SYSTEMATIC REVIEW PROTOCOL

Anti-inflammatory compounds to reduce infarct size in large-animal models of myocardial infarction: A meta-analysis

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ABSTRACT

Targeting the inflammatory response after myocardial infarction (MI) could potentially prevent infarct expansion, resulting in a preservation of cardiac function. Despite extensive testing in large-animal models of MI, anti-inflammatory therapeutics are not incorporated in daily clinical practice. Methodological review of the literature describing the effects of anti-inflammatory compounds in large-animal models of MI may provide useful insights into the reasons for the translational failure from preclinical to clinical studies. Moreover, systematic review of these preclinical studies may allow us to determine which anti-inflammatory agents have the greatest potential to successfully treat MI in the clinic and guide which preclinical setting appears most appropriate to test these future treatment strategies in. The current systematic review protocol provides a detailed description of the design of this systematic review of studies investigating the effects of anti-inflammatory compounds in large-animal models of MI.

Keywords: myocardial infarction, anti-inflammatory, infarct size reduction, large animals

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Background

Cardiac damage after myocardial infarction (MI) induces a sterile immune response that leads to infarction after the initial ischaemic event.1–3 Owing to the release of pro-inflammatory mediators from the damaged myocardium post-MI, circulating cells are drawn to the infarcted tissue to clear out dead cardiac resident cells and promote infarct healing. Paradoxically, these circulating cells are known to also target viable tissue and greatly amplify the initial inflammatory response, thereby inducing infarct enlargement regardless of the application of revascularization therapies in patients with MI.4,5 Therefore, targeting the immune response could potentially prevent infarct expansion, resulting in a preservation of cardiac function and a prevention of heart failure in patients suffering from ischaemic heart disease.6

Before effectiveness of new anti-inflammatory therapeutics can be tested in clinical trials, novel compounds are commonly tested in large-animal models of MI. These large-animal models have shown to possess additive translational value due to comparable haemodynamics, similar heart size and corresponding coronary physiology.7–9 Moreover, large-animal models enable clinical treatment

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regimens, delivery route and identical function-related measurements and therefore can provide an evidence base for clinical trial design. Over the last five decades, numerous pharmacological therapies that target the inflammatory response have been tested in large-animal models of MI. Unfortunately, none of these treatments have made it past the clinical trials into daily practice.

Methodological review of the preclinical literature describing the effects of anti-inflammatory compounds in large-animal models of MI may provide useful insights into the reasons for the translational failure from preclinical to clinical studies. Systematic review of these preclinical studies may also allow us to determine which anti-inflammatory agents have the greatest potential to successfully treat MI in the clinic and guide which preclinical setting appears most appropriate to test these future treatment strategies in.

Objectives of this SR

**SPECIFY THE DISEASE/HEALTH PROBLEM OF INTEREST**

The mortality due to MI has decreased over the past 30 years. This can be mainly attributed to optimized revascularization therapy. However, MI still accounts for a large amount of cardiovascular deaths worldwide and is expected to increase again due to an increased prevalence of obesity and diabetes in the western world. Moreover, as more patients survive, the prevalence of heart failure—a direct consequence of MI—dramatically increases. These high mortality numbers, combined with the societal and economic burden of heart failure, call for improved treatment after acute MI.

There is considerable heterogeneity regarding the way MI is applied in the different studies performed during the past few decades (e.g. open/closed chest, permanent/temporary ligation). In the current systematic review we have included studies that satisfy the following definition: "An intervention that leads to permanent or temporary total occlusion of a coronary artery, disabling blood flow for a period long enough (>30 min) to induce permanent damage to the myocardium."

**SPECIFY THE POPULATION/SPECIES STUDIED**

In this systematic review we will focus on large-animal models. We specifically want to study this group of animals because the cardiac physiology and anatomy (e.g. haemodynamics, coronary anatomy) combined with the immune response post-MI is considered relatively similar to that of humans. Also, route of drug administration and treatment regimens in large-animal models allow protocols that resemble clinical treatment. In this review, we focus on four species: pigs, dogs, sheep and goats because these species are mostly used and widely applied for the validation of novel anti-inflammatory treatments of MI.

