Umbilical cord blood-derived mesenchymal stem cells: New therapeutic weapons for idiopathic dilated cardiomyopathy?

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Abstract

Dilated cardiomyopathy is the most frequent etiology of non-ischemic heart failure. In a majority of cases the causal mechanism is unknown, giving rise to the term ‘idiopathic’ dilated cardiomyopathy (IDCM). Major pathological derangements include patchy interstitial fibrosis, degenerated cardiomyocytes, and dilatation of the cardiac chambers, but recent evidence suggests that disease progression may also have the signature of cardiac endothelial dysfunction. As we better understand the molecular basis of IDCM, novel therapeutic approaches, mainly gene transfer and cell-based therapies, are being explored. Cells with regenerative potential have been extensively tested in cardiac diseases of ischemic origin in both pre-clinical and clinical settings.

This article is a concise summary of cell therapy studies for IDCM, with a focus on recent advances that highlight the vascular potential exhibited by umbilical cord blood-derived mesenchymal stem cells (UCBMSCs). We also provide an overview of cardiac vasculature as a key regulator of subjacent myocardial integrity and function, and discuss the potential mechanisms of UCBMSC amelioration of IDCM myocardium. Consideration of these issues shows that these cells are conceivably new therapeutic agents for this complex and elusive human disorder.

1. Introduction: idiopathic dilated cardiomyopathy

Dilated cardiomyopathy is the most frequent etiology of non-ischemic heart failure and is characterized by ventricular chamber enlargement (adverse remodeling) or dilatation and systolic dysfunction with normal left ventricular (LV) wall thickness [1,2]. With remarkable annual prevalence (1:2500) and incidence (1:15,000–18,000) rates in adults [4], dilated cardiomyopathy affects either sex and people of any ethnic origin, who suffer a progressive decrease in LV contractility and often sudden death; approximately 50% of individuals are reported to die within 5 years of diagnosis [5]. In a majority of cases the causal mechanism is unknown, giving rise to the term ‘idiopathic’ dilated cardiomyopathy (IDCM). Causes are multifactorial and include genetic or environmental factors, which can manifest clinically at a range of ages [4]. Despite higher survival rates with evidence-based drugs, devices, and surgery [5], the only definitive treatment is heart transplantation, which is often restricted to a select minority due to the scarcity of organ donations. The health costs of IDCM are great worldwide [6]. Histologically, the major pathological derangements include patchy interstitial fibrosis, degenerated cardiomyocytes, and dilatation of the cardiac chambers, but recent evidence suggests that disease progression may also have the signature of cardiac endothelial dysfunction [7].

New therapeutic approaches are being explored to counteract irreversible myocardial damage and subsequent alterations in remodeling in IDCM. For example, the application of cells with regenerative potential is currently being explored [8,9] with an increased understanding of the molecular basis of disease [10–12] and supplementary therapies, such as those based on gene transfer [13]. In both pre-clinical and clinical settings, cell-based therapies for cardiac diseases of ischemic origin (i.e., acute myocardial infarction and chronic ischemic heart disease) keep the stakes very high. In contrast, the potential benefit of cell therapy for IDCM is still being evaluated.

This article is a concise summary of cell therapy studies for IDCM, with a focus on recent advances that highlight the vascular potential exhibited by umbilical cord blood-derived mesenchymal stem cells (UCBMSCs). We also provide an overview of cardiac vasculature as a key regulator of subjacent myocardial integrity and function and discuss the potential mechanisms of UCBMSC amelioration of IDCM.
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2. IDCM: a myocardial disease arising from alterations in the cardiac endothelium

Histological examination of the myocardium in IDCM reveals some characteristic macroscopic hallmarks, including altered cardiac muscle integrity, myocyte atrophy, and increased deposition of collagen and lipids around myocardial filaments (Fig. 1) [14–16]. Notably, cardiac endothelial dysfunction has been associated with progression and poor prognosis of the disease [7]. Over the past few years, research in this field has been aided, in part, by the implementation of sophisticated imaging techniques, such as angiography and computer tomography, which show the mismatch between artery size and left ventricular mass, clear side branch paucity, and shorter, thinner epicardial arteries in IDCM [17,18]. More importantly, reduced and sparse microvasculature has been detected in both patients and animal models that present with disease characteristics resembling those in humans (Fig. 1) [18–21].

