic modifications, 3) reports on renal ischemia-reperfusion injury and outcome measures related to kidney injury or function, and 4) examined the effect of remote and/or local ischemic postconditioning. Subsequently, the full-text manuscripts of eligible studies were reviewed for inclusion. Studies involving co-medication other than anaesthetics or analgesics, or a co-intervention other than collateral nephrectomy (*e.g.* renal transplantation) were excluded. In both phases, references were independently assessed for inclusion by two reviewers (KW and SJ). In case of discrepancies, consensus was reached through discussion. Authors of eligible conference abstracts were contacted through e-mail in order to retrieve the full manuscript if available. If there was no reply within three weeks after sending a reminder, the study was excluded from analysis.

2.4 Study characteristics and data extraction

The following study characteristics were extracted: bibliographical data (author, year, title, language), animal characteristics (species, strain, sex, age, weight), experimental groups, number of animals per group, duration of index ischemia, and details of the IPostC protocol (site of IPostC, number of cycles, duration of ischemia and reperfusion). One reviewer extracted the data (SJ) and a second reviewer (TM) checked the data for inconsistencies. Based on their clinical relevance, we selected the following outcome measures for analysis: serum creatinine, BUN and renal histology scores (Jablonski [18] or comparable). Data was collected as mean and standard deviation (SD). For serum creatinine and BUN, all data was recalculated to the two same unit of measurement (umol/L for creatinine and mmol/L for BUN). For renal histology, scores were expressed as a percentage on the grading scale used. If an outcome was measured at several time-points, data was extracted for the time-point of greatest efficacy. If a study reported data from several experimental groups, it was extracted as separate comparisons and the number of animals in the control group was corrected (number of animals divided by number of comparisons). If data was only presented graphically, it was extracted using digital imaging software (ImageJ, National Institutes of Health, USA). Authors were contacted to provide additional information in case of unreported or unclear data. If there was no reply within three weeks after sending a reminder, a conservative estimate was made.

2.5 Risk of bias and study quality

Two reviewers (SJ and TM) independently assessed the risk of bias and study quality of each included study. In case of discrepancies, consensus was reached by discussion with a third reviewer (KW). Risk of bias was assessed using SYRCLE's Risk of Bias tool [19]. Reporting bias (item #9) was not assessed, since none of the studies reported the

use of a study protocol predefining primary and secondary outcomes. When assessing selection bias, groups within a study were considered similar at baseline if sex and baseline serum creatinine did not significantly differ between groups (or, if baseline creatinine was unavailable, body weight). To assess whether studies were free of other risks of bias, addition of animals to groups during the experiment and a possible conflict of interest were taken into account. We also assessed reporting of the following study quality items: any randomization, any blinding, regulation of body temperature within 3°C variation, a sample size calculation and a conflict of interest statement.

2.6 Data analysis

Data was analyzed using Stata/SE (StataCorp, Texas, USA). For the outcome measures serum creatinine and BUN, meta-analysis was performed on the NMD, which allows us to correct for baseline kidney injury by relating the magnitude of the effect of treatment to a baseline measured in untreated animals [20]. For histology, the MD was used. A random effects model was used to account for expected between-study heterogeneity. To assess heterogeneity, the I^2 and adjusted R^2 statistics were determined. To examine potential sources of heterogeneity, predefined subgroup analyses were performed on subgroups containing data from at least three studies. For the duration of IPostC ischemia, studies were categorized using increments of 0.7 log, which resulted in categories of 26–125, 126–630 and 631–3162 seconds of ischemia. For the duration of index ischemia, studies were categorized using increments of 15 minutes, resulting in categories of 16–30, 31–45, 46–60, 61–75 (no studies) and 76–90 minutes. Differences between subgroups were determined by calculating the difference in NMD and MD respectively and the 95% confidence intervals (CI) of the difference. Results are reported as a NMD or MD [95% CI], unless stated otherwise. For each outcome measure, the significance level for subgroup analyses was adjusted for the number of analyses using the Bonferroni-Holm method [21].

Publication bias was assessed for each outcome measure by visual evaluation of funnel plots, Duval and Tweedie's trim and fill analysis and by performing Egger's test for small study effects. Sensitivity analyses were carried out for creatinine and BUN using a fixed time point of outcome assessment (24 hours). For histology, a sensitivity analysis was performed using only Jablonski histology scores.

Table S3.1. Complete search strategy for Medline (via PubMed) and EMBASE

Medline (via PubMed)	Kidney	"kidney"[MeSH Terms] OR "acute kidney injury"[MeSH Terms] OR "kidney transplantation"[MeSH Terms] OR "nephrology"[MeSH Terms] OR "kidney"[Tiab] OR "kidneys"[Tiab] OR "renal"[Tiab] OR "nephrology"[Tiab]
	Postconditioning	"ischemic postconditioning"[MeSH Terms] OR "postconditioning"[tiab] OR "post conditioning"[tiab] OR "post-conditioning"[tiab] OR "IPostC"[tiab] OR "RIPostC"[tiab] OR "IPOC"[tiab] OR "RIPOC"[tiab] OR "IPC"[tiab] OR "RIPC"[tiab] OR "postcon"[tiab] OR "brief ischemia"[tiab] OR "brief ischaemia"[tiab] OR "transient ischaemia"[tiab] OR "transient ischemia"[tiab] OR "intermittent ischaemia"[tiab]OR "intermittent ischemia"[tiab] OR "continuous ischemia"[tiab] OR "continuous ischaemia"[tiab] OR "IPost" [tiab] OR "RIPost" [tiab] OR "rPostC" [tiab] OR "PostC" [tiab]
	Animals	Laboratory animal search filter [16]
February 4th 2015: 213 hits NB: adding additional abbreviations of ischemic postconditioning "POC"[tiab] OR "IPO"[tiab] did not generate additional relevant hits		
EMBASE	Kidney	exp kidney/ or exp acute kidney failure/ or exp kidney transplantation/ or exp kidney allograft rejection/ or (renal or kidney or kidneys or nephrology).ti,ab.
	Postconditioning	ischemic postconditioning/ or (brief ischemia or brief ischaemia or postconditioning or post conditioning or post-conditioning or transient ischaemia or transient ischemia or intermittent ischaemia or intermittent ischemia or continuous ischemia or continuous ischaemia or IPost or RIPost or IPOC or RIPOC or IPostC or RIPostC or IPC or RIPC or postcon or rPostC or PostC). ti,ab.
	Animals	Laboratory animal search filter [17]
February 4th 2015: 272 hits		

Table S3.2. Study quality and risk of bias assessment, individual scores

	Reporting						Risk of bias								
	Any randomization	Any blinding	Sample size calculation	Conflict of interest statement	Temperature regulation	Overall (times Y out of 5)	Random group allocation (selection)	Groups similar at baseline (selection)	Blinded group allocation (selection)	Random housing (performance)	Blinded interventions (performance)	Random outcome ass. (detection)	Blinded outcome ass. (detection)	Reporting of drop-outs (attrition)	Other biases
Chen 2008 [41]	Y	Y†	N	N	Y	3	?	?#	?	?	?	?	?	?	?
Chen 2011 [42]	Y	N	N	N	N	1	?	?#	?	?	?	?	?	?	?
Chen 2014 [43]	Y	Y†	N	N	Y	3	?	?#	?	?	?	?	?	?	?
Chen 2015 [44]	Y	Y†	N	Y	Y	4	?	?#	?	?	?	?	?	H	L
Eldaif 2010 [45]	N	Y†	N	N	Y	2	?	?#	?	?	?	?	?	H	?
Fan 2009 [46]	Y	N	N	N	N	1	?	?#	?	?	?	?	?	L	?
Guo 2014 [47]	N	N	N	N	N	0	?	?#	?	?	?	?	?	?	?
Ji 2012 [48]	Y	N	N	N	Y	2	?	?#	?	?	?	?	?	L	?
Jiang 2010 [49]	Y	Y†	N	N	Y	3	?	?#	?	?	?	?	?	L	?
Jiang 2014 [50]	Y	N	N	Y	N	2	?	?#	?	?	?	?	?	L	L
Kadkhodaee 2011 [22]	Y	N	N	Y	N	2	?	?#	?	?	?	?	?	?	L
Kadkhodaee 2014 [51]	Y	N	N	Y	Y	3	?	?#	?	?	?	?	?	?	L
Lemoine 2015 [52]	N	Y†	N	Y	Y	3	?	?#	?	?	?	?	?	?	L
Li 2010 [53]	Y	N	N	N	Y	2	?	?#	?	?	?	?	?	?	?
Li 2012 [54]	Y	N	N	N	N	1	?	?#	?	?	?	?	?	L	?
Liu 2007 [55]	N	Y†	N	N	Y	2	?	?#	?	?	?	?	?	?	?
Mahfoudh-Boussaid 2012 [24]	Y	Y†	N	Y	Y	4	?	?#	?	?	?	?	?	L	L
Mahmoudi 2014 [35]	Y	N	N	N	N	1	?	?#	?	?	?	?	?	?	?
Miklós 2012 [26]	N	Y†	N	N	N	1	?	L	?	?	?	?	?	?	?
Serviddio 2008 [27]	N	Y†	N	Y	N	2	?	L	?	?	?	?	?	?	L
Shokeir 2012 [56]	Y	N	N	Y	N	2	?	L	?	?	?	?	?	?	L
Shokeir 2014 [57]	Y	N	N	Y	N	2	?	L	?	?	?	?	?	L	L
Szwarc 2007 [58]	N	N	N	N	Y	1	?	L	?	?	?	?	?	L	?
Tan 2013 [59]	N	N	N	Y	Y	2	?	?#	?	?	?	?	?	?	L
Tang 2008 [60]	Y	N	N	N	N	1	?	?#	?	?	?	?	?	L	?
Tao 2012 [61]	Y	N	N	Y	N	2	L	L	?	?	?	?	?	L	L
Wang 2010 [62]	Y	Y†	N	N	Y	3	?	?#	?	?	?	?	?	?	?
Weng 2012 [28]	Y	Y†	N	Y	Y	4	?	?#	?	?	?	?	?	L	L
Wever 2012 [23]	Y	Y†	N	Y	N	3	?	?#	?	?	?	?	?	L	L
Xia 2014 [25]	Y	N	N	Y	N	2	?	?#	?	?	?	?	?	L	L
Yun 2009 A [63]	Y	Y†	N	N	Y	3	?	?#	?	?	?	?	?	L	?
Yun 2009 B [64]	Y	Y†	N	N	Y	3	?	?#	?	?	?	?	?	?	?
Zhang 2011 [65]	Y	N	N	N	N	1	?	?#	?	?	?	?	?	L	?
Zhu 2008 [66]	Y	N	N	N	N	1	?	?#	?	?	?	?	?	L	?
Zhuang 2009 [67]	N	Y†	N	N	N	1	?	?#	?	?	?	?	?	?	?
Total Y/L	26	16	0	14	17		1	6	0	0	0	0	0	16	14

Y = yes, N = no, ? = unclear risk of bias, H = high risk of bias, L = low risk of bias; †Only blinded for histology; #groups were similar only for sex

Table S3.3. Subgroup analysis serum creatinine local IPoC

	# pub	# comp	NMD	[95% CI]
All (T^2 539, I^2 74.7%)	29	34	45.0	[33.4, 56.6]
Species p=0.14, adj. R^2 12.8%				
Dog	1	3	79.3	[29.0, 129.6]
Mouse	3	4	19.5	[-13.1, 52.0]
Rat	25	27	47.2	[33.8, 60.5]
Sex p=0.21, adj. R^2 10.4%				
Female	3	3	20.9	[-18.3, 60.2]
Male	27	31	47.8	[35.1, 60.6]

# cycles 	
p=0.26, adj. R^2 8.8%				

3 cycles	10	11	39.6	[21.3, 58.0]
4 cycles	4	5	28.3	[-5.8, 62.4]
6 cycles	14	16	51.3	[31.4, 71.3]
10 cycles	2	2	86.2	[36.4, 136.0]
IPoC ischemia p=0.12, adj. R^2 8.7%				
26–125 sec	23	26	41.8	[27.5, 56.1]
126–630 sec	4	5	82.0	[45.4, 118.5]
631–3162 sec	3	3	35.9	[5.6, 66.3]
Index ischemia p=0.15, adj. R^2 7.7%				
16–30 min	4	5	20.2	[-10.7, 51.1]
31–45 min	20	22	43.7	[28.2, 59.1]
46–60 min	4	6	72.3	[41.5, 103.1]
76–90 min	1	1	58.3	[7.5, 109.1]
Delay (linear) p=0.20, adj. R^2 6.4%	30	34		

Total # comparisons = 6, corrected p<0.009; IPoC = ischemic postconditioning, pub = publications, comp = comparisons, NMD = normalized mean difference, adj. = adjusted. Protocol ischemia; amount of total ischemia time within IPoC protocol, delay; amount of delay between index ischemia and start IPoC protocol.

Table S3.4. Subgroup analysis blood urea nitrogen local IPoC

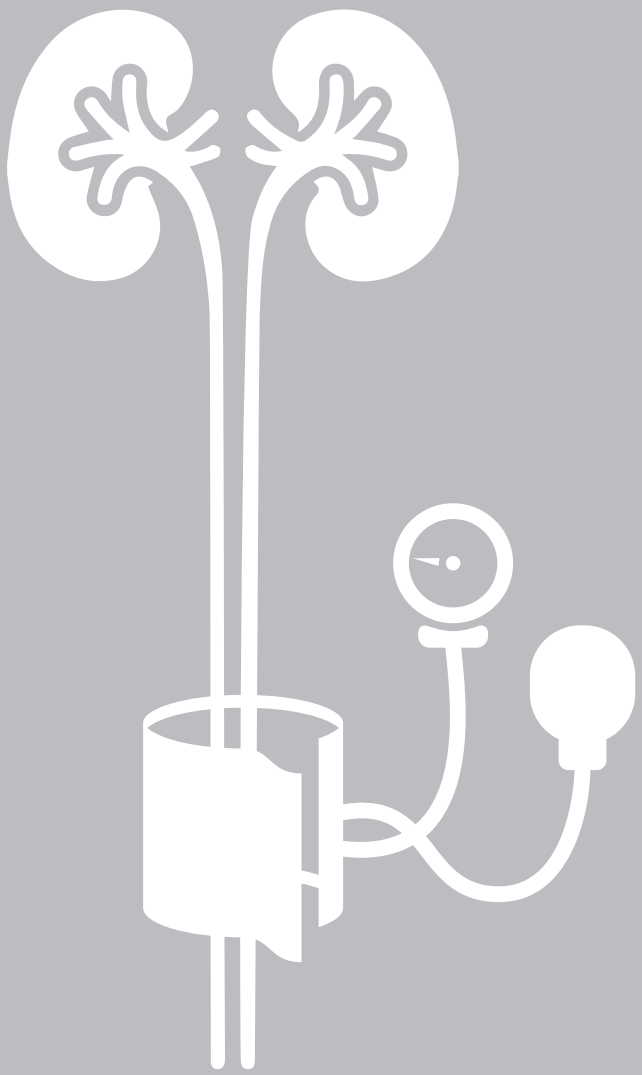
	# pub	# comp	NMD	[95% CI]
All (T^2 703, I^2 71.6%)	28	33	43.4	[30.8, 56.1]
Species *Not analyzed*				
Dog	1	3	74.4	[9.6, 139.3]
Mouse	2	3	4.2	[-22.7, 31.0]
Rat	25	27	48.3	[36.5, 60.0]
Sex *Not analyzed*				
Female	2	2	30.6	[-15.3, 76.6]
Male	27	31	44.0	[31.5, 56.5]
Cycles p=0.05, adj. R^2 31.9%				
3 cycles	8	9	28.1	[12.0, 44.2]
4 cycles	4	5	29.5	[-2.4, 61.5]
6 cycles	15	17	56.2	[39.1, 73.4]
10 cycles	2	2	81.9	[35.4, 128.3]
Protocol ischemia p=0.12, adj. R^2 8.5%				
26–125 sec	22	25	42.6	[29.0, 56.2]
126–630 sec	4	5	78.2	[39.1, 117.2]
631–3162 sec	3	3	25.2	[-3.2, 53.7]
Index ischemia p=0.007, adj. R^2 44.5%				
16–30 min	3	4	6.2	[-17.4, 29.9]
31–45 min	19	21	43.1	[29.8, 56.4]
46–60 min	5	7	73.2	[48.5, 97.9]
76–90 min	1	1	47.1	[8.8, 85.5]
Delay (linear) p=0.05, adj. R^2 14.4%	28	33		

Total # comparisons = 4, corrected p<0.012; IPoC = ischemic postconditioning, pub = publications, comp = comparisons, NMD = normalized mean difference, adj. = adjusted. Protocol ischemia; amount of total ischemia time within IPoC protocol, delay; amount of delay between index ischemia and start IPoC protocol.

Table S3.5. Subgroup analysis renal histology local IPoC

	# pub	# comp	MD	[95% CI]
All (T^2 369, I^2 96.2%)	18	23	27.8	[18.4, 37.2]
Species				
Not analyzed				
Dog	1	3	59.7	[31.7, 87.6]
Mouse	2	3	17.3	[-1.5, 36.1]
Rat	15	17	26.2	[17.3, 35.1]
Sex				
Not analyzed				
Female	1	1	3.6	[-29.1, 36.3]
Male	18	22	28.8	[20.6, 37.0]
Cycles				
p=0.84, adj. R^2 -17.0%				
3 cycles	4	5	23.4	[5.5, 41.3]
4 cycles	2	3	22.2	[-1.0, 45.3]
6 cycles	12	14	29.5	[18.1, 40.9]
10 cycles	1	1	37.5	[2.7, 72.3]
Protocol ischemia				
p=0.63, adj. R^2 -5.7%				
26–125 sec	15	18	25.5	[16.2, 34.7]
126–630 sec	3	4	36.1	[14.7, 57.4]
631–3162 sec	1	1	35.0	[-5.6, 75.5]
index ischemia				
p=0.13, adj. R^2 3.3%				
16–30 min	3	4	15.5	[-1.6, 32.6]
31–45 min	11	13	25.9	[14.6, 37.1]
46–60 min	4	6	39.9	[24.4, 55.3]
Delay (linear)				
p=0.22, adj. R^2 2.45%	18	23		

Total # comparisons = 4, corrected p<0.012; IPoC = ischemic postconditioning, pub = publications, comp = comparisons, MD = mean difference, adj. = adjusted. Protocol ischemia; amount of total ischemia time within IPoC protocol, delay; amount of delay between index ischemia and start IPoC protocol.



4

Local and remote ischemic postconditionings have synergistic protective effects on renal ischemia-reperfusion injury

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Transplantation. 2012 Jul 15;94(1), letter

In the current era of meticulous surgical technique and modern immunosuppressive therapy, ischemia-reperfusion injury (IRI) is one of the major determinants of early and longterm allograft function after kidney transplantation [1, 2]. In an experimental model of renal IRI, we showed that remote ischemic preconditioning using the hind limb as the remote organ is effective in reducing IRI [3]. Kadkhodaee et al. [4] recently reported that remote ischemic perconditioning and remote ischemic postconditioning (RIPostC) also significantly reduce renal IRI in a comparable model.

Here, we report the first data on the combined effect of local IPostC (LIPostC) and RIPostC on renal IRI. Male Sprague-Dawley rats weighing approximately 300 g were randomized into five groups before surgery. All animals underwent nephrectomy of the left kidney. Five sham-operated animals served as a baseline control (sham). All other animals were subjected to 25 minutes of renal ischemia (by clamping the renal artery and vein of the right kidney) with 48 hr of reperfusion. Eight animals underwent renal IRI only (no IPostC). In nine animals, three cycles of RIPostC by brief hind limb ischemia were induced directly after clamp release, by inflating small blood pressure cuffs around both proximal thighs for five min, followed by five minutes of reperfusion (RIPostC). Successful hind limb occlusion (loss of pulse and strong decrease of saturation) was confirmed by means of a pulse oximeter clip placed on the foot. In another nine animals, LIPostC was induced by six cycles of eight sec of ischemia, followed by eight sec of reperfusion (LIPostC). Seven animals underwent both postconditioning procedures (RIPostC plus LIPostC). After 48 hr, blood, urine, and renal tissue samples were analyzed to assess renal function and damage.

Renal IRI caused a decline in renal function, as reflected by an increase in plasma creatinine level, plasma urea level, and fractional excretion of sodium. These detrimental effects were only partially reduced by RIPostC or LIPostC alone. However, the combined application of RIPostC and LIPostC significantly reduced the IRI-induced decrease in renal function (Figure 4.1A-C). Furthermore, a similar synergistic effect of RIPostC plus LIPostC was observed for renal histologic damage, as assessed by scoring periodic acidYSchiffYstained sections of renal cortex on a 0 to 5 scale by an investigator blinded to treatment allocation (Figure 4.1D) [3].

In contrast with a previous report [3], RIPostC did not significantly reduce plasma creatinine and urea levels in our model. This may be explained by the use of a shorter sustained ischemic stimulus (25 *vs.* 45 min) and hind limb occlusion by blood pressure cuff, rather than clamping of the iliac vessels. Nevertheless, we demonstrate that RIPostC effectively prevents an IRI-induced increase in fractional excretion of sodium. More

importantly, we show that RIPostC and LIPostC have synergistic protective effects on IRI of the kidney. Although both strategies have been shown to influence the status of the mitochondrial permeability transition pore, it has been postulated that LIPostC does so by delaying the normalization of the intracellular pH [5], whereas RIPostC is believed to cause the release of various signalling molecules, such as adenosine, opioids, and cytokines, which act on the mitochondrial permeability transition pore through the activation of the cyclic guanosine monophosphate, Protein Kinase G (cGMP/PKG), Reperfusion Injury Salvage (RISK), or Survivor Activating Factor Enhancement (SAFE) pathway [6].

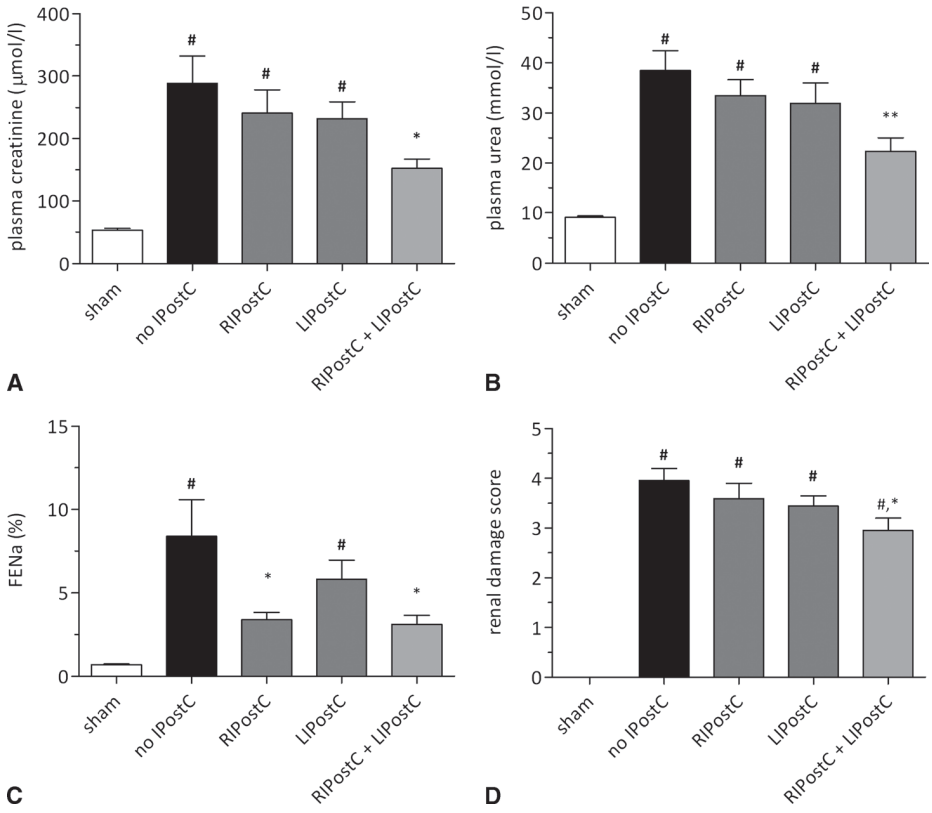

Figure 4.1. Remote ischemic postconditioning (RIPostC) and local ischemic postconditioning (LIPostC) reduce renal damage after ischemia-reperfusion injury.
When compared with sham-operated animals, rats subjected to 25 minutes of renal ischemia and 48 hours of reperfusion (no IPostC) experienced a decline in renal function, as reflected by an increase in plasma creatinine level (A), plasma urea level (B), and fractional excretion of sodium (FENa) (C). These detrimental effects were only partially reduced by RIPostC (three cycles of 5-min hind limb occlusion) or LIPostC (six cycles of 8-sec renal reocclusion) alone. Combined application of RIPostC and LIPostC significantly reduced the ischemia-reperfusion injury induced decrease in renal function (A–C). A similar synergistic effect of RIPostC plus LIPostC was observed for the degree of renal histologic damage (D). # PG0.01 vs. sham. * PG0.05, ** PG0.01 vs. no IPostC by one-way analysis of variance followed by Tukey multiple comparison posttest. n=5 for sham, n=8 for no IPostC, n=9 for RIPostC, n=9 for IPostC, and n=7 for RIPostC plus IPostC.

Our present finding supports the theory that the mechanisms of action could be different for LIPostC *vs.* RIPostC. For the implementation of IPostC into the clinical practice of kidney transplantation, we believe that its efficacy should be tested further in animal models of renal transplantation. Our data suggest that the combination of LIPostC and RIPostC is a highly interesting approach for further preclinical studies.