**SPECIFY THE INTERVENTION/EXPOSURE**

The intervention applied for the treatment of MI should in this case be a pharmacological treatment with an anti-inflammatory drug. Any route of drug delivery or treatment regimen of anti-inflammatory compounds (intra-muscular; intra-venous, subcutaneous, orally) is possible. Studies were included in the analyses if the interventions applied met the following criteria:

1. A compound that is Food and Drug Administration-approved for its anti-inflammatory mechanisms of action.

**AND/OR**

2. A compound that directly targets a (recently discovered) mechanism, which plays a proven role in inflammation.

In this perspective, it is possible that certain compounds may have multiple mechanisms of action and pleiotropic effects besides being anti-inflammatory. To exclusively investigate anti-inflammatory compounds, we chose to exclude interventions that have relevant and evident pleiotropic effects. Also, if the mechanism of action was unclear regardless of the effect on inflammatory parameters, these interventions were excluded. Finally, the treatment had to be solely pharmacological. According to these criteria, the following interventions were excluded from analysis:

- Stem-cells, biomaterials, pro-inflammatory compounds, statins, angiotensin converting enzyme-inhibitors, β-blockers, calcium-channel antagonists, adenosine analogues, prostacyclin analogues, l-arginine analogues, endothelin-1 analogues, thromboxane A2-antagonists, omega-3 fatty acids, gene therapy, anaesthetics, extracorporeal treatments, aspirin in dosages <6.25 mg/kg/day (anti-platelet therapy), flavonoids, flavonols and map-kinase inhibitors.

**SPECIFY THE CONTROL POPULATION**

The control population is a group of animals that receive the above-defined MI without any additional (anti-inflammatory) treatment and is preferably placebo controlled. If studies use multiple control groups, the control group that resembles the interventional group the most in terms of vehicle use and administration route was chosen.

**SPECIFY THE OUTCOME MEASURES**

**Primary outcome measures**

- Myocardial infarct size (IS) measured as a percentage of the area at risk (AAR)

**Secondary outcome measures**

1. Myocardial IS measured as a percentage of the total left ventricle (LV)
2. Left ventricular ejection fraction (LVEF)
3. Myocardial scar thinning given in millimetres or as a ratio, divided by the opposite, non-infarcted wall
4. Left ventricular end diastolic volume (LVEDV) and left ventricular end systolic volume (LVESV) in mL
5. Mortality after therapy administration

**STATE YOUR RESEARCH QUESTION**

What is the effect of anti-inflammatory compounds on mortality, IS, cardiac function and myocardial scar thinning in large-animal models of MI, when compared to placebo-treated large-animal models of MI?

**Methods**

**SEARCH AND STUDY IDENTIFICATION**

**Identify literature databases to search**

Pubmed and Embase.

**Define electronic search strategy**


AND


AND


**Embase.** “ischemic heart disease’/exp OR “myocardial infarction”/ab,ti OR “myocardial infarction”/ab,ti OR “myocardial infarct”/ab,ti OR “myocardial infarcts”/ab,ti OR “myocardial ischemia”/ab,ti OR “myocardial ischemias”/ab,ti OR “myocardial ischaemia”/ab,ti OR “myocardial ischaemias”/ab,ti OR “myocardial reperfusion”/ab,ti OR coronary occlusion”/ab,ti OR “coronary occlusions”/ab,ti OR (myocardial/ab,ti AND reperfusion/ab,ti AND injury/ab,ti)

AND

“swine’/exp OR “dog’/exp OR “sheep’/exp OR “goat’/exp OR “large model”/ab,ti OR “large animal”/ab,ti OR “swine”/ab,ti OR “pigs”/ab,ti OR “pig”/ab,ti OR “porcine”/ab,ti OR “suidae”/ab,ti OR “hog”/ab,ti OR “minipig”/ab,ti OR “minipigs”/ab,ti OR “dogs”/ab,ti OR “dog”/ab,ti OR “canis”/ab,ti OR “canine”/ab,ti OR “hound”/ab,ti OR “sheep”/ab,ti OR “ovis”/ab,ti OR “ovine”/ab,ti OR “goat”/ab,ti OR “goats”/ab,ti OR “capra”/ab,ti OR “capras”/ab,ti

