SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Ischemic postconditioning of the kidney – a systematic review of animal studies	
2.	Authors (names, affiliations, contributions)	• S.J. Jonker† – Design search strategy, in- and exclusion, data extraction, data-analysis, quality and risk of bias assessment, writing paper • T.P. Menting‡ - data extraction, quality and risk of bias assessment • prof.dr. M. Ritskes-Hoitinga† – Critical revision of manuscript • dr. K.E. Wever† – Overall supervision, design search strategy, in- and exclusion, data-analysis, supervising research, writing paper † Department of SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE), Radboudumc, Nijmegen ‡ Department of Surgery, Radboudumc, Nijmegen	
3.	Other contributors (names, affiliations, contributions)	A. Tillema, Medical library Radboud University, Nijmegen, Design search strategy	
4.	Contact person + e-mail address	K.E. Wever, Kim.Wever@radboudumc.nl	
5.	Funding sources/sponsors	None	
6.	Conflicts of interest	None	
7.	Date and location of protocol registration	12-02-2015 Nijmegen	
8.	Registration number (if applicable)	NA	
9.	Stage of review at time of registration	Search conducted, study screening by title and abstract completed, full-text inclusion ongoing.	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	The application of a brief period of ischemia and reperfusion (I/R) after a prolonged episode of ischemia, so-called ischemic postconditioning (IPoC), is a protective strategy against ischemia reperfusion injury (IRI). The conditioning stimulus has been shown to be effective when applied either to the target itself (local IPoC; LIPoC) or to a remote organ or tissue (remote IPoC; RIPoC)[1,2]. Since their first description, both LIPoC and RIPoC have been successfully reproduced in a variety of animal species, using various organs, e.g. heart, brain and kidney[3]. Thus, IPoC poses a promising alternative to existing treatments for IRI in humans, since current strategies to reduce this important and common clinical problem are inadequate.	

		The kidney is one of the major organs of interest for clinical application of IPoC, since renal IRI is a major cause of kidney injury in *e.g.* renal transplantation[4]. Even though the protective effect of LIPoC and RIPoC on renal IRI has been shown in animal studies, translation of IPoC to the clinic has, as yet, not been successful. It is unclear if and how factors pertaining to the IPoC protocol (e.g. timing and duration) and the animals/patients under investigation (e.g. gender, comorbidities) influence IPoC efficacy. As a result the IPoC stimulus could have been suboptimal or incorrectly applied in clinical trials, or unsuitable for the patient population. Previously, meta-analysis and systematic review of preclinical (animal) studies have been used to optimize experimental animal models and to improve the design of clinical trials[5-7]. Performing a systematic review of animal studies on IPoC of the kidney provides a detailed, systematic overview of current knowledge on this topic, as well as an assessment of the quality of preclinical research in this field. In addition, meta-analysis allows us to synthesize novel data on the influence of variables on treatment efficacy, such as IPoC timing and duration. Combined, the outcome of this project can be used to optimize animal models and improve the design of future clinical trials. References: 1. Zhao ZQ, Corvera JS, Halkos ME, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol 2003; 285: H579. 2. Kerendi F, Kin H, Halkos ME, et al. Remote postconditioning. Brief renal ischemia and reperfusion applied before coronary artery reperfusion reduces myocardial infarct size via endogenous activation of adenosine receptors. Basic Research in Cardiology. 2005;100(5):404-412 3. Zhao ZQ. Postconditioning in reperfusion injury: a status report. Cardiovasc Drugs Ther 2010; 24: 265. 4. Van den Akker EK, Manintveld OC, Hesselink DA, et al. Protection Against Renal Ischemia-Reperfusion Injury by Ischemic Postconditioning. Transplantation Journal. 2013; 95(11):1299-1305. 5. Van der Worp HB, Macleod MR, Kollmar R. Therapeutic hypothermia for acute ischemic stroke: ready to start large randomized trials? J Cereb Blood Flow Metab. 2010;30:1079-1093. 6. Van der Worp HB, Sena ES, Donnan GA, et al. Hypothermia in animal models of acute ischaemic stroke: a systematic review and meta-analysis. Brain. 2007;130:3063-3074. 7. Pound P, Ebrahim S, Sandercock P, et al. Where is the evidence that animal research benefits humans? BMJ. 2004;328:514-517.	
	Research question		
11.	Specify the disease/health problem of interest	Renal ischemia reperfusion injury	
12.	Specify the population/species studied	Animals	
13.	Specify the intervention/exposure	Ischemic postconditioning	
14.	Specify the control population	No ischemic postconditioning, sham surgery	

