Cardiac stem cell treatment in myocardial infarction: protocol for a systematic review and meta-analysis of preclinical studies

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ABSTRACT

Cardiac-derived stem or progenitor cells (CSCs) have emerged as a possible therapeutic intervention for myocardial infarction, potentially ameliorating the devastating effects caused by inadequate blood flow to the heart. The first human clinical trials using these myocardial-derived cells have recently started, but scientific controversy exists regarding the efficacy and origin of some of these stem cells in preclinical animal models. Systematic review of the current literature on CSCs in ischaemic cardiomyopathy can provide useful additional information on the use of CSCs in preclinical trials. By combining all available data, we can adequately compare the different types of cells being used and possibly identify factors that influence cardiac stem cell therapy in general. This protocol provides a thorough description of the methodology that will be used in our systematic review and meta-analysis of all preclinical animal studies involving cardiac stem cell treatment for ischaemic cardiomyopathy.

Keywords: cardiac stem cells, myocardial infarction, animal models

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General

The structure of this protocol is adapted from and based on the Systematic Review Protocol for Animal Intervention Studies.1

Title of the Systematic Review

Cardiac stem cell treatment in ischaemic cardiomyopathy: a systematic review and meta-analysis of preclinical studies

Stage of the Project at Time of Protocol Submission

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<th>Stage of process</th>
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<td>Piloting study selection</td>
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<td>Formal screening with final search criteria</td>
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Background

Cardiovascular diseases are the leading causes of death in current medical practice, with more than 7 million people dying from ischaemic heart disease in 2012. Because therapy for coronary heart disease is ever-evolving and improving, chronic disease burden is increasing due to the ageing population and longer survival after an initial ischaemic event. Stem cell therapy has been proposed as an additive therapeutic after myocardial infarction (MI), aiming at stimulating or contributing to regenerative effects. In particular, cardiac-derived stem or progenitor cells (CSCs) hold great potential as they already originate from the heart, can differentiate into all cardiovascular lineages and have the potential to stimulate regeneration of the heart through several mechanisms. Different CSC types have been discovered over the past decade; the c-kit + CSC, the cardiospheres and cardiosphere-derived cell (CDC), the Sca-1+ CSC, the islet-1+ CSC and the side population (SP) cells have all been isolated from adult myocardial tissue.

Human trials (SCIPIO for c-kit + CSCs and CADUCEUS for CDCs) have been started recently and the initial results look promising. Nonetheless, researchers have recently questioned the origin of the cardiac stem cell and its potential. With not all preclinical studies reaching positive outcomes for the CSCs, a solid and complete overview of all preclinical studies conducted with CSCs is still lacking. By combining all available data we can make accurate comparisons between the different CSC types, which are currently being used. Furthermore, all preclinical studies combined will provide us with additional information on study design and effectiveness of CSCs in MI models and might help us in optimizing our treatment strategies and translation towards clinical studies. Additionally, performing a meta-analysis of these studies will provide information on possible factors that influence efficacy of CSCs in animal models of MI.

Objectives of the Systematic Review and Meta-Analysis

Specify the Disease/Health Problem of Interest

In the current study, myocardial infarction is defined as ischaemia resulting in permanent damage to the myocardium, caused by the disruption of adequate blood flow. In real life, this is usually caused by obstruction of one or more coronary arteries due to rupture of an unstable atherosclerotic lesion. In preclinical models, mechanical obstruction of one of the coronary arteries is most commonly used.

Specify the Population/Species Studied

Research regarding CSCs as a therapeutic agent to stimulate cardiac performance started in 2003. Since then, several studies have been published in many different animal models. We will include all placebo-controlled preclinical studies using the following animals: mice, rats, guinea pigs, rabbits, goats, sheep, dogs and pigs.

Specify the Intervention/Exposure

The intervention of interest is the administration of CSCs. A cardiac stem or progenitor cell is defined as a stem cell, showing (some degree of) clonogenicity, residing in the adult heart with the ability to commit to cell types of the cardiovascular lineage (cardiomyocytes, smooth muscle cells and endothelial cells) Five different CSCs harvested from the adult heart have been repetitively identified and will therefore be included in our systematic review:

- C-kit + CSC
- cardiosphere/CDC
- Sca-1+ CSC
- Islet-1+ CSC
- SP cell

Specify the Control Population

Studies will be included when using placebo treatment—phosphate buffered saline (PBS), vehicle solution (e.g. culture medium) or cells of another origin as a control. Sham animals or affected animals without administration of a placebo will be excluded.