AND

“anti-inflammatory agent’/exp OR “immunosuppressive agent’/exp OR “immunosuppressive agent’/exp OR “corticosteroid”/exp OR “anti-inflammatory”/ab,ti OR “anti inflammatory”/ab,ti OR “anti inflammatory”/ab,ti OR “anti-inflammatory drugs”/ab,ti OR “anti-inflammatory drug”/ab,ti OR “inflammation”/ab,ti OR “inflammation”/ab,ti OR “pro inflammatory”/ab,ti OR “immunosuppressive”:ab,ti OR “immunosuppressive”:ab,ti OR “immuno suppressive”:ab,ti OR “immuno suppressive”:ab,ti OR “immunosuppressant”:ab,ti OR “immunosuppressant”:ab,ti OR “immunosuppression”:ab,ti OR “immunosuppression”:ab,ti OR “radicals”:ab,ti OR “radicals”:ab,ti OR “radical”:ab,ti OR “metalloproteinase”:ab,ti OR “complement”:ab,ti OR “cyclosporin”:ab,ti OR “corticosteroids”:ab,ti OR “corticosteroid”:ab,ti OR “cyclooxygenase”:ab,ti OR “cox”:ab,ti OR “nsaid”:ab,ti OR “cox 2”:ab,ti OR “nsaids”:ab,ti OR “leukocyte”:ab,ti OR “leukocytes”:/exp OR “leukocytes”:ab,ti OR “neutrophil”:ab,ti OR “neutrophils”:ab,ti OR “monocyte”:ab,ti OR “monocytes”:ab,ti OR “macrophage”:ab,ti OR “macrophages”:ab,ti OR “cytokines”:ab,ti OR “cytokines”:ab,ti OR “cyclosporin”:ab,ti OR “corticosteroids”:ab,ti OR “corticosteroid”:ab,ti OR “cyclooxygenase”:ab,ti OR “cox”:ab,ti OR “nsaid”:ab,ti OR “cox 2”:ab,ti OR “nsaids”:ab,ti OR “leukocyte”:ab,ti OR “leukocytes”:/exp OR “leukocytes”:ab,ti OR “neutrophil”:ab,ti OR “neutrophils”:ab,ti OR “monocyte”:ab,ti OR “monocytes”:ab,ti OR “macrophage”:ab,ti OR “macrophages”:ab,ti OR “cytokines”:ab,ti OR “cytokines”:ab,ti
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cytokine:ab,ti OR chemokines:ab,ti OR chemokine:ab,ti OR interleukin:ab,ti OR interleukins:ab,ti OR integrin:ab,ti OR integrins:ab,ti OR tlr:ab,ti OR “toll-like receptor”/exp OR “toll-like receptor”:ab,ti OR “toll-like receptors”:ab,ti OR “tlrs”:ab,ti OR “inflammasome”:ab,ti

Identify other sources for study identifications
None.

Define search strategies for these sources
Not applicable.

STUDY SELECTION PROCEDURE

Define screening phases
1. Title/abstract screening phase
2. Full-text screening phase

Specify number of observers per screening phase
Two observers per reference per phase: G.P.J. van Hout and S.J. Jansen of Lorkeers.

STUDY SELECTION CRITERIA

Type of study design
Inclusion criteria: Controlled study/Cohort study.
Exclusion criteria: Other study types including non-controlled studies and case reports.

Type of animal/population
Inclusion criteria: Pigs, dogs, sheep and goats subjected to MI as defined previously. Co-medication that potentially influences IS (e.g. platelet inhibitors/β-blockade) is no exclusion criteria as long as the control group also receives the co-intervention.
Exclusion criteria: Transgenic animals. All other species of animals different from the ones previously mentioned or animals from the same species but different disease models. In vitro and ex vivo studies are also excluded.

Type of intervention, e.g. doses, time, frequency
See the previously stated definition of the intervention. The timing, delivery route and frequency of the anti-inflammatory compounds are no exclusion criteria for this systematic review.

Outcome measures
Inclusion criteria, studies are included in the analysis if they reported at least one of the following outcome measures:

1. Primary outcome measurement: Myocardial IS measured as a percentage of the area at risk (IS/AAR)
2. Myocardial IS measured as a percentage of the total left ventricle (IS/LV)
3. Left ventricular ejection fraction (LVEF)
4. Myocardial scar thinning given in millimetres or as a ratio, divided by the opposite, non-infarcted wall

Exclusion criteria, studies are excluded in absence of the following outcome measures:

1. The absence of inclusion criteria 1–4: IS/AAR, IS/LV, LVEF or myocardial scar thinning

Language restrictions
None.

Publication date restrictions
None.

Other
Inclusion criteria: Full-text original papers only, meaning no congress abstracts.
Exclusion criteria: Congress abstracts.

Sort and prioritize your exclusion criteria per selection phase
Selection phase title/abstract
1. No MI
2. No anti-inflammatory compound
3. No original data (e.g. review)
4. No large animals
5. Not in vivo

Selection phase full text
1. No full-text publication
2. No MI
3. No anti-inflammatory compound
4. No original data
5. No large animals
6. Not in vivo
7. No correct endpoints (EF, IS/AAR, IS/LV or scar thinning)
8. Lack of control group

STUDY CHARACTERISTICS TO BE EXTRACTED

Study ID
First author, corresponding author, journal, publication year and source of funding.

Study design characteristics
Number of animals per groups and experimental groups.
Animal model characteristics
Species, gender, age, weight, location of infarct, method of induction of injury (e.g. ligation, balloon occlusion), model (I/R or permanent), anaesthetics, ventilation, duration of occlusion, surgical procedure (closed chest, lateral sternotomy, medial sternotomy) and co-morbidity.

Intervention characteristics
Name compound, treatment group (e.g. NSAIDs, free radical scavenger, corticosteroids) dosage, time of delivery, number of administrations, bolus versus continuous administration, duration of delivery, duration of follow up, route of delivery and co-treatment.

Outcome measures
Outcome data for any of the following
1. Myocardial IS measured as a percentage of the AAR
2. Myocardial IS measured as a percentage of the total LV
3. LVEF
4. Myocardial scar thinning given in millimetres or as a ratio, divided by the opposite, non-infarcted wall
5. LVEDV and LVESV
6. Mortality after therapy administration

Risk of bias assessment
Scoring for the risk of bias is performed based on the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies checklist.20

- Publication in a peer-reviewed journal
- Reporting of random allocation
- Reporting of blinding of the operator
- Reporting of blinded assessment of outcome
- Use of co-morbid animals
- Reporting of a sample size calculation
- Reporting of compliance with animal welfare regulations
- Reporting of a potential conflict of interest

Data collection
- Infarct size: IS/AAR in %
- Infarct size: IS/LV in %
- LVEF in %
- Scar thinning in millimetres and/or ratio
- LVESV and LVEDV in mL
- Mortality: number of animals that died after therapy administration

Methods of data extraction/retrieval
Primarily we extract data from the results section of the manuscript. When data is unavailable in text or tables, data will be extracted electronically from graphs. In the absence of these data in graphs, authors will be contacted through e-mail. In case of no response after 2 weeks, we will exclude the study from the analysis.

DATA ANALYSIS AND DATA SYNTHESIS

Data combination
Data will be combined in a systematic review with a forest plot followed by a meta-analysis.

