Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease

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Aims
Stem cell therapy is a treatment strategy for ischaemic heart disease patients. Meta-analysis of randomized human trials showed <5% improvement in left ventricular ejection fraction (LVEF). Meta-analysis of available pre-clinical data of ischaemic heart disease could provide important clues to design human clinical trials.

Methods and results
Random-effects meta-analysis was performed on pig, dog, or sheep studies investigating the effect of cardiac stem cell therapy in ischaemic cardiomyopathy (52 studies; n = 888 animals). Endpoints were LVEF and death. Ischaemia/reperfusion infarction was performed in 23 studies and chronic occlusion in 29 studies. Pooled analysis showed a LVEF difference of 7.5% at follow-up after cell therapy vs. control (95% confidence interval, 6.2–8.9%; P < 0.001). By exploratory multivariable meta-regression, significant predictors of LVEF improvement were: cell type [bone marrow mononuclear cells (BM-MNC) showed less effect than other cell types, e.g. mesenchymal stem cells; P = 0.040] and type of infarction (left anterior descending artery 8.0 vs. left circumflex artery 5.8%; P = 0.045). Cell therapy was not associated with increased mortality (P = 0.68). Sensitivity analysis showed trends towards more improvement with higher cell number (≥10^7), chronic occlusion models, and late injections (>1 week).

After follow-up of 8 weeks, the effect of cell therapy decreased to 6%.

Conclusion
This meta-analysis showed that large animal models are valid to predict the outcome of clinical trials. Our results showed that cell therapy is safe and leads to a preserved LVEF. Future trials should focus on cell types other than BM-MNC, large infarction, and strategies to obtain sustained effects.

Keywords
Meta-analysis • Myocardial infarction • Animal models • Cell therapy • Stem cells

1. Introduction
Coronary heart disease is a major public and economic health problem leading to more than 7 million deaths worldwide each year.1,2 Myocardial infarction (MI) is characterized by loss of cardiomyocytes, scar formation, and ventricular remodelling, and it can develop into end-stage heart failure. Optimal pharmacological treatment and coronary reperfusion therapy have led to improved survival of patients with coronary artery disease, even if current medical therapies cannot replace dysfunctional cardiomyocytes. Cell therapy has emerged as a potential therapeutic strategy. The ultimate goals of cell therapy are myocardial regeneration and revascularization, thus, re-establishing synchronous contractility and bioelectrical conductivity to achieve overall clinical improvement of cardiac function without severe adverse effects.

Many large animal studies in acute MI and ischaemic cardiomyopathy have been performed, mostly with heterogeneous design and conflicting outcomes. These pre-clinical results led to the initiation of clinical trials and showed at best marginal results.3 Nevertheless, pre-clinical studies are mandatory to assess risk of a new therapy and predict safety, feasibility, and efficacy. Moreover, they address unresolved issues regarding clinical cell therapy (i.e. choice of cell type, etc.).

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cell number, method of delivery, time of delivery, and follow-up after cell transplantation), which have been outlined by the task force of the European Society of Cardiology on stem cell repair of the heart. Therefore, large animal models are valid and relevant for clinical practice, and have important clues regarding these yet unanswered questions. Similar to the human cardiac stem cell therapies, large number of animal studies have been performed including relative small number of animals. We hypothesize that meta-analysis of these pre-clinical data might be helpful to design future clinical studies similarly to the meta-analysis of human cardiac stem cell trials.

We performed a systematic overview of the pertinent literature including a quantitative meta-analytical pooling of the data to assess the effects of stem cell transplantation in large animals with acute or chronic ischaemic cardiomyopathy. A pre-specified sub-analysis is performed to focus on afore-mentioned unresolved issues.

2. Methods

2.1 Eligibility criteria

Acute MI or chronic ischaemic cardiomyopathy models in large animals were screened. Randomized controlled (RCT) and cohort studies investigating the effect of stem cell therapy on cardiac function as determined by left ventricular ejection fraction (LVEF) were analysed. In addition, a placebo- or sham-operated control group had to be included in the study. Trials that only investigated transplanted or genetically engineered stem cells altering cell behaviour, or studies using conditioned medium were excluded, but studies using reporter genes (solely for stem cell imaging purposes) were included. Reviews, editorials, comments, reports from scientific sessions and discussions were excluded.