Specify the Outcome Measures

Primary outcome
Ejection fraction (EF).

Secondary outcomes
End systolic volume (ESV), end diastolic volume (EDV), wall thickness (WT), fractional shortening (FS), infarct size (IS) (per area at risk (IS/AAR) and per left ventricle (IS/LV)).

State Your Research Question

What is the effect of CSC therapy in MI animal models when compared to placebo-treated controls?

Methods

Search and Study Identification

Identify literature databases to search

Based on previous experience, we chose to search the Pubmed and Embase databases.
Define electronic search strategy (final searches conducted on November 5, 2014)

**Pubmed.** (“cardiac stem cell” OR “cardiac stem cells” OR “cardiac progenitor” OR “cardiac progenitors” OR “cardiomyocyte progenitor” OR “cardiomyocyte progenitors” OR cardiosphere OR cardiospheres OR CMPC OR CSC OR CPC OR CDC).  
AND (cardiac OR heart OR myocardial OR infarction OR ischaemic).  
AND (pig OR dog OR canine OR sheep OR goat OR porcine OR swine OR ovine OR mice OR mouse OR rat OR rats OR murine OR rabbit∗ OR “guinea pig”).

**Embase.** (“cardiac stem cell” OR “cardiac stem cells” OR “cardiac progenitor” OR “cardiac progenitors” OR “cardiomyocyte progenitor” OR “cardiomyocyte progenitors” OR cardiosphere OR cardiospheres OR CMPC OR CSC OR CPC OR CDC).  
AND (cardiac or heart or myocardial or infarction or ischaemic).  
AND (pig OR dog OR canine OR sheep OR goat OR porcine OR swine OR ovine OR mice OR mouse OR rat OR rats OR murine OR rabbit∗ OR “guinea pig”).

**Other sources for study identifications**
Reference lists of included studies and relevant reviews.

**STUDY SELECTION PROCEDURE**
Define screening phases and number of observers (two observers per phase)

1. Title/abstract screening (PPZ/AV)
2. Full-text screening (PPZ/AV)

In both phases, the two observers try to reach consensus on inclusion by discussion. In case of no consensus among the two primary observers, a third reviewer (JS) is consulted.

**STUDY SELECTION CRITERIA**

**Type of study design**
Inclusion: placebo-controlled randomized trial, placebo-controlled (cohort) study.  
Exclusion: review, editorial, case report, case series, protocol paper, study without placebo-control group.

**Type of animals/population (e.g. age, sex, disease model)**
Inclusion: any animal MI model by coronary occlusion >10 min (rodents) or >30 min (non-rodents) by ligation, balloon occlusion, microembolization, coil embolization, sponge embolization or any other temporary or permanent occlusion method of an animal’s coronary artery. Cut-off points for rodents and non-rodents, respectively, are 10 and 30 min based on literature on myocardial stunning and preconditioning, which we want to exclude.14,15

Exclusion: foetal ischaemia models (in utero), ischaemia less than 10 min for rodents or less than 30 min for non-rodents. Studies that perform co-interventions will be excluded.

**Type of intervention**
Inclusion treatment: CSCs as previously defined.  
Exclusion criteria: since we solely want to determine the effect of CSCs that naturally reside in the myocardium, we choose to exclude genetically modified cells, pretreated cells, cells in/on scaffolds/patches/beads, fully differentiated cardiomyocytes, embryonic-derived cardiac progenitors and treatment with cell-derived material like conditioned medium or extracellular vesicles.

**Outcome measures**
Studies will be included in the analysis if they reported the primary outcome measure EF or a combination of both individual ESV and EDV (from which the individual and mean EF can be calculated). If a study uses an imaging modality generally used for EF measurements, but did not report these, authors will be emailed to ask to provide possible data. If a study mentions quartiles instead of means in combination with a standard deviation (SD) or standard error of the mean (SEM), authors will be emailed to ask to provide the raw data or means and SDs or SEMs. Authors will also be emailed if the number of animals per group is not stated and asked to provide the information.

Exclusion criteria: studies will be excluded if data on EF or the number of animals per group could not be obtained (either through extraction from the paper or after repetitive email contact).

**Language restrictions**
Inclusion: English.  
Exclusion: any language other than English.

**Publication date restrictions**
We did not include any date restrictions in our search. We will discard papers published before 2002 since discovery of the first CSC being used for therapy was published in 2003.5

**Other**
Inclusion criteria: full-text original papers.  
Exclusion criteria: congress abstracts.

In case experimental groups and data are used repeatedly in different studies (e.g. to answer different hypothesis), we will include these data only once.
ORDER OF PRIORITY EXCLUSION CRITERIA PER SCREENING PHASE

Order for title/abstract screening
1. No CSC treatment
2. No MI
3. No original data (e.g. review, editorial)
4. No animal study
5. In utero ischaemia model

Order for full-text screening
1. No full-text paper
2. No CSC treatment
3. No MI
4. No original data (e.g. review, editorial)
5. No animal study
6. In utero ischaemia model
7. No imaging modality suitable for EF measurement
8. No placebo-control
9. Number of animals per group not stated

STUDY CHARACTERISTICS TO BE EXTRACTED

One reviewer will extract study characteristics and all data input will be checked by another reviewer in the database.

Study ID
DOI, first author, corresponding author, journal, publication year, source of funding.

Study design
The number of animals per group will be extracted. If the exact number per group is not mentioned (but for example only a range) the lowest number of animals will be used for data analysis.

CSC treatment, when reported as either the primary treatment or as a control treatment when testing for improved therapy, will be extracted. Information on the use of immunosuppression or immune-compromisation will be extracted.

Animal model
Animal type (rodent or non-rodent), species, breed/strain, sex, age, weight, method of induction of injury (ligation, balloon occlusion, embolization), ischaemia model (permanent or ischaemia-reperfusion), duration of occlusion, comorbidity.

Intervention characteristics
Type of CSC, cell dose, time of delivery relative to time of induction of ischaemia and reperfusion, route of delivery, duration of follow-up and time of functional cardiac assessment after cell delivery, cell characteristics (2D/3D culture, autologous/syngeneic/allogeneic/xenogeneic, comorbidity), CSC-group used as primary intervention of the study or as a control for another treatment. In case of multiple time-points, the latest time point will be included for uniformity and since this has the most clinically relevant implication.

Outcome measurements and data collection
1. Method of functional outcome assessment
2. Left ventricular EF as percentage
3. ESV and EDV in mL
4. IS/AAR and IS/LV as percentage
5. WT in mm
6. FS as percentage

All data will be extracted as a mean with SD or SEM for database input.

Risk of bias assessment
Risk of bias is assessed based on the CAMARADES checklist that includes the following criteria:
1. Publication in a peer reviewed journal
2. Reporting of random allocation
3. Reporting of blinding of the operator
4. Reporting of blinded assessment of outcome
5. Use of comorbid animals
6. Reporting of a sample size calculation
7. Reporting of compliance with animal welfare regulations
8. Reporting of a potential conflict of interest

Moreover, attrition bias will be measured using a specific part of the SYRCLE’s risk of bias tool.

Methods of data extraction and retrieval
Data is preferably extracted from either text or tables in the results section of the manuscript of interest. When the data is not available in text or tables, data will be extracted electronically from available graphs using the Image J® software, version 1.48 (ImageJ, U.S. National Institutes of Health, Bethesda, MD, http://imagej.nih.gov/ij/, 1997–2015). If an imaging modality capable of measuring EF is being used, with no mentioning in the manuscript of an EF, authors will be contacted for the data by email. In case of no response after 4 weeks, including a reminder, manuscripts will be excluded from the analysis. If only individual data is present, mean and SD will be calculated from these values.

DATA ANALYSIS AND DATA SYNTHESIS

Data gathering
All data will be inserted in the CAMARADES database (data available upon request).
Data combination

Data will be combined in a systematic review, forest plot and subsequent meta-analysis.

Specify if and when data combination is appropriate

We expect to include over 40 studies. We choose a minimum number of 25 studies to be included; we need at least 25 studies to make sure we can adequately determine publication bias and conduct our meta-analysis.

First, we will pool all data for our general outcomes. We expect to encounter differences in effect between rodents and non-rodents (e.g. large animals) for our analyses, since this has been reported previously in research involving therapeutic modalities in preclinical cardiac disease models. Therefore, we will stratify these groups upfront and pool these data separately for additional meta-regression analyses.

We expect the different ischaemia models and outcome measures to be uniform and widely used in the same way. Furthermore, all three major CSC (c-kit+, CDC and Sca-1+) types have shown comparability in cell characteristics when cultured under the same circumstances. Therefore, we think it is feasible to pool our data for a combined analysis for each separate outcome measurement.

Our interest is in parameters that influence our primary outcome in study, animal and/or cell characteristics. Direct comparison in our eyes is feasible when groups contain five or more studies. To explore sources of heterogeneity in our included studies, we will conduct a meta-regression; significant predictors will be further investigated based on the outcome of the meta-regression. The number of parameters, tested by meta-regression, is 1 parameter for every 10 included studies. For the primary outcome (EF) no correction will be applied, with a p value <0.05 regarded as a significant difference. For all secondary outcome measures, we will correct for the number of parameters tested with a Bonferroni-Holm correction.

IF META-ANALYSIS IS FEASIBLE

Specify effect measures to be used

We expect the values to be heterogeneous with regard to animal sizes and imaging modalities for our study. Most of our outcomes are measured in percentages/ratios, so for EF, IS and FS we will use raw mean difference as our effect measures since these modalities are already corrected for size of the animal. Since animal size will vary between studies, absolute measures (mL, mm) will vary as well. In order to combine these data, a standardized mean difference analysis will be performed for ESV, EDV and WT. Depending on the reported values, we will extract the reported value with the additional SEM or SD. Studies reporting median will be excluded.

Outcome measurements

1. EF: raw mean difference
2. EDV/ESV: standardized mean difference
3. IS/AAR/IS/LV: raw mean difference
4. WT: standardized mean difference
5. FS: raw mean difference

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

1. Cell type (c-kit + CSC, cardiosphere/CDC, Sca-1+ CSC, Islet1+ CSC, SP cell)
2. Immunosuppression (yes/no)
3. Cells being used as control or ultimate treatment
4. Cell characteristics upon administration
   (a) two-dimensional or three-dimensional cultured
   (b) Comorbidity (diseased vs. healthy)
   (c) Autologous versus syngeneic versus allogeneic versus xenogeneic
5. Animal characteristics
   (a) Age of recipient animal
   (b) Sex of recipient animal (male/female/mixed/unknown)
   (c) Animal species
   (d) Strain or breed within species
6. Timing of therapy
7. Timing of assessment
8. Randomization (yes/no)
9. Blinding
   (a) Allocation concealment (yes/no)
   (b) Assessment of outcome (yes/no)

Specify statistical model of analysis

Our data will be heterogeneous since we include studies using different study designs (i.e. animal species, cell type) and therefore we will use a random effects model for analysis. We will quantify the extent of heterogeneity present in our data set by determining the Tau^2 and I^2 statistics. Statistical analysis will be performed using Stata Statistical Software: Release 13 (College Station, TX: StataCorp LP).

Methods for assessing risk of publication bias

Risk of publication bias will be assessed using funnel plotting and Egger’s regression analysis. Missing studies will be identified using Tweedie and Duval trim and fill analysis.

Sensitivity analysis

A sensitivity analysis will be performed for time of outcome measurement. Clinically, the latest timepoint seems most relevant to us. However, there might be considerable variation in the timepoints of outcome assessment. Therefore, we will compare these outcomes to outcomes closest to the commonly used timepoint (in case of multiple measurements) in our studies (most likely around 3–4 weeks).
CSCs in MI: preclinical meta-analysis protocol

**Expected possible limitations of this systematic review**

The data might be too heterogeneous to make adequate subgroups (>five studies) and to do an adequate meta-regression. It could also be that the number of articles will be less than expected; again, a meta-analysis might not be feasible in that case.

**Conflict of Interest**

The authors declare that there are no conflicts of interest.

**REFERENCES**


