Vascular stem cells—potential for clinical application

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Abstract

Introduction: Cell therapy is a growing area of research as an alternative to pharmaceuticals or surgery for the treatment of ischaemic disease. Studies are focusing on delivering tissue-derived cells into damaged organs to promote vascular regeneration or gain of function.

Sources of data: Pubmed, clinicaltrials.gov, BHF website.

Areas of agreement: Stem cells have the potential to become a viable treatment for many diseases, as indicated by the numerous pre-clinical studies demonstrating therapeutic benefit.

Areas of controversy: The mechanisms of action for transplanted stem cells are still open to debate. Proposed mechanism includes direct cell incorporation and paracrine action. Additionally, the secretome produced by transplanted cells remains largely unknown.

Growing points: Initial studies focused on delivering stem cells by injection; however, current research is utilizing biomaterials to target cell delivery to specific areas.

Areas timely for developing research: Whilst stem cell research in the laboratory is expanding rapidly, transition into clinical studies is hindered by the availability of equivalent clinical grade reagents.

Key words: vascular stem cells, myocardial infarction, cell therapy, scaffolds
Introduction

Vascular stem cell therapy is currently an active area of research as a potential treatment of ischaemic heart disease. Around 188 000 cases of myocardial infarction (MI) occur in the UK each year, resulting in approximately 57 000 deaths. Current therapies for ischaemic disease focus on surgical intervention, angioplasty or pharmacological treatment; however, there is increasing evidence that stem cell therapy would be a suitable treatment by promoting revascularization and tissue regeneration of the affected organ. Tissues and organs are able to undergo endogenous repair by mobilization of stem cell reserves to the site of injury to promote repair; however, age and disease-states (e.g. diabetes) have been demonstrated to decrease the number and efficacy of these cells. Therefore, ways of isolating and delivering exogenous stem cells directly to areas of injury is currently an active area of research.

The definition of a stem cell is an undifferentiated cell that can self-renew and give rise to a progeny of functionally mature cells. The ideal cell type for stem cell therapies needs to be easily accessible, easily derived, disease free, able to expand rapidly in vitro, but do not form tumours in vivo. Preferably, they also need to be immune-compatible, but this is not essential. Autologous cells are collected, expanded and re-administered to the same person. They are the most favourable option as it means there is no potential for an immune response against the cells; however, it is not always feasible or advantageous to collect cells from an individual who is not healthy. Allogeneic cells, in which cells are collected from a healthy donor, expanded, then administered to one or multiple individuals, overcome this issue; however, the recipient may need to take immunosuppressant drugs to prevent rejection of the donor cells. Some cells, like mesenchymal stem cells (MSCs), possess immunomodulatory properties and thereby escape the immune response of recipient organism.

In the embryo, blood vessels are formed from endothelial progenitor cells (EPCs), or angioblasts, in a process known as vasculogenesis. For many years, it was believed that the only mechanism for formation of new blood vessels in the adult was by angiogenesis—the proliferation, migration and remodelling of pre-existing endothelial cells. However, Asahara et al. presented evidence that peripheral blood contains progenitor cells that could differentiate into endothelial cells, therefore demonstrating vasculogenesis could occur in the adult. Since this initial discovery, the following two decades have revealed the vascular wall to be a niche for a number of undifferentiated cells, which contain the potential to differentiate into specific cell types that could promote vasculogenesis. This review will discuss the identified sources of vascular stem cells and summarize pre-clinical data demonstrating the potential of these cells for promoting revascularization and tissue regeneration. Mechanisms of action and delivery of cells to damaged tissues will also be discussed. Finally, the issues associated with developing cell therapy from the lab to the clinic and current clinical trials will be reviewed.

Sources of vascular stem cells

Endothelial progenitor cells

Asahara et al. identified a source of CD34+ cells in the peripheral blood, which also expressed the vascular endothelial growth factor receptor Flk1 and had the ability to form tube-like structures when seeded on fibronectin-coated plates. These cells were found to differentiate into endothelial cells in vitro, expressing the endothelial markers Flk1, CD31 and eNOS and were therefore defined as peripheral blood EPCs. In vivo, injection of EPCs into the tail vein of a mouse model of limb ischaemia (LI) caused these cells to mobilize to the area of ischaemia and appeared to promote vasculogenesis. Subsequently, Ingram et al. demonstrated EPCs that could be isolated from the umbilical vein and Zengin et al. identified a source of CD34+ cells that residing in the vascular wall, located between the smooth muscle and adventitial layers. These cells were negative for CD31, but expressed VEGFR2 and Tie2. In vitro, these cells expressed endothelial cell markers VE-cadherin and occludin and like circulating EPCs,
formed tube-like structures when cultured in collagen gel.\textsuperscript{12} Whilst there is some discussion as to how to truly identify an EPC (reviewed in Madonna et al.\textsuperscript{13}), there has been a number of publications demonstrating EPCs can promote neovascularization \textit{in vitro} and \textit{in vivo}.\textsuperscript{2,10,14} The advantages of using EPCs for cell therapy are that they are easily isolated using a minimally invasive procedure and the cells collected can be used as an autologous cell source. However, EPCs are relatively scarce (Case et al.\textsuperscript{15} found that only 0.0084\% of cells from peripheral blood were EPCs) and this may mean that it is difficult to acquire the required number for cell therapy.

**Mesenchymal stem cells**

MSCs can be isolated from the bone marrow or found in other tissues such as heart,\textsuperscript{16} blood,\textsuperscript{17} skeletal muscle,\textsuperscript{18} adipose tissue\textsuperscript{19} and blood vessels.\textsuperscript{20} They are a heterogeneous subtype of stromal cells. In order to distinguish a clear criteria for defining stem cells of mesenchymal origin, the International Society for Cellular Therapy issued a position statement in 2006\textsuperscript{21} to address this issue. It was proposed MSC populations must express CD90, CD105 and CD73 at >95\% of the total cell population when quantified by flow cytometry.\textsuperscript{21} Additionally, MSC must be capable of differentiating into multiple lineages, including adipocytes, chondrocytes and osteoblasts.\textsuperscript{21} Obtaining MSCs from patients involves processes of varying invasiveness. MSCs can be easily isolated from blood, umbilical cord or bone marrow with minimum disturbance to the patient, whereas collection of blood vessels would involve a surgical procedure. Once collected, cells are isolated from tissue by enzymatic digestion, followed by magnetic sorting to only select MSCs, then expansion \textit{ex vivo}. Therefore, MSCs can be either autologous or allogeneic. \textit{In vivo} injection of MSCs into animal models of MI promotes a reduction in infarct size and improved vascularization.\textsuperscript{4,5} Additionally, it has been proposed that some MSCs also have the potential to differentiate into vascular smooth muscle cells. Both Hu et al.\textsuperscript{22} and Rodriguez-Menocal et al.\textsuperscript{23} provided evidence that suggests MSCs found in the vascular adventitia can also differentiate into vascular smooth muscle cells.

In our lab, we have identified a source of vascular progenitor cells—adventitial progenitor cells (APCs), which express typical pericyte (NG2, PDGFR\(\beta\)), mesenchymal (CD44, CD90, CD105) and stemness (c-kit, GATA-4) markers.\textsuperscript{20} These cells are isolated from saphenous vein remnants from coronary artery bypass surgery.\textsuperscript{20} We plan to use these cells as an autologous source of stem cells for treatment of refractory angina as APCs play an important role in angiogenesis and vascular stabilization, are easily isolated from tissue and expand well \textit{in vitro}. We have demonstrated injection of APCs into mouse models of LI or MI results in increased angiogenesis.\textsuperscript{4,20} Injection of APCs into the LI model caused a significant increase in the time to recover vessel perfusion and increased numbers of capillaries and arterioles when compared to controls.\textsuperscript{20} Similarly, in the mouse MI model injection of APCs resulted in increased myocardial blood flow and increased numbers of capillaries and arterioles 14-days post-MI when compared to controls. A decrease in vascular permeability was also observed. Furthermore, Dil-labelling of the APCs revealed the cells to be found beside capillary endothelial cells.\textsuperscript{4} Currently, we are refining the cell isolation protocol so we are able to isolate APCs to conform to Good Manufacturing Practice (GMP) in preparation for use in a first-in-man clinical trial.\textsuperscript{24} Using the same protocol, we have identified and expanded a similar cell population from the heart of children undergoing repair of congenital cardiac defects. These cells express the previously described pericyte, mesenchymal and stemness markers and can differentiate into vascular smooth muscle cells. \textit{In vitro} experiments demonstrated a pro-angiogenic response when co-cultured with endothelial cells.\textsuperscript{16}

**Other cell sources**

Other sources of cells with vasculogenic potential have been identified. In particular, bone marrow-derived multipotent stem cells are a widely investigated cell type due their ease of extraction, autologous nature and ability to differentiate into a number of...
vascular cell types—endothelial cells, pericytes and smooth muscle cells. Pre-clinical studies have demonstrated their pro-vasculogenic potential and already a number of clinical studies have been performed with these cells (see “Clinical trials” section).

Mechanisms of action
The mechanism of therapeutic action for stem cells is still a matter of some debate. Proposed mechanisms include the direct incorporation of these cells into tissues and their differentiation into the appropriate cell type, paracrine action of pro-angiogenic factors secreted by transplanted cells that stimulate the local micro-environment to promote neovascularization and the recruitment of resident stem cells to the site of injury. Some in vivo studies have reported a significant reduction in number of transplanted stem cells at the site of delivery post-injection, therefore giving more weight to the paracrine action theory. A number of papers have described expression of secreted factors that could promote neovascularization or recruitment of other cells; however, the full secretome produced by these cells remains largely unknown. More recently, it has been suggested that the paracrine action is not just restricted to secretion of soluble factors, but also the action micro-RNAs (small nucleotide sequences capable of modifying gene expression) secreted from the transplanted cells. Katare et al. demonstrated secretion of miR-132 by vascular-derived stem cells enhanced angiogenesis in a mouse model of LI. De Luca et al. recently demonstrated the existence of MSC-derived cellular vesicles that are capable of transporting micro-RNAs that can influence gene expression in surrounding cells, whilst Teng et al. have shown injection of MSC-derived exosomes (small vesicles secreted by cells, thought to play a role in cell–cell communication by transporting RNA or protein) in a mouse of model of MI reduced infarct size and increased capillary density. These recent findings demonstrate significantly that more work is required to elucidate the exact mechanism of action of transplanted stem cells.

Multiple cell-type therapy
As tissues consist of more than one cell type, it may be that the greatest therapeutic benefit would be seen if two or more cells types were administered together. This may enhance regenerative capacities by allowing secretion of a mixture of growth factors, cytokines and/or miRNA that could be missing if one cell line is not present. Currently, there has only been two reports detailing the therapeutic benefit of using more than one cell type. Williams et al. administered both human MSCs and cardiac tissue-derived stem cells (CSCs) into a pig model of MI and found the combination cell therapy caused a 2-fold decrease in infarct size compared to when a single cell type was administered. Similarly, Avolio et al. have demonstrated co-delivery of human CSCs and APCs in vitro resulted in enhanced paracrine activities, whilst in vivo the combination of cells additively reduced the infarct size and promoted vascular proliferation, thus demonstrating combined cell therapy is a viable option, which requires further investigation.

Mode of delivery
In light of the recent developments in stem cell therapy for the treatment of patients with MI, the interest in cell delivery techniques is increasing day by day. Many pre-clinical and clinical trials have delivered cells by direct injection into the heart (intra-myocardial injection), which allows for a targeted approach, but still may involve open heart surgery. Other trials have used intra-coronary infusion of cells. This technique is less complex, offers a more repeatable procedure and avoids the risk of ventricular arrhythmia that is associated with intra-myocardial injection. However, this technique does require the migration of cells into the myocardium. Currently, these mechanisms of delivery are not efficient enough to ensure the desired retention and survival of cells in the target area. A number of studies have demonstrated a significant decrease in cell number after transplantation, some in as little as 48 hours. Additionally, the presence of the transplanted cells has been found in...
other various organs, e.g. liver, lungs and kidney in addition to the target organ. Pre-clinical studies are focusing on new cell delivery systems by exploiting the interdisciplinary methodologies of cell therapy, nanotechnologies and tissue engineering to achieve the therapeutic goal of revascularization and vessel regeneration.

Many methods of delivery are based on cell encapsulation, in which cells are suspended in microparticles of a viscous polymer solution. Pericytes encapsulated in alginate beads have been demonstrated in vitro to stimulate vessel structure formation mediated by the release of paracrine factors. In vivo trials using MSCs and EPCs encapsulated in alginate or arginine-glycine-aspartic acid conjugated alginate demonstrated an increase in arterial collaterals and activation of vascular endothelial growth factor or hepatocyte growth factor pathways. There is however concern about this technique based upon the large modification of the injection area due to the high volume of micro-particles required to reach a beneficial number of cell release.

Other studies have focused on innovative approaches consisting of attaching stem cells on to a delivery platform made by pre-designed scaffolds or injectable hydrogels. Synthetic polymers have been utilized, in particular polycaprolactone (PCL) fibres and PCL nanofibres conjugated with fibronectin, to deliver EPCs and MSCs. Naturally occurring scaffolds have also been utilized. Mesenchymal progenitor cells (MPCs) have been combined with the natural scaffold from decellularized myocardium and fibrin hydrogel resulting in the basal recovery of left ventricular (LV) dimensions and the formation of a micro-vasculature in the infarcted area. Alternatively, researchers are also testing the ability of commercially available prosthetic patches, already used is surgery, as cell carriers. The patches are impregnated with cells then stitched into the damaged area, allowing specific delivery of the therapeutic cells, but also potentially encouraging migration of resident cells. Meanwhile, the number of papers in literature concerning injectable scaffolds is rapidly increasing due to the potential to be easily delivered with the use of catheter. Bone marrow-derived stem cells have been used for treatment of MI delivered in injectable materials such as polyethylene-glycol (PEG), fibrin glue and polysaccharides-based chitosan hydrogel, whilst Portalska et al. recently showed the beneficial effect of dextran-hyaluronic acid hydrogel combined with MSCs for the treatment of ischaemic disease. At first glance, the pre-designed scaffolds represent only a solid carrier for the delivery of cells. However, they offer many other potential benefits such as mechanical properties, chemical cues, and shape and surface patterning, which can vary widely depending on regenerative aim. The intention being the hydrogels or scaffolds will stimulate vasculogenesis as well as deliver cells. All these approaches are summarized in Table 1.

Pathway to the clinic

The difficulties in preparing proof-of-concept cell therapy studies and then translating this to a clinical trial are covered in detail in the following reviews. Here, we outline the immediate issues faced by clinicians and scientists regarding transferring stem cell therapy to the clinic.

Cell therapy products are defined as Advanced Therapy Medicinal Products, which need to be manufactured following strict, centralized procedures resulting in a more complex and less routine regulatory pathway than used for bringing synthetic drugs to the clinic. For example, unlike synthetic drugs, cell therapy products could be susceptible to contamination by bacteria or fungi, allowing accidental introduction of infectious agents. There is also the need to be sure that the introduction of cells does not lead to malignant transformation. Additionally, it is relatively simple to maintain purity and potency in a synthetically manufactured product; however, it is more difficult to maintain product consistency when a different cell donor is used each time. This calls for the use of biomarkers that can predict the therapeutic efficacy of cell products. We have recently demonstrated that the epigenetic profile of APCs does correlate with therapeutic outcome when tested in a mouse model of LI.

Stem cells must be manufactured to comply with GMP. GMP covers the isolation of cells from tissues
and the expansion to the required number of cells. All reagents used must comply with GMP regulations by following Standard Operating Procedures, being free from animal materials and manufactured in a GMP-certified clean room. The cell product must then undergo safety and efficacy testing in an animal model and quality control testing before the cell product can progress to clinical trials (process summarized in Fig. 1). Additionally, any scaffold or hydrogel used to deliver/support the cells must also be GMP certified.

The transfer from laboratory grade cell product to clinical grade can be problematic, for example the necessary reagents may not be available as clinical grade products. Additionally, before commencing GMP manufacture it is necessary to demonstrate all clinical grade reagents used work as efficiently as the research grade reagents and do not cause alteration of the cell characteristics in any way.

**Clinical trials**

Despite the challenges in the transition from laboratory concept to the clinic, a number of clinical trials involving stem cells to treat MI have taken place. Currently, worldwide 139 clinical trials using stem cells for treatment of MI are registered on clinicaltrials.gov, a service of the US National Institute of Health, demonstrating that this is an active area of research. Here, we focus on clinical trials using cells with vasculogenic potential; however, there have also been promising studies performed using cardiac-derived stem cells, which are outside the scope of this review.51,52

A number of trials (summarized in Table 2) have tested the safety and efficacy of MSCs as a treatment for MI, although at present the majority of the cells used was isolated from the bone marrow rather than directly from the vasculature. Hare et al.53 investigated the safety and efficacy of injection of allogeneic human MSCs in patients with MI. They found increased LV function leading to reverse remodelling in treated patients compared to controls. In 2012, a similar phase I/II randomized control study (POSEIDON) was performed to compare the efficacy of injection of allogeneic and autologous MSCs in patients with LV dysfunction. Again this study showed promising ventricular remodelling, and also demonstrated that allogeneic MSCs did not stimulate significant immune reactions.54 The C-CURE study55 isolated bone marrow-derived MSC and exposed them in vitro to a cardiogenic cocktail to form cardiopoietic stem cells, which were then injected into patients. The primary objectives of this study were feasibility and safety, with the secondary objectives being improvement in cardiac function. No safety issues were found. Additionally, LV function was improved and associated with a reduction

**Table 2 Methods used to delivery cell therapy**

<table>
<thead>
<tr>
<th>Delivery technique</th>
<th>Stem cell types</th>
<th>Biomaterials</th>
<th>Clinical application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intro-myocardial infusion</td>
<td>BMDSCs, MSCs, CSCs and pericytes</td>
<td></td>
<td>MI1,27,36</td>
<td></td>
</tr>
<tr>
<td>Intra-coronary infusion</td>
<td>MPCs</td>
<td></td>
<td>MI37</td>
<td></td>
</tr>
<tr>
<td>Intra-muscular infusion</td>
<td>Pericytes</td>
<td></td>
<td>Li20</td>
<td></td>
</tr>
<tr>
<td>Intravenous infusion</td>
<td>MSCs</td>
<td></td>
<td>Li48</td>
<td></td>
</tr>
<tr>
<td>Encapsulation</td>
<td>MSCs</td>
<td>Alginate/arginine-glycine-aspartic acid</td>
<td>MI5,37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EPCs</td>
<td>Alginate</td>
<td>Li40</td>
<td></td>
</tr>
<tr>
<td>Scaffold</td>
<td>BMDSCs</td>
<td>PEG, fibrin glue, chitosan hydrogels</td>
<td>MI44-46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MSCs</td>
<td>Dextran-hyaluronic acid hydrogel</td>
<td>Ischaemic vascular system47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MPCs, EPCs, MSCs</td>
<td>Fibrin, PCL fibres, PCL/fibronectin fibres</td>
<td>MI51-53</td>
<td></td>
</tr>
</tbody>
</table>

BMDSCs, bone marrow-derived stem cells; MSCs, mesenchymal stem cells; CSCs, cardiac stem cells; MPCs, mesenchymal progenitor cells; EPCs, endothelial progenitor cells; PEG, polyethylene-glycol; PCL, polycaprolactone; MI, myocardial infarction; LI, limb ischaemia.
in LV end-systolic volume in cell therapy group compared to controls. Finally, the TAC_HFT study\textsuperscript{56} compared the effect of bone marrow-derived MSCs against whole bone marrow cells. Over 1 year, the Minnesota Living with Heart Failure score significantly improved in MSC and bone marrow groups compared to controls; however, infarct size was only reduced in MSC group compared to controls. In a phase II randomized trial, Losordo\textit{et al.}\textsuperscript{57} isolated circulating EPCs and injected them into the myocardium to treat refractory angina. The assessed outcomes were safety, frequency of angina post-injection and exercise tolerance. There was significantly reduced frequency of angina in the EPC-treated group along with significantly improved exercise tolerance tests. A phase III trial of this therapy, which will increase study participant numbers to over 400, is currently being undertaken.\textsuperscript{58}

Table 2 Summary of clinical trials using MSCs to treat ischaemic heart disease

<table>
<thead>
<tr>
<th>Study</th>
<th>Cell type</th>
<th>Disease</th>
<th>Cell number ($10^6$)</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hare\textit{et al.}\textsuperscript{53}</td>
<td>BM-MSC</td>
<td>MI</td>
<td>Three groups: 0.5, 1.5 and 5 per kg body weight</td>
<td>Significantly increased LV function and remodelling compared to controls</td>
</tr>
<tr>
<td>POSEIDON\textsuperscript{54}</td>
<td>BM-MSC</td>
<td>MI</td>
<td>20</td>
<td>Significant ventricular remodelling</td>
</tr>
<tr>
<td>C-CURE\textsuperscript{55}</td>
<td>BM-MSC</td>
<td>MI</td>
<td>733</td>
<td>Significantly improved LV function, reduction in LV end-systolic volume</td>
</tr>
<tr>
<td>TAC_HFT\textsuperscript{56}</td>
<td>BM-MSC and whole bone marrow cells</td>
<td>MI</td>
<td>200</td>
<td>Significant improvement in Minnesota living with heart failure score. No change in LV function</td>
</tr>
<tr>
<td>Williams\textsuperscript{59}</td>
<td>BM-MSC</td>
<td>MI</td>
<td>100–200</td>
<td>Significantly improved LV function</td>
</tr>
<tr>
<td>Losordo\textit{et al.}\textsuperscript{57}</td>
<td>Circulating EPCs</td>
<td>Refractory angina</td>
<td>0.1 or 0.5 cells/kg</td>
<td>Significantly reduced frequency of angina.</td>
</tr>
<tr>
<td>SCIPIO\textsuperscript{51}</td>
<td>Cardiac stem cells</td>
<td>MI</td>
<td>Maximum of 1</td>
<td>Improved LV function and decreased infarct size</td>
</tr>
<tr>
<td>CADCEUS\textsuperscript{52}</td>
<td>Cardiosphere-derived cells</td>
<td>MI</td>
<td>12.5–25</td>
<td>Decreased scar size</td>
</tr>
<tr>
<td>BONAMI\textsuperscript{60}</td>
<td>BM-MSC</td>
<td>MI</td>
<td>98</td>
<td>Improved myocardial viability</td>
</tr>
<tr>
<td>REGENT\textsuperscript{61}</td>
<td>BM-MSC</td>
<td>MI</td>
<td>178</td>
<td>No change in LV function</td>
</tr>
<tr>
<td>CASTELLANI\textsuperscript{62}</td>
<td>CD133+ (from peripheral blood)</td>
<td>MI</td>
<td>5.9</td>
<td>Significant decrease in infarct size, no change in LV function</td>
</tr>
<tr>
<td>Gao\textsuperscript{63}</td>
<td>BM-MSC</td>
<td>MI</td>
<td>3.08</td>
<td>No significant change in LV function</td>
</tr>
<tr>
<td>Lipiec\textsuperscript{64}</td>
<td>BM-MSC</td>
<td>MI</td>
<td>CD133 + 0.33CD34 + 0.36</td>
<td>No significant change in LV function</td>
</tr>
<tr>
<td>Quyyumi\textsuperscript{65}</td>
<td>BM-MSC</td>
<td>MI</td>
<td>Three doses: 5, 10 and 15, respectively</td>
<td>Trend for increase in LV function</td>
</tr>
</tbody>
</table>

BM-MSCs, bone marrow-derived MSCs.
Overall therapeutic potential

Meta-analysis of stem cell clinical therapy currently at best shows a modest benefit and again is mainly restricted to bone marrow-derived cells. Meta-analysis of eight trials by Liu et al. found that bone marrow stem cell therapy significantly improved Left Ventricular Ejection Fraction (LVEF) by 3.2%. Likewise similar conclusions were drawn in a separate analysis by Clifford et al. These authors found that in addition to improved LVEF, the observed effects were sustained long term. However, Gyongyosi et al. recently published a meta-analysis of 12 clinical trials using stem cells to treat MI and found that there was no beneficial effect. The primary endpoints of the studies examined were to prove that stem cell treatment was safe and did not cause major adverse cardiac events and this was proven. In terms of secondary endpoints, such as improvement in LV function, this meta-analysis found no benefit. The authors acknowledge that there were limitations in this analysis that could be confound the outcome. The analysis was based on several studies combining several different cell sources. Additionally, not all studies were included, this analysis did not include allogeneic cell trials, only autologous. The challenges in analysing data with significant clinical heterogeneity are discussed by Huang et al. Additionally, most of these current studies are relatively small; thus, the power may not be great enough to demonstrate efficacy, particularly when multiple comparisons are made. This issue is discussed in detail by Simari et al.

Conclusions

There are numerous pre-clinical studies indicating the beneficial effects of vascular stem cell therapy for the treatment of ischaemic disease; however, the results seen in the clinic are more modest. The conclusive outcome from recent clinical studies is that stem cell therapies (regardless of cell source: bone marrow, MSC or EPC) are a safe option for treatment of MI. However, at present the produced results do not match the promise of pre-clinical studies and so it is not possible to make a definitive statement on clinical effect. The field of stem cell therapy is still in its infancy and so there are still many areas where optimization is required. There is still uncertainty regarding the best cell type to use (most current trials have tested bone marrow-derived cells), and clinical data on stem cells derived directly from the vasculature are still relatively scarce compared to the available pre-clinical data. Also, the number of cells used therapeutically in clinical trials varies enormously and much more work is still needed on the mechanism of action of cell therapy. What soluble factors are produced by transplanted cells, the contents of exosomes they produce and how these factors interact with resident cells are still unanswered questions. Also, the effect of administering a combination of cell types needs more investigation. Additionally, the best way to deliver the cells is at present a very active research area. It is recognized that injected cells do not remain at the site of injury and so the use of scaffolds may be of great therapeutic benefit, not only for keeping the transplanted cells in situ or possibly allowing resident cells to incorporate into the scaffold, but also the scaffold itself may contribute to vasculogenesis.

In summary, the current evidence suggests that vascular stem cells continue to offer a viable option for the treatment of ischaemic disease but further, larger scale clinical trials, along with additional laboratory-based investigations into mechanisms of action and delivery methods, are required to address the present modest therapeutic benefit.

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