Medical research has been advanced by the development of animal models with pathological traits similar to those seen in patients. In contrast to myocardial infarction [22–26], IDCM research has been hampered because none of the early animal models completely conveyed the complex traits of heart failure, including fibrosis, inflammation, and apoptosis, which evolve over a period of months and years [7,27]. Small animal models commonly generated to investigate disease etiology and progression and to assess possible novel therapeutic targets fall into two different categories: non-genetic and genetic [7]. Remarkably, in 2010, Huang et al. reported a mouse model of chemically induced cardiomyopathy that exhibited similar reduced blood flow, coronary branching, and capillary density as found in patients [21]. This model, which culminates in the development of life-threatening cardio-myopathy after exposure to the anticancer anthracycline doxorubicin [28], has been proposed for the assessment of functionally improved IDCM-derived circulating myeloid cells, which exhibit impaired stromal cell-derived factor 1-mediated migration and enhanced myocardial revascularization and cardiac function [12].

Studies in large animals are pivotal for progressing towards clinical translation. Pacing-induced heart failure models (in dogs and pigs) are frequently used because they result in myocardial remodeling and chamber dilation [29,30]. Rapid ventricular pacing induces a low output cardiomyopathic state and neurohormonal activation similar to that seen in human IDCM. Further experiments in this model have provided insights into the molecular and cellular basis of IDCM [31], and opportunities to test the efficacy of potential treatments [30,32]. Rapid pacing-inducing tachycardia leads to marked but reversible alterations in the discontinuation of cardiac stimulation, whereas coronary microembolization establishes a refractory heart failure [29].
Taken together, small and large animal studies demonstrate that alterations in both cardiac muscle and endothelia contribute to the contractile deficiency of and pump failure in the IDCM myocardium [7].

3. Cell-based studies for IDCM

A ‘stem’ or progenitor cell, by definition, is an undifferentiated cell able to proliferate via self-renewal to produce a large number of differentiated progeny and regenerate tissue [33]. For decades the heart was widely accepted to be a terminally differentiated organ without the capacity of self-renewal after injury. Subsequently, key studies of cardiomyocyte turnover, cardiac chimerism after pregnancy or the recipients of hearts donated by other humans, and resident cardiac progenitor cells showed great promise for launching a new era in the treatment of cardiac diseases [34]. However, this endogenous capacity for regeneration is insufficient to mediate repair after severe cardiac injury. Thus, the ability of cardiac tissue to recruit highly plastic extracardiac cells would be beneficial to regeneration. In this context, during the past 20 years, great effort has been made to improve cardiac regeneration through cell-based therapies, primarily in ischemic heart disease. Advanced therapeutic options are being developed to generate new myocardial tissue and blood vessels using cells, either alone (cellular cardiomyoplasty) or, more recently, with biological and/or synthetic materials and growth, proangiogenic, and differentiation factors (cardiac tissue engineering) [35,36]. These approaches have been demonstrated to be safe with encouraging signs of functional improvement in a variety of cardiac diseases in the pre-clinical setting [37–40]. Scarce evidence is available regarding the potential benefit of regenerative cells in IDCM due to the limited number of clinical trials conducted to date [8]. The majority of these studies have been designed for the use of bone marrow-derived cells. Although being multipotent and an autologous source for cell infusion, there are several obstacles to recognize these cell suspensions as fully efficient products for therapy. These limitations include high cell heterogeneity and restricted capacity of expansion ex vivo [34]. Accordingly, in terms of positive cell selection, specific antibodies against cell surface markers such as CD34 have been applied to implant more homogeneous cell suspensions [41,42,49].

Table 1 summarizes the major clinical trials in IDCM. In this context, Vrtovec et al. reported the results of intracoronary transplantation of bone marrow CD34+ cells [41]. This study, which was based on cell mobilization with granulocyte colony-stimulating factor (G-CSF) and subsequent magnetic separation of the cells from peripheral blood, reported improved LV ejection fraction (LVEF), better exercise tolerance, and increased survival. The same authors further investigated the long-term effects and relationship between intramyocardial cell homing and clinical response [42]. In this separate study, the improvement in LVEF was more significant in patients with higher myocardial homing of the injected cells. During the follow-up, 25% of patients died and 8% underwent heart transplantation. However, the total mortality was lower in patients who received cells (14%) than in controls (35%). Using the same technical procedure, Bocchi et al. confirmed the short-term feasibility, safety, and beneficial effects of G-CSF administration associated with intracoronary infusion of an enriched suspension of CD34+ cells [43]. In contrast, the Autologous Bone Marrow Cells in Dilated Cardiomyopathy (ABCD) trial reported a significant improvement in NYHA functional class by at least one degree in 63% of patients in the treatment arm. LVEF also increased substantially (from 22.5 ± 8.3% to 28.4 ± 1.8%) due to a significant change in LV end-systolic volume (LVEVS), which is a major determinant of adverse remodeling, in cell-treated patients (from 137.3 ± 62.6 mL to 120 ± 52 mL) [44]. Furthermore Fischer-Rasokat et al. and Martino et al. were able to demonstrate that intracoronary administration of unselected bone marrow cells was associated with LVEF and quality improvements, respectively [45,46]. In the study of Brazilians, four patients died between 6 and 12 weeks after implantation procedure [46]. The authors, however, concluded that the number of deaths was compatible with the disease state presented by the patients (mean LVEF was below 25% as determined by echocardiography and 20% by magnetic resonance imaging) and was identical to that reported by Seth et al. [44].

Other studies have assessed the effects of additional cell types and/or implantation techniques. For instance, direct intramyocardial implantation of fetal-derived stem cells was studied by Benetti et al. in patients with heart failure due to non-ischemic, non-chagasic dilated cardiomyopathy [47]. The authors concluded that this procedure was...
feasible and improved LVEF, quality of life, and exercise tolerance at 40 months. Intramyocardial transplantation of bone marrow cell suspensions was also demonstrated by Kalil et al. to be feasible and safe and resulted in early improvements in symptoms and LV performance in nine patients [48]. However, medium-term evaluation of cell-treated patients revealed a regression of LV function. Compared to the intracoronary route, transendocardial transplantation exhibited greater myocardial CD34+ cell retention 18 h after the procedure (19.2 ± 4.8 versus 4.4 ± 1.2%, P < 0.01), and improved LVEF (+8.1 ± 4.3 versus +4.2 ± 2.3%, P = 0.03), and LVEF demonstrated a regression of LV function. Compared to placebo at 12 months (628 ± 211 versus 315 ± 133 pg/mL, P = 0.04), and 6-minute walk test distance (125 ± 33 versus 86 ± 13 m, P = 0.03) at 6 months [49].

Collectively, these clinical trials indicate that, similar to ischemic heart disease, intracoronary administration of bone marrow-derived cells is safe and improves surrogate endpoints in IDC. Notably, although great heterogeneity is present in the study characteristics, the methodological quality is often limited, and the disease evolution time prior to study enrollment is not indicated. These reports conclude that cell therapy is an option to pursue in large prospective double-blind placebo-controlled studies with long-term follow-up. However, further investigation of arrhythmia occurrence, mortality, and optimal cell and infusion systems is needed. Thus, more details from ongoing trials are awaited with interest. In particular, the Poseidon-DCM Study (NCT01392625) has been designed to compare the 1-year potential benefits of transendocardial injection of autologous (N = 18) versus allogenic (N = 18) bone marrow mononuclear cells isolated by plastic adherence and expanded in cell culture. The REGENERATE-DCM trial (NCT01302171) is also evaluating the effect of intracoronary injection of bone marrow mononuclear cells compared to placebo at 12 months in patients pre-treated with G-CSF (n = 60) [50].

In the following pages of this review we recount the facts, the pros and cons, and the reasons for using mesenchymal stem cells (MSCs) from umbilical cord blood (UCB) instead of bone marrow mononuclear cells or other potential cell types for therapy in IDC. Particularly, the great vascular capacity, potential mechanisms and benefit of these therapeutically valuable cells are discussed.

4. Vascular potential of umbilical cord blood-derived mesenchymal stem cells

A large body of evidence shows that the endothelium plays critical roles in organism homeostasis and the movement of nutrients, gasses, and waste products to and from cells. Thus, therapeutic procedures capable of restoring the blood supply to injured tissues, including the myocardium in IDC, may be more valuable for regenerative medicine. As the identification of genuine vascular precursors has been the subject of great controversy [51–55], alternative cell sources and lineages with vascular potential are under intense scrutiny [56]. Vascular growth properties have been documented for bone marrow progenitors [57,58], adipose tissue-derived progenitor cells [59,60], embryonic stem cells [61], induced pluripotent stem cells (iPSCs) [62], and UCBMSCs [63,64].

MSCs are central to the major mechanisms underlying blood vessel repair [65]. Cells with multi-potentiality for forming adipogenic, chondrogenic, and osteogenic lineages after clonal expansion in vitro, now termed mesenchymal, were identified over four decades ago by Friedenstein and colleagues [66,67] and further characterized by Marshak’s group [68]. Although MSCs are best known by their extraction from bone marrow, similar cells have been isolated from a variety of other postnatal and perinatal tissues, including peripheral blood, fat, dental pulp, cartilage, skeletal muscle, dermis, lung, Wharton’s jelly, amniotic fluid, placenta, periosteum, and synovial fluid and membrane [65]. MSCs have the advantage to be homogenous and expandable to high cell numbers ex vivo in comparison with both selected (e.g. CD34+) and unselected bone marrow cells [34]. MSCs also exhibit low immunogenicity, e.g. those derived from UCB which are therefore immunologically safe for use in allogeneic clinical applications [69]. In terms of disadvantages, sometimes the MSC isolation procedure is invasive and painful (i.e. from bone marrow) and their cardiac application involves adverse reactions such as calcification and bone formation [34]. Alternatively, iPSCs represent a potentially unlimited source for generation of both cardiomyocytes and endothelial cells [62]. However, although there are no ethical objections to the use of iPSCs and their derivatives compared to totipotent embryonic stem cells, teratoma formation and immunoreactivity are still active areas of testing before any clinical use of these promising cells [62,70].

The absence of ethical concerns and unlimited cell supply due to the continuous growth of the world population explain the increasing interest in using UCB for multiple clinical settings. Indeed, UCB is considered as the most replete reservoir of regenerative cells. Although used mainly for cell transplantation against blood disorders, the spectrum of diseases where UCB is effective has been expanded to non-hematopoietic alterations and as a form of regenerative cell therapy and immune modulation [64,71,72]. Moreover, UCB can be safely and painlessly extracted, long-term cryopreserved without loss of basic properties, and has a lower risk of transmitting viral infections or somatic mutations than adult cell sources [64]. The most marked limitation in the use of UCB is its low progenitor cell concentration, albeit transplantation of double partially HLA-matched UCB units is recognized as a simple approach for overcoming cell dose limitation [73].

As mentioned above UCB also contains MSCs [74], which play key roles in the regulation of blood vessel formation [75]. UCBMSCs were initially characterized as spontaneously exhibiting cardiomyogenic traits, such as abundant expression of α-actinin, connexin-43, and SERCA-2 [74]. Subsequent studies demonstrated that they do not differentiate into functional cardiac muscle-like cells following a broad range of in vitro stimuli or by co-culture with neonatal rat cardiomyocytes [76]. However, Roura et al. recently examined the intracellular regulatory mechanisms involved in the angiogenic potential exhibited by UCBMSCs and reported the existence of a conserved molecular machinery controlling angiogenesis between multipotent MSCS and mature endothelial cells [77]. These authors first observed that UCBMSCS have the ability to develop well-organized vascular-like networks with high amounts of nuclear early growth response factor (Egr)-3 in a standard Matrigel-based assay of angiogenesis [75]. Further experiments demonstrated that this angiogenic capacity is regulated by a PKC/MAPK/ERK signaling pathway, which efficiently controls Egr-3 protein levels in these cells [77]. Moreover, using non-invasive bioluminescence imaging (BLI) [78], genetically-modified UCBMSCS have been assessed in a mouse model of angiogenesis by inducing the expression of chimeric luciferase/fluorescent proteins [75]. Interestingly, implanted cells migrated and self-organized into new functional blood vessels connected with the host circulatory system.

Despite the administration of isolated cell suspensions being proved feasible and safe in clinical settings, the degree of newly regenerated tissue and subsequent functional recovery are modest at best. As such, tissue engineering procedures have emerged as novel, advanced options to increase the regeneration of whole organs or locally damaged tissue [36,79]. In this setting, UCBMSCs embedded in a fibrin patch have been employed as a myocardial bioprosthesis to exert beneficial effects following acute myocardial infarction in mice [75]. Implanted cells were reported to be retained for several weeks over the infarcted myocardium, proliferating early and differentiating into the endothelial lineage, inducing light and fluorescence emission through the activation of inducible and constitutive promoter-regulated luciferases and fluorescent proteins, as well as forming vascular-like networks newly expressing the endothelial-specific surface marker CD31 (Fig. 2). Animals treated with this newly designed myocardial bioprosthesis exhibited reduced infarct scarring and larger vessel density than control animals. More recently, Kang et al. demonstrated that fibronectin-immobilized polycaprolactone nanofibers are effective carriers for UCBMSCs
transplanted following myocardial infarction [80]. Although it is not yet known whether these effects can be extrapolated to IDCM, it is intuitive that patients may gain deep clinical benefit by the delivery of any stem cell type with a great capacity of forming and/or repairing vasculature and extremely low immunogenicity (Fig. 3).

5. Potential mechanisms of umbilical cord blood-derived mesenchymal stem cells in IDCM

Transplantation of UCBMSCs represents a new and promising treatment for ischemic diseases, in which revascularization is meaningful [81]. Although IDCM is not associated with an ischemic etiology, the data described above suggest that UCBMSC application could be useful and even more beