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Pericytes from human veins for treatment of myocardial ischemia

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Abstract

Stem cell therapies promise to regenerate the infarcted heart through the replacement of dead cardiac cells and stimulation of neovascularization. New research from our laboratory shows the transplantation of stem cells from human veins helps heart healing after an acute ischemic insult. Using a mouse model, we demonstrated that pericytes expanded from redundant human leg veins relocate around the vessels of the peri-infarct zone and release factors that promote reparative angiogenesis and cardiomyocyte survival and inhibit interstitial fibrosis. We plan to perform a first-in-man clinical trial with human pericytes in patients with refractory myocardial ischemia in the next 5 years.

Introduction

Coronary artery disease (CAD) is a leading cause of death in the Western world. Although mortality rates continue to fall, the number of death is still staggeringly high. In the UK alone, there are ~124,000 cases of myocardial infarction (MI) every year, 88,000 of which are fatal. Critical limb ischemia (CLI) is estimated to affect 1 in 2500 of the UK population annually.

Current care includes lifestyle modifications and drugs to reduce blood cholesterol, glucose and hypertension. Angioplasty or bypass surgery is used to remove the arterial blockage. However, revascularization is not amenable to all the patients, is too-often followed by restenosis, and does not halt the underlying pathology. Progression of CAD eventually leads to heart failure (HF), which currently affects more than 750,000 people in the UK and has a worse prognosis than many cancers. Major limb amputation is often needed as an ultimate remedy in CLI patients. A second amputation becomes necessary in 30% of cases and death occurs in 25% within the first year post-amputation. The social and economic burden of cardiovascular disease is enormous. Hence new cost-effective treatments are urgently required.

To reverse this trajectory, in the 1990s researchers began investigating non-invasive treatments that could trigger the formation of new blood vessels. The very first attempt consisted of treating a CLI patient with an angioplasty balloon coated with the vascular endothelial growth factor-A (VEGF-A) gene. This promoted the formation of new collaterals and improved blood flow, thus introducing the new medical concept of

therapeutic angiogenesis (Isner et al., 1996). Data accumulated over the following 20 years showed that angiogenic gene therapies have an acceptable safety profile. However, their efficacy proved to be inconsistent in phase II–III clinical trials (Gupta et al., 2009), including two multi-centre studies using hypoxia-inducible factor 1a (HIF-1a, Genzyme) (Creager et al., 2011) and fibroblast growth factor 1 (FGF1, Sanofi) (Belch et al., 2011). The reasons for these failures remain incompletely understood.

In the meantime, cell therapy has made its grand entry, promising to provide a new means to restore the integrity of damaged tissues. Contemporary approaches consist of subsidizing the ischemic heart and limb muscles with boluses of stem cells (SCs) with the hope that encouraging the formation of new blood vessels may eventually result in myocyte salvage/regeneration (Dimmeler et al., 2005; Behfar et al., 2008; Gupta et al., 2009; Belch et al., 2011; Creager et al., 2011). Meta-analyses of recent clinical trials indicate that autologous cell therapy is safe and provides a modest benefit in patients with acute MI or HF (Martin-Rendon et al., 2008; Chavakis et al., 2010). Transplantation of bone marrow (BM) cells reportedly benefits patients with CLI (Mackie and Losordo, 2011).

So far, some 1500 cardiovascular patients were treated with adult SCs worldwide. Moreover, several biotech companies are moving their proprietary cell products to the clinic. Nonetheless, underlying mechanisms remain elusive, spanning from cell differentiation to a more realistic promotion of cell survival and angiogenesis through the release of growth factors, cytokines and microRNAs (Dimmeler et al., 2005; Burchfield and Dimmeler, 2008; Gneccchi et al., 2008; Loffredo et al., 2011).

Although first-generation cell therapy proved to be feasible and generally safe, clinical efficacy is significantly inferior compared to the success reported in animal models. One reason for these modest results is that current therapies are unable to build a robust, long-lasting neovascularization. This review article highlights recent evidence indicating that pericytes could be the missing piece of the regenerative puzzle.

Topography and antigenic characteristics of pericytes

Pericytes, also known as Rouget cells or mural cells, are adjacent to capillaries in a variety of tissues (Hirschi and D'Amore, 1996). Typical microvascular pericytes are shaped with a prominent nucleus and a tiny cytoplasm, which protrudes into long extensions encircling the capillary wall. Pericytes are embedded within and contribute to the production of components of the vascular basement membrane. Under homeostatic conditions, pericytes provide physical strength and nurturing signals to adjacent endothelial cells. Moreover, during angiogenic sprouting, pericytes instruct neovessels to organize in a stable and efficient tubular network. Among their diverse functions, pericytes are also thought to be multipotent, being capable of differentiating into adipocytes, osteoblasts and phagocytes. Recent studies used immunohistochemistry and flow cytometry to identify CD146^{high} (pericyte/endothelial marker), CD34[−] (vascular cell marker), CD45[−] (leukocyte marker), CD56[−] (satellite cell marker) and CD144[−] (endothelial marker) pericytes in adult and fetal human tissues (Crisan et al., 2008; Corselli et al., 2010). In addition, we identified multipotent pericytes in the adventitia of arteries and veins (Campagnolo et al., 2010). These adventitial cells express *in situ* typical pericyte markers (NG2 and PDGFR β) and mesenchymal markers (CD44, CD90, CD73, and CD29) as well as stem cell antigens (Oct-4 and Sox-2), but are negative for myogenic, hematopoietic and endothelial markers, including CD31 and CD146. Moreover, adventitial pericytes natively express and can be sorted by the use of the CD34 antigen. However, CD34 expression is lost during cell isolation and expansion (Campagnolo et al., 2010). This antigenic phenotype was confirmed after cloning and sub-cloning of single sorted adventitial pericytes (Campagnolo et al.,

2010). Therefore, substantial antigenic differences emerge from comparison of “classical microvascular pericytes” and adventitial pericytes. It remains unknown to date if the two populations are the progeny of a common ancestor cell and if one is the precursor of the other.

Therapeutic activity of adventitial cells from human fetal aorta

An initial study identified the presence of mesenchymal stem cells in the rat dorsal aorta at early embryonic stages (De Angelis et al., 1999). A few years later, Alessandri et al. reported that similar cells are present in the aorta adventitia of 11- to 12-week-old human fetuses (Alessandri et al., 2001). These cells co-express the stem cell marker CD133 and hematopoietic/endothelial markers, like CD34 and VEGF receptor-2 (VEGFR2/KDR). In culture systems containing VEGF and platelet-derived growth factor- β (PDGF- β), fetal aorta (FA)-derived adventitial cells give rise to a mixed population of mature endothelial and mural cells. In 3D collagen, they grow as non-adherent cells and reorganize into cohesive cellular cords that resemble mature vascular structures. Upon transplantation into ischemic limb muscles of immunodeficient mice, FA-derived adventitial cells markedly improve neovascularisation, inhibit ischemia-induced apoptotic cell loss, and stimulate myocyte regeneration. These combined actions lead to acceleration of perfusion recovery and reduction of toe necrosis (Invernici et al., 2007).

We next evaluated the healing potential of human FA-derived CD133⁺ progenitor cells and their conditioned medium in a mouse model of ischemic diabetic ulcer (Barcelos et al., 2009). Type 1 diabetic mice underwent bilateral limb ischemia and punch wounding at the level of the calf. One wound was covered with collagen containing 20,000 cells or vehicle (collagen) and the contra-lateral was covered with vehicle as an internal control. We found that FA-derived CD133⁺ progenitor cells accelerate wound closure as compared with vehicle and promoted peri-wound angiogenesis, through stimulation of endothelial cell proliferation, migration and survival by paracrine secretion of VEGF-A and interleukin (IL)-8. Consistently, application of CD133⁺ progenitor cell conditioned medium improves wound healing, with benefit being abrogated by VEGF-A or IL-8 inhibition or antagonism of Wnt signaling (Barcelos et al., 2009). *In vitro*, these effects were recapitulated following exposure of high-glucose-primed human umbilical vein endothelial cells to the cell conditioned medium, resulting in stimulation of migration, angiogenesis-like network formation and induction of Wnt expression (Barcelos et al., 2009). We concluded that the human FA-derived progenitor cells represent a useful means for treatment of ischemic ulcers.

Searching for similar regenerative cells in adult vessels

FA-derived cells used in our initial studies were obtained from abortive material. Hence, we were concerned that ethical considerations might hinder the development of a universally accepted cell product. Therefore, we next investigated if saphenous vein leftovers from coronary artery bypass graft (CABG) surgery could be a suitable source of analogous regenerative elements. Indeed, we found that CD34⁺ cells coexpressing pericyte markers are localized around adventitial *vasa vasorum* (Campagnolo et al., 2010). After dissection of the vein from surrounding tissues and enzymatic digestion, CD34⁺/CD31⁻ cells were isolated by selective culture, immunomagnetic beads, or fluorescence-assisted cell sorting. In the presence of serum, CD34⁺/CD31⁻ cells give rise to a highly proliferative population that express pericyte/mesenchymal antigens together with the stem cell marker Sox2 and show clonogenic and multilineage differentiation capacities (Campagnolo et al., 2010). We called this population “saphenous vein-derived progenitor cells” (SVPs). Interestingly, at variance with FA-derived cells, SVPs grow as adherent cells, do not form spheroids and are less

abundant in CD133. Furthermore, they do not express endothelial and myogenic lineage markers. In culture, SVPs integrate into networks formed by endothelial cells and support angiogenesis through paracrine mechanisms, involving SVP-secreted Angiopoietin-1 and VEGF-A, which stimulate endothelial cell proliferation, and endothelial cell-secreted PDGF- β , which activates SVP recruitment (Campagnolo et al., 2010). Moreover, we showed that SVPs express and secrete the pro-angiogenic microRNA-132 (miR-132) and that miR-132 is then taken up by endothelial cells in co-culture systems (Katare et al., 2011). The transfer of miR-132 is instrumental to the *in vitro* angiogenic action of SVPs, because silencing miR-132 in SVPs prevents their ability to enhance endothelial network formation (Katare et al., 2011).

We performed a series of preclinical studies in animal models to verify the efficacy and safety of SVP transplantation. In a murine model of limb ischemia, we showed that intramuscular injection of SVPs improves angiogenesis and accelerates limb reperfusion (Campagnolo et al., 2010). By contrast, transplantation of early-culture endothelial blood monocytes, used as a cellular control, improved postischemic neovascularization, without accelerating blood flow recovery (Campagnolo et al., 2010). Next, we tested SVP transplantation in immunodeficient mice with acute MI (Katare et al., 2011). Of interest, intra-myocardial injection of SVPs led to preservation of left ventricular ejection fraction (LVEF) and cardiac output (CO). Dosage titration experiments showed that intra-myocardial injection of 300,000 SVPs is sufficient to reach maximal effects without incremental benefit using 1,000,000 cells (Katare et al., 2011).

Unpublished data indicate that challenging SVPs against allogeneic lymphocytes results in low levels of cyto-toxicity. This suggests that SVPs do not trigger cell-mediated immune response, thus opening the possibility of allogeneic cell therapy. Therefore, we next investigated if injection of SVPs in the infarct border zone of immunocompetent mice induces benefit similar to that seen in immunodeficient mice. We observed that transplantation of SVPs promotes a long-lasting improvement of cardiac function, increases myocardial blood flow and blunts the effect of ischemia on vascular permeability, thereby reducing myocardial edema (Katare et al., 2011). Moreover, SVP transplantation stimulates reparative neovascularization and reduces infarct size and interstitial fibrosis (Katare et al., 2011) (Fig. 1).

Our investigation was then directed to explore the mechanisms underlying cardiac protection by SVP therapy (Fig. 2). We observed that SVPs attract other regenerative elements to the infarct border zone, namely VEGF-B expressing monocytes and cardiac progenitor cells (CPCs) (Katare et al., 2011). Moreover, we found that SVPs increase miR-132 levels in the infarcted heart (*via* paracrine release and stimulation of miR-132 production by the recipient's cells) and thereby inhibit the expression Ras-GTPase activating protein (RasGAP) (Katare et al., 2011), a validated miR-132 target (Anand et al., 2010). By removing RasGAP inhibition, miR-132 stimulates Ras-dependent angiogenesis. Furthermore, SVP-induced miR-132 inhibits methyl-CpG-binding protein 2 (MeCP2) and angiotensin-1 AT1 receptor, which are implicated in adverse cardiac remodeling and fibrosis (Mann et al., 2010; Jin et al., 2012). Notably, SVPs transfected *ex vivo* with a miR-132-silencing vector failed to improve cardiac function, reparative angiogenesis and fibrosis (Katare et al., 2011). This indicates that miR-132 secreted by SVPs acts as a pleiotropic mechanism to improve different aspects of post-ischemic remodeling.

Preclinical studies were reassuring on safety, with no calcification or tumors being observed to date (Campagnolo et al., 2010; Katare et al., 2011). Comparison with BM-derived mesenchymal SCs showed that SVPs are superior in terms of efficacy and safety, as they

produced a greater improvement in cardiac function and no myocardial calcification in mice with MI. This latter side effect was common with mesenchymal SCs (Katare et al., 2011).

Conclusions

SVPs accomplish important requirements for cell therapy, being present in an easily accessible adult tissue, expandable *ex vivo* and able to engraft into host neovascularization, herein maintaining their native specialized function. This exciting discovery is one more step towards mending broken hearts. It shows that ‘one man’s trash could be another’s treasure’: cells from leftover vein normally binned after heart bypass surgery or saphenectomy could infact repair heart damage in an allogeneic recipient. However, we initially envision the use of SVPs for autologous transplantation. Our plan is to develop a clinical grade cell product for a first-in-man study in patients not amenable of revascularization. While it is early days, this research shows that pericytes may have a significant potential for the treatment of ischemic disease.

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Fig. 1. Representative confocal microscopy images showing the saphenous vein derived pericytes transplanted into mouse infarcted hearts. Transplanted pericytes (red) are localized along the isolectin stained blood vessels (green) in the myocardium at 5 days post-MI. Scale bars are 50 μm .

Fig. 2.

Cartoon summarizing the mechanisms of pericyte-induced therapeutic action. CM: cardiomyocyte, MF: myofibroblasts, CPC: cardiac progenitor cell, EC: endothelial cell. Secreted factors are highlighted in red and factors inhibited by microRNA-132 are highlighted in orange and overlaid by a cross. VEGF and Angiopoietin stimulate EC proliferation and cardiomyocyte survival. VEGF-B and HGF stimulate CPC migration; furthermore, MCP-1 attracts angiogenic monocytes (not shown in the cartoon). MiR-132 inhibits Ras-GAP, angiotensin 1 receptor (AT1R) and MeCP2, thereby promoting angiogenesis and inhibiting cardiac hypertrophy and fibrosis.