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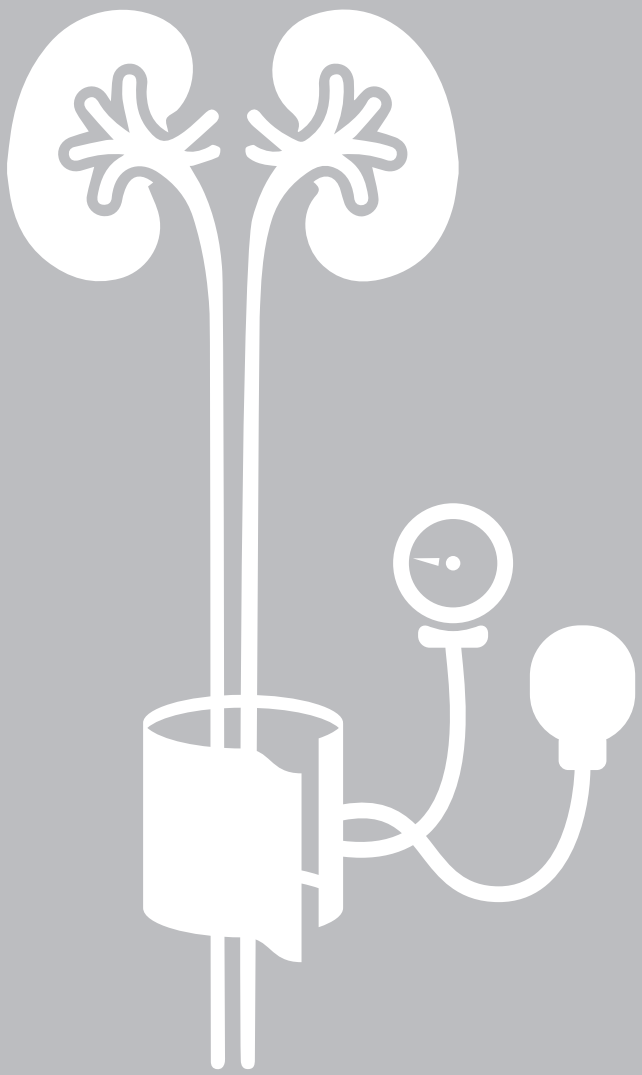

5

Repeated remote ischemic preconditioning and isoflurane anesthesia in an experimental model of renal ischemia-reperfusion injury

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ABSTRACT

Background

In animal studies, remote ischemic preconditioning (RIPC) and anesthetic preconditioning are successful in reducing renal ischemia reperfusion injury (IRI), however the protective effect of RIPC may be improved by repeating the RIPC stimulus.

Methods

Sprague-Dawley rats underwent unilateral nephrectomy followed by 30 minutes of renal pedicle clamping. Animals were allocated into six groups: sham, control (IRI), RepISO (daily isoflurane anesthesia), RIPC (single dose isoflurane anesthesia and single dose RIPC), RepISO+RIPC (7-day isoflurane anesthesia and single dose RIPC) and RepISO+RepRIPC (7-day isoflurane anesthesia with 7-day RIPC). RIPC was applied by 3x 5 minutes of cuff inflation on both thighs. Serum creatinine and urea levels were measured and histology was obtained at day two.

Results

RepISO diminished renal IRI, as reflected by a significant reduction in serum creatinine levels as compared to the control group, 170 ± 74 resp. 107 ± 29 µmol/L. The other preconditioning protocols showed similar reduction in serum creatinine levels as compared to the control group. No significant differences were observed between the different preconditioning protocols. For urea levels, only RepISO+RIPC resulted in significantly lower levels as compared to the control group, 14 ± 4 resp. 22 ± 7 mmol/L (p=0.010). In the preconditioning groups only RepISO showed less histological damage as compared to controls 1.73 ± 1.19 resp. 2.91 ± 1.22 (p=0.032).

Conclusions

In this study no additional protective effect of repeated ischemic preconditioning was observed as compared to single dose RIPC. Repeated administration of isoflurane provided stronger protection against renal IRI as compared to single dose isoflurane.

INTRODUCTION

Ischemia reperfusion injury (IRI) is tissue damage caused by the restoration of blood flow after a period of deprived circulation of that tissue [1]. The deficit of oxygen and nutrients during the ischemic phase creates a condition in which the return of blood flow induces oxidative stress, inflammation and results in apoptosis of the cell [2]. This may lead to tissue damage and loss of organ function [3]. The kidney is an organ especially vulnerable to IRI, due to its high-energy demand and delicate microcirculation. IRI of the kidney is a significant clinical problem in shock, renal transplantation and major cardiac or vascular surgery [4]. A promising method to diminish IRI was first described in 1986 by Murry [5], he discovered that short harmless periods of ischemia can protect the heart against a prolonged ischemic period; this phenomenon is called ischemic preconditioning (IPC). It was later described that the interruption of blood flow to an organ different than the target organ could also have a protective effect on IRI. This phenomenon is known as remote ischemic preconditioning (RIPC) [6]. Although the exact mechanism of RIPC is unknown, prevention of apoptosis by closure of the mitochondrial permeability transition pores (mPTP), seems to play a pivotal role [3]. A limb is often used as the remote organ for the application of the RIPC stimulus as the blood flow can safely and easily be obstructed by insufflation of a blood pressure cuff around an arm or leg. Experimental studies have shown that RIPC does not only protect against IRI in the heart, but also in other organs, including the kidney [7].

Not only a distant ischemic impulse can cause renal protection from IRI, some anesthetics also protect the kidney against IRI. In myocardial and renal animal studies [8], anesthetics have shown to reduce IRI in a similar signaling cascade as RIPC, known as anesthetic preconditioning (APC). Volatile anesthetics have extensively been tested for their APC effectiveness in cardiac studies: isoflurane, sevoflurane, desflurane [9, 10], halothane [11] and ether derived anesthetics [12] have proven clinical and preclinical cardioprotective effects. Experiments with intravenous anesthetics, propofol, barbitarates and ketamine [13–15] show no protective effect and have been demonstrated to inhibit mKATP channels which is an indication these anesthetics might diminish the protective effect of APC or RIPC [16]. The effects of multiple periods of anesthetics on IRI are unknown.

In general, animal studies show that RIPC is effective in reducing renal IRI [17]; however, human studies show disappointing results, with a small or non-significant protective effect [18, 19]. Cumulating evidence exists that in cardiac IRI models, repeating the RIPC stimulus over a period of multiple days, repeated RIPC (RepRIPC), could be more effective as compared to single dose RIPC [13, 20, 21]. It is unclear if this holds true for

renal IRI. In this study we test whether the null-hypothesis could be rejected that single dose and repeated RIPC are equally effective in an experimental model of renal IRI.

MATERIALS AND METHODS

The Committee for Animal Experiments of the Radboud Medical Center, Nijmegen approved all procedures (registration number 20149), and the experiment was conducted according to the ARRIVE criteria. Fifty- nine male Sprague–Dawley rats (Harlan Laboratories, Eystrup, Germany) were brought into the facility two weeks before the start of the experiment to acclimatize. Rats from different groups were housed randomly in the same room and under standard specific pathogen-free housing conditions. The environmental temperature was regulated at 22°C, with a relative humidity of 45% and a 12/12h day/night cycle. At the start of the experiment the animals weight was 311 ± 21g, at the age of ten weeks.

Blinding

Group assignment of each rat was done by computer-generated randomization. The surgeon, caregivers and the analysts performing creatinine, urea and histology measurements were blinded for group assignment of the animals.

Study design

All animals were anesthetized using isoflurane for the same period of time and all animals underwent right nephrectomy. Animals were randomly divided in six groups (Figure 5.1): Group 1 and 2 underwent no preconditioning. The sham group (n=4, group 1) underwent a laparotomy, including the resection of the right kidney. The control group (n=11, group 2) underwent 30 minutes of left renal ischemia (IRI stimulus) during right kidney resection. Groups 3–6 were the experimental, preconditioning groups, all undergoing 30 minutes of left renal ischemia at the day of surgery and a specific preconditioning stimulus: In group 3; repeated isoflurane (RepISO, n=11), the animals underwent seven days of isoflurane anesthesia for 25 minutes prior to the day of the operation. In group 4; single RIPC (RIPC, n=11); the animals underwent 3x 5 minutes of cuff inflation and five minutes of reperfusion prior to the operation. Cuff inflation was initiated by using human toe pressure cuffs, inflating them simultaneously to 200mmHg on both thighs. RIPC required 25 minutes of anesthesia as the last five minutes of reperfusion did not require anesthesia. In group 5; repeated isoflurane and a single RIPC stimulus (RepISO+RIPC,

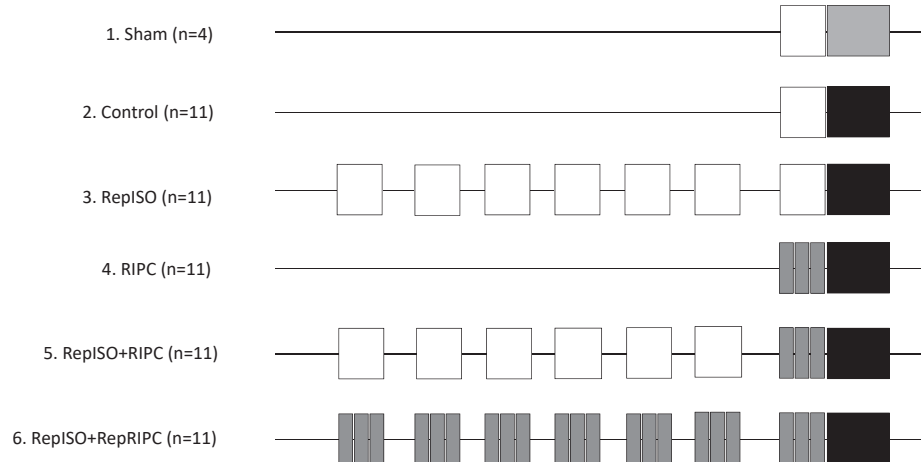

Figure 5.1. Schematic protocol of the animal groups were the line is a non linear timeframe of seven days. The open boxes indicate a period of anesthesia alone, gray boxes a period or RIPC and black boxes a period of renal ischemia. Animals were randomly allocated into six groups: sham, control (IRI), RepISO (daily isoflurane anesthesia), RIPC (single dose isoflurane anesthesia and single dose RIPC), RepISO+RIPC (7-day isoflurane anesthesia and single dose RIPC) and RepISO+RepRIPC (7-day isoflurane anesthesia with 7-day RIPC). RIPC was applied by 3x 5 minutes of cuff inflation on both thighs.

n=11), the animals underwent seven days of anesthesia for 25 minutes prior to the day of the operation. On the day of surgery, during isoflurane anesthesia, 3x 5 minutes of cuff inflation on both thighs and five minutes of reperfusion was performed. In group 6; repeated isoflurane and repeated RIPC (RepISO+RepRIPC, n=11), the animals underwent seven days of anesthesia for 25 minutes together with seven days of 3x 5 minutes of cuff inflation on both thighs and five minutes of reperfusion.

Surgical procedures

All experiments were randomly performed between 8.00 and 16.00h on Mondays and Tuesdays. Preoperative analgesic [Carprofen, 5 mg/kg body weight (b.w.)] was administered subcutaneously 30 minutes prior to surgery. Surgical procedures were conducted using standard aseptic surgical techniques and all microsurgical instruments were sterilized using a dry bead sterilizer (Inothech, Dottikon, Switzerland). Animals were placed on a sterile drape overlying a heating pad to maintain body temperature at 36–38°C, monitored continuously using a rectal thermometer. Body weights were recorded prior to surgery, prior to blood collection and at the end of the experiment. Anesthesia was induced with 5% isoflurane in pressurized air and maintained at 2.5–3%. Depth of anesthesia was assessed by toe and tail pinch. Preconditioning was done by TM. All operations were done

by an experienced microsurgeon (RL), renal ischemia was initiated by blunt dissection of the left renal hilus, and an atraumatic vascular clamp was used to obstruct the venous and arterial blood flow of the kidney. Complete obstruction was confirmed by visualization of the kidney gaining the typical ischemic dark purple color; complete revascularization after removal of the clamp was also visualized before closure of the abdomen. Closure of the abdomen was done by a running suture, securing both ends with a metal clip to prevent opening of the wound by the animal. One day post-operatively, an analgesic (Carprofen, 5 mg/kg b.w.) in 5 mL saline was administered subcutaneously.

Renal function analysis and histology

At baseline, day one and day two blood samples were collected and stored. Blood samples were collected in EDTA tubes and centrifuged for 15 minutes at 3000g to obtain plasma. Plasma was snap frozen in liquid nitrogen and stored at -80°C until further use. For the histology, tissue from the remaining kidney was taken two days after surgery and was fixed in 4% paraformaldehyde for at least 48 hours. For light microscopy of the renal cortex, kidneys were dehydrated and embedded in paraffin. To score renal damage, sections of 4 µm were stained with periodic acid-Schiff. Of each kidney, four sections were taken at different latitudes and scored for damage of the renal cortex and averaged. Damage scoring was performed by a blinded investigator, on a scale from 0 to 4 according to the Jablonski scale [22], with 0: no proximal tubule damaged, and 4: all tubules damaged.

Statistical and power analysis

Serum creatinine levels were used as the primary outcome measure. Previous experiments have shown that in our model of 30 minutes renal injury, serum creatinine levels in control animals 48 hours post-operative are on average 290 µmol/L, with an average standard deviation of 103 µmol/L [23, 24]. We aim to detect a difference in serum creatinine between the RepISO+RepRIPC and all the other experimental groups including the control group of 100 µmol/L. Since there are five comparisons we have adjusted our level of significance for five comparisons, using Bonferonni correction: 0.05/5=0.01. In order to achieve a statistical power of at least 80%, we require 11 animals per group. Previous experiments have shown that the standard deviation in sham-operated animals is low (average serum creatinine 48 hours post-operative = 46 ± 8). Therefore four animals in the sham group were required. Although the animals were obtained from a different supplier, we estimated that the susceptibility to renal IRI would be similar because the strain, age, sex and weight were identical as in the previously mentioned experiments.

All data are presented as mean ± SD unless otherwise specified. The means of the different groups were compared using the Student-t test. The level of statistical significance was set at $p<0.05$. Data were assessed and SPSS 22 and GraphPath 5.03 plotted graphs.

RESULTS

Peri-operative complications

Fifty-nine rats were randomly assigned to six different groups. Two rats died during anesthesia. A third animal was excluded at day two of the experiment due to intestinal rotation with obstruction. A fourth rat was excluded because the remaining kidney contained a large tumor, which filled one third of the kidney's volume. The excluded rats belonged to different groups: control, RepISO, RIPC and RepISO+RepRIPC. The weight of the animals at baseline and the average weight loss at day two in the different groups were not significantly different between the groups.

Renal function analysis

Serum creatinine (Figure 5.2) and serum urea concentrations (Figure 5.3) were measured at baseline (ten days before surgery) and on postoperative day one and two. All baseline outcome measures were not significantly different.

In comparison with the control group, all groups showed a significantly lower level of creatinine; control 170 ± 74 µmol/L *vs.* sham and experimental group 1 and 3–6 respectively; 71 ± 16 µmol/L (p=0.023), 107 ± 29 µmol/L (p=0.022), 107 ± 45 µmol/L (p=0.032), 96 ± 22 µmol/L (p=0.007) and 102 ± 37 µmol/L (p=0.023). For the experimental groups only serum creatinine levels of RepISO on day 1 were significantly higher than sham creatinine levels; 107 ± 29 µmol/L resp. 71 ± 16 µmol/L (p=0.039). On day two the creatinine concentrations were reduced compared with day one and on day two there was no significant difference between sham and the experimental groups. The control animals showed significantly higher creatinine concentrations compared with the experimental groups, RepISO, RepISO+RIPC and RepISO+RepRIPC respectively: 102 ± 29 µmol/L *vs.* 63 ± 21 µmol/L (p=0.036), 49 ± 8 µmol/L (p=0.006) and 57 ± 21 µmol/L (p=0.028).

For urea levels, all groups showed significantly higher levels on day one as compared to sham: 9 ± 1 mmol/L *vs.* control 22 ± 7 mmol/L (p=0.000), *vs.* RepISO 18 ± 5 mmol/L (p=0.000), *vs.* RIPC 17 ± 8 mmol/L (p=0.010), *vs.* RepISO+RIPC 14 ± 4 mmol/L (p=0.002)

and *vs.* RepISO+RepRIPC 17 ± 7 mmol/L (p=0.047). Compared to control operated animals, only serum urea levels in RepISO+RIPC were significantly lower, 14 ± 4 *vs.* 22 ± 7 mmol/L (p=0.010).

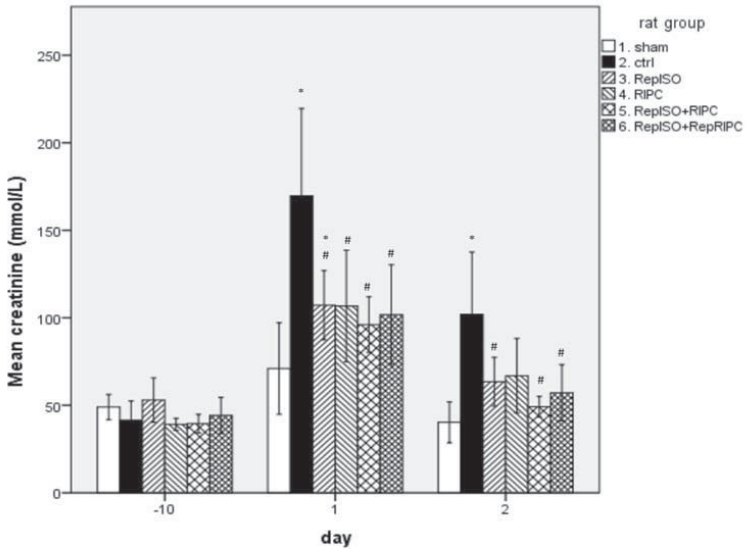

Figure 5.2. Serum creatinine.
Day -10 (baseline), 1 and 2 postoperative (* significantly different from sham, # significantly different from control group).

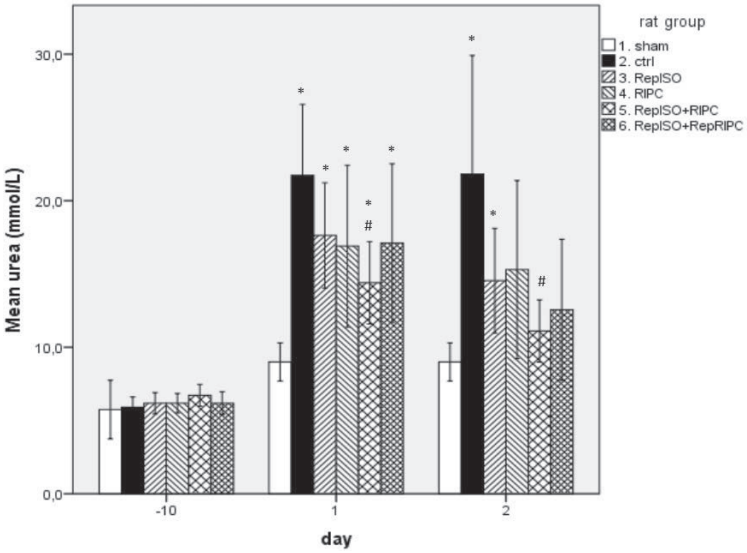

Figure 5.3. Serum urea.
Day -10 (baseline), 1 and 2 postoperative (* significantly different from sham, # significantly different from control group).

Histology

Histology, according to the Jablonski score, showed significantly more renal damage in the control group 2.91 ± 1.22 as compared to sham 0.75 ± 0.96 (p=0.007). In the preconditioning groups only RepISO, 1.73 ± 1.19 (p=0.032), showed significantly less damage as compared to control (Figure 5.4).

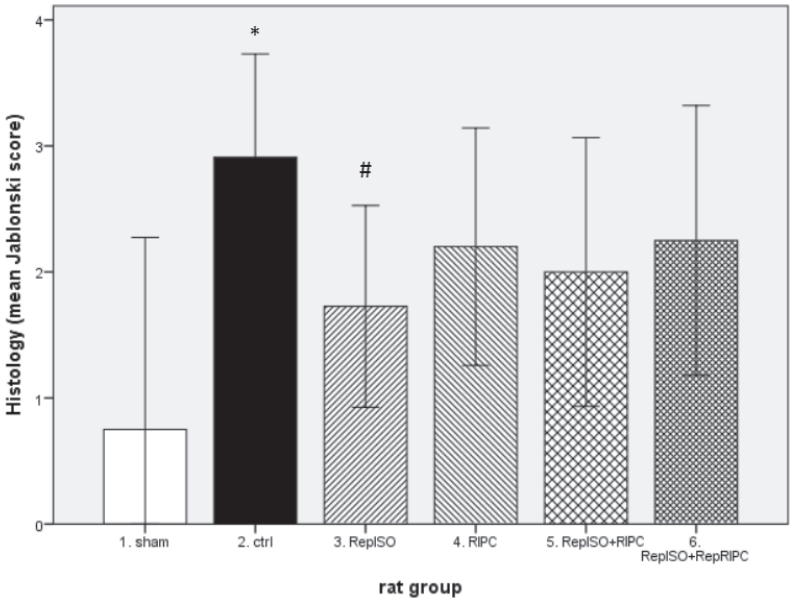

Figure 5.4. Histology.
Day 2 postoperative (* significantly different from sham, # significantly different from control group).

DISCUSSION

To our knowledge this is the first experiment of RepRIPC compared to single dose RIPC in an experimental renal IRI model. With regard to the primary hypothesis, we were not able to demonstrate an additive protective effect of a repeated ischemic preconditioning stimulus in this experiment. However the question whether an additive protective effect of RepRIPC does not exist or the unanticipated large reduction in renal IRI by repeated isoflurane blurred the additional protective effects of repeated RIPC, remains unanswered.

Results show that all different preconditioning protocols, including RepRIPC, showed a significant reduction in serum creatinine at day one, which was the primary outcome

measure. However it is important to note that the observed differences in serum creatinine levels at day one between the different preconditioning protocols and the control group (single dose APC) were smaller than the difference used for the power calculation (100 µmol/L). This indicates that a smaller difference in serum creatinine levels would have been more appropriate to reduce the risk of a type I error. With regard to serum urea levels, only RepISO+RepRIPC showed a significant reduction as compared to the control group, receiving a single period of isoflurane. Probably the number of animals per group was too small to detect differences in serum urea levels between RepISO and controls. With regard to the histology data, only animals in the RepISO group had lower scores for renal injury as compared to controls. This finding supports the main observation of this study, repeated administration of isolfurane provides stronger protection against renal IRI as compared to single dose isolfurane.

In this study isoflurane was chosen as an anesthetic because it is safe, has little side effects and is widely used in animal studies and in patients. The downside of using isoflurane in this experiment is the protective effect of isoflurane on renal IRI. One previous study [8] showed that single dose isoflurane preconditioning ameliorated IRI of the kidney. In our study we showed that a 7-day repeated isoflurane preconditioning provided significantly more protection against renal IRI as compared to single dose isoflurane in the control group. The smallest number of daily repeated isoflurane preconditioning cycles providing maximum protection remains unknown. To our knowledge, the strong protective effects of repeated isoflurane administrations over multiple days has not been described previously.

Another remarkable observation is that 30 minutes of pedicle clamping induced less renal injury as compared to our previous experiments [23, 24]. As the amount of renal injury varied between this experiment and previous observations, it would have been better to include more animals to control for this variation in our experimental model. The most likely explanation for the difference with the previous experiments is that our animals were obtained from a different supplier. Despite the fact that we used the same strain, there may have been differences in the genetic makeup leading to a lower susceptibility to renal IRI. This phenomenon is supported by studies showing that different strains of mice have a different susceptibility to cardiac IRI [25, 26]. In this experiment 30 minutes of IRI was chosen, despite the fact that 45 minutes is more commonly used in similar experiments [17]. The reason to shorten the IRI period is that the amount of IRI in most animal studies is relatively large as compared to clinical trials [17–19, 21, 27]. In our view the induction of a lower amount of IRI results in a more realistic animal model for the translation into clinical practice.

CONCLUSION

IPC has been a promising phenomenon since its discovery in 1986 [5]; however, the vast amount of IRI protection by IPC, shown in animal studies, cannot be translated into clinical trials [17, 19]. Accumulating evidence indicate that RepRIPC is a promising tool to provide a more effective and robust RIPC stimulus. RepRIPC was successfully studied in animal heart models [28, 29], endothelial dysfunction models in healthy humans [21], coronary artery bypass grafting [30] and after stroke [20]. Nevertheless our results show that it is difficult to establish additional protection of a repeated RIPC stimulus as compared to single dose RIPC in animal studies reducing renal IRI.

In future animal studies investigating the mechanisms and/or efficacy of repeated RIPC and APC, the strong protective effects of the repeated administration of (volatile) anesthetics, *i.e.* isoflurane, should be taken into account.

DECLARATIONS

Ethics approval and consent to participate

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution and practice at which the studies were conducted, in this case the Committee for Animal Experiments of the Radboud Medical Center Nijmegen approved all procedures (registration number 20149), and the experiment was conducted according to the ARRIVE criteria. (https://www.radboudumc.nl/Research/Organisationofresearch/Departments/cdl/Pages/FacilitiesAndServices.aspx).

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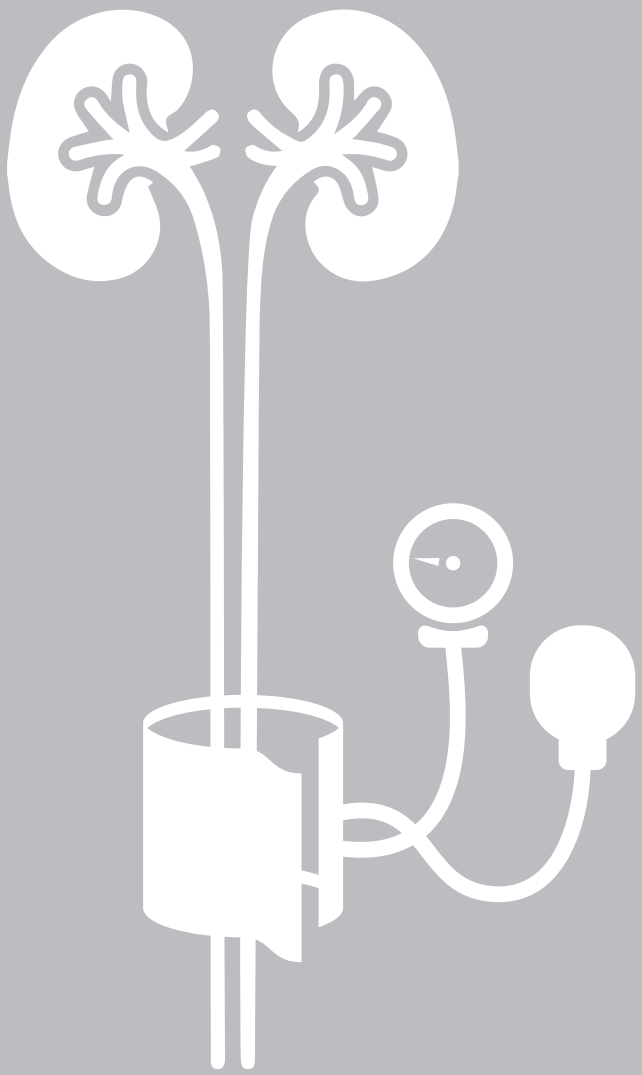

6

Ischaemic preconditioning for the reduction of renal ischaemia reperfusion injury, a Cochrane systematic review and meta-analysis

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JA Van der Vliet, MM Rovers, MC Warlé

Cochrane Database Syst Rev. 2017 Mar 4;3

ABSTRACT

Background

Ischaemia reperfusion injury can lead to kidney dysfunction or failure. Ischaemic preconditioning is a short period of deprivation of blood igurupply to particular organs or tissue, followed by a period of reperfusion. It has the potential to protect kidneys from ischaemia reperfusion injury.

Objectives

This review aimed to look at the benefits and harms of local and remote ischaemic preconditioning to reduce ischaemia and reperfusion injury among people with renal ischaemia reperfusion injury.

Search methods

We searched Cochrane Kidney and Transplant's Specialised Register to the eighth of August 2016 through contact with the Information Specialist using search terms relevant to this review.

Selection criteria

We included all randomised controlled trials measuring kidney function and the role of ischaemic preconditioning in patients undergoing a surgical intervention that induces kidney injury. Kidney transplantation studies were excluded.

Data collection and analysis

Studies were assessed for eligibility and quality; data were extracted by two independent authors. We collected basic study characteristics: type of surgery, remote ischaemic preconditioning protocol, type of anaesthesia. We collected primary outcome measurements: serum creatinine and adverse effects to remote ischaemic preconditioning and secondary outcome measurements: acute kidney injury, need for dialysis, neutrophil gelatinase-associated lipocalin, hospital stay and mortality. Summary estimates of effect were obtained using a random-effects model, and results were expressed as risk ratios (RR) and their 95% confidence intervals (CI) for dichotomous outcomes, and mean difference (MD) and 95% CI for continuous outcomes.

Main results

We included 28 studies which randomised a total of 6,851 patients. Risk of bias assessment indicated unclear to low risk of bias for most studies. For consistency regarding the direction of effects, continuous outcomes with negative values, and dichotomous outcomes with values less than one favour remote ischaemic preconditioning. Based

on high quality evidence, remote ischaemic preconditioning made little or no difference to the reduction of serum creatinine levels at postoperative days one (14 studies, 1,022 participants: MD -0.02 mg/dL, 95% CI -0.05 to 0.02; I2=21%), two (9 studies, 770 participants: MD -0.04 mg/dL, 95% CI -0.09 to 0.02; I^2=31%), and three (6 studies, 417 participants: MD -0.05 mg/dL, 95% CI -0.19 to 0.10; I^2=68%) compared to control.

Serious adverse events occurred in four patients receiving remote ischaemic preconditioning by iliac clamping. It is uncertain whether remote ischaemic preconditioning by cuff inflation leads to increased adverse effects compared to control because the certainty of the evidence is low (15 studies, 3,993 participants: RR 3.47, 95% CI 0.55 to 21.76; I^2=0%); only two of 15 studies reported any adverse effects (6/1,999 in the remote ischaemic preconditioning group and 1/1,994 in the control group), the remaining 13 studies stated no adverse effects were observed in either group.

Compared to control, remote ischaemic preconditioning made little or no difference to the need for dialysis (13 studies, 2,417 participants: RR 0.85, 95% CI 0.37 to 1.94; I^2=60%; moderate quality evidence), length of hospital stay (8 studies, 920 participants: MD 0.17 days, 95% CI -0.46 to 0.80; I^2=49%, high quality evidence), or all-cause mortality (24 studies, 4,931 participants: RR 0.86, 95% CI 0.54 to 1.37; I^2=0%, high quality evidence).

Remote ischaemic preconditioning may have slightly improved the incidence of acute kidney injury using either the AKIN (eight studies, 2,364 participants: RR 0.76, 95% CI 0.57 to 1.00; I^2=61%, high quality evidence) or RIFLE criteria (three studies, 1,586 participants).

Authors' conclusions

Remote ischaemic preconditioning by cuff inflation appears to be a safe method, and probably leads to little or no difference in serum creatinine, adverse effects, need for dialysis, length of hospital stay, death and in the incidence of acute kidney injury. Overall we had moderate-high certainty evidence; however the available data does not confirm the efficacy of remote ischaemic preconditioning in reducing renal ischaemia reperfusion injury in patients undergoing major cardiac and vascular surgery in which renal ischaemia reperfusion injury may occur.

PLAIN LANGUAGE SUMMARY

Short periods of limb blood flow obstruction to reduce kidney injury

What is the issue?

The kidney is highly sensitive to shortage in blood flow and thus oxygen supply. This may cause irreversible kidney injury leading to haemodialysis or death. Kidney injury does not only relate to the temporary lack of oxygen supply, but is also due to the re-saturation of blood flow. At this stage, toxic products are released and initiate a reaction of the body causing further cellular damage within the kidney, the so called 'ischaemia-reperfusion injury'. A lack of oxygen supply to the kidney injury may have many different causes, for example blood pressure changes that may occur during major surgery.

What did we do?

Our hypothesis is that short harmless periods (five minutes) of blood flow obstruction to an organ can reduce injury in this particular organ (local ischaemic preconditioning), but can also reduce injury in other organs at a distance (remote ischaemic preconditioning). A blood flow obstruction can easily and safely be achieved in a limb by inflating blood pressure cuff around the upper arm or leg. The mechanism of this remote ischaemic preconditioning is not precisely known, it is assumed that a protective signal from the remote organ to the kidney is transferred through the blood stream or nervous system.

In this analysis our primary goal is to investigate whether remote ischaemic preconditioning is safe and effective in reducing kidney injury in patients undergoing a (surgical) procedure in which kidney injury may occur. Kidney injury after kidney transplantation may have a different underlying pathophysiology and therefore these studies are not taken into account. The impact of remote ischaemic preconditioning on the need for dialysis, hospital stay and mortality will be assessed.

What did we find?

We performed a search off all available literature on the eighth of August 2016 to find all randomised controlled studies. 28 studies including 6,851 patients were included in this analysis. Five studies included children undergoing cardiac surgery. Adult studies included patients undergoing major vascular surgery (three studies), cardiac surgery (nine studies), coronary bypass surgery (ten studies) and partial kidney resection (one study). The overall quality of the studies was acceptable.

Twenty studies were funded without economical interest. One study was funded from a source with commercial interest. The other seven studies did not report funding.

Remote ischaemic preconditioning performed with a blood pressure cuff appears to be safe as no side effects were reported. However remote ischaemic preconditioning by vascular clamping may cause vascular complications. Kidney injury in patients undergoing (surgical) procedures in which kidney injury may occur, was not reduced by remote Ischaemic preconditioning measured at day one, two or three after surgery. The need for dialysis, hospital stay and death were not reduced by remote ischaemic preconditioning.

Conclusion

Although remote ischaemic preconditioning by cuff inflation is safe, available data do not confirm the efficacy of remote ischaemic preconditioning in reducing kidney injury.

BACKGROUND

Description of the condition

Ischaemia reperfusion injury is defined as damage to an organ that occurs after a critical period of ischaemia, followed by restoration of blood supply. This can happen spontaneously, such as in stroke or myocardial infarction, or during transplantation and other types of surgery. Cells become deprived of oxygen in the ischaemic phase, and as a result, metabolism switches from aerobic to anaerobic glycolysis, leading to cell swelling, acidosis, ATP depletion, intracellular sodium (Na^+) and calcium ion (Ca^{2+}) overload and inhibition of the mitochondrial respiration chain. This leads to cell death in minutes to hours, depending on the cell type. Restoration of blood flow after ischaemia is therefore essential for cell survival. However, reperfusion of ischaemic tissue invokes paradoxical effects that are detrimental, rather than beneficial, to cells. This particularly holds true for sudden restoration of oxygen (which leads to oxidative stress), pH (which can induce cell death), and evoked inflammatory response. Inflammatory response induces adhesion of cytokine-releasing leukocytes, which attracts neutrophils, macrophages, lymphocytes and dendritic cells to the site. This may cause further release of reactive oxygen species and microvascular dysfunction [30, 44, 60].

Ischaemia reperfusion injury can lead to organ dysfunction or failure and is a significant clinical problem in transplantation, shock and major surgery. The high metabolism and vascular anatomy of the kidney is particularly sensitive to ischaemia reperfusion injury. The critical ischaemic period is organ-dependent: 15 to 20 minutes of ischaemia has been shown to cause irreversible damage to the kidney [40, 48, 50].

Description of the intervention

Ischaemic preconditioning is a short and harmless period of deprivation of blood supply to particular organs or tissue, followed by a period of reperfusion [31, 37, 61]. Preconditioning stimulus is applied before onset of ischaemia reperfusion injury to a target organ. In 1986 it was shown that ischaemic preconditioning on the heart can reduce ischaemia reperfusion injury, (local ischaemic preconditioning) [43], and has since been reproduced in many other target organs. Later on, studies have shown that ischaemic preconditioning of remote organs and tissues at a distance can protect the target organ from ischaemia reperfusion injury as well (remote ischaemic preconditioning) [45]. Use of the limbs as remote tissue offers many advantages, since skeletal muscle is less susceptible to ischaemia reperfusion injury compared to visceral tissues.

A typical schedule of five minute periods of ischaemic preconditioning, separated by five minutes of reperfusion, applied directly before the ischaemia reperfusion injury period of the target organ, is used in most clinical studies. Numerous variations to this schedule have been studied in animals and the efficacy of the ischaemic preconditioning has been shown to vary, depending on the animal sex, animal species, preconditioned tissue volume, length of ischaemic preconditioning, reperfusion and time between ischaemic preconditioning and ischaemia reperfusion injury. The optimal schedule is still unclear and is probably different for different target organs and species [29, 32, 58].

How the intervention might work

Several endogenous molecules have been implicated in local and remote ischaemic preconditioning signalling, most of which are known to have cytoprotective effects. Downstream, the ultimate protective step in ischaemic preconditioning signalling appears to be inhibition of mitochondrial permeability transition pore opening, which prevents cell death. Remote and local ischaemic preconditioning appear to be similar in terms of invoking mitochondrial permeability transition pore inhibition, and many signalling molecules seem to be similar to those implicated in local ischaemic preconditioning signalling. The theoretical difference is that remote ischaemic preconditioning requires transduction of the protective signal from the remote organ or tissue to the target organ. The protective effects of ischaemic preconditioning are found both directly after application of the stimulus (early window of protection), and in the days or weeks following (second window of protection). In animal models, both windows of protection have been shown to reduce renal ischaemia reperfusion injury [58]. Although there are similarities in the mechanisms underlying early and second windows of protection, the second window of protection has been found to require de novo protein synthesis of distal mediators such as iNOS and COX-2. However, remote ischaemic preconditioning signalling has been most extensively studied in the early window of protection, where three major pathways have been indicated in this process (Figure 6.1): the humoral route, the neurogenic pathway and alteration of immune cells. Signalling via the humoral route (upper route, Figure 6.1) requires release of signalling molecules such as adenosine or endorphins from the remote organ into the bloodstream, which are then carried to the target organ to exert their protective effects via their respective receptors. The nervous system also appears to play a role in some models of remote ischaemic preconditioning: denervation or ganglion blockade inhibit the protective effect of remote ischaemic preconditioning (middle route, Figure 6.1). Activation of the neurogenic pathway by peptides released from the remote organ may cause systemic factor release (combined

humoral-neurogenic route), lead to local factor release or activation of central reflexes. Both the humoral and the neurogenic pathways are thought to induce various kinase cascades and eventually prevent opening of the mitochondrial permeability transition pore in the target organ cells, thereby reducing cell death. Thirdly, remote ischaemic preconditioning has been shown to modulate gene and receptor expression on immune cells, which therefore pose a third signalling pathway that presumably reduces damage by altering the inflammatory response (lower route, Figure 6.1) [54].

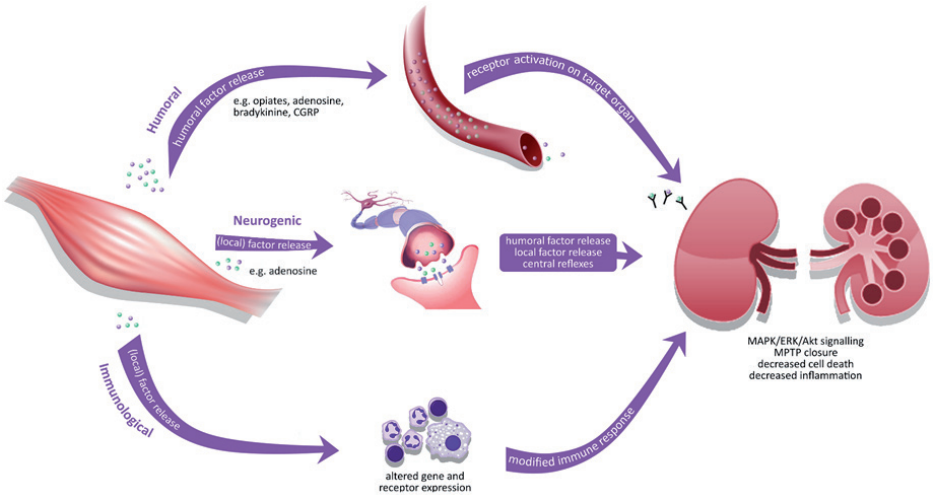

Figure 6.1. Remote ischaemic preconditioning signalling pathways.
Source: KE Wever. Novel protective approaches in ischemia-reperfusion injury *in vivo* studies in animals and humans. PhD thesis, p. 16.

Why it is important to do this review

Despite that the efficacy of ischaemic preconditioning has been acknowledged since described by [43], the technique was introduced into clinical studies only relatively recently; however, results to date have not been consistently positive [1, 3, 25, 28, 57]. Although experimental data show promise, the mechanism underlying ischaemic preconditioning signalling remains unclear and the optimal preconditioning protocol remains unknown [58].

The kidney is very sensitive to ischaemia reperfusion injury, and therefore, is an organ system that can benefit from ischaemic preconditioning. Furthermore, kidney function and damage are very well documented and can be tested using robust endpoints. The

kidney is therefore an ideal target organ to investigate the protective effects of ischaemic preconditioning on renal ischaemia reperfusion injury.

OBJECTIVES

This review aimed to look at the benefits and harms of local and remote ischaemic preconditioning to reduce ischaemia and reperfusion injury among people with renal ischaemia reperfusion injury.

METHODS

Criteria for considering studies for this review

Types of studies

All randomised controlled trials (RCTs) looking at the role of ischaemic preconditioning *vs.* no ischaemic preconditioning among patients undergoing interventions that result in ischaemic kidney damage were eligible for inclusion. There was no restriction on publication status, language, or sample size. Quasi-RCTs (RCTs in which allocation to treatment was obtained by alternation, use of alternate medical records, date of birth or other predictable methods) were excluded.

Types of participants

We included all patients who underwent any intervention for any indication that resulted in ischaemic kidney damage (*e.g.* extracorporeal membrane oxygenation, open aneurysm repair, coronary artery bypass grafting, aortic surgery and any other type kidney surgery). Liver, lung and peripheral bypass surgeries in which kidney injury was highly unlikely were excluded. Studies investigating ischaemic preconditioning in kidney transplantation or patients at risk for contrast-induced nephropathy, including endovascular aneurysm repair, were excluded.

Types of interventions

The ischaemic preconditioning protocol could include remote and/or local ischaemic preconditioning stimulus applied before the intervention and the remote stimulus could be applied to any organ. Preconditioning stimuli could be continuous (one continuous ischaemic pulse followed by reperfusion) or fractioned (two or more cycles of brief ischaemia and reperfusion). The ischaemic preconditioning stimulus could be applied

directly before index ischaemia or some time, even days, before. The control condition of no ischaemic preconditioning could be no intervention or mock ischaemic preconditioning, that is, application of a tourniquet, blood pressure cuff or other means of occlusion without actually interrupting blood flow.

Types of outcome measures

Primary outcomes

- Serum creatinine on days one, two or three postoperative
- Complications and adverse effects related to ischaemic preconditioning

Secondary outcomes

- Need for dialysis following kidney-related ischaemia
- Acute kidney injury (AKI) as defined by KDIGO, AKIN and RIFLE criteria [41, 42, 47]
- Blood urea nitrogen (BUN)
- Serum/urine neutrophil gelatinase-associated lipocalin (NGAL)
- Serum/urine kidney injury molecule-1 (KIM-1)
- Mortality
- Quality of life
- Length of hospital stay

Search methods for identification of studies

Electronic searches

We searched the Cochrane Kidney and Transplant Specialised Register up to the eighth of August 2016 through contact with the Information Specialist using search terms relevant to this review. The Specialised Register contains studies identified from the following sources.

1. Monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL)
2. Weekly searches of MEDLINE OVID SP
3. Handsearching of kidney-related journals and the proceedings of major kidney conferences
4. Searching of the current year of EMBASE OVID SP
5. Weekly current awareness alerts for selected kidney journals
6. Searches of the International Clinical Trials Register (ICTRP) Search Portal and ClinicalTrials.gov

Studies contained in the Specialised Register are identified through search strategies for CENTRAL, MEDLINE, and EMBASE based on the scope of Cochrane Kidney and Transplant. Details of these strategies, as well as a list of handsearched journals, conference proceedings and current awareness alerts, are available in the Specialised Register section of information about Cochrane Kidney and Transplant.

See Appendix 6.1 for search terms used in strategies for this review.

Searching other resources

1. Reference lists of review articles, relevant studies, and clinical practice guidelines.
2. Letters seeking information about unpublished or incomplete studies to investigators known to be involved in previous studies.

Data collection and analysis

Selection of studies

The search strategy described was used to obtain titles and abstracts of studies that may be relevant to the review. Titles and abstracts were screened independently by two authors, who discarded studies that were not applicable; however studies and reviews thought to include relevant data or information on studies were retained initially. Two authors independently assessed retrieved abstracts and if required assessed the full text of these studies to determine which satisfied the inclusion criteria.

Data extraction and management

Data extraction was carried out independently by two authors using standard data extraction forms. Studies reported in non-English language journals were to be translated before assessment. Where more than one publication of one study existed, reports were grouped together and the publication with the most complete data was used in the analyses. Where relevant outcomes were only published in earlier versions these data were used. Any discrepancy between published versions was to be highlighted.

Assessment of risk of bias in included studies

- The following items were independently assessed by two authors using the risk of bias assessment tool [39] (see Appendix 6.2).
- Was there adequate sequence generation (selection bias)?
- Was allocation adequately concealed (selection bias)?
- Was knowledge of the allocated interventions adequately prevented during the study?
  - Participants and personnel (performance bias)

  - Outcome assessors (detection bias)
- Were incomplete outcome data adequately addressed (attrition bias)?
- Are reports of the study free of suggestion of selective outcome reporting (reporting bias)?
- Was the study apparently free of other problems that could put it at a risk of bias?

Measures of treatment effect

For dichotomous outcomes (*e.g.* need for dialysis and death) results were expressed as risk ratio (RR) with 95% confidence intervals (CI). Where continuous scales of measurement had been used to assess the effects of treatment (*e.g.* serum creatinine), the mean difference (MD) was used, or the standardised mean difference (SMD) if different scales were used.

Dealing with missing data

Any further information required from the original author was requested by written correspondence (*e.g.* e-mailing or writing to corresponding authors) and any relevant information obtained in this manner was included in the review. Evaluation of important numerical data such as screened, randomised patients as well as intention-to-treat, as-treated and per-protocol population was carefully performed. Attrition rates, for example dropouts, losses to follow-up and withdrawals were investigated. Issues of missing data and imputation methods (for example, last-observation-carried-forward) were critically appraised [39].

Assessment of heterogeneity

Heterogeneity was analysed using a Chi^2 test on N-1 degrees of freedom, with an alpha of 0.05 used for statistical significance and with the I^2 test [38]. I^2 values of 25%, 50% and 75% correspond to low, medium and high levels of heterogeneity.

Assessment of reporting biases

If possible, funnel plots were to be used to assess for the potential existence of small study bias [39].

Data synthesis

Data were pooled using the random-effects model but the fixed-effect model was also to be used to ensure robustness of the model chosen and susceptibility to outliers.

Subgroup analysis and investigation of heterogeneity

Subgroup analysis was used to explore possible sources of heterogeneity (*e.g.* preconditioning site, number of ischaemic preconditioning stimuli, early *vs.* late windows of protection). Heterogeneity among participants related to age and gender. Heterogeneity in treatments related to the type of intervention such as major aorta surgery or coronary artery bypass surgery. Adverse effects were to be tabulated and assessed using descriptive techniques. Where possible, the risk difference with 95% CI was to be calculated for each adverse effect, either compared with no treatment or another agent.

Sensitivity analysis

We performed sensitivity analyses to explore the influence of the following factors on effect size.

- Repeating the analysis excluding unpublished studies
- Repeating the analysis taking account of risk of bias, as specified
- Repeating the analysis excluding any very long or large studies to establish how much they dominate the results
- Repeating the analysis excluding studies using the following filters: diagnostic criteria, language of publication, source of funding (industry *vs.* other), and country.

SUMMARY OF FINDINGS TABLES

We presented the main results of the review in 'Summary of findings' tables. These tables present key information concerning the quality of the evidence, the magnitude of the effects of the interventions examined, and the sum of the available data for the main outcomes [51]. The 'Summary of findings' tables also include an overall grading of the evidence related to each of the main outcomes using the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) approach [36]. The GRADE approach defines the quality of a body of evidence as the extent to which one can be confident that an estimate of effect or association is close to the true quantity of specific interest. The quality of a body of evidence involves consideration of within-trial risk of bias (methodological quality), directness of evidence, heterogeneity, precision of effect estimates and risk of publication bias [52]. We presented the following outcomes in the 'Summary of findings' tables.

- Serum creatinine on days one, two and three
- Adverse events of remote ischaemic preconditioning

- Need for dialysis
- AKI defined by the AKIN score
- Mortality

RESULTS

Description of studies

A detailed description of all studies can be found in Characteristics of included studies and Characteristics of excluded studies.

Results of the search

The search identified 1,381 unique records (Appendix 6.1). Two independent review authors screened each reference for inclusion on the basis of title and abstract, and subsequently assessed full-text copies of all publications eligible for inclusion. For five studies presented only as abstract, full text publications were obtained by contacting authors by e-mail. We identified 55 studies (70 records). Of these, 28 studies (38 records) were included and 27 excluded (32 records) (Figure 6.2).

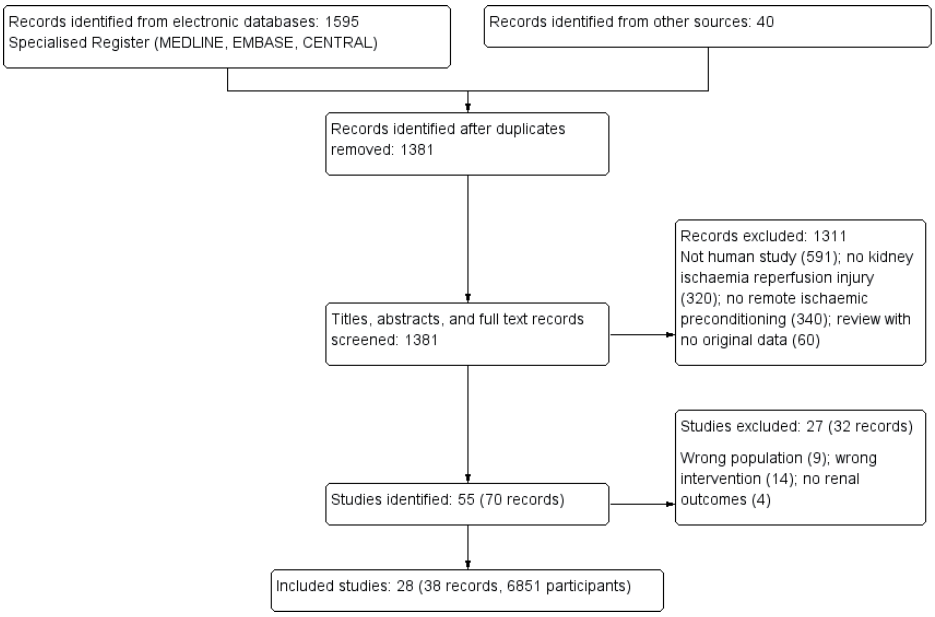

Figure 6.2. The flow diagram of references.

Included studies

We included 28 studies ([1–4, 4a, 5–28]) reporting data from a total of 6,851 patients undergoing a surgical procedure associated with renal ischaemia and reperfusion (I/R). [4] and [4a] are the same study, however we have analysed this as two separate studies because the outcomes for two different patient populations (stable and unstable angina) were reported separately.

Patients were randomised to undergo remote ischaemic preconditioning (remote ischaemic preconditioning; n=3,441) or a control intervention consisting of sham remote ischaemic preconditioning or no treatment (n=3,441). All 28 studies reported at least one kidney outcome measure. See Characteristics of included studies (for details on sample size, procedure, patient characteristics, inclusion and exclusion criteria, intervention and reported outcomes.

Participants

Five studies included only children, all aged <17 years old, scheduled for heart surgery ([10, 14–17]). Other studies included adult cardiovascular patients scheduled for a coronary artery bypass graft (11 studies), cardiac surgery (nine studies) or aortic aneurysm repair (three studies). One study included kidney cancer patients undergoing partial nephrectomy [8].

Methods of remote ischaemic preconditioning induction: site, timing and protocols

The intervention of interest in all studies was remote ischaemic preconditioning, which was compared to a control intervention. All studies applied preconditioning only, except for [6], [7] and [9], in which the conditioning protocol was applied both before and after surgery, thereby inducing a combination of remote ischaemic preconditioning and remote ischaemic postconditioning.

Cuff inflation was the preferred method of remote ischaemic preconditioning induction: 25 studies used a blood pressure cuff to induce ischaemia in an upper arm (15 studies), lower limb (nine studies) or both [2]. [25] applied their remote ischaemic preconditioning protocol to each lower limb, one after the other. [1] and [25] induced remote ischaemic preconditioning by clamping of the common iliac artery. In one study, ([4]; [4a]) the (suprarenal) ascending aorta was clamped, thereby inducing both remote ischaemic preconditioning and local ischaemic preconditioning.

The remote ischaemic preconditioning stimulus was applied directly after anaesthetic induction in all studies, except for [15], in which remote ischaemic preconditioning was induced 24 hours before surgery, making this the only study investigating the so-called second window of protection by ischaemic preconditioning.

Seven different remote ischaemic preconditioning protocols were used among the 28 studies. The most frequently used protocol consisted of three cycles of five minutes of occlusion of the remote organ, interspersed with five minutes of reperfusion (3 x 5'/5' I/R), ([8, 11, 12, 18, 19, 21–24, 27, 28]). Other protocols were 4 x 5'/5' I/R ([5–7, 10, 13–17, 20, 26]), 3 x 10'/10' I/R ([3, 9]), 2 x 10'/10' I/R ([1]), 2x 5 minutes ([2]), 2 x 2'/3' I/R ([4, 4a]) and cross-clamping the right common iliac artery ten min, thereafter clamping the left common iliac artery ([25]).

Outcome measures

The included studies reported a variety of outcome measures. Kidney impairment (*e.g.* postoperative creatinine, NGAL, AKIN score and RIFLE score) was the primary outcome in seven studies (25%) ([3, 8, 16, 25–28]). [20] reported a composite primary end point of death, myocardial infarction, stroke and AKI. Cardiac outcome measures were the primary endpoint in ten studies ([1, 2, 5, 6, 7, 10, 18, 19, 22, 23]), and pulmonary outcome measures were the primary endpoint in three studies ([9, 11, 13]). [14] reported hospital stay, [15] reported IL4, and [21] reported myocardial microRNA expression as primary outcome measures. In the remaining four studies there were no specified primary or secondary outcome measures ([4, 4a, 12, 17, 24]). Mortality was reported in 24 studies, duration of hospital stay in eight studies, and incidence of adverse effects of remote ischaemic preconditioning in 13 studies. Other reported outcome measures were myocardial injury, pulmonary injury, stroke, ICU stay, inotropic support, and a wide variety of non-kidney molecular markers (*e.g.* IL-8, CK-MB and NF-kB).

Kidney outcome measures differed substantially among studies. Serum creatinine was published in 15 studies at 13 different time points postoperatively. Serum NGAL was reported in two studies ([3, 16]) at day one, two and three after surgery. Incidence of AKI was reported in ten studies, of which [16], [20] and [26] assessed AKI incidence according to the RIFLE criteria, and [2], [3], [5], [18], [24], [27], and [28] used the AKIN criteria.

Excluded studies

Studies on surgical procedures unlikely to cause kidney ischaemia reperfusion injury [62–69], studies without remote ischaemic preconditioning applied [70–74], studies relating to transplantation [75], and studies investigating iodine contrast [76–84] were excluded. Studies in patients undergoing kidney transplantation or intravenous contrast administration were excluded because of the differences in pathophysiology of the inflicted kidney injury.

Studies with a different primary outcome measure than kidney injury and with an intervention causing kidney injury were included if they published kidney outcome measures. However, not all studies reported kidney outcome measures, we emailed the authors of those studies for kidney data and if no reply was received, we attempted to contact them again after three weeks. Studies without published kidney outcome measures were excluded after this second email attempt [85–88].

Risk of bias in included studies

Risk of bias assessment was performed by two independent review authors and summarised in Figure 6.3 and Figure 6.4.

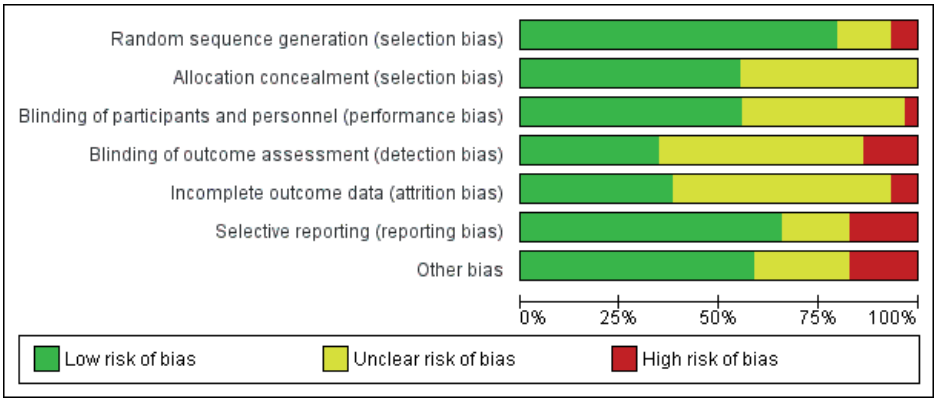

Figure 6.3. Risk of bias graph.
Review authors' judgements about each risk of bias item presented as percentages across all included studies.

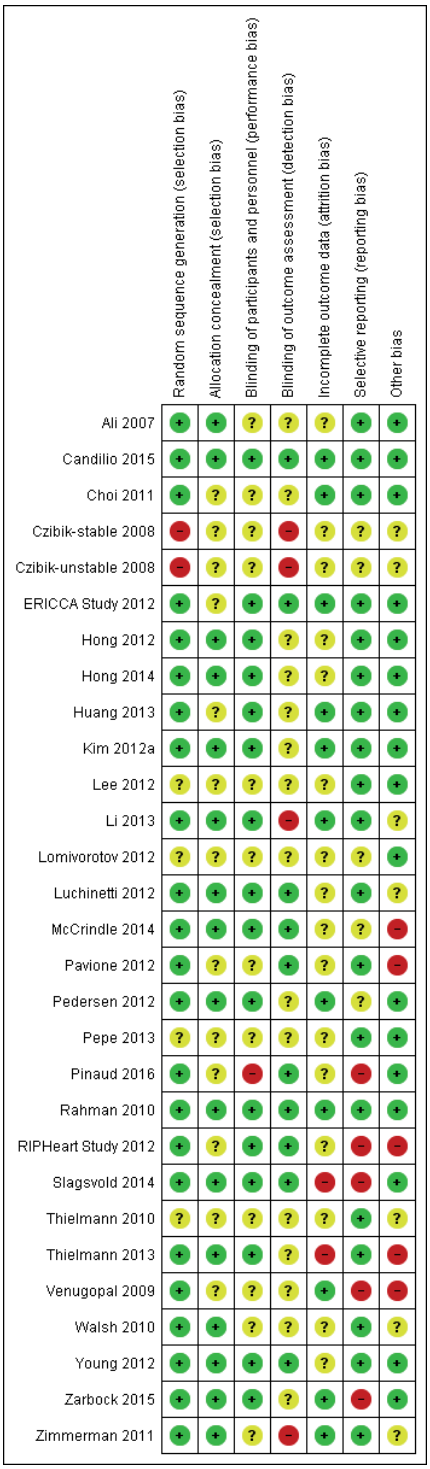

Figure 6.4. Risk of bias summary.
Review authors' judgements about each risk of bias item for each included study.

Allocation (selection bias)

Most included studies reported adequate methods of random sequence generation for patient allocation, which was assessed at low risk of selection bias. A computer generated random number sequence was used in 22 studies; [28] used manually shuffled blocks which we also reported as low risk of bias. In one study ([4, 4a]) randomisation was not reported, leading to a high risk of selection bias. [10], [12], [17] and [22] reported randomisation, but did not report the method used, and were therefore assessed to be at unclear risk of selection bias.

There were 16 studies reporting blinding of allocation using numbered, sealed, opaque envelopes, which was assessed to introduce a low risk of selection bias. The remaining 12 studies did not mention whether the patient allocation was blinded. For these studies ([3, 4, 4a, 5, 8, 10, 12, 15, 17, 18, 22, 24]), the risk of selection bias was assessed as unclear.

Blinding (performance bias and detection bias)

Many studies did not adequately report measures to reduce performance and detection bias. Only 16 studies reported adequate blinding of investigators and involved personnel, such as by hiding the blood pressure cuff and using an independent investigator to perform the remote ischaemic preconditioning protocol. The risk of performance bias in these studies was therefore assessed to be low. [18] reported their study as single blinded and therefore was assessed as high risk of bias. The remaining 11 studies had unclear risk of bias as blinding was not described. Patients were anaesthetised during remote ischaemic preconditioning in all studies except for [15]; however, anaesthesia does not ensure that patients are fully blinded for the allocated intervention. All studies therefore were assessed at unclear risk of bias.

Ten studies ([2, 5, 13, 14, 15, 18–21, 26]) reported adequate blinding of outcome assessors and were therefore rated as being at low risk of detection bias. Fifteen studies did not adequately report blinding of outcome assessors and were assessed at unclear risk of detection bias. Three studies did not mention blinding of outcome assessors and were assessed at high risk of bias ([4, 4a, 11, 28]).

Incomplete outcome data (attrition bias)

Risk of attrition bias was unclear in 15 studies ([1, 4, 4a, 6, 7, 10, 12–15, 17, 18, 20, 22, 25, 26]) and high in two studies ([21, 23]). Eleven studies ([2, 3, 5, 8, 9, 11, 16, 19, 24, 27, 28]) reported missing data and prespecified outcome measures, indicating a low risk of attrition bias.

Selective reporting (reporting bias)

The risk of reporting bias due to selective outcome reporting was assessed to be low in most studies. This was based on the fact that, in most studies, the outcome measures specified in the introduction matched those presented in the results of the article (19 studies). In six studies ([18, 20, 21, 23, 24, 27]) the risk of reporting bias was assessed to be high, due to the incoherency between reported outcome measures in the method section of the articles and the results.

Performing funnel plots for the primary outcome measures, there is a high suspicion of underreporting negative small studies as can be seen in the funnel plots of serum creatinine on days one and two (Figure 6.5; Figure 6.6).

Other potential sources of bias

Five studies were classified as high risk of bias for the following reasons.

- [15] failed to adequately report the patient inclusion and exclusion criteria
- [23] and [24] studies reported changes in the anaesthesia protocol during the study
- [20] recalculated the sample size during the inclusion period and reduced the total number of patients to include
- The authors of [14] were shareholders in a company producing a remote ischaemic preconditioning device.

[4], [4a], [11], [13], [22], [25], and [28] did not report their funding source and were classified as unclear risk of bias. All remaining 21 studies adequately reported their funding sources and there were no other concerns.

Effects of interventions

See: Summary of findings for the main comparison Summary of findings table. For consistency regarding the direction of effects, continuous outcomes with negative values, and dichotomous outcomes with values less than one favour remote ischaemic preconditioning.

Primary outcome measures

Serum creatinine

Serum creatinine levels were reported in 15 studies ([1, 3, 4, 4a, 8–10, 13–17, 21, 22, 24, 25]), measured at 13 different time points postoperatively. In total, serum creatinine was measured in 707 patients undergoing remote ischaemic preconditioning and in 704

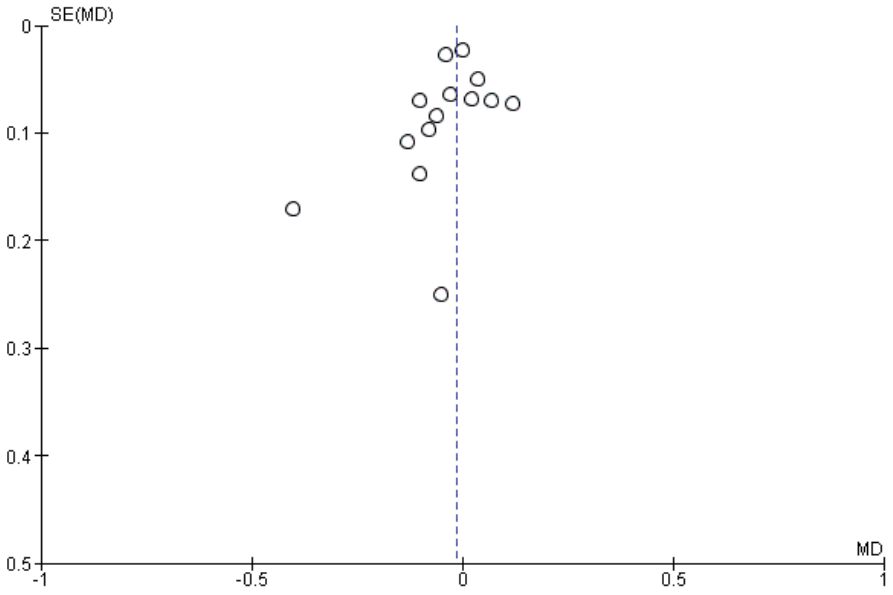

Figure 6.5. (Analysis 1.6). Funnel plot of comparison.
Serum creatinine, outcome: 1.6 Creatinine day 1 [mg/dL].

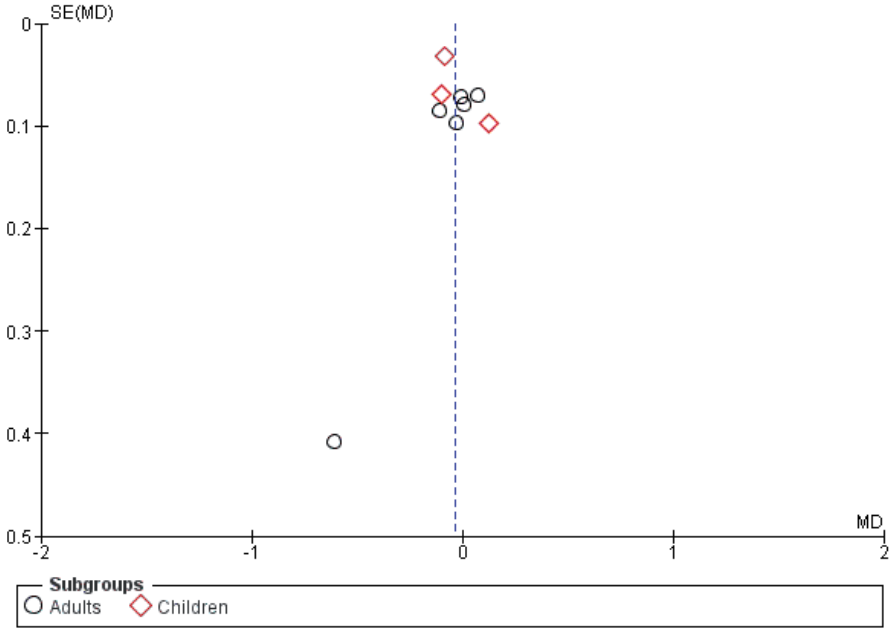

Figure 6.6. (Analysis 1.8). Funnel plot of comparison.
1 Serum creatinine, outcome: 1.8 Creatinine day 2 [mg/dL].

patients undergoing a control intervention. Our meta-analysis shows no difference in serum creatinine between patients undergoing remote ischaemic preconditioning *vs.* controls on postoperative days one (Analysis 1.6 (14 studies, 1,022 participants): MD -0.02 mg/dL, 95% CI -0.05 to 0.02; I^2=21%), two (Analysis 1.8 (9 studies, 770 participants): MD -0.04 mg/dL, 95% CI -0.09 to 0.02; I^2=31%), and three (Analysis 1.10 (6 studies, 770 participants): MD -0.05 mg/dL, 95% CI -0.19 to 0.10; I^2=68%).

We found a reduction in peak postoperative creatinine during the first three days in patients undergoing remote ischaemic preconditioning (Analysis 1.16 (3 studies, 365 participants): MD -0.10 mg/dL, 95% CI -0.20 to -0.01; I^2=0%).

Complications and adverse effects related to ischaemic preconditioning

Data on adverse effects of the preconditioning stimulus were reported in 15 studies ([1–4, 4a, 5–9, 12, 15, 17, 23, 25]), including a total of 1,999 patients receiving remote ischaemic preconditioning and 1,994 patients undergoing a control intervention. Overall there was no significant difference between the two groups (Analysis 2.1 (15 studies, 3,993 participants): RR 3.47; 95% CI 0.55 to 21.76; I^2=0%). No serious adverse effects were reported in studies using a blood pressure cuff for remote ischaemic preconditioning induction. [5] reported skin petechiae at the time of the intervention (35/801 participants; 4.4%), which is considered to be a minor side effect and is therefore not included in this review. Severe adverse effects occurred only in patients in the experimental arm of the study by [25], in which four patients receiving remote ischaemic preconditioning developed lower limb ischaemia due to the traumatic effects of vascular clamping of the iliac artery.

Secondary outcome measures

Need for dialysis

The need for dialysis was reported in 13 studies ([1, 3, 6, 7, 11–13, 16, 18, 19, 24, 27, 28]), including 1,211 remote ischaemic preconditioning-treated patients and 1,206 patients undergoing a control intervention. Forty-four patients in the remote ischaemic preconditioning group required dialysis *vs.* 46 patients in the control group. There was no difference between the two groups (Analysis 3.1 (13 studies, 2,417 participants): RR 0.85; 95% CI 0.37 to 1.94; I^2=60%).

Acute kidney injury (KDIGO, AKIN, RIFLE criteria)

There were no studies reporting AKI according to the KDIGO criteria.

Eight studies ([2, 3, 5, 9, 18, 24, 27, 28]) used the AKIN criteria to assess the incidence of AKI, including 1,170 remote ischaemic preconditioning-treated patients and 1,194 patients undergoing a control intervention. The incidence of AKI did not differ overall (Analysis 4.1 (8 studies, 2,364 participants): RR 0.76, 95% CI 0.57 to 1.00; I^2=61%), or when stratified for AKIN grade 1 (Analysis 4.2 (5 studies, 2,135 participants): RR 0.72, 95% CI 0.47 to 1.11; I^2=67%) grade 2 (Analysis 4.3 (5 studies, 2,135 participants): RR 0.71, 95% CI 0.41 to 1.24; I^2=26%) and grade 3 (Analysis 4.4 (5 studies, 2,135 participants): RR 0.63, 95% CI 0.37 to 1.07; I^2=11%).

Three studies ([16, 20, 26]) reported data on the incidence of AKI according to the RIFLE criteria for 794 remote ischaemic preconditioning-treated patients and 792 patients undergoing a control intervention. The incidence of AKI did not differ overall (Analysis 5.1 (3 studies, 1,586 participants): RR 0.91, 95% CI 0.75 to 1.12; I^2=0%) or when stratified for Risk (Analysis 5.2 (3 studies, 1,586 participants): RR 0.77, 95% CI 0.58 to 1.03; I^2=0%) Injury (Analysis 5.3 ((RR 1.09, 95% CI 0.59 to 2.02)) or Failure (Analysis 5.4 (3 studies, 1,586 participants): RR 1.09; 95% CI 0.59 to 2.02; I^2=0%). There were no events in either group for RIFLE criteria Loss and End-stage kidney failure.

Blood urea nitrogen (BUN)

Blood urea nitrogen was not reported as an outcome measure by any of the included studies.

Neutrophil gelatinase associated lipocalin (NGAL)

Two studies ([3, 16]) reported serum NGAL levels measured post-operatively on day one for 92 preconditioned and 89 control patients. Our meta-analysis showed no difference in serum NGAL between the groups (Analysis 6.1 (2 studies, 181 participants): MD 0.57 ng/mL, 95% CI -2.65 to 3.79; I^2=0%).

[16] also measured serum NGAL post-operatively at six and 12 hours, and on day two and three, and reported no difference between the groups for any of these time points.

[27], reported urine NGAL was reduced in patients undergoing remote ischaemic preconditioning, when measured four, 12 and 24 hours after surgery (Analysis 7.1; Analysis 7.2; Analysis 7.3).

Kidney injury molecule-1 (KIM-1)

KIM-1 was not reported as an outcome measure by any of the included studies.

Mortality

Twenty-four studies ([1, 2, 4, 4a, 5–7, 9, 11–17, 19, 21–28]) reporting mortality in 2,467 patients receiving remote ischaemic preconditioning and 2,464 control patients. We included data on all-cause mortality to 30 days and all-cause in-hospital mortality in our analysis. When both in-hospital mortality and 30-day mortality were reported, [27] in-hospital mortality was used in the analysis. Mortality after a longer postoperative period and mortality due to a specific condition or disease were excluded. Our analysis showed no difference in mortality between the groups (Analysis 8.1 (24 studies, 4,931 participants): RR 0.86, 95% CI 0.54 to 1.37; I^2=0%).

Quality of life

Quality of life was not reported as an outcome measure by any of the included studies.

Length of hospital stay

Eight studies ([1, 2, 3, 6, 9, 22–24]) report data on the length of hospital stay, including a total of 457 remote ischaemic preconditioning-treated patients and 463 patients undergoing a control intervention. The length of hospital stay did not differ between the groups (Analysis 9.1 (8 studies, 920 participants): MD 0.17 days, 95% CI -0.46 to 0.80; I^2=49%).

Subgroup analyses

We aimed to perform subgroup analyses for our primary outcome measures, for all subgroups containing two or more studies. Adverse effects occurred only in six patients in two studies ([5, 25]), and as a consequence, RR could only be calculated for these two studies. Therefore, no subgroup analysis was performed for this outcome measure. The following subgroup analyses therefore only concern serum creatinine on postoperative day one, two and three. All subgroup analysis results are shown in Table 6.1.

Age

We stratified the included studies according to participants' age (child or adult). There was no effect of remote ischaemic preconditioning on serum creatinine on day one, two or three postoperatively in any of the subgroups, and no difference in treatment effect between subgroups. Compared to the overall analysis of serum creatinine on postoperative day two, heterogeneity was reduced in the subgroup of studies performed in adults, while heterogeneity in the subgroup of children increased to high. No change in heterogeneity was observed on for serum creatinine on day one and three.

Sex

Subgroup analysis could not be performed, since none of the studies reported separate outcomes for men and women and no individual patient data were retrieved.

Remote ischaemic preconditioning protocol

There were seven different remote ischaemic preconditioning protocols used in the studies, but only three different remote ischaemic preconditioning protocols were used in more than one study. We stratified the protocols according to the total duration of ischaemia and the number of remote ischaemic preconditioning cycles (see Table 6.1).

For total duration of ischaemia, the subgroups of 15, 20 and 30 minutes contained two or more studies and were included in the analysis. The subgroup of four minutes total ischaemia was not analysed since it contained two comparisons from the same study ([4, 4a]). There was no effect of treatment on serum creatinine on day one, two or three postoperatively in any of the subgroups, and no difference in treatment effect between subgroups.

Compared to the overall analysis, heterogeneity was reduced in the 15 minutes subgroup, but increased in the other subgroups, on all three postoperative days.

For the number of preconditioning cycles, the subgroups of two, three, and four cycles contained two or more studies and were included in the analysis. In the subgroup of two preconditioning cycles, serum creatinine was reduced on postoperative day one, while there was no effect of treatment on this day in the subgroups of three and four cycles. However, the confidence intervals overlapped between all subgroups. No effect of treatment and no differences between subgroups were observed for serum creatinine on postoperative days two and three.

Heterogeneity in the subgroups was similar to the overall analysis on day one, was increased in the four cycles subgroup on day two, and was reduced in all subgroups on day three.

Method of remote ischaemic preconditioning induction

The type and amount of tissue used to induce the remote ischaemic preconditioning stimulus may influence its efficacy in reducing kidney damage. Often, the preconditioning stimulus is applied to an extremity, by inflating a blood pressure cuff around an upper or lower limb. Alternatively, atraumatic vascular clamping of the aorta or iliac artery may be used. We therefore stratified the studies according to the method of remote ischaemic preconditioning induction, creating three subgroups: blood pressure cuff on

Table 6.1. Subgroup analyses

	Creatinine day 1 post-op (mg/dL)			Creatinine day 2 post-op (mg/dL)			Creatinine day 3 post-op (mg/dL)		
Subgroups	Number of studies	MD [95% CI]	I^2	Number of studies	MD [95% CI]	I^2	Number of studies	MD [95% CI]	I^2
Total	14	-0.02 [-0.05 to 0.02]	21%	9	-0.04 [-0.09 to 0.02]	31%	6	-0.05 [-0.19 to 0.10]	68%
Age									
Adults	10	-0.02 [-0.09 to 0.05]	28%	6	-0.01 [-0.08 to 0.06]	0%	5	-0.07 [-0.24 to 0.09]	73%
Children	4	-0.02 [-0.06 to 0.02]	22%	3	-0.05 [-0.16 to 0.06]	55%	1	–	NA
Total duration of ischemia									
15 minutes	3	-0.02 [-0.10 to 0.06]	0%	3	-0.04 [-0.14 to 0.06]	0%	2	-0.09 [-0.28 to 0.09]	41%
20 minutes	7	-0.02 [-0.07 to 0.03]	42%	5	-0.03 [-0.13 to 0.08]	60%	3	-0.09 [-0.44 to 0.27]	82%
30 minutes	2	0.04 [-0.16 to 0.25]	49%	1	–	NA	1	–	NA
Number of RIPC cycles									
2 cycles	4	-0.14 [-0.27 to -0.02]	0%	1	–	NA	1	–	NA
3 cycles	5	0.01 [-0.06 to 0.08]	0%	4	-0.03 [-0.11 to 0.05]	0%	3	-0.05 [-0.15 to 0.06]	12%
4 cycles	5	-0.01 [-0.05 to 0.03]	24%	4	-0.02 [-0.12 to 0.08]	63%	2	0.12 [-0.00 to 0.24]	0%
Method of RIPC induction									
Blood pressure cuff upper arm	4	-0.03 [-0.08 to 0.01]	0%	4	-0.08 [-0.13 to -0.02]	0%	2	-0.09 [-0.28 to 0.09]	41%
Blood pressure cuff lower limb	6	0.01 [-0.04 to 0.06]	25%	4	0.01 [-0.08 to 0.10]	35%	3	0.06 [-0.03 to 0.15]	0%
Aortic or iliac artery clamping	4	-0.14 [-0.27 to -0.02]	0%	1	–	NA	1	–	NA
Type of surgery									
Abdominal aortic aneurysm repair	2	-0.27 [-0.60 to 0.06]	25%	1	–	NA	1	–	NA
Coronary artery bypass grafting	6	-0.02 [-0.08 to 0.05]	0%	4	-0.00 [-0.08 to 0.08]	0%	3	-0.01 [-0.19 to 0.16]	66%
Other types of cardiac surgery	6	-0.01 [-0.05 to 0.04]	34%	4	-0.05 [-0.13 to 0.03]	42%	2	0.01 [-0.10 to 0.13]	0%

the upper arm, blood pressure cuff on the lower limb or clamping of the aorta or iliac artery (see Table 6.1).

On postoperative day one, there was no effect of treatment in the subgroups using blood pressure cuff occlusion, but serum creatinine was reduced in the subgroup of studies using vascular clamping. On postoperative day two, serum creatinine was reduced in the subgroup using blood pressure cuff occlusion of the upper arm, but not in the lower limb subgroup. On postoperative day three, there was no effect of treatment in any of the groups. Importantly, the confidence intervals of all subgroups overlapped on each postoperative day.

Heterogeneity in the subgroups was similar to the overall analysis for postoperative day one and two, and reduced in both subgroups on day three.

Type of surgical procedure

The type of surgery is a major determinant of the risk and severity of perioperative kidney injury, which may influence remote ischaemic preconditioning efficacy. The type of surgery may also correlate to a type of patient with a specific susceptibility to kidney injury. Studies were stratified according to the type of surgery performed, including abdominal aortic aneurysm repair, coronary artery bypass grafting, and other types of cardiac surgery (see Table 6.1). Only [8] reported partial nephrectomy and was excluded from this subgroup analysis.

There was no effect of treatment on serum creatinine on day one, two or three postoperatively in any of the subgroups, and no difference in treatment effect between subgroups.

Compared to the overall analysis, heterogeneity in the subgroup of cardiac surgery was increased on day one and day two, but decreased on day three. Heterogeneity in the other subgroups and days was similar or decreased compared to the overall analysis.

Sensitivity analysis

Excluding studies of lower quality with regard to the primary outcome measures had no significant effect on the results of this meta-analysis. Excluding studies of less than 30 patients in each group showed no significant difference in primary outcome measures.

Publication bias

We aimed to construct funnel plots to assess publication bias for the primary outcome measures. For adverse events and creatinine on postoperative day three, there were ≤ six data points, which were considered insufficient to reliably assess funnel plot asymmetry. Our assessment of the funnel plot of serum creatinine data on postoperative day one and two (Figure 6.5; Figure 6.6) showed signs of publication bias, with small studies predominantly showing positive results.

SUMMARY OF FINDINGS TABLE

The summary of findings table can be found at: Summary of findings table S6.1.

DISCUSSION

Summary of main results

Our search identified 28 studies investigating the effect of remote ischaemic preconditioning on kidney outcomes in patients undergoing surgery associated with kidney ischaemia reperfusion injury. Our primary endpoints showed no protective effect for remote ischaemic preconditioning application. Adverse effects were found in one study after vascular clamping to induce remote ischaemic preconditioning. The general method using cuff inflation on the upper arm or thigh showed no serious adverse effects. Furthermore, the secondary outcomes – need for dialysis, AKI indicated by RIFLE or AKIN criteria, serum NGAL, mortality and length of hospital stay – were unchanged in patients undergoing remote ischaemic preconditioning when compared with controls. One study reported urinary NGAL was significantly reduced in the remote ischaemic preconditioning group [27].

We found that a significant effect was seen regarding the method of remote ischaemic preconditioning induction. The subgroup of Invasive clamping of arteries to induce remote ischaemic preconditioning is more effective compared to non-invasive cuff inflation. However, this observation should be interpreted with care, since invasive remote ischaemic preconditioning induction included only three studies, one of which [1] introduced high heterogeneity in the data-set. Subgroup analysis indicated that age, type of surgery, site of preconditioning, number of cycles and duration of the remote ischaemic preconditioning protocol did not influence remote ischaemic preconditioning efficacy.

Overall we conclude that after major surgery associated with kidney ischaemia reperfusion injury, remote ischaemic preconditioning does not significantly reduce kidney injury. Based on these data, routine use of remote ischaemic preconditioning in major surgery cannot be justified.

Overall completeness and applicability of evidence

The heterogeneity of the outcome measurements and surgical procedures makes them difficult to compare. Of note, local ischaemic preconditioning, which has been shown to be highly effective in animal models ([58]), has not been studied in patients and its feasibility therefore remains unclear.

Because of expected differences in the underlying mechanism of kidney injury, studies including patients undergoing kidney transplantation or interventions using nephrotoxic contrast media were excluded from this review. Despite inclusion of 28 studies, the primary outcome measures were heterogeneous and this severely hampered our meta-analysis. Therefore, we advocate a more standardised primary outcome measure in future studies.

Many studies, as well as our meta-analysis, focused on serum creatinine as the primary outcome. However, the use of this outcome measure as a gold standard for kidney injury is under debate [56] and its relationship with the long-term quality of life is not straightforward. Only a few studies assessed the effect of remote ischaemic preconditioning on long-term outcome measures such as quality of life and long term mortality.

Quality of the evidence

Overall, the quality of the evidence provided by the included studies was acceptable. Including data from studies of lower quality may lead to an overestimation of treatment effects, since low-quality studies generally overstate efficacy. Excluding studies of lower quality did not affect the primary outcomes. Therefore, after performing the risk of bias assessment we included all available data in our analysis.

One important aspect in the quality of the evidence and potential bias is the funding of the studies which might influence the outcome. Influential funding was reported by one study and seven studies did not report their funding sources, which is a source of potential bias.

Potential biases in the review process

We attempted to identify all relevant studies through comprehensive, systematic searching of the literature in multiple databases, as well as contacting authors to obtain additional publications and data. Still we could not exclude the presence of publication bias in our data-set.

Data extraction was completed independently by two authors without conflicts of interest regarding the outcome, thereby avoiding potential bias. We performed several subgroup analyses. However, since our approach is observational rather than experimental, their results should be regarded as hypothesis generating. This meta-analysis was slightly hampered by missing data, resulting from studies reporting outcome data as medians and interquartile ranges. We were unable to obtain raw data for a number of these studies, and this may have biased our analysis.

In summary, we consider this review to be influenced by missing data and an unclear risk of publication bias.

Agreements and disagreements with other studies or reviews

The results of this review are comparable with other meta-analyses in the literature ([33, 34, 59]) despite differences in inclusion criteria (*i.e.* others included contrast studies and did not include studies in children). The negative result from this review contradicts the overall beneficial effect of remote ischaemic preconditioning in animal models of kidney ischaemia reperfusion injury. A meta-analysis of animal studies by [58] showed that remote ischaemic preconditioning was successful in reducing kidney ischaemia reperfusion injury. Possible explanations for the discrepancy between human and animal studies are:

The lower amount of kidney injury in human studies compared with experimental models of kidney ischaemia reperfusion injury. This review (Analysis 1.6; Analysis 1.8; Analysis 1.10) showed that postoperative serum creatinine levels among control groups were comparable with normal values serum creatinine. When there is limited kidney damage, very large numbers of patients are required to show a significant reduction by remote ischaemic preconditioning. The ischaemia reperfusion injury applied to the kidney in the animal models is much more severe compared to the amount of kidney injury in human studies. Most animal studies have a single kidney model where the pedicle is clamped for 45 minutes [58]. For such an amount of kidney damage there is no comparable human patient group. Although kidney transplant recipients were not addressed in this review,

kidneys from deceased donors are exposed to prolonged periods of (cold) ischaemia. Therefore, it may be interesting to pursue remote ischaemic preconditioning efficacy in those patients.

Animal studies generally use healthy young animals ([58]); studies included in this review mostly recruited aged patients with significant comorbidity. Aging and comorbidity significantly reduce the effectiveness of remote ischaemic preconditioning in human models of ischaemia reperfusion injury ([35, 53, 55]). Experimental studies using animals with diabetes and hypertension confirm a decreased effectiveness of remote ischaemic preconditioning compared with healthy animals [46]. Furthermore, studies in the present review may have included patients suffering from pathologies which induce temporary (mild) ischaemia of remote tissues, *e.g.* unstable angina or claudication. Such episodes could induce a protective remote preconditioning effect on kidney's ischaemia reperfusion injury, which may have abolished the protective effect of the experimental preconditioning stimulus.

AUTHORS' CONCLUSIONS

Implications for practice

Although remote ischaemic preconditioning by cuff inflation is safe, available data do not confirm the efficacy of remote ischaemic preconditioning in reducing kidney ischaemia reperfusion injury. Remote ischaemic preconditioning applied by currently used protocols should not be used routinely in clinical practice to reduce kidney ischaemia reperfusion injury in patients undergoing major surgery in which kidney injury may occur.

Implications for research

Future RCTs should focus on patients undergoing a procedure that is associated with significant kidney ischaemia reperfusion injury, such as open abdominal aortic or kidney transplant surgery. Furthermore, fundamental research is required to be able to predict the protective effect of remote ischaemic preconditioning and to increase the efficacy of the preconditioning protocol in humans.

Future studies should be adequately powered and designed with undisputed endpoints such as need for dialysis, mortality and/or quality of life, rather than short-term kidney function. Markers of (subtle) ischaemic kidney injury are useful for research purposes, but should be secondary to long-term clinically pertinent outcome measures.

Studies need to report methods of allocation, blinding and outcome data in detail and should publish a predefined study protocol. Doing so will increase study quality and make the conclusions more applicable to clinical practice.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Where outcome measure data were presented graphically, data were extracted using digital image analysis software ([49], ImageJ; imagej.nih.gov). When data distributions were presented as median and interquartile range, or both, we attempted to obtain data as mean ± SD or SEM by contacting authors by email. In case of no response after two attempts or data not available the study was excluded from the analysis.

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Table S6.1. Summary of findings table

Ischaemic preconditioning for the reduction of renal ischaemia reperfusion injury (Review)						
Patients or population: patients undergoing a surgical intervention that induces kidney injury **Setting:** perioperative hospital setting **Intervention:** ischemic preconditioning **Controle:** no treatment						
Outcomes	**Absolute effect* (95% CI)**		**Relative effect (95% CI)**	**Number of patients (studies)**	**Quality of the evidence (GRADE)**	**Comments**
	Risk with no treatment	**Risk with RIPC**				
Serum creatinine: day 1	The mean serum creatinine on day 1 was 0 mg/dL	MD was **0.02 mg/dL lower** (0.05 mg/ dL lower to 0.02 mg/ dL higher)	-	1,022 (14 RCTs)	⊕⊕⊕⊕ High	Funnel plot assessment suggests publication bias. Analysis without small studies showed no significant differences.
Serum creatinine: day 2	The mean serum creatinine on day 2 was 0 mg/dL	MD was **0.04 mg/dL lower** (0.09 mg/ dL lower to 0.02 mg/ dL higher)	-	770 (9 RCTs)	⊕⊕⊕⊕ High	Funnel plot assessment suggests publication bias, however due to a limited amount of studies these results should be interpreted with care. Analysis without small studies showed no significant differe
Serum creatinine: day 3	The mean serum creatinine on day 3 was 0 mg/dL	MD was **0.05 mg/dL lower** (0.19 mg/dL lower to 0.1 mg/ dL higher)	-	417 (6 RCTs)	⊕⊕⊕⊕ High	
Adverse events related to RIPC	Study population 1 per 1,000	2 per 1,000 (0 to 11)	RR 3.47 (0.55 tot 21.76)	3,993 (15 RCTs)	⊕⊕⊕⊕ High	Adverse effects due to remote ischaemic preconditioning only occurred in traumatic clamping of arteries. There were no reports of adverse events when applying remote ischaemic preconditioning by cuff

Table S6.1. ***Continued***

Outcomes	Absolute effect* (95% CI)		Relative effect (95% CI)	Number of patients (studies)	Quality of the evidence (GRADE)	Comments
	Risk with no treatment	Risk with RIPC				
Need for dialysis	Study population 38 per 1,000	32 per 1,000 (14 to 74)	RR 0.85 (0.37 tot 1.94)	2,417 (13 RCTs)	⊕⊕⊕⊕ High	The low incidence of dialysis results in a moderate Grade of evidence
Acute kidney injury defined by AKIN	Study population 369 per 1,000	281 per 1,000 (211 to 369)	RR 0.76 (0.57 tot 1.00)	2,364 (8 RCTs)	⊕⊕⊕⊕ High	This outcome regards the overall AKIN score, when subdivided in grade 1, 2 and 3 there is no significant effect
Mortality	Study population 17 per 1,000	15 per 1,000 (9 to 23)	RR 0.86 (0.54 to 1.37)	4,931 (24 RCTs)	⊕⊕⊕⊕ High	

The risk in the intervention group (and the 95% confidence interval) is based on the risk in the control group and the **relative effect** of the intervention.
CI: Confidence interval; **RR:** Risk ratio; **OR:** Odds ratio.
GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Appendix 6.1. Electronic search strategies

Database	Search terms
CENTRAL	1. MeSH descriptor Ischemic Preconditioning explode all trees 2. MeSH descriptor Reperfusion Injury explode all trees 3. (ischemi*):ti,ab,kw in Trials 4. (reperfusion):ti,ab,kw in Trials 5. (renal or kidney*):ti,ab,kw in Trials 6. (#2 OR #3 OR #4 OR #5) 7. (#1 AND #6)
MEDLINE (OVID SP)	1. exp Ischemic Preconditioning/ 2. exp Reperfusion Injury/ 3. ischemi$.tw. 4. reperfusion$.tw. 5. (renal or kidney).tw. 6. or/2-5 7. and/1,6
EMBASE (OVID SP)	1. ischemic preconditioning/ 2. reperfusion injury/ 3. (renal or kidney).tw. 4. or/1-2 5. and/1,4

Appendix 6.2. Risk assessment tool

Potential source of bias	Assessment criteria
Random sequence generation Selection bias (biased allocation to interventions) due to inadequate generation of a randomised sequence	*Low risk of bias:* Random number table; computer random number generator; coin tossing; shuffling cards or envelopes; throwing dice; drawing of lots; minimization (minimization may be implemented without a random element, and this is considered to be equivalent to being random). *High risk of bias:* Sequence generated by odd or even date of birth; date (or day) of admission; sequence generated by hospital or clinic record number; allocation by judgement of the clinician; by preference of the participant; based on the results of a laboratory test or a series of tests; by availability of the intervention. *Unclear:* Insufficient information about the sequence generation process to permit judgement.
Allocation concealment Selection bias (biased allocation to interventions) due to inadequate concealment of allocations prior to assignment	*Low risk of bias:* Randomisation method described that would not allow investigator/participant to know or influence intervention group before eligible participant entered in the study (*e.g.* central allocation, including telephone, web-based, and pharmacy-controlled, randomisation; sequentially numbered drug containers of identical appearance; sequentially numbered, opaque, sealed envelopes). *High risk of bias:* Using an open random allocation schedule (*e.g.* a list of random numbers); assignment envelopes were used without appropriate safeguards (*e.g.* if envelopes were unsealed or non-opaque or not sequentially numbered); alternation or rotation; date of birth; case record number; any other explicitly unconcealed procedure. *Unclear*: Randomisation stated but no information on method used is available.
Blinding of participants and personnel Performance bias due to knowledge of the allocated interventions by participants and personnel during the study	*Low risk of bias*: No blinding or incomplete blinding, but the review authors judge that the outcome is not likely to be influenced by lack of blinding; blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken. *High risk of bias*: No blinding or incomplete blinding, and the outcome is likely to be influenced by lack of blinding; blinding of key study participants and personnel attempted, but likely that the blinding could have been broken, and the outcome is likely to be influenced by lack of blinding. *Unclear*: Insufficient information to permit judgement

Appendix 6.2 continues on next page.

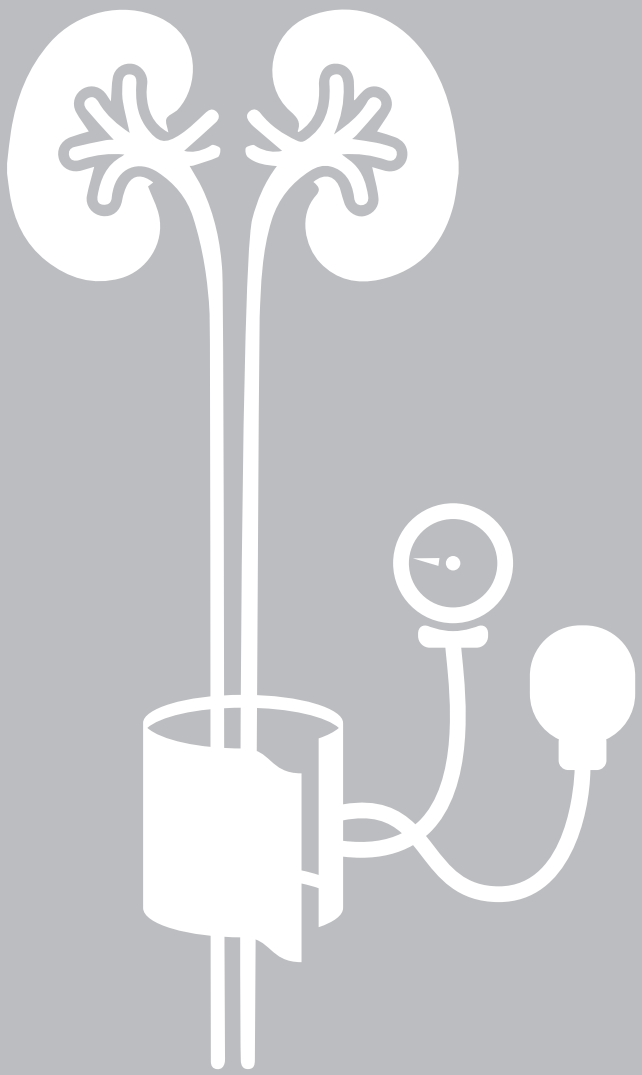
Appendix 6.2. ***Continued***

Potential source of bias	Assessment criteria
Blinding of outcome assessment Detection bias due to knowledge of the allocated interventions by outcome assessors.	*Low risk of bias:* No blinding of outcome assessment, but the review authors judge that the outcome measurement is not likely to be influenced by lack of blinding; blinding of outcome assessment ensured, and unlikely that the blinding could have been broken. *High risk of bias:* No blinding of outcome assessment, and the outcome measurement is likely to be influenced by lack of blinding; blinding of outcome assessment, but likely that the blinding could have been broken, and the outcome measurement is likely to be influenced by lack of blinding. *Unclear:* Insufficient information to permit judgement
Incomplete outcome data Attrition bias due to amount, nature or handling of incomplete outcome data.	*Low risk of bias:* No missing outcome data; reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size; missing data have been imputed using appropriate methods. *High risk of bias:* Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; 'as-treated' analysis done with substantial departure of the intervention received from that assigned at randomisation; potentially inappropriate application of simple imputation. *Unclear:* Insufficient information to permit judgement

Appendix 6.2 continues on next page.

Appendix 6.2. ***Continued***

Potential source of bias	Assessment criteria
Selective reporting Reporting bias due to selective outcome reporting	*Low risk of bias:* The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way; the study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon). *High risk of bias:* Not all of the study's pre-specified primary outcomes have been reported; one or more primary outcomes is reported using measurements, analysis methods or subsets of the data (*e.g.* subscales) that were not pre-specified; one or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); one or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis; the study report fails to include results for a key outcome that would be expected to have been reported for such a study. *Unclear:* Insufficient information to permit judgement
Other bias Bias due to problems not covered elsewhere in the table	*Low risk of bias:* The study appears to be free of other sources of bias. *High risk of bias:* Had a potential source of bias related to the specific study design used; stopped early due to some data-dependent process (including a formal-stopping rule); had extreme baseline imbalance; has been claimed to have been fraudulent; had some other problem. *Unclear:* Insufficient information to assess whether an important risk of bias exists; insufficient rationale or evidence that an identified problem will introduce bias.



7

Remote ischemic preconditioning to reduce contrast-induced nephropathy: study protocol for a randomized controlled trial

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Trials. 2014;15:119

ABSTRACT

Background

Despite the increasing use of pre- and posthydration protocols and low-osmolar instead of high-osmolar iodine-containing contrast media, the incidence of contrast-induced nephropathy (CIN) is still significant. There is evidence that contrast media cause ischemia-reperfusion injury of the medulla. Remote ischemic preconditioning (RIPC) is a non-invasive, safe, and low-cost method to reduce ischemia-reperfusion injury.

Methods

The RIPCIN study is a multicenter, single-blinded, randomized controlled trial in which 76 patients at risk of CIN will receive standard hydration combined with RIPC or hydration with sham preconditioning. RIPC will be applied by four cycles of five minutes ischemia and five minutes reperfusion of the forearm by inflating a blood pressure cuff at 50 mmHg above the actual systolic pressure. The primary outcome measure will be the change in serum creatinine from baseline to 48 to 72 hours after contrast administration.

Discussion

A recent pilot study reported that RIPC reduced the incidence of CIN after coronary angioplasty. The unusual high incidence of CIN in this study is of concern and limits its generalizability. Therefore, we propose a randomized controlled trial to study whether RIPC reduces contrast-induced kidney injury in patients at risk for CIN according to the Dutch guidelines.

Trial registration

Current Controlled Trials ISRCTN76496973.

BACKGROUND

Iodine-containing contrast media are often used for diagnostic and therapeutic procedures and their use is the leading cause of hospital-acquired acute kidney injury [1]. Prospective studies demonstrate that contrast media are responsible for approximately 15% of acute kidney injury cases [2, 3]. Despite the increasing use of pre- and posthydration protocols and low-osmolar instead of high-osmolar iodine-containing contrast media, the incidence of contrast-induced acute kidney injury is still significant [4, 5]. This so called contrast-induced nephropathy (CIN) is defined as an absolute rise of ≥0.5 mg/dL and/or a relative increase of ≥25% in serum creatinine compared to baseline within 48 to 72hoursafter contrast administration without an alternative cause of kidney injury [6]. CIN is strongly associated with morbidity and mortality [7, 8]. In patients with CIN, 8% need dialysis treatment and between 22% and 34% die during the index hospitalization [3, 9–11]. In accordance with international guidelines, all patients who receive iodine-containing contrast are screened for risk factors of CIN, including measures of renal function (estimated glomerular filtration rate, based upon the MDRD formula) [12–15]. High-risk patients receive pre- and posthydration by saline solution infusion for four to 12 h. Furthermore, 48 to 72 hours after contrast administration, serum creatinine should be measured [16]. Despite the identification of high-risk patients and the use of hydration protocols, the incidence of CIN still varies between 2% and 13% [17–20]. The exact mechanism underlying CIN remains to be elucidated. There is evidence to suggest that contrast media have direct toxic effects on the tubular cells resulting in altered mitochondrial function and apoptosis [21]. Moreover, ischemia-reperfusion injury of the medulla has been shown to play an important role [22]. The outer part of the medulla has an area with a high oxygen demand and is located at a distance from the vasa recta which supplies the medulla of blood. Contrast-induced vasoconstriction of the vasa recta induces ischemia-reperfusion injury of the medulla which contributes significantly to the pathophysiology of CIN. Remote ischemic preconditioning (RIPC) is a short and harmless discontinuation of blood supply to particular organs or tissue, followed by reperfusion [23, 24]. A preconditioning stimulus is applied before the onset of prolonged ischemia. In animal models it has been found to reduce ischemia-reperfusion injury of the kidney [25]. Although the precise mechanism of RIPC remains unknown, two major pathways may play a pivotal role: the humoral and neurogenic pathways. Both are thought to induce various kinase cascades and eventually prevent opening of the mitochondrial permeability transition pore in the target organ, thereby reducing cell death [26]. A retrospective cohort study by Whittaker et al. indicated that multiple balloon inflations during coronary angioplasty (as a remote stimulus) might

reduce CIN [27]. Furthermore, a recent pilot study by Er et al. showed that RIPC reduced CIN in high-risk patients undergoing elective coronary angiography [28]. However, there was an unusually high incidence of CIN (40%) in the control group. The question arises whether protection by RIPC, as an adjunct to standard preventive measures (that is, hydration and discontinuation of nephrotoxic drugs), also holds for patients with a lower risk of CIN. As generalizability of the results by Er et al. is confined to a selected group of patients with an unusual high risk of CIN, we propose a randomized controlled trial to study whether RIPC reduces contrast-induced kidney injury in patients at risk of CIN according to the Dutch guideline [14].

METHODS/DESIGN

A multicenter, single-blinded, randomized controlled trial will be performed at the Radboud University Nijmegen Medical Centre and Slingeland Hospital Doetinchem. Inclusion will be performed by the physician researcher after written informed consent.

Study population

A total of 76 patients will be randomized. Sealed envelopes are used to randomly assign consecutive patients in a 1:1 ratio to receive either sham preconditioning or RIPC (Figure 7.1). The study population consists of patients at risk of CIN according to criteria adopted from the Dutch guidelines: (1) eGFR <45 mL/min/1.73 m^2; (2) eGFR <60 mL/min/1.73 m^2; (3) eGFR <60 mL/min/1.73 m^2 and two additional risk factors (that is, peripheral vascular disease, heart failure, >75 years of age, anemia, dehydration, use of diuretics and/or NSAIDs). Patients undergoing contrast procedures for diagnostic and/or treatment purposes are eligible. As patients receiving less than 100 mL of iodinated contrast media may not have an increased risk of contrast-induced kidney injury, an expected use of at least 100 mL was used as inclusion criterion [3, 29]. Inclusion criteria

1. Patients undergoing an interventional or diagnostic radiological procedure in which they receive an expected >100 mL intravascular contrast including:

    - Thoracic and/or abdominal endovascular aortic repair
    - Endovascular aortic repair
    - Digital subtraction angiography
    - Percutaneous transluminal angioplasty
    - Percutaneous intentional extraluminal revascularization
    - Carotic artery stenting

- Percutaneous coiling/embolization procedures
- Computed tomography

2. Patients who comply with the risk criteria for CIN according to the Dutch guidelines [14]
   - Peripheral vascular disease, heart failure, >75 years, anemia (Ht <0.39 men and <0.36 women, dehydration, diuretics and/or NSAID use)

3. Written informed consent.

Exclusion criteria

- Age <18 years
- Hemodialysis or peritoneal dialysis
- Simultaneous participation in another interventional study
- Percutaneous coiling/embolization procedures of the kidney
- Impossibility to perform RIPC, due to pathology of both arms (for example, dystrophy, recent trauma, chronic wounds)

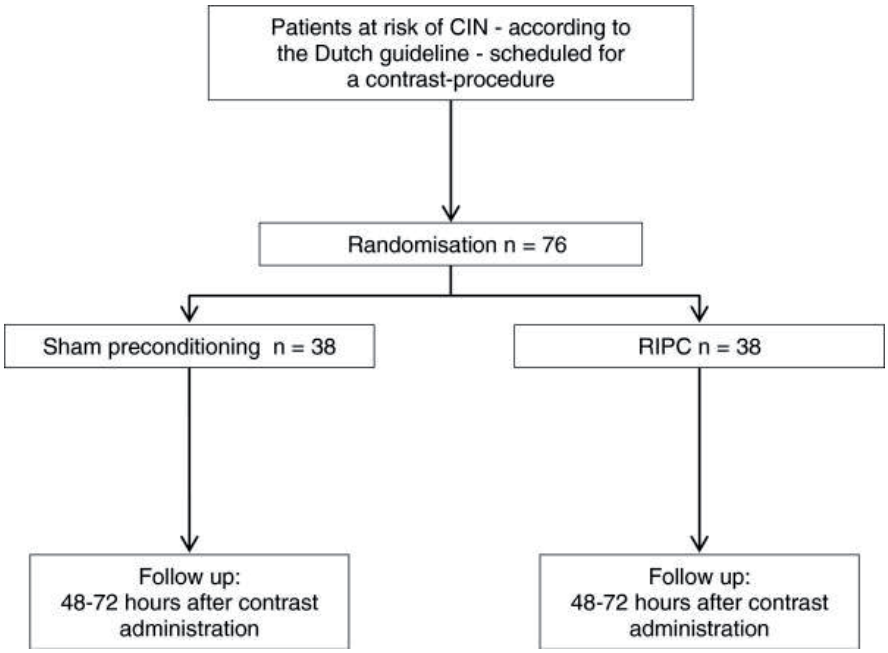

Figure 7.1. Study flow chart.

Study protocol

All participating patients will receive the standard hydration schedule consisting of an infusion with saline 0.9% solution 3 to 4 mL/kg/h for four hours prior to and four hours after contrast administration. In patients with congestive heart failure or MDRD <30 mL/min/1.73 m^2 a long schedule is used with an infusion of saline 0.9% solution 1 mL/kg/h for 12 hours prior to and 12 hours after the contrast administration. Nephrotoxic drugs (for example, metformin and diuretics) are discontinued at least 24 hours before and after contrast administration [14]. Patients in the experimental group of the study will receive RIPC by four cycles of ischemia and reperfusion of the forearm by inflating a blood pressure cuff around the upper arm at 50 mmHg above the actual systolic pressure during five minutes followed by five minutes of reperfusion. In the control group, patients receive sham preconditioning by inflating the blood pressure cuff to ten mmHg below the actual diastolic pressure during five minutes followed by five minutes of reperfusion (four cycles). The time between the last inflation cycle and the start of the intervention isplanned within 45 min. In the interest of blinding, the investigator ensures that the inflation pressure is not visible for both the patient and the (interventional) radiologist. All patients receive Xenetrix 300 (0.6 to 0.85 Osmol/kg H_2O), a low osmolar, non-ionic, and hydrophilic contrast medium [30, 31]. Patients will complete a questionnaire to obtain all relevant baseline characteristics such as age, weight, previous contrast procedures, diabetes, vascular-related diseases, and (discontinuation of) medication. Chart review will be performed to complement and double check this information. Blood and urine samples are taken at baseline and four to six hours after contrast administration. A final blood sample is taken 48 to 72 hours after contrast administration. According to the Dutch guidelines, monitoring of renal function in highrisk patients is recommended within 48 to 72 hours after contrast administration. All samples will be number coded before analysis to ensure blinding of the independent investigator performing the analyses.

Primary endpoint

The primary endpoint is change in serum creatinine form baseline to serum creatinine within 48 to 72 hours after contrast administration.

Secondary endpoint

The secondary endpoints are the incidence of CIN (defined as an absolute rise of ≥0.5 mg/dL or a relative increase of ≥25% in serum creatinine over baseline within 48 to 72

hours after contrast administration), rehospitalization, hemodialysis, and mortality within six weeks after contrast administration.

Ethics, informed consent

An independent ethics committee, the Central Committee on Research involving Human Subjects, Arnhem-Nijmegen, approved the protocol. Oral and written informed consent from the patient will be obtained prior to inclusion.

Adverse events

Although RIPC by repeated insufflations of a blood pressure cuff around the upper arm is considered safe, serious adverse events possibly related to the application of RIPC will be reported to the ethical committee. Mild adverse events are: transient discomfort due to compression and/or ischemia and the formation of ecchymosis (upper arm) or petechia (lower arm).

Power analysis

In this randomized study, the change of serum creatinine from baseline to 48 to 72 hours after contrast administration will be compared between the experimental and control group. Using serum creatinine change as continuous response variable increases the power of the study. In a previous retrospective cohort study at our center including 2,169 patients at risk for contrast-induced nephropathy, serum creatinine values decreased from 120 µmol/L at baseline to 118 µmol/L at 48 to 72 hours after contrast administration due to adequate hydration protocols [17]. This mean change in serum creatinine (-2 µmol/L) was normally distributed with a standard deviation of 23 µmol/L. Based on existing evidence we assume that RIPC with hydration may provide a further decrease in mean serum creatinine from baseline to 48 to 72 hours of approximately 14 µmol/L as compared to hydration only. This corresponds with approximately 60% of the effect that was found by Er et al. [28]. If the true difference in the experimental and control means is 14 µmol/L, we will need to study 34 experimental and 34 controls to be able to reject the null hypothesis with a power of 0.80 and an alpha of 0.05 calculated with a one-sided independent t-test. Based on existing animal [25] and human studies [32, 33] investigating the influence of RIPC on renal ischemiareperfusion injury, we assume that RIPC does not negatively affect renal function. Therefore, one-sided testing would be appropriate for this study. Expected lost to follow-up (for example, blood sampling not realized between 48 to 72 h) is approximately 5%. For this reason 38 patients will be included in both the experimental and control arm.

Statistical analysis

The analysis will be performed on the basis of intentionto-treat principles. Student's t-test will be used to compare normally distributed variables, and Mann-Whitney U test will be used to compare not-normally distributed continuous data. Categorical variables will be compared with the chi-square test. If univariable analysis reveals a significant difference in baseline characteristics, then a multivariable linear regression analysis will be used to assess its impact on the primary outcome measure (that is, change in serum creatinine between baseline and 48 to 72 hours after contrast administration). A subgroup analysis will be performed to assess whether the impact of RIPC on the primary outcome measure is affected by the Mehran risk score. For this analysis patients will be divided into three equal groups (that is, tertiles) according to their Mehran risk score. Statistical analyses will be performed with SPSS 20.0. A probability value of <0.05 is considered to indicate statistical significance and 95% confidence intervals will be calculated. The RIPCIN study is registered at: http://www.controlled-trials.com/ISRCTN76496973.

DISCUSSION

In this study, we hypothesize that RIPC reduces the occurrence of CIN in patients at risk of acute kidney injury due to the use of contrast media. A recent randomized pilot study suggested that RIPC reduced contrastinduced kidney injury, however this study was performed in patients with an unusual high risk of CIN. A comment on this study by Mehta Oza et al. clarified that based on the reported Mehran risk score the incidence of CIN should lie between 26% and 30% instead of 40% as reported by Er et al. [34]. The authors stated that this high incidence of CIN could be attributed to a high prevalence of heart failure and diabetes mellitus in their cohort. However, if standard measures to prevent CIN, that is, hydration with saline and discontinuation of nephrotoxic drugs, were not carried out appropriately, then the incidence of CIN would also be increased. As compliance to standard preventive measures against CIN was not described by Er et al. their results do not fully justify the conclusion that RIPC, as an adjunct to standard preventive measures, effectively reduces CIN. Another important issue to address is the fact that the incidence of CIN varies with the criteria used [35]. Er et al. defined CIN as an absolute or relative increase in serum creatinine, whereas some evidence exists that both an absolute and a relative increase in serum creatinine more accurately predicts adverse events after coronary angioplasty. To overcome the flaws related to the use of different definitions of CIN, we will use the change in serum creatinine from

baseline to 48 to 72 hours after contrast administration as that primary endpoint in the proposed trial. As serum creatinine levels generally peak between 48 and 72 hours after contrast administration, it would be ideal to measure serum creatinine at both 48 and 72 h. However, this would not be in line with Dutch and international guidelines which recommend checking renal function once between 48 and 72 hours after contrast administration. In practice most patients are discharged within 24 hours after contrast administration and for many it is already difficult to realize one blood sample between 48and 72 hours after contrast administration. In our view, it is appropriate for proof-of-concept studies investigating new strategies to reduce contrast-induced kidney injury to use the change in serum creatinine from baseline to 48 to 72 hours as the primary endpoint. Once the efficacy of a new strategy against contrast-induced kidney injury has been confirmed, much larger clinical trials should be conducted with adverse effects after the use of contrast-media (for example, dialysis and/or death) as the primary endpoint.

COMPETING INTERESTS

The authors of this manuscript have no competing interests to disclose.

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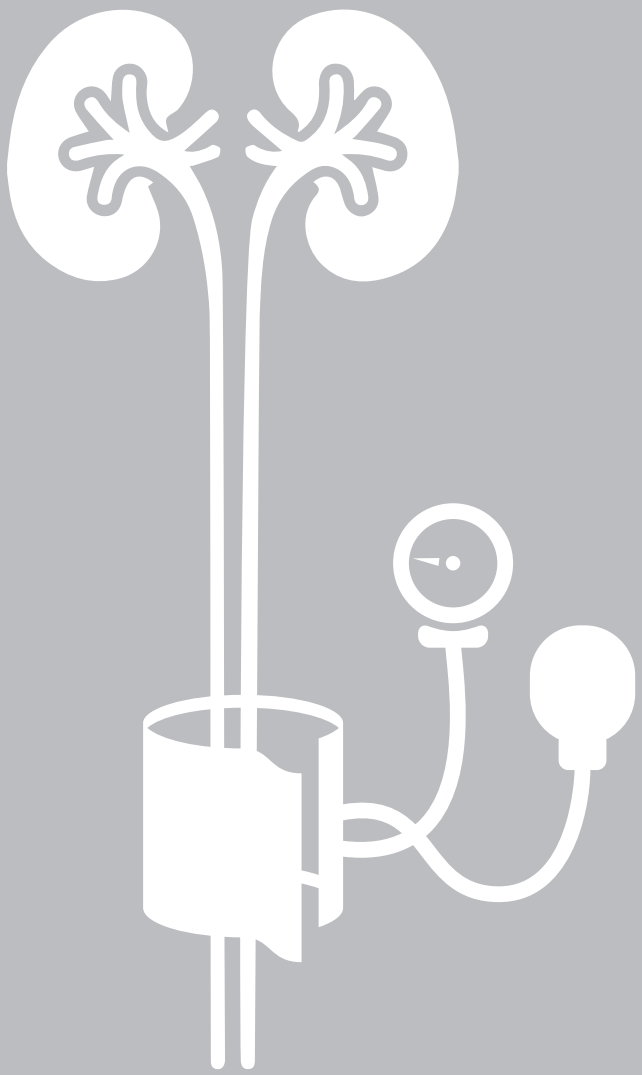

8

Remote ischemic preconditioning to reduce contrast-induced nephropathy: a randomized controlled trial

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ABSTRACT

Background

Despite the increasing use of pre- and post-hydration protocols and low osmolar instead of high osmolar iodine containing contrast media, the incidence of contrast induced nephropathy (CIN) is still significant. There is evidence that contrast media cause ischemia reperfusion injury of the renal medulla. Remote ischemic preconditioning (RIPC) is a non-invasive, safe, and low cost method to reduce ischemia reperfusion injury. The aim of this study is to investigate whether RIPC, as an adjunct to standard preventive measures, reduces contrast induced acute kidney injury in patients at risk of CIN.

Methods

The RIPCIN study is a multicenter, single blinded, randomized controlled trial in which 76 patients at risk of CIN received standard hydration combined with RIPC or hydration with sham preconditioning. RIPC was applied by four cycles of five minutes ischemia and five minutes reperfusion of the forearm. The primary outcome measure was the change in serum creatinine from baseline to 48 to 72 hours after contrast administration.

Results

With regard to the primary endpoint, no significant effect of RIPC was found. CIN occurred in four patients (two sham and two RIPC). A pre-defined subgroup analysis of patients with a Mehran risk score ≥11, showed a significantly reduced change in serum creatinine from baseline to 48 to 72 hours in patients allocated to the RIPC group (Δ creatinine -3.3 ± 9.8 mmol/L) compared with the sham group (Δ creatinine +17.8 ± 20.1 mmol/L).

Conclusion

RIPC, as an adjunct to standard preventive measures, does not improve serum creatinine levels after contrast administration in patients at risk of CIN according to the Dutch guideline. However, the present data indicate that RIPC might have beneficial effects in patients at a high or very high risk of CIN (Mehran score ≥11). The RIPCIN study is registered at: http://www.controlled-trials.com/ISRCTN76496973.

What this paper adds

Remote ischemic preconditioning, as an adjunct to standard preventive measures, does not improve serum creatinine levels after contrast administration in patients at risk of contrast induced nephropathy according to the Dutch guideline.

INTRODUCTION

The use of iodine containing contrast media for diagnostic and therapeutic procedures is the leading cause of hospital acquired acute kidney injury [1]. Despite the increasing use of low osmolar instead of high osmolar iodine containing contrast media and hydration protocols, the incidence of contrast induced acute kidney injury (CI-AKI) is still significant (2–13%) [2–7]. This so called contrast induced nephropathy (CIN) is defined as an absolute rise of ≥0.5 mg/ dL and/or a relative increase of ≥25% in serum creatinine compared with baseline within 48–72 hours after contrast administration without an alternative cause of kidney injury [8]. In patients with CIN, 8% require dialysis treatment and between 22% and 34% die during the index hospitalization [2, 9–11] In accordance with international guidelines, all patients who receive iodine containing contrast media are screened for risk factors of CIN, including measures of renal function (estimated glomerular filtration rate [eGFR], based on the MDRD formula) [12, 13]. Patients with an eGFR <45 mL/ min/1.73 m^2, an eGFR ≤60 mL/min/1.73 m^2 with diabetes mellitus, or an eGFR ≤60 mL/ min/1.73 m^2 with two or more additional risk factors are at high risk of CIN according to the Dutch guideline [14]. For high risk patients, the Dutch guideline recommends the use of pre- and post-hydration by saline infusion and the discontinuation of nephrotoxic medication. Furthermore, 48–72 hours after contrast administration serum creatinine should be measured. Despite the identification of high risk patients and the use of hydration protocols, the incidence of CIN still varies between 2% and 13% [2–7].

Although the precise mechanism underlying CIN remains unknown, evidence exists that contrast media have direct toxic effects on the tubular cells resulting in altered mitochondrial function and apoptosis. Moreover, solid evidence exists from experimental models that renal ischemia, resulting from contrast induced vasoconstriction, plays a key role in the pathogenesis of contrast induced kidney injury [15, 16]. The outer part of the medulla has an area with high oxygen demand and is therefore vulnerable to contrast induced vasoconstriction of the vasa recta. When vasoconstriction resolves and the oxygen supply is restored, post-ischemic cells produce free oxygen radicals. The formation of free radicals contributes at least in part to the renal tubular cell injury [17]. Upon reperfusion, ischemic cells may become apoptotic because of the opening of mitochondrial permeability transition pores [18]. Remote ischemic preconditioning (RIPC) is a short and harmless discontinuation of blood supply to an organ or tissue, followed by reperfusion, which is applied before the onset of prolonged ischemia to a distant organ or tissue [19, 20]. It has been shown that RIPC induces various kinase cascades that eventually prevent the opening of mitochondrial permeability

transition pores in the target organ cells, thereby reducing cell death [18]. Moreover, evidence exists that RIPC reduces oxidative stress by improving antioxidative defence mechanisms (*e.g.* increased superoxide dismutase activity and glutathione peroxidase) and/or decreasing the generation of free radicals (*e.g.* decreased xanthine oxidase activity) [21].

In animal models and in some clinical trials, RIPC reduces ischemia reperfusion injury of the kidney [22, 23]. A pilot study by Er et al. indicated that RIPC reduced the incidence of CIN in high risk patients undergoing elective coronary angiography [24]. However, there was an unusually high incidence of CIN (40%) in the control group in that study [25]. Another recent study showed that RIPC reduced urinary liver type fatty acid binding protein (L-FABP), a biomarker for tubulointerstitial damage, 24 hours after contrast administration in patients at low-moderate risk of CIN [26]. However, RIPC did not show beneficial effects on eGFR or the incidence of CIN in this study. Although, patients enrolled in the study by Er et al. had a lower mean baseline eGFR (41 vs. 48 mL/min/1.73 m^2), there was considerable overlap in the integer CI-AKI risk score between the studies. Therefore, the difference in baseline integer CI-AKI risk score may not fully explain the discrepancies between the studies. Given the inconclusive data in literature, a randomized controlled trial was performed to study whether RIPC, as an adjunct to standard preventive measures, reduces CI-AKI in patients at risk of CIN.

METHODS

A multicenter, single blinded, randomized controlled trial was performed at the Radboud University Medical Center Nijmegen and the Slingeland Hospital Doetinchem. The RIPCIN study is registered at: http://www.controlled-trials.com/ISRCTN76496973 and the study protocol has been published previously [27].

Study population

A total of 76 patients were enrolled after written informed consent was obtained. Sealed envelopes were used to randomly assign consecutive patients using a 1:1 ratio to receive either sham preconditioning or RIPC. The study population consisted of patients at risk of CIN according to criteria adopted from the Dutch guidelines. Inclusion criteria were:

1. patients undergoing an interventional or diagnostic radiological procedure in which they receive an expected >100 mL intravascular contrast; and

2. patients who comply with the risk criteria for CIN according to the Dutch guidelines [14]: (a) eGFR <45 mL/min/1.73 m^2; (b) eGFR <60 mL/min/1.73 m^2 with diabetes mellitus; (c) eGFR <60 mL/min/1.73 m^2; and two additional risk factors from peripheral vascular disease, heart failure, age >75 years, anemia, dehydration, diuretics, and non-steroidal anti-inflammatory drug use. Exclusion criteria were: (1) age <18 years; (2) hemodialysis or peritoneal dialysis;
3. simultaneous participation in another interventional study;
4. percutaneous coiling/embolization procedures of the kidney;
5. impossibility to perform RIPC, caused by pathology in both arms (*e.g.* dystrophy, recent trauma, chronic wounds); and
6. no written informed consent.

An independent regional ethics committee, the central committee on research involving human subjects, Arnhem-Nijmegen, approved the protocol (number: 41890.091.12; date: October 16, 2012).

Study protocol

All participating patients received the standard hydration schedule consisting of intravenous infusion with saline 0.9% solution 3–4 mL/kg/h for four hours prior to and four hours after contrast administration. In patients with congestive heart failure or eGFR <30 mL/min/1.73 m^2, a long schedule was used with an infusion of saline 0.9% solution 1 mL/kg/h for 12 hours prior to and 12 hours after the contrast administration. Nephrotoxic drugs (*e.g.* diuretics) and metformin were discontinued 24 hours before and after contrast administration. Patients in the experimental group of the study received RIPC by four cycles of ischemia and reperfusion of the forearm by inflating a blood pressure cuff around the upper arm at 50 mmHg above the actual systolic pressure for five minutes, followed by five minutes of reperfusion. In the control group, patients received sham preconditioning by inflating the blood pressure cuff to ten mmHg below the actual diastolic pressure during five minutes, followed by five minutes of reperfusion (four cycles). The time between the last inflation cycle and the start of the intervention was planned to be within 45 minutes; this time window allowed performance of the sham or RIPC procedure under calm conditions on the ward. In the interest of blinding, the investigator ensured that the inflation pressure was not visible to either the patient or the (interventional) radiologist. All patients received Xenetrix 300 (0.6–0.85 Osmol/kg H_2O), a low osmolar, non-ionic, and hydrophilic contrast medium. Patients completed a questionnaire to obtain all relevant baseline characteristics including (discontinuation of) medication. Chart review was performed to complement and double check this

information. Blood samples were taken at baseline and four to six hours after contrast administration. A final blood sample was taken 48–72 hours after contrast administration.

Endpoints

The primary endpoint was change in serum creatinine from baseline to serum creatinine 48–72 hours after contrast administration. The secondary endpoints were the incidence of CIN (defined as an absolute rise of ≥0.5 mg/dL or a relative increase of ≥25% in serum creatinine over baseline within 48–72 hours of contrast administration), rehospitalization, hemodialysis, and mortality within six weeks of contrast administration.

Power analysis

In this randomized study, the change of serum creatinine from baseline to 48–72 hours after contrast administration was compared between the experimental and control groups. Using serum creatinine change as the continuous response variable increased the power of the study. In a previous retrospective cohort study at the authors' center including 2,169 patients at risk of contrast induced nephropathy, serum creatinine values decreased from 120 µmol/L at baseline to 118 µmol/L at 48–72 hours after contrast administration as a result of adequate hydration protocols [1, 28]. This mean change in serum creatinine (-2 µmol/L) was normally distributed with a standard deviation of 23 µmol/L. Based on existing evidence, it was assumed that RIPC with hydration may provide a further decrease in mean serum creatinine from baseline to 48–72 hours of approximately 14 µmol/L compared with hydration only. This corresponds with approximately 60% of the effect found by Er et al. [24]. If the true difference between the experimental and control means is 14 mmol/L, then 36 experimental participants and 36 control participants would be required for the study to be able to reject the null hypothesis with a power of 0.80 and an α of 0.05 calculated using a one-sided independent t test. In addition, the expected loss to follow up (*e.g.* blood sampling not done between 48 and 72 hours) was approximately 5%. Therefore, a total of 76 patients was required for inclusion in the study.

Statistical analysis

The analysis was performed on the basis of intention to treat principles. Student t test was used to compare normally distributed variables, and Mann-Whitney U test was used to compare non-normally distributed continuous data. In a previously published trial protocol [23], a subgroup analysis was defined to assess whether the impact of RIPC on the primary endpoint is affected by the Mehran risk score [29]. For this analysis patients

were divided into three groups according to their Mehran risk score (*i.e.* (1) risk score ≤5; (2) risk score 6–10; (3) risk score ≥11). Statistical analyses were performed with SPSS 20.0. A probability value of <.05 was considered to indicate statistical significance.

RESULTS

A total of 102 patients was assessed for eligibility, but 26 patients did not fulfill the inclusion criteria (Figure 8.1). Seventy-six patients were randomly allocated to receive sham preconditioning or RIPC in a 1:1 ratio. Four patients, two in the sham group and two in the RIPC group, were excluded after randomization because blood sampling 48–72 hours after contrast administration was not performed. A total of 72 patients completed follow up of the study. There were no significant differences in baseline characteristics or cardiovascular medication between the experimental and control groups (Tables 8.1 and 8.2).

Trial outcomes

With regard to the primary study endpoint (*i.e.* change in serum creatinine from baseline to 48–72 hours after contrast administration), no significant effect of RIPC was found

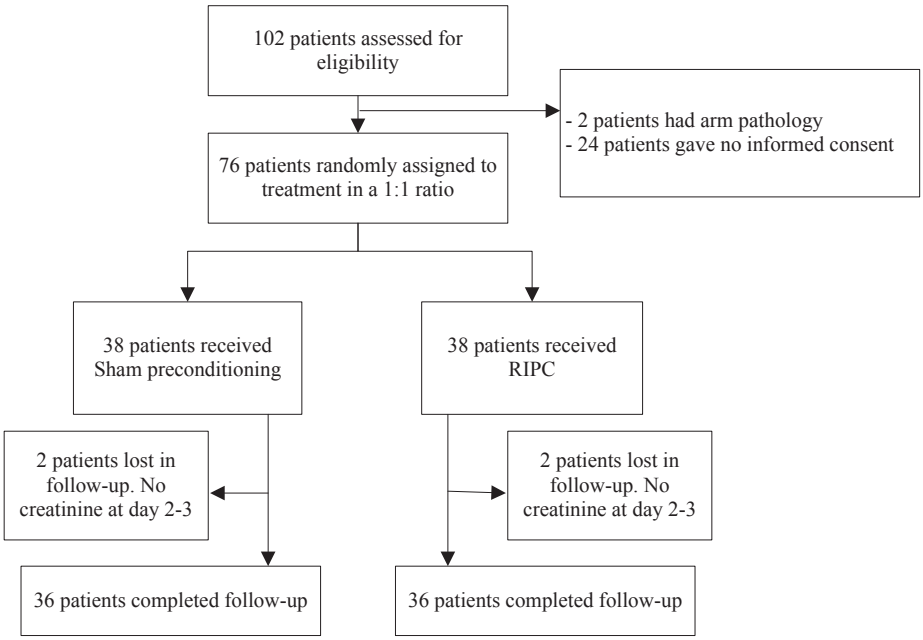

Figure 8.1. Study flow chart.

Table 8.1. Baseline characteristics

	Sham group (n=36)	RIPC group (n=36)	p-value
Age, years	73 ± 8.5	71 ± 11	0.37
Male sex, n (%)	21 (58)	14 (39)	0.16
BMI kg/m²	27 ± 4.7	29 ± 5.6	0.22
Contrast administration			
Diagnostic	18 (50)	21 (58)	
Treatment	18 (50)	15 (42)	0.64
Underlying disease			
Diabetes	10 (28)	8 (22)	0.58
Peripheral vascular disease	21 (58)	20 (56)	0.64
Myocardial infarction	16 (44)	13 (36)	0.46
Heart failure	8 (22)	7 (19)	0.78
Brain infarction	5 (14)	4 (11)	0.73
TIA	7 (19)	6 (17)	0.76
Anemia	5 (14)	3 (8)	0.47
Hypertension	24 (67)	28 (78)	0.59
Malignancy	8 (22)	7 (19)	0.77
Vital signs			
Systolic (mmHg)	137 ± 17	144 ± 20	0.12
Diastolic (mmHg)	75 ± 10	76 ± 11	0.82
Heart rate (beats/min)	68 ± 13	69 ± 12	0.75
Hydration protocol			
Short	31 (86)	31 (84)	
Long	5 (14)	5 (14)	0.60
Baseline serum creatinine, µmol/L	119 ± 32	115 ± 27	0.50
Baseline eGFR ml/min/1.73m²	52 ± 13	51 ± 11	0.87
Volume of contrast used (mL)	98 ± 29	99 ± 29	0.85
Time between last inflation and contrast administration, minutes	44 ± 36	46 ± 23	0.83
Integer CI-AKI risk score			
Mean (Q1–Q3)	6 (2–9)	6 (2–9)	1.00
Integer CI-AKI risk score			
<5	15 (42)	17 (47)	
6–10	16 (44)	13 (36)	
>11	5 (14)	6 (17)	0.91

Data given as mean ± SD, n (%) or as specified. BMI = body mass index; CI-AKI, contrast-induced acute kidney injury; eGFR = estimated glomerular filtration rate; TIA = transient ischemic attack; y = years.

Table 8.2. Baseline cardiovascular medication

	Sham group (n=36)	RIPC group (n=36)	p-value
Platelet aggregation inhibitors	20 (56)	14 (39)	0.24
Coumarin	9 (25)	8 (22)	1.00
NSAID	0 (0)	2 (3)	0.49
Beta-blocker	23 (64)	24 (67)	1.00
Isosorbide dinitrate	8 (22)	3 (8)	0.19
Calcium channel blocker	8 (22)	12 (33)	0.43
Angiotensin-converting enzyme inhibitor	13 (36)	9 (25)	0.44
Angiotensin II receptor blocker	11 (31)	8 (22)	0.59
Loop/thiazide diuretics	24 (67)	15 (42)	0.10
Spironolactone	3 (8)	3 (8)	1.00

Data given as n (%).
RIPC = remote ischaemic preconditioning; NSAID = Non-Steroidal AntiInflammatory Drug.

Table 8.3. Trial outcomes

	Sham group (n=36)	RIPC group (n=36)	p-value
Primary endpoint			
Change in serum creatinine from baseline to 48–72 hours (μmol/L)	-0.3 ± 14.7	0.25 ± 14.6	0.87
Secondary endpoints			
Change in serum creatinine from baseline to 4–6 hours (μmol/L)	-14.5 ± 11.8	-11.4 ± 11.0	0.26
Contrast-induced nephropathy n (%)	2 (6)	2 (6)	1.00
Rehospitalization within 6 wk	0 (0)	0 (0)	n.a.
Dialysis within 6 wk	0 (0)	0 (0)	n.a.
Mortality within 6 wk	2 (6)	0 (0)	0.49

Data given in mean ± SD, n (%). RIPC = remote ischaemic preconditinong; wk = weeks.

(Table 8.3). CIN occurred in four patients (two sham and two RIPC) and their respective Mehran risk scores were 1, 2, 6, and 14. None of the patients required dialysis or rehospitalization. Two patients (one with CIN) died within six weeks of the intervention. No adverse events occurred because of the preconditioning protocols. A subgroup analysis, in which all patients were classified according to their Mehran risk score, showed a significantly reduced change in serum creatinine in patients with a Mehran risk score ≥11 allocated to the RIPC group compared with the sham group (Table 8.4).

Table 8.4. Change in serum creatinine per group divided by Mehran risk score

Mehran risk score		Group	Change in serum creatining (mean ± SD)	p
<5	n=32	Sham (n=15)	- 0.2 ± 14.2	
		RIPC (n=17)	0.9 ± 13.1	0.83
6–10	n=29	Sham (n=16)	-6.1 ± 8.2	
		RIPC (n=13)	1.1 ± 18.6	0.18
≥11	n=11	Sham (n=5)	17.8 ± 20.1	
		RIPC (n=6)	-3.3 ± 9.8	0.048

RIPC = remote ischaemic preconditioning.

DISCUSSION

The present study demonstrates that RIPC, induced by intermittent upper arm ischemia before diagnostic and therapeutic intravascular contrast procedures, does not reduce contrast induced kidney injury in patients who are at risk of developing CIN according to the Dutch guideline [14]. However, the data indicate that a subgroup of patients who are at high to very high risk of developing CI-AKI, may benefit from RIPC as an adjunctive preventive measure. Therefore, the findings do not necessarily contradict results from previous studies in which RIPC was found to alleviate CI-AKI [24, 26]. Er et al. investigated the effects of RIPC in patients with a (very) high risk of developing CIN according to the risk classification system developed by Mehran et al. This best validated risk score includes both clinical and procedural variables and is divided into four risk classes of developing CI-AKI: low (risk score ≤5), moderate (risk score 6–10), high (risk score 11–15), and very high (risk score ≥16). In the study by Er et al., 60% of the participants were at high or very high risk of developing CI-AKI, whereas Igarashi et al. included only 6% of these (very) high risk patients [24, 26]. In the present study, 15% were at high or very high risk of developing CI-AKI. In line with these findings, Igarashi et al. did not find a reduction in creatinine based CI-AKI. Although their finding, that RIPC reduced the incidence of L-FABP based CI-AKI, provides interesting proof of concept evidence, its clinical relevance remains to be established. With regard to the study by Er et al., it should be noted that based on the reported Mehran risk score, the incidence of CIN should lie between 26% and 30% rather than 40% in the control group [25]. Although the authors attributed this discrepancy to the high prevalence of heart failure and diabetes in their cohort, the question arises whether the standard preventive measures (*i.e.* hydration and discontinuation of nephrotoxic drugs) were carried out appropriately. Nevertheless, the results of the present subgroup analysis support the

hypothesis that RIPC reduces the incidence of creatinine based CI-AKI in patients who are at high or very high risk of developing CI-AKI.

With regard to the preconditioning protocol, the present study used a similar protocol to that of the studies by Er et al. [24] and Igarashi et al. [26], consisting of four cycles of five minutes inflation of a blood pressure cuff around the upper arm and five minutes deflation. This protocol is relatively "standard" and has been used in many clinical trials. A recently performed meta-analysis investigating the effect of RIPC on ischemia reperfusion injury on animal kidneys, did not reveal a significant difference in efficacy between RIPC protocols using repeated short cycles of ischemia (fractionated stimulus) and those using one (usually) longer continuous ischemic stimulus [22]. To date, human data comparing the efficacy of different RIPC protocols are very scarce and there is no convincing evidence that justifies the use of an alternative RIPC protocol for clinical trials.

The major strengths of this study are related to its design as a blinded, sham controlled study with a registered and previously published study protocol. All physicians, nursing staff, and radiology personnel were blinded to the allocation of treatment. This minimized the risk of bias because of altered adherence to other preventive measures including saline infusion, and discontinuation of diuretics and metformin, as both patients and nursing staff were unaware of the allocation of treatment. Physicians and radiology personnel, responsible for the amount of contrast used, were also blinded. As inclusion criteria were not confined to patients with low to moderate or high to very high risk of developing CIN, the present study cohort reflects routine clinical practice very well.

There are several limitations to this study. First, as serum creatinine levels generally peak between 48 and 72 hours after contrast administration, it would have been ideal to measure serum creatinine at both 48 and 72 hours. However, in practice most patients were discharged within 24 hours of the contrast procedure. Those patients were asked to visit the outpatient clinic or their general practitioner for blood sampling at 48 or 72 hours after contrast administration. Sampling at both time points would severely increase the burden for the participants. It is also important to note that blood samples taken by the general practitioner were analyzed outside the authors' institution. Although slight variability between the different laboratories cannot be ruled out, it is not expected that this influenced results significantly. Second, this was a relatively small study with small numbers available for subgroup analysis. Although this subgroup analysis has been described in a previously published study protocol, its outcome should be interpreted with care. Third, a certain influence of the sham RIPC treatment, by inflating a cuff ten mmHg below the actual diastolic pressure, cannot be ruled out. As the sham

inflation around the upper arm only restricts blood flow to a very limited extent, this theoretical disadvantage does not outweigh the risk of bias related to a study design without adequate blinding. Finally the incidence of CIN was not used as the primary endpoint. Given the low incidence of CIN in patients who are at risk according to the Dutch guidelines (approximately 2%), use of this as the endpoint would require a trial with a very large number of participants. Therefore, the change in serum creatinine from baseline to 48–72 hours was used as the primary endpoint. However, the clinical relevance of improved serum creatinine levels in those patients remains to be elucidated.

In conclusion, the results from this randomized controlled trial show that RIPC, as an adjunct to standard preventive measures, does not reduce CI-AKI in patients at risk of CIN according to the Dutch guideline. However, it is proposed that RIPC might have clinical benefits in patients with a high or very high risk of CIN, and this should be pursued in new clinical trials.

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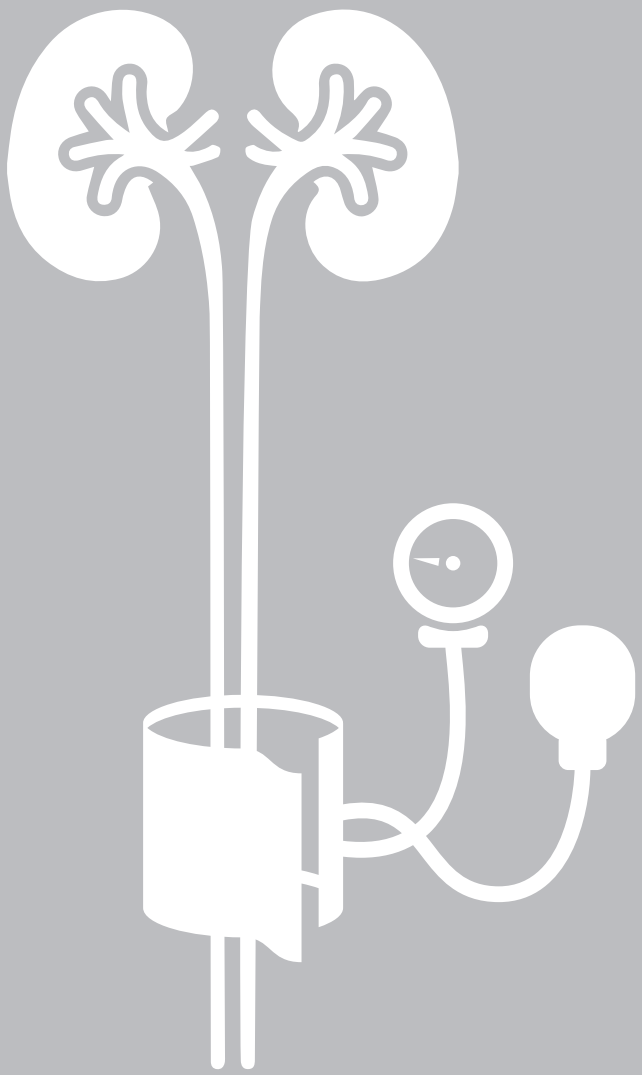

9

General discussion and future perspectives

In this thesis we identified and discussed the translation of ischemic preconditioning (IPC) from animal models to clinical trials. We started by analysing all renal IPC studies reported in literature. These analyses revealed unanswered questions which were addressed in subsequent animal and human studies. We hypothesized that a combination of conditioning protocols could increase the efficacy of IPC and used a previously successful renal IPC rat model available in our group to test this hypothesis. This specific rat model was meant to be converted into a transplantation model in future studies. It was decided to diminish the amount of renal ischemia-reperfusion injury (IRI) to better represent the clinical situation. A systematic review and meta-analysis of human studies was performed to select the proper patient population and the proper outcomes before designing a clinical trial. Results of this analysis were disappointing, not providing clear guidance for a future successful clinical trial. We subsequently selected reduction of contrast induced nephropathy (CIN) as a model to clinically study IPC, although to date the mechanism of IRI in CIN is not fully understood. Patients prone to CIN are ideal to study clinical benefit of IPC. It comprises a single intervention related to renal IRI, and the magnitude (the amount of iodine contrast) and timing of IRI can be determined rather precisely. We expanded the indication from high risk patients, as previous studies did, to all patients requiring renal protection from CIN according to our national guidelines. Results revealed no significant protective effect. Subgroup analysis, however, indicated that patients at very high risk of CIN allocated to the remote ischemic preconditioning (RIPC) group had significantly lower serum creatinine levels after contrast administration.

There are two main explanations for the lack of translation in the studies presented in this thesis: the differences between animals and patients and methodological issues. There are many differences between animals and patients included in experimental and human studies respectively. For instance, animal studies usually include young adolescent, healthy animals, whereas patients are typically elderly with medication and comorbidities. In the literature there is evidence that ageing, medication use and comorbid conditions can result in a smaller protective effect of IPC [1]. To compensate for the differences between patients and animal models, a limited number of animal studies have included aged animals [2–4], animals with comorbidities [5, 6] or medication [7] and these studies confirm that IPC does indeed have a smaller protective effect in these animals. Within translational medicine [8], another explanation for the problem of translation of IPC from animal models to clinical trials is related to sex differences. Specifically, male animals, used in the majority of studies, are better protected from renal IRI by RIPC when compared to female animals (Chapter 2). In theory, female patients included in clinical trials may have skewed the overall protective effect of RIPC. There is,

however, no physiological explanation for the difference between sexes in reducing renal IRI by RIPC to date. Besides sex and comorbidity, Chapter 2 showed that the protective effects of IPC were larger for the smallest animals, *i.e.* mice, compared to rats, dogs and pigs. Although no strong experimental evidence is available, it has been suggested that a higher metabolic rate in smaller animals could result in a larger effect of IPC [9]. This might explain why humans, having a slower metabolic rate compared to mice, benefit less from the protective effects of IPC.

In addition to the differences between humans and animals, methodological choices made by researchers are also likely to contribute to translational difficulties. An important issue in this respect is the relatively small amount of renal damage inflicted in clinical trials when compared to animal studies. This difference is reflected in a much higher rise in serum creatinine levels in animal models when compared to the amount of renal injury that is observed after for example major cardiovascular surgery. The differences in serum creatinine rise indicate that the amount of renal injury in animal models does not necessarily represent clinical practice. Another methodological issue is related to the use of anesthetics, which can have strong protective effects against (renal) IRI. Anesthetics are mandatory in animal experiments and interfere with the amount of renal IRI inflicted in the experiment. It has also been shown that anesthetics can interfere with the protective effects of RIPC [10]. For animal experiments regarding RIPC, the ideal anesthetic has no influence on RIPC or IRI. Currently, little evidence indicates that barbiturates have little or no influence on RIPC and IRI, and therefore, could be the anesthetic of choice for future animal experiments [11]. The protective effects of anesthetics have also been described in human trials [12, 13]. The use of these agents in clinical trials investigating the effectiveness of RIPC may partially explain the discrepancies between animal and human studies.

The interval between RIPC and index ischemia is often well defined in animal studies and crucial for its efficacy. The meta-analysis of Chapter 2 demonstrated that IPC in the second window of protection provided better protection against renal IRI in animal studies when compared to the first window of protection. In human studies the efficacy of RIPC in the second window of protection has not been established yet. Another, more recent and extensive meta-analysis on cardiac IPC in animal models [14] revealed that there is an interval between the first and second window of protection in which IPC is less effective, ranging from 45 minutes to four hours prior to the index ischemia. Although this 'ineffective' window of protection may also exist for RIPC in models of renal IRI, this could not be confirmed in our meta-analysis of animal studies reducing renal IRI by IPC due to insufficient data for this timeframe. An ineffective window may

also have played a role in our randomized trial, where the mean interval between RIPC and contrast administration was 46 minutes. Therefore, a substantial number of patients were possibly preconditioned in the ineffective window, providing a possible explanation for the fact that RIPC did not significantly affect the primary endpoint in this study. This can be considered a design error that future researchers should take into account. Our human meta-analysis could not show the existence of an ineffective window because the interval between IPC and index ischemia was not well defined in most trials. IPC is usually started directly after induction of anesthesia. As induction is not the start of renal IRI, the exact timing of IRI remains unclear, which means that the RIPC stimulus could fall within the ineffective window of protection and diminish the effectiveness of RIPC in those clinical trials [15, 16].

Future perspectives

This thesis supports the need for future animal experiments to further explore the mechanism of IPC and optimization of the IPC stimulus. Such experiments for example could help to provide insight into the ineffective window of protection of RIPC in reducing renal IRI.

With regard to future human studies, this thesis supports further exploration of RIPC as an adjunct to standard measures in the prevention of CIN. Support for further studies comes from a recent meta-analysis of five randomized clinical trials showing that RIPC as adjunct to saline is highly effective in reducing the incidence of CIN [17]. When future studies confirm the results of this meta-analysis RIPC as adjunct to saline hydration for patients at high-risk of CIN, RIPC should be incorporated in (inter)national guidelines for the prevention of CIN. Further optimization of CIN prevention will probably replace the saline hydration by the hydration with sodium bicarbonate as it has been reported that hydration with one hour sodium bicarbonate, and hydration with four hours administration of saline before and after contrast administration are equally effective [18–20]. As one-hour hydration with sodium bicarbonate does not require hospital admission, it is a highly attractive strategy to reduce the burden for patients and healthcare costs. Therefore, a future trial should establish whether RIPC as adjunct to one-hour sodium bicarbonate provides similar protection in patients with the highest risk of CIN when compared to standard hydration with saline.

In conclusion, a wide range of factors influences successful translation of IPC from animal studies to clinical trials. This thesis has contributed to the knowledge on factors that influence translation of IPC to reduce renal IRI. This knowledge should be taken into

account when designing future animal studies and clinical trials. In future animal studies, the influence of anesthesia on the efficacy of RIPC should be elucidated by comparing the efficacy of RIPC under different types of anesthetic protocols. A future clinical trial should study the efficacy of RIPC as adjunct to sodium bicarbonate hydration in patients at high risk of CIN.

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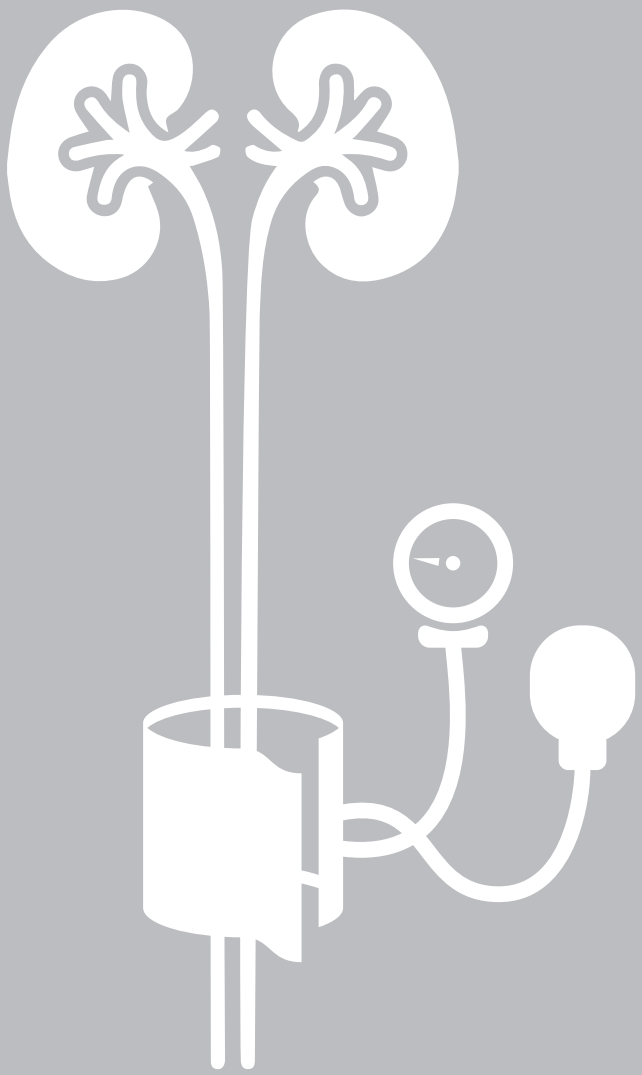

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Summary

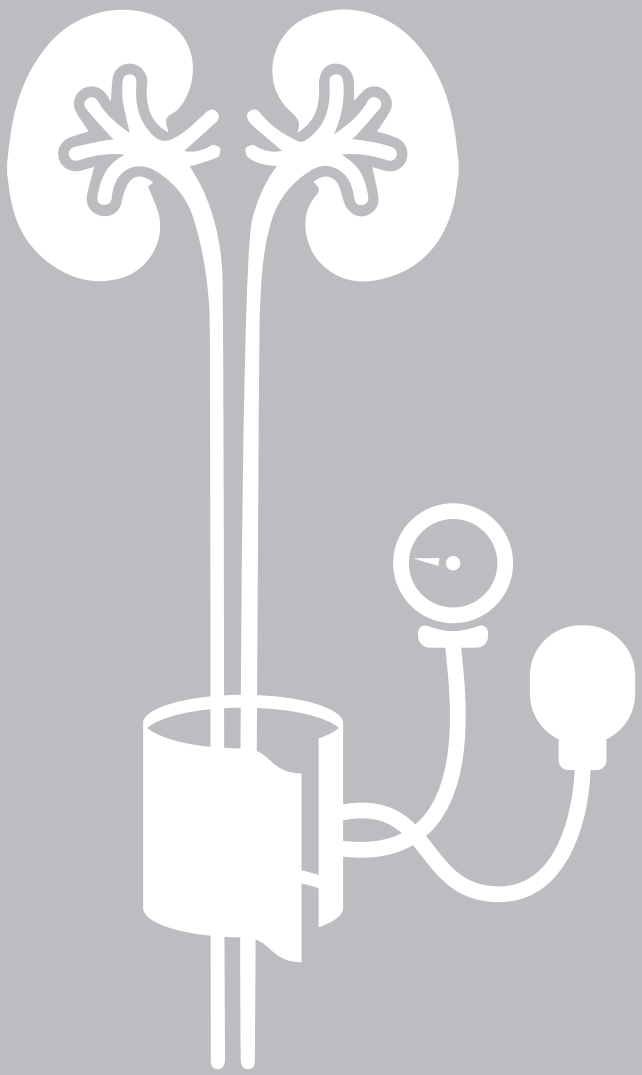
This thesis has explored factors that are important for the efficacy of ischemic preconditioning (IPC) to reduce renal ischemia reperfusion injury (IRI) and has presented difficulties and possible solutions that arise from the translation of preconditioning protocols in animals to patients.