15.	Specify the outcome measures	Kidney injury and renal function	
16.	State your research question (based on items 11-15)	1. What is the effect of ischemic postconditioning on kidney injury and function in animals subjected to renal ischemia reperfusion injury? 2. How do different factors related to animal characteristics, the postconditioning protocol and study quality influence treatment efficacy?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	☒ MEDLINE via PubMed ☐Web of Science ☐SCOPUS ☒ EMBASE ☐Other, namely: ☐Specific journal(s), namely:	
18.	Define electronic search strategies (*e.g.* use the **{**HYPERLINK "http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3265183/pdf/LA-11-087.pdf"**}**and animal search filters [**{**HYPERLINK "http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3104815/pdf/LA-09-117.pdf"**}** **{**HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/23836850"**}**])	When available, please add a supplementary file containing your search strategy: A search was performed on PubMed and EMBASE using the keywords 'kidney' and 'postconditioning'. Also in both databases, an animal search filter (**{**HYPERLINK "http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3104815/pdf/LA-09-117.pdf"**}**, **{**HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/23836850"**}**) was used. The complete search can be found in the next file:**{**HYPERLINK "file:///G:\\02%20Onderzoeksstage%20feb-april%202015\\Search\\Zoekstrategie\\PubMed%20en%20EMBASE%20Search.docx"**}**	
19.	Identify other sources for study identification	☒Reference lists of included studies ☐Books ☒Reference lists of relevant reviews ☐Conference proceedings, namely: ☒Contacting authors/ organisations, namely: in case of included abstract to retrieve original data / full publication ☐Other, namely:	
20.	Define search strategy for these other sources	1. Check each reference list from included studies for possible relevant titles which were not found by our search in PubMed and EMBASE. 2. Identify relevant reviews and check reference list for possible relevant titles which were not found by our search in PubMed and EMBASE 3. E-mail the authors in order to retrieve original data or the full publication corresponding to an included abstract	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	After removal of duplicates: 1. Prescreening based on title and abstract 2. Full text evaluation for inclusion	
22.	Specify (a) the number of reviewers per screening phase and (b) how	Two reviewers for both phases (KW and SJ). In case of discrepancies a discussion between two reviewers will	

	discrepancies will be resolved	take place to reach consensus.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: - Primary study with unique data - Presence of a control group Exclusion criteria: - Not a primary study with unique data, e.g. (systematic) reviews, editorials, comments. - No control group present, e.g. case studies	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: - All *in vivo* animals with or with or without comorbidities Exclusion criteria: - All non-*in vivo* studies: human, *in vitro* (cells/tissue), isolated kidney model, *in silico*, only use of genetically modified animals	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: - Local or remote ischemic postconditioning timed after a period of renal ischemia Exclusion criteria: - Local or remote ischemic pre- or preconditioning and non-ischemic postconditioning (e.g. pharmacological, radiation etc.).	
26.	Outcome measures	Inclusion criteria: - All outcomes related to kidney injury and kidney function (e.g. histology, serum creatinine) Exclusion criteria: - No outcomes available related to kidney injury or function	
27.	Language restrictions	Inclusion criteria: All languages Exclusion criteria: None	
28.	Publication date restrictions	Inclusion criteria: All publication dates Exclusion criteria: None	
29.	Other	Inclusion criteria: - postconditioning in bilateral or unilateral kidney models Exclusion criteria: - Co-medication and/or a co-intervention other than collateral nephrectomy (e.g. transplantation)	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: Pre-screening on title and abstract 1. Excluding non primary studies like (systematic) reviews, editorials and comments 2. Excluding all studies that are not an *in vivo* animal study in not genetically modified animals 3. Excluding all studies not reporting on the kidney 4. Excluding all studies which don't examine the effects of ischemic postconditioning. Selection phase: Full text evaluation for inclusion Same as in pre-screening phase with addition of 5. Excluding all studies with co-medication and co-	