Specify when data combination is appropriate
Based on our inclusion and exclusion criteria, we expect the models and outcome measures to be uniform enough to pool data for a combined analysis for each separate outcome measurement. Anti-inflammatory compounds have been tested for several decades in large-animal models of MI and we therefore expect to include over 100 different studies. We chose 25 as a cut off for the number of studies to be included in order to allow reliable determination of publication bias and meta-analysis.21

In the current systematic review, we also aim to determine whether study-related parameters influence outcome (e.g. surgical approach, time of compound delivery and type of anti-inflammatory compound). In our opinion, direct comparison of these subgroups is feasible when groups contain at least five independently conducted studies. Meta-regression will be used to explore heterogeneity between included studies. Significant predictors will be further investigated by subgroup analysis. If sufficient studies are included, within-subgroup analyses will also be performed for different treatment groups of anti-inflammatory compounds (i.e. NSAIDs, free radical scavengers) to determine if study-related parameters influence outcome in these particular groups of anti-inflammatory compounds. The number of parameters tested by meta-regression is based on the number of included studies per outcome measure and will be one parameter for every 10 studies. For the primary outcome measurement (IS) no correction will be applied (p < 0.05 will be regarded as significant). For all the secondary outcome measures, a Bonferroni correction will be applied, based on the number of parameters tested.

IF META-ANALYSIS SEEMS FEASIBLE

Specify the effect measures to be used
We expect the reporting of the selected outcome measurements to be uniform since our primary outcome measures (IS/AAR, IS/LV, LVEF, scar thinning) are reported on a relative scale (ratio/percentage) in the vast majority of cases. For this reason it is assumable we will be able to use the raw difference in means for these parameters. For each study group we will extract the reported mean combined with either the standard deviation or standard error of the mean, depending on the parameter provided by the different studies.
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Effect measures used for each individual parameter

- Mortality—Odds ratio
- IS/AAR—Raw difference in means
- IS/LV—Raw difference in means
- LVEF—Raw difference in means
- Scar thinning—Raw difference in means or standardized difference in means, depending on the uniformity of the data
- LVEDV and LVESV—Standardized difference in means

Specify which study characteristics will be analysed as possible sources of heterogeneity

1. Treatment group (e.g. NSAIDs, free radical scavengers, complement inhibitors)
2. Ischaemia-reperfusion/permanent occlusion
3. Occlusion duration
4. Timing of therapy
5. Timing of assessment
6. Year of publication
7. Animal species
8. Surgical procedure (sternotomy vs. no sternotomy)
9. Location of injury (which coronary artery was occluded)
10. Gender
11. Randomization
12. Blinding
13. Methods of infarct/function measurement
14. English/non-English articles

Specify subgroups and comparisons of interests

Subgroup analysis will be performed for significant predictors of outcome, based on meta-regression. No sensitivity analysis will be performed.

Method of analysis

Because of anticipated heterogeneity among included studies, a random effects model will be appropriate. We will also determine the extent of heterogeneity in our dataset by assessing the T² and I² statistics.

Method for assessing risk of publication bias

We will assess the risk of publication bias through the construction of a funnel plot and a subsequent Egger’s regression for testing the symmetry in funnel plot and detecting small study bias. Duval and Tweedie’s “trim and fill analysis” will be performed to identify missing studies.

Other

Possible limitations

1. Inclusion of studies is based on LVEF, IS and scar thinning; this means that studies that do not report this will be excluded, enabling possible selection bias.

2. Our definition of “anti-inflammatory treatment” is based on the working mechanism and clinical usage of certain compounds, requiring arbitrary choices. In our study, possible anti-inflammatory therapies with unknown working mechanisms or major pleiotropic effects are excluded. On the other hand, compounds that will be included in the final analysis could, to some extent, also have pleiotropic effects possibly clouding the sole effect of inhibiting the inflammatory response post MI.

3. In the current study design we have not taken into account the commercialization potential of certain drug types. While clinical testing of certain compounds may seem to be very promising based on this systematic review, it should be noted that we have not taken the economic position (e.g. patent, costs) into consideration, which is also essential for eventual drug development.

Conflict of Interest

The authors have declared no conflicts of interest for this article.

REFERENCES


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