2.2 Search strategy

A PubMed search was performed (January 1980–March 2010) using the following search terms: ‘pig OR porcine OR swine OR canine OR dog OR sheep OR ovine’ AND (stem cells OR progenitor cells OR bone marrow) AND (myocardial infarction OR heart failure OR coronary artery disease OR cardiac repair OR myocardial regeneration)’. Only English and published reports were included. The complete search strategy is available on request.

2.3 Data abstraction

Two reviewers (T.I.G.S. and S.J.J.L.) independently screened abstracts, and the resulting manuscripts were approved by a third reviewer (S.A.J.C.). The following information was extracted from the complete manuscripts of the qualified studies: basal characteristics of the study, LVEF, end-diastolic volume (EDV), end-systolic volume (ESV), and mortality. If necessary, data were estimated from graphics or recalculated by available data: LVEF was recalculated as follows: \( \frac{EDV - ESV}{EDV} \times 100\% \). Accordingly, standard deviations were determined or recalculated from standard errors. Volume data were recalculated for body weight. For final analysis, we preferably used magnetic resonance imaging (MRI) data. Alternatively, data derived by echocardiography, nuclear imaging, left ventricle angiography, or pressure–volume (PV) loops, respectively, were used in the absence of MRI data. In case of missing data, corresponding authors were contacted. Thirty-six emails were sent, and 18 authors responded. Standard guidelines for quality assessment of clinical trials could not be completely applied in these pre-clinical experiments. Therefore, we used modified criteria to assess selection, performance, and detection bias: randomization (yes/no), adequate allocation (y/n), adequate method of randomization (y/n), blinding of the operator (y/n), and blinding of the functional analysis (y/n).

2.4 Data analysis

Our primary outcome was difference in mean LVEF (reported in %) at follow-up between control and treated animals. Secondary endpoints were difference in EDV and ESV (reported as volume in millilitre) at follow-up and mortality after treatment. In case of multiple measurements over time, data measured at the longest duration of follow-up were used for analysis. A random-effect model was applied. Continuous variables were reported as weighted mean differences with 95% confidence intervals (CIs) between the cell-treated animals and control groups. In case of dichotomous data, the pooled estimate of effect was presented as odds ratio (OR) with 95% CI. In case of multiple experimental groups next to one control group within one study, the number of animals in the control group was divided equally by the number of experimental groups. Details of enrolled subgroups are provided in Supplementary material online, Table S1. Unadjusted P-values are reported throughout, with hypothesis testing set at the two-tailed 0.05 level. Heterogeneity was considered significant at \( P < 0.10 \). Inconsistency was estimated by using the \( I^2 \) statistic; values of 25, 50, and 75% were considered low, moderate, and high inconsistency, respectively.

Based on clinical scenario, a multivariate analysis was performed for: MI model (ischaemia/reperfusion or chronic occlusion); location of infarct-related artery (left anterior descending artery (LAD) or left circumflex artery (LCX)); type of animal (pig, dog, and sheep); cell type; number of cells injected; method of cell delivery (retrograde coronary transvenous injection, surgical, intracoronary (ic), and trans-endocardial (TE) delivery); timing of cell therapy after acute MI, and follow-up after cell therapy. Furthermore, from a clinical point of view, the following sub-group analyses were performed: MI model (ischaemia/reperfusion or chronic occlusion); type of infarction (LAD or LCX); cell type (bone marrow mononuclear stem cells (BM-MNC) or mesenchymal stem cells (MSC)); number of cells injected (\( < 10^2 \), \( 10^2 – 10^3 \), \( 10^3 – 10^4 \), or \( \geq 10^4 \)), timing of cell therapy after acute MI (\( \leq 1 \) day, 1–7 days, \( > 7 \) days), and follow-up after cell therapy (1–4 weeks, 5–8 weeks, 9–12 weeks, >12 weeks). A funnel plot was drawn for LVEF to explore publication bias. A power analysis for future studies in ischaemic heart disease was performed. All analyses were performed with Review Manager version 5 (The Nordic Cochrane Center, København, Denmark) and SPSS 17.0 (SPSS, Chicago, IL, USA).

3. Results

3.1 Included study characteristics

The electronic database search identified 304 articles, among which 52 articles were eligible for review (34 RCT and 18 cohort studies; Figure 1). In total, 1251 animals were described in the included articles, but 888 animals met our inclusion criteria and were analysed. Characteristics of the enrolled studies are depicted in Table 1. Most studies used a porcine model (41 studies). In 23 studies, ischaemia/reperfusion was used as a MI model. MI was mainly induced in the left anterior descending coronary artery (38 studies), but site of ligation/constriction of the vessel (proximal, mid, or distal) varied. Ten different cell types have been studied. In most cases, surgical or ic delivery was performed. Timing of cell therapy after induction of MI was \( < 1 \) day (15 studies), 1–7 days (11 studies), or \( > 7 \) days (26 studies). Median and inter-quartile range of time to follow-up imaging was 6 weeks (4–8 weeks). Functional endpoints were assessed by MRI (18 studies), echocardiography (23 studies), nuclear imaging (five studies), left ventricle angiography (four studies), or
PV-loop (two studies). Volume data were reported in 25 studies and mortality in 32 studies.

3.2 Quality of included studies

Data in Supplementary material online, Table S2 show the methodological quality of the enrolled studies. Blinded analysis of LVEF was performed in 12 RCT and 10 cohort studies. The operator was blinded in five studies. One article reported the method of randomization. Thirty-six studies (69%) were published in journals with an impact factor $\geq 3.0$.

3.3 Meta-analyses

Pooled analysis showed a LVEF difference of 7.5% at follow-up after cell therapy vs. control (95% CI, 6.2–8.9%; $P < 0.001$) with significant heterogeneity ($P < 0.01$) and inconsistency ($I^2: 77$%; Figure 2). At follow-up, mean LVEF after cell transplantation and control was 56 and 48%, respectively. Consistently, an ESV difference of −7.4 mL (95% CI, −12.9 to −1.8 mL; $P = 0.01$) and EDV difference of −5.3 mL (95% CI, −12.7 to 2.1 mL; $P = 0.16$) was found with significant heterogeneity ($P < 0.001$ for both) and inconsistency ($I^2 > 90\%$ for both). Overall, no significant difference in LVEF at baseline between the control group and cell-treated group was found ($P = 0.31$); however, only 69% of the studies reported these baseline data. No significant differences were found in mortality after cell transplantation: 9.5% (36 of 380) in cell-treated group vs. 8.4% (21 of 251) in the control group [OR 1.13 (0.63–2.02), $I^2 = 0\%$; $P = 0.68$]. The majority of deaths were due to arrhythmias (data not shown).

3.4 Sensitivity analyses

A multivariable meta-regression analysis showed that cell type ($P = 0.040$) and type of infarction ($P = 0.045$) are the only independent significant predictors of LVEF improvement. A trend was observed (Figure 3) towards more improvement of cell therapy regarding: anterior infarction with LAD as infarct-related artery, high cell number ($\geq 10^7$), and late injections (>1 week after MI). BM-MNC showed less effect than MSC. In addition, less benefit was observed in ischaemia/reperfusion MI models compared with chronic MI models. During follow-up, the effect of cell therapy appeared to decline over time. No trend in LVEF improvement was
Table 1 Study characteristics

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<td>LAD</td>
<td>No I/R</td>
<td>BM-MNC</td>
<td>1.0 × 10^6</td>
<td>MI*</td>
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<td>Wang et al.68</td>
<td>12</td>
<td>Pig</td>
<td>RCT</td>
<td>LAD</td>
<td>No I/R</td>
<td>BM-MNC</td>
<td>1.0 × 10^6</td>
<td>MI*</td>
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<tr>
<td>Yang et al.69</td>
<td>12</td>
<td>Pig</td>
<td>RCT</td>
<td>LAD</td>
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<td>BM-MNC</td>
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<td>Yang et al.70</td>
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<td>BM-MNC</td>
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<td>Yang et al.71</td>
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<td>BM-MNC</td>
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**Continued**
observed regarding animal model \((P = 0.49)\) and route of cell delivery \((P = 0.90)\). The funnel plot for LVEF suggests a lack of publication bias as values were evenly distributed around the overall estimate (Figure 4).

3.5 Power calculation

Based on our results, we performed a sample size calculation for future studies in ischaemic heart disease. To obtain a power of at least 80% in a two-sided two-sample \(t\)-test with an alpha of 0.05, 11 animals needed to be included in each group to detect a significant difference of 8% in LVEF.

4. Discussion

The current analysis comprises data of 52 published pre-clinical studies involving large animals treated with cardiac stem cells in order to investigate the effects of cell therapy for ischaemic heart disease. The main findings are: (i) cell therapy improves LVEF by 7.5% due to a significant decrease in ESV; (ii) there is no increased mortality after cell treatment; (iii) cell type and type of infarction are important predictors of functional outcome; (iv) sensitivity analysis suggests that MSC, LAD infarction, chronic occlusion MI models, a higher number of cells \((\geq 10^8)\), and cell injection at least 1 week after MI have a beneficial effect on LVEF; (v) no effect on animal type and route of delivery was found.

4.1 Safety and efficacy of cardiac stem cell therapy in pre-clinical trials

Safety of cell therapy is still an important issue,\(^8\) regarding the no reflow after ic cell injections and myocardial perforation by intramyocardial application. In spite of cell delivery associated adverse events, human trials did not report increased mortality. Similarly, the present meta-analysis of pre-clinical trials showed no significant difference in death in animals receiving cell transplantation compared with controls, although only 32 studies (61%) addressed this issue.

Although global LV function improves after cell therapy, no significant difference in EDV \((-1.92 \text{ mL})\) was documented, indicating that cell therapy led to increase in contractility, but did not prevent ventricular remodelling. Similar result was observed in two clinical meta-analyses.\(^9\) This could be due to the relative short-term follow-up (<4 months) of the enrolled studies in our analysis. However, it is possible that structural myocardial changes and effects on diastolic filling occur after 4 months.

Transplantation of higher number of cells \((\geq 10^8)\) appears to have a more pronounced impact on improvement in LVEF. Our results are in agreement with clinical meta-analysis in that significant effect on LVEF may only be achieved when infusing doses are higher than 10\(^8\) cells.\(^3\) Moreover, meta-regression analysis showed that choice of cell type is an important predictor for LVEF. Sub-analysis revealed a trend towards larger benefit in case of transplantation of MSC when compared with BM-MNC. Scarce evidence is available that these cell types can regenerate new cardiomyocytes in vivo. This suggests the stimulation of an endogenous regenerative capacity of the heart upon cell transplantation, by release of growth factors, cytokines, and other paracrine molecules by the transplanted and host cells, enhancing angiogenesis and reducing apoptosis.\(^10^-1^2\)

Unfortunately, no complete data on infarct size were reported. However, our meta-regression analysis showed that type of infarction is an important significant independent predictor for clinical outcome. In detail, LAD-related anterior wall infarction showed more benefit after stem cell therapy compared with LCX infarction (LAD 8.0 vs. LCX 5.8%). Interestingly, there was no important difference in ratio of MI model for LAD and LCX infarction. Therefore, the observed effect may be caused by a greater degree of expansion after LAD infarction\(^1^3\) leading to lower LVEF and a higher risk for mortality therefore more benefit from cell therapy is expected in this patient group. Indeed, post hoc analyses of the REPAIR-AMI trial database and a clinical meta-analyses suggested that the effects of bone marrow cells were significantly higher in the subgroup with a baseline LVEF <49% who may have a tendency to develop heart failure.\(^1^4^-1^5\)

Ischaemia/reperfusion MI models were associated with less improvement in LVEF compared with chronic occlusion models (LVEF; 6.3 vs. 8.3%) although there was a higher incidence of permanent ligation animal studies. No reliable insight in baseline LVEF was available between these groups. In theory, percutaneous ischaemia/reperfusion models are considered most reliable for translational research as it mimics more closely the clinical practice of primary percutaneous coronary intervention. Timing of injection is important with more...
Figure 2: Forest plot showing the impact of stem cell therapy on LVEF improvement compared with controls. RCT, randomized controlled trial; 95% CI, 95% confidence interval.

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pronounced benefit if applied 7 days after MI. Our findings are comparable with clinical studies.3,16 In the acute setting (<24 h), cellular retention and survival are likely influenced by the local hostile microenvironment.

In large animals, the effect of cell therapy fades away 8 weeks after cell injection. This phenomenon is in accordance with initial observations in patient studies.17 This finding should trigger researchers towards novel applications and strategies of stem cell therapy (e.g. slow release agents, genetic engineering of stem cells, or repetitive injections overtime).

4.2 Effect of study design on study outcome

An overall beneficial effect of cardiac stem cell therapy has been observed in this analysis. However, this effect appears to be more pronounced in cohort studies when compared with RCT (LVEF; RCT 6.5 vs. cohorts 8.9%). It is conceivable that cohort studies are designed for practical reasons and might systematically overestimate the effect of cell therapy.

The capability of animal studies to predict human clinical outcome have been questioned by some authors.18,19 However, the results

Figure 3 Sensitivity analysis by visual inspection showed a trend towards more improvement of cell therapy compared with control regarding: (A) high cell number (≥10⁷; P = 0.52), (B) other cell types than bone marrow (P = 0.040), (C) late injections (>1 week; P = 0.68), (E) chronic oclusion model (P = 0.70), and (F) LAD infarction (P = 0.045). After 8 weeks follow-up (D), the effect of cell therapy fades away (P = 0.11). LVEF, left ventricular ejection fraction; LAD, left anterior descending artery; LCX, left circumflex artery; I/R, ischaemia/reperfusion; BM-MNC, bone marrow mononuclear stem cells; MSC, mesenchymal stem cells; P-values are derived from the multivariate analysis.
from the animal RCT studies are comparable to clinical meta-analyses20 (RCT; LVEF 6.5 vs. 4%) indicating that ischaemic large animal models are relevant for translational purposes.

4.3 Recommendations for future translational stem cell research

In view of clinical practice, it is mandatory that pre-clinical studies are performed according to high standards. In our opinion, the following items should be reported in pre-clinical studies for establishing standards for translational stem cell research bearing in mind the clinical horizon: randomized study design; blinded functional analysis; number of animals used in the study protocol must be clear and include the measured data before treatment and at the follow-up, mortality after treatment and during follow-up.

Over the next few years, adequately powered large animal studies and clinical trials should focus on transplantation of ≥10^7 stem cells, other cell types than BM-MNC, and later time point of injection (>1 week after MI). To maintain the beneficial effect on LVEF over time, repeated cell injections or the use of biomaterials to enhance survival of transplanted cells should be evaluated. No difference between species was observed, and we therefore recommend the use of pigs to evaluate the effect of cell therapy since many studies are available for comparison. We suggest the use of pig in the setting of acute MI and TE for chronic MI since no difference was found in our meta-analysis between these transplantation techniques.

Meta-analyses of animal studies are not common, yet they are recommended in several settings21 and can often guide research and clinical endeavours.22 Performing pre-clinical meta-analysis may also be attractive to evaluate the effect of other therapies to design future (pre-) clinical trials.

4.4. Limitations

Limitations of meta-analysis are well known.23 In particular, in our study, the diversity in animal type, incidence of permanent occlusion, delivery method, time of injection after MI, follow-up after cell therapy, and number of cells may play a role in the observed outcomes in the present study. However, multivariate analysis (used as an exploratory tool) showed no differences, but should be used with caution to generate new hypothesis. Heterogeneity may be present due to the extremely sensitive endpoints chosen (all continuous: LVEF, EDV, and ESV). By using random-effect analysis, the risk of finding erroneous estimates is minimized. Although various imaging modalities have been used to measure our endpoints, univariate analysis showed no significant difference between these techniques (P = 0.44). Our analysis was based on study outcomes, and we did not have access to individual data. Accordingly, we provided mean values. As some studies did not report all data necessary for the analysis, effort was made to contact corresponding authors to complete the database: only five studies were finally excluded due to incomplete data.

To date, numerous human clinical trials have already been conducted in order to assess the efficacy and safety aspects of cardiac stem cell therapy.3 Obviously, differences exist between large animal models and clinical practice. Healthy young large animals differ from older patients with long-standing coronary artery disease, and frequently co-morbidities (e.g. diabetes, hypertension, renal failure) are present. Consequently, many patients are routinely treated with other drugs (e.g. angiotensin-converting enzyme-inhibitors, beta-blockers, anti-diabetic medication), in contrast to research animals. Furthermore, autologous stem cells extracted from young large animals are ‘fresh’, whereas cells from patients are ‘aged’. Finally, duration of follow-up is relatively short in animal studies. Despite these differences, we have shown that pre-clinical data are highly relevant to predict outcome for clinical trials.

4.5 Conclusions

To the best of our knowledge, this is the first systematic review and meta-analysis in large animal models to evaluate the effect of cell therapy in ischaemic heart disease. This analysis showed that large animal models are valid to predict outcome of clinical trials. Moreover, the results showed that cardiac cell therapy is safe, led to an improved LVEF, and revealed important clues for designing (pre-) clinical trials.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

Conflict of interest: none declared.

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