Chapter 1 provides the current knowledge of the pathophysiology of IRI and working mechanisms of all types of ischemic preconditioning (IPC). Although a large quantity of (pre)clinical data was present regarding the efficacy of IPC, the translation from effective animal studies to clinical practice is still not evident. New animal experiments and human trials are necessary to fill the knowledge gaps, however these studies have to be performed after a systematic review and meta-analysis is done to identify those gaps. Therefore, **Chapter 2** gives a systematic review and meta-analysis of IPC in animal studies, providing evidence that both local ischemic preconditioning (LIPC) and remote ischemic preconditioning (RIPC) are highly effective in reducing serum creatinine levels after IRI. It also demonstrated that IPC stimuli in the late window of protection were more effective in reducing renal IRI than stimuli applied in the early window of protection. In **Chapter 3** a systematic review and meta-analysis is presented on local ischemic postconditioning (LIPostC) and remote ischemic postconditioning (RIPostC) in animal studies. Results showed that both LIPostC and RIPostC are effective in reducing renal IRI, as reflected by lower serum creatinine levels, blood urea nitrogen levels and renal histological damage scores. A subgroup analysis indicated that the efficacy of IPostC increased with the duration of index ischemia.

To fill important gaps in our knowledge regarding the most effective IPC protocol, we conducted two animal experiments in a rat model of renal IRI. In **Chapter 4** an experiment in which rats underwent a combination of RIPostC and LIPostC directly after renal IRI is described. Unexpectedly, renal injury was not significantly reduced by RIPostC or LIPostC alone. However, the combined application of RIPostC and LIPostC significantly reduced renal injury, indicating that local and remote IPostC act synergistically. Another gap in the current knowledge is related to the question whether the efficacy of RIPC in reducing renal IRI could be increased by repeating the RIPC stimulus. In **Chapter 5** a RIPreC protocol was evaluated in which RIPC was repeated daily for seven days prior to renal IRI. Daily RIPC was applied in rats under isoflurane anesthesia. This experiment did not show an additive protective effect of repeating the preconditioning stimulus. However, a control group receiving daily administration of isoflurane anesthesia, showed that repeated anesthetic preconditioning (APC) was highly effective in reducing renal IRI. The phenomenon of APC is well known; however, repeated administration of isoflurane protected against renal IRI to a much larger extent than anticipated.

Chapter 6 a systematic review and meta-analysis of IPC for the reduction of renal IRI in patients undergoing cardiac or vascular surgery is provided. It included twenty-eight clinical studies investigating the effect of RIPC on renal function in patients undergoing surgery associated with renal IRI. No protective effect of RIPC was found on the primary outcome measure: serum creatinine levels in the early postoperative phase. In **Chapter 7** the methods of a randomized controlled trial were described. In this clinical trial, **Chapter 8**, RIPC was applied as an adjunct to standard protective measures (i.e. hydration with intravenous saline) in patients who were at risk of contrast induced nephropathy (CIN). RIPC did not reduce contrast induced renal injury in patients undergoing diagnostic or therapeutic intravascular contrast procedures. However, a subgroup analysis indicated that patients at very high risk of CIN (Mehran score ≥11) allocated to the RIPC group had significantly lower serum creatinine levels 48–72 hours after contrast administration. In **Chapter 9** we presented our view on the current literature and expected future developments in the field of IPC.

In conclusion, both IPC and IPostC are highly effective in reducing renal IRI in animal studies. The effectiveness of IPostC may be improved by combining local and remote postconditioning. Repeating RIPC does not provide additive protection, where the repeated administration of isoflurane anesthesia provides strong protection against renal IRI. Available human data do not confirm the efficacy of RIPC in reducing renal IRI in patients undergoing major cardiac or vascular surgery. However, RIPC as adjunct to standard measures to prevent CIN might be effective in patients who are at high risk of CIN.



Appendices

Nederlandse samenvatting
Dankwoord
Curriculum Vitae
List of publications
RIHS PhD portfolio

NEDERLANDSE SAMENVATTING

Nierschade kan op verschillende manieren ontstaan en kan variëren van een tijdelijk, subklinisch nierfunctieverlies tot blijvend nierfalen. In het kader van dit proefschrift zijn wij geïnteresseerd in de nierschade die optreedt door een tijdelijke, ontoereikende doorbloeding. Dit wordt ook wel ischemie reperfusie-schade (IRI) van de nier genoemd en kan optreden bijvoorbeeld na grote hart- en vaatoperaties, niertransplantaties, sepsis en na het toedienen van jodiumhoudende contrastvloeistof voor radiologisch onderzoek en/of behandeling. De huidige methoden om IRI te verminderen, namelijk het verkorten van de duur van de schade of het koelen van de nier, zijn beperkt toepasbaar en veelal onvoldoende effectief.

Een nieuwe manier om IRI te beperken is enkele malen kortdurend de doorbloeding van de nier te blokkeren; dit heet ischemische preconditionering (IPC). De nier wordt als het ware voorbereid op een periode met onvoldoende bloedvoorziening. Uit onderzoek blijkt ook dat het onderbreken van de doorbloeding van een ander orgaan of lichaamsdeel de IRI van de nier reduceert; dit heet ischemische preconditionering op afstand (RIPC). Een veel gebruikte methode hiervoor is een bloeddrukband om een arm drie á vier keer gedurende vijf minuten op te blazen. Als het moment van IRI niet te voorspellen is, bijvoorbeeld bij een hart- of een herseninfarct is ischemische preconditionering niet mogelijk. Het beschermende effect van het kortdurend stoppen van de doorbloeding werkt echter ook wanneer dit wordt toegepast direct na de periode van ischemie; dit heet ischemische postconditionering (IPostC). Ook hiervoor geldt dat eenzelfde bescherming kan worden bereikt wanneer een ander orgaan dan de nier wordt gebruikt voor de conditionering; ischemische postconditionering op afstand (RIPostC).

In experimentele diermodellen zijn veel positieve resultaten van bescherming van de nierfunctie door conditionering beschreven, bij patiënten zijn de effecten vaak minder duidelijk. In dit proefschrift hebben wij onderzoek gedaan naar de effectiviteit van IPC in dierstudies en in patiëntenstudies. Daarnaast hebben we ons gericht op het optimaliseren van de IPC-methode.

Hoofdstuk 1 beschrijft het probleem van IRI, hoe dit ontstaat en welke processen hieraan ten grondslag liggen. Hierbij wordt ook duidelijk hoe het concept van IPC is ontstaan en hoe dit idee is vormgegeven in veel verschillende methoden om met IPC orgaanschade te verminderen. Een aantal cruciale stappen van IPC is bekend, maar het exacte werkingsmechanisme van IPC is nog niet opgehelderd. Ook de optimale toediening van een IPC-stimulus is nog onbekend.

In **hoofdstuk 2** werden alle dierstudies waarin het effect van IRI door (R)IPC was onderzocht systematisch geanalyseerd op de mate van effect en factoren die mogelijk het effect bepaalden. Door middel van een systematische zoekstrategie werden 523 studies gevonden. Hiervan waren 58 studies bruikbaar voor een meta-analyse. Het bleek dat (R)IPC een beschermend effect had op de nierfunctie gemeten aan de hand van het serum ureum en creatinine. Dit effect was ook zichtbaar bij microscopische analyse van nierschade. Bij subgroep analyse bleek dat muizen meer beschermd worden dan ratten en dat een interval van meer dan 24 uur tussen de IPC-stimulus en de IRI effectiever is dan een korter interval. Het bleek dat zowel IPC als RIPC effectief zijn en er was geen verschil in de mate van effectiviteit.

In **hoofdstuk 3** wordt een systematische analyse van dierstudies beschreven op het gebied van (R)IPostC. We vonden 39 artikelen geschikt voor meta-analyse, in vier studies werd RIPostC onderzocht, in de overige studies IPostC. Zowel IPostC als RIPostC hebben een beschermende werking op de nierfunctie op basis van het serum creatinine en ureum, en de histologie. Het beschermende effect is groter naarmate de periode van IRI langer is.

Voor zowel de meta-analyse in hoofdstuk 2 als in hoofdstuk 3 geldt dat de beschrijving van de methoden in de geïncludeerde artikelen matig was. Ook zijn subgroepen die belangrijk zijn voor translatie van uitkomsten naar de klinische praktijk, zoals vrouwelijke proefdieren en dieren met comorbiditeit, ondervertegenwoordigd in de studies.

In **hoofdstuk 4** hebben we de combinatie van IPostC en RIPostC in een rattenmodel onderzocht. In dit model werd nierschade veroorzaakt door de niervaten gedurende 25 minuten af te klemmen. IPostC werd uitgevoerd door zes keer acht seconden de niervaten af te klemmen direct na het langdurig klemmen. RIPostC werd in dit experiment uitgevoerd door het opblazen van bloeddrukbandjes om beide achterpoten van de rat gedurende drie keer vijf minuten. Het serum creatinine en ureum, de natrium uitscheiding en de histologie werden gebruikt als parameters voor optreden van nierschade. In de dieren die alleen IPostC ondergingen werd geen beschermend effect aangetoond in vergelijking met de controle ratten. Voor de dieren die alleen RIPostC ondergingen werd alleen een significant effect in de natrium uitscheiding gevonden. De combinatie van IPostC en RIPostC had een beschermend effect op de nierfunctie en niermorfologie. Op basis van deze resultaten concluderen we dat de toepassing van IPostC lokaal en op afstand elkaars effect versterken. Verder onderzoek moet uitwijzen of de resultaten reproduceerbaar zijn en kunnen worden toegepast bij patiënten.

Eén van de uitkomsten van de meta-analyse beschreven in hoofdstuk 2 was de hogere effectiviteit bij proefdieren waarbij het interval tussen de IPC-stimulus en IRI lang was (meer dan 24 uur). Mede hierdoor ontstond de hypothese dat een herhaalde RIPC-stimulus (RepRIPC), zowel korte als langere tijd voor IRI, effectiever is dan een enkele RIPC-stimulus. Deze hypothese werd getest in de dierstudie die is beschreven in **Hoofdstuk 5**. In een aaneengesloten periode van zeven dagen werd iedere dag een IPC-stimulus toegediend van drie keer vijf minuten door het afknellen van beide achterpoten, waarna ischemie van 30 minuten werd toegediend door tijdelijk een vaatklem op de bloedvaten van de nier te zetten. IPC en IRI vonden plaats onder algehele narcose met isofluraan. Om het effect van IRI, IPC en narcose goed van elkaar te kunnen onderscheiden werden in totaal 6 groepen onderzocht: Sham (hier werd alleen een laparotomie verricht, controle (met alleen nierschade), herhaalde narcose (RepISO), RIPC, RepISO+RIPC en RepISO+RepRIPC. De vier laatste groepen (interventie-groepen) gaven in vergelijking met de controlegroep een vergelijkbare bescherming tegen IRI van de nier op basis van lagere serum creatinine waarden ten opzichte van de controlegroep. In deze studie gaf herhaalde RIPC geen extra bescherming ten opzichte van eenmalige RIPC. Het beschermende effect van herhaalde isofluraan-anesthesie was groter dan voorzien. Bij toekomstige experimenten zal met het beschermende effect van isofluraan en eventueel ook andere anesthetica rekening gehouden moeten worden. Het is noodzakelijk om de beschermende effecten van verschillende narcosemiddelen verder te onderzoeken, enerzijds om het effect van (R)IPC beter te kunnen onderzoeken, anderzijds om te beoordelen in welke mate narcosemiddelen nierschade kunnen verminderen.

In **Hoofdstuk 6** worden resultaten beschreven van een systematische review en meta-analyse van gerandomiseerde klinische studies waarin het klinisch effect en de veiligheid van (R)IPC werd onderzocht. Het betrof studies bij patiënten die een operatie ondergingen waarbij nierschade kan ontstaan, zoals hartoperaties, open aorta aneurysma operaties en nieroperaties. 28 artikelen werden geïncludeerd met in totaal 6851 patiënten. De studies waren over het algemeen van goede kwaliteit. Slechts bij vier patiënten werden ernstige bijwerkingen van RIPC gezien, dit waren patiënten bij wie met een vaatklem de slagader naar het been werd afgesloten. RIPC door een bloeddrukband liet geen relevante bijwerkingen zien. Serum creatinine op dag 1, 2 en 3 na de operatie zijn niet significant verschillend tussen RIPC en de controle groep. Ook de secundaire uitkomstmaten: dialysebehoefte, opname duur en ziekenhuissterfte, zijn niet significant verschillend. RIPC geeft mogelijk wel een iets lagere kans op nierschade zoals gedefinieerd door de AKIN of RIFLE criteria.

Ondanks het gebruik van hydratieprotocollen om de nieren te beschermen tegen intraveneus jodiumcontrast komt contrastgeïnduceerde nierschade (CIN) regelmatig voor.

Onderzoek laat zien dat CIN voor een deel wordt veroorzaakt door IRI. Op basis hiervan zou de incidentie van CIN kunnen worden gereduceerd door RIPC. Er zijn enkele studies gepubliceerd met een gunstig effect van RIPC op nierschade bij jodiumcontrasttoediening. In **Hoofdstuk 7** is het protocol opgenomen van de studie waarvan de resultaten in **Hoofdstuk 8** worden beschreven. Er werden 76 patiënten gerandomiseerd in een controle groep en een RIPC-groep. RIPC werd uitgevoerd door een bloeddrukband tot een druk van 50 mmHg boven de systolische bloeddruk op te blazen. De band werd vier keer gedurende vijf minuten opgeblazen binnen 45 minuten voor de contrasttoediening. In beide groepen werd standaard hydratie met fysiologisch zout gehanteerd. Er was geen verschil tussen beide groepen in de primaire uitkomstmaat, het serum creatinine. Ook de secundaire uitkomstmaten, incidentie van CIN, heropname in het ziekenhuis, dialyse en sterfte verschilden niet. Wel was er een beschermend effect van RIPC op de nierfunctie in de subgroep met het hoogste risico op nierschade. Het toevoegen van RIPC aan een hydratieprotocol met fysiologisch zout voorafgaand aan het toedienen van een jodiumhoudend contrastmiddel is mogelijk zinvol bij patiënten voorafgaand aan contrasttoediening die een hoog á priori risico hebben op het ontwikkelen van nierschade.

In **Hoofdstuk 9** worden de studies in een bredere context bediscussieerd en vergeleken met huidige literatuur. Ook worden voorstellen gedaan voor vervolgonderzoek. Hoofdstuk 10 bevat de Engelse versie van deze samenvatting.

DANKWOORD

Dit proefschrift was er nooit gekomen zonder hulp van velen. Ieder die hieraan heeft bijgedragen wil ik bedanken en een aantal mensen in het bijzonder.

Geachte prof. van Goor, beste Harry,

Bijna kon ik een fulltime promotietraject bij je beginnen op het gebied van adhesies. Voor het onderwerp van dit manuscript was mijn belangstelling echter groter. Mooi dat ik nu toch bij je mag promoveren. Dank voor alle kansen die je me gegeven hebt, ook buiten het onderzoek, bij de chirurgie en het simulatieonderwijs. Je open, kritische blik naar alles wat er om je heen gebeurt en de drang om dat te verbeteren is een grote inspiratiebron voor mij.

Beste Michiel,

Jij bent de inspirator achter ischemische preconditionering. Jouw bevlogenheid en enthousiasme zorgden dat we altijd met honderd nieuwe plannen uit een overleg kwamen, eigenlijk is het boekje nog lang niet af. Hopelijk kunnen we ook in de toekomst nog een paar van die plannen verwezenlijken.

Beste Kim,

Een 'die hard' bioloog in de chirurgenwereld, ik heb er altijd van genoten en ik heb er veel van geleerd. Wat fijn als iemand zo met onderzoek omgaat als jij. De tijd in het dierenlab was onvergetelijk.

Geachte leden van de manuscriptcommissie, prof. dr. N.P. Riksen, prof. dr. W.J. Morshuis, prof. dr. J.N.M. IJzermans,

Veel dank voor uw kritische beoordeling van mijn proefschrift.

Lieve Dora en Joep, paranimfen,

Al tientallen jaren staan jullie mij bij, mooi dat dat nu ook zo is.

Coauteurs; M. Rovers, J.A. van der Vliet, G.A. Rongen, R. Masereeuw, M. Ritskes-Hoitinga, C.R. Hooijmans, S.J. Jongker, M. Ergun, M.H.D. Bruintjes, R.M.L.M. Lomme, D.M.D. Ozdemir van Brunschot, Y. de Waal, R. Donders, M.S. Lemson, J.F. Wetzels, L.J. SchulzeKool, T.B. Sterrenborg,

Bedankt voor jullie harde werk, kritische blik en enorme hoeveelheid kennis van zaken.

Beste medewerkers van het dierenlab; SPF unit, beste Daphne, Denise, Linda,

Of het nu weekend of avond was, de dierexperimenten moesten doorgaan. We hebben veel plezier gehad in het lab.

Beste chirurgen Slingeland Ziekenhuis,

Van jullie heb ik geleerd een goede dokter te zijn en hoe je opereert. Bedankt voor de mogelijkheden die jullie mij gegeven hebben. Naar het Slingeland gaan voelt nog steeds als thuiskomen.

Beste chirurgen Radboud UMC,

Nevelsteen, niertransplantatie of multi-orgaan donatie, niets was te gek. Ook het geluid van de traumahelikopter geeft nog altijd een kick. Ik heb het naar mijn zin gehad en heb veel mogen leren.

Beste chirurgen Rijnstate Ziekenhuis,

Bij jullie mag ik vaatchirurg worden, hoe geweldig is dat! Nog bijna twee prachtige jaren voor de boeg, om alles van jullie te mogen leren.

Collega's van het Slingeland, Radboud en Rijnstate,

Hard werken en zoveel meer: Cash, Vaatdagen, chirurgencup, cabaret, chirurgendagen, skiën, assistentenuitje, Aesculaaf, St. Anneke, Nescio of Ruimzicht. Bedankt voor de collegialiteit en gezelligheid. Waar en wanneer is onbekend, maar we komen elkaar weer tegen.

Beste prof. dr. R.M.H. Wijnen, dr. T.J. Blokhuis en dr. M. van Deuren,

Mogelijk weten jullie niet meer wie ik ben. Mijn drang om onderzoek te doen begon al vroeg in de geneeskundeopleiding, maar was toen nog minder doelgericht. Ik ben niet gepromoveerd op congenitale hernia diafragmatica, botgenezing bij osteoporose of het complementsysteem. Toch hebben jullie mij warm gemaakt voor het onderzoek, bedankt hiervoor.

Beste Wout en Mark,

Nooit had ik oudere broers, maar in jullie heb ik die toch gevonden. Jullie pad op weg naar chirurg-zijn, heeft mede mijn pad bepaald. Iets afspreken met onze vrouwen en steeds meer kinderen blijft vreselijk moeilijk en steevast komt de datumplanner zonder

geschikte datum, maar zowel in het vak als met datumplanners blijven we doorgaan tot succes bereikt is.

Beste Bart,

Via onze vrouwen leerden we elkaar kennen, er was eindelijk iemand in Nijmegen die niet in de medische wereld zit, fijn om te borrelen en niet over het ziekenhuis te praten. Hopelijk kan ik nu het wielrennen oppakken.

Bram, Joep, John en Pieter; 'De Mannen',

Al meer dan 20 jaar Echte Vrienden! Wat had ik jullie nodig; er is niets beter dan samen in Kroatië te zeilen of samen een biertje doen. Met z'n vijven weten en kunnen we alles.

Familie van Werven, de koude kant,

Jullie zijn echt familie geworden. Weekendje Valencia, city-run of diesfeest bij het DSC, maar vooral zeilen op Loosdrecht is zoveel waard.

Dora,

Lieve zus. Leuk dat we, nu er kindjes zijn, elkaar weer zo veel vaker zien. Samen de wereld analyseren en belachelijk maken.

Pa en Ma,

'Wanneer is dat onderzoekje eens af…', ik heb het vaak moeten horen. Zonder jullie geduld de afgelopen 34 jaar was het niet gelukt. Dank voor jullie enthousiasme, jullie stimulerende woorden.

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Wat hebben wij de afgelopen 14 jaar veel moois meegemaakt: de zeeën bezeild, Mount Kilimanjaro beklommen, een huis verbouwd. Het begon allemaal met een ijsje eten. Laten samen we nog veel ijsjes eten, dan kunnen we elke uitdaging aan. Jij bent de beste.

CURRICULUM VITAE

Theo P. Menting was born the 6th of April 1983 in Gorredijk, Friesland. At a young age he was already intrigued by nature, animals, plants and the whole world around him. After moving to the south of the Netherlands, Vessem, and graduating high school at Sondervick College, Veldhoven (VWO), he wanted to explore the fundamentals of nature by studying applied physics at the Technical University of Eindhoven.

After one year he decided his true fascination was medicine, the human body, and switched to the Radboud University Nijmegen. He completely merged into student life and became a fanatic rower (NSRV Phocas) and president of the medical students association (MFVN). Theo's exploring nature made him join the student exchange project IFMSA for two months to G. Alvaro hospital, Santos, Brazil and one month to Hospital Bezmialem Vakif, Istanbul, Turkey. The last three months of his internship were situated in Biharamulo Hospital, Tanzania.

After obtaining his medical degree he started working as a surgical resident in Slingeland Hospital Doetinchem and developed his passion for surgery. Subsequently he worked as an ANIOS at the Radboud University Medical Centre, Nijmegen where he started his PhD project. In 2012 he started his surgical training at the Slingeland Hospital, Doetinchem (dr. F.M. van Lammeren, dr. M.S. Lemson) and later on at the Radboud University Medical Centre, Nijmegen (dr. B. Verhoeven) again. Since 2017 he is specializing in vascular surgery at Rijnstate Hospital, Arnhem (dr. J.W. Lardenoije) and will do so for two more years.

Theo lives in Arnhem with his wife Hannah, their daughter Linde (2014) and son Dorus (2016). Next to his passion for surgery, he loves to sail.

LIST OF PUBLICATIONS

Menting TP, Wever KE, Ozdemir van Brunschot DMD, van der Vliet DJA, Rovers MM, Warlé MC. Ischaemic preconditioning for the reduction of renal ischaemia reperfusion injury. Cochrane Database Syst Rev. 2017 Mar 4;3.

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Menting TP, Tan ECTH. Overreden door vrachtwagen, Thiersch plastiek biedt uitkomst. NtvTraumatologie. 2012;22(6):151-3.

Menting TP, Staal JF. A girl with a painful hip after a splits. NtvGeneeskunde. Ntv-Geneeskunde. 2012;156(21):A3320.

RIHS PHD PORTFOLIO

Name PhD student: T.P. (Theo) Menting
Department: surgery
Research School: Radboudumc Institute for Health Sciences
PhD period: 2011–2017
Promotor: prof. dr. H. van Goor
Co-promotors: dr. M.C. Warlé and dr. K.E. Wever
Mentor: prof. dr. G.A.P.J.M. Rongen

Training activities	EC points
Courses and Workshops	
Cochrane workshop, AMC Amsterdam, 2012	0.8
PhD training course Hartstichting, Papendal, 2015	1.75
EVAR for dummies, Amsterdam, Medtronic, 2014	0.8
Endovascular surgery, JBZ, 's Hertogenbosch, 2015	0.8
Workshop academic writing, Nijmegen, 2016	0.25
Private course academic writing, Amsterdam, 2016	1.75
Themabijeenkomst Vascular Damage, oral presentation, 2012, 2013	2
How to write a veni grant, RIMLS, 2015	0.25
Symposium IRI, 9-4-2015	0.25
EVC Maastricht, 2016	0.25
Symposia and congresses	
Chirurgendagen NVvH, Veldhoven, 2012–2016	0.5
Najaarsvergadering NVvH, 2012–2016	0.5
Najaarsvergadering NVvV, oral presentation	0.25
Vaatdagen, oral presentation, 2012	0.25
Vaatdagen, 2013–2016	0.25
Course Damage control surgery, 2015	0.2
Cash vascular surgery	0.2
TTS, Berlin, 2x poster presentation, 2012	1
ESSR, Lille, 2x oral presentation, 2012	1
Morbidity and mortality conference, 2015–2016	1
Multidisciplinairy complication meeting, 2015–2016	1
Vascular surgery paper review talks, 2015–2016	1
Vascular rounds, oral presentation, Huntinglodge Rozendaal, 2012–2016	0.8
Proefdiercursus en stralingscursus, 2011	5.25
Other	
Principal Udcd member	4
Teaching activities	
Student meets patient, Nijmegen, 2016	0.5
Vascular anastomosis training on cadaver pigs, 2014	0.4
Cash Vascular surgery	0.7
Human patient simulator training, Radboud University, 2012–2013	5
Total EC points	32.7