		interventions other than collateral nephrectomy 6. Unretrievable papers	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Authors, year, journal, language	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Experimental groups, number of animals (per group)	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Species, strain, gender, age, weight, comorbidities	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	- Duration of index ischemia - Delay between index ischemia and IPoC - Duration of each IPoC ischemia/reperfusion cycle - Number of IPoC ischemia/reperfusion cycles - Remote or local IPoC - If remote IPoC: remote organ used - Duration of final renal reperfusion - Unilateral or bilateral IRI - Collateral nephrectomy Y/N	
35.	Outcome measures	- All outcomes related to kidney injury and kidney function (e.g. histology, serum creatinine) - At which time point the outcome measures were collected/measured	
36.	Other (*e.g.* drop-outs)	- Body temperature during surgery - Sample size calculation reported Y/N - Conflict of interest statement Y/N	
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	Two reviewers (SJ and TM) will assess risk of bias and study quality. In case of discrepancies a discussion between two reviewers will take place to reach consensus.	
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	☐By use of **{**HYPERLINK "http://www.biomedcentral.com/1471-2288/14/43/abstract"**}** ☒ By use of SYRCLE's Risk of Bias tool, adapted as follows: Addition of the following study quality indicators: - Body temperature regulated Y/N - Sample size calculation Y/N - Conflict of interest statement Y/N - Randomisation reported Y/N - Blinding reported Y/N ☐By use of **{**HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/15060322"**}** ☐By use of CAMARADES' study quality checklist, adapted as follows: ☐Other criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	Kidney injury: - Histology (all scoring systems); continuous; arbitrary scales Kidney function:	

		- Serum creatinine or creatinine clearance; continuous; mg/dL(/min) or umol/L)(/min) - Blood urea nitrogen; continuous; mg/dL or mmol/L	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	1. Direct extraction of data from tables, text and figures 2. Extraction from graphs using digital screen ruler 3. Contact authors by e-mail for original data if data not reported or unclear All data will be collected as mean and standard deviation (SD). Standard error of the mean will be recalculated to SD. In case the number of animals is unclear, a conservative estimate will be made. In case the data are reported as median and interquartile range, the authors will be contacted for raw data. In case an outcome was measured at multiple time points, the measurement of greatest efficacy will be chosen. In case of missing data and no author contact details, or no response from authors within 3 weeks including a reminder, the study will be omitted from analysis.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	One reviewer will extract the data, a second reviewer will check the extracted data for inconsistencies.	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	If possible, a meta-analysis will be performed for all outcome measures (serum creatinine, blood urea nitrogen, histology). If meta-analysis is not possible, data will be reported on by a descriptive summary	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	A meta-analysis will be performed if ≥4 studies report on a specific outcome measure. For subgroup analysis a minimum of 3 studies per subgroup is required.	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	Standardized mean difference for all outcome measures	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	(residual) I^2 and adjusted R^2	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	- Duration of index ischemia (linear regression) - Site of IPoC (stratified local vs remote vs both) - Animal species (stratified per species) - Gender (stratified m vs f vs mixed vs not reported) - Delay between index ischemia and IPoC (linear regression) - Number of cycles IPoC protocol (stratified per # cycles) - Total time of ischemia in IPoC protocol (stratified)	
48.	Any sensitivity analyses you propose to perform	Choose 1 specific time-point for outcome measure, instead of choosing the time-point of greatest efficacy.	
49.	Other details meta-analysis (*e.g.*	We need to perform a Holm-Bonferroni correction for	

	correction for multiple testing, correction for multiple use of control group)	multiple testing. For 7 tests, this gives a corrected p of 0,007. If one or more subgroup analyses cannot be performed due to insufficient data, the p-value will be adjusted accordingly. Also correction for multiple use of control group will be performed by dividing the number of animals in the control group by the number of comparisons performed with this control group.	
50.	The method for assessment of publication bias	Produce funnel plots and visual analysis of these plots for outcome measures containing 20+ studies. We are aware that funnel plots of SMD are susceptible to distortion and will omit the assessment of publication bias if this is suspected for our dataset. In addition, we aim to perform Egger's test for small study effects for outcome measures containing 20+ studies	
Final approval by (names, affiliations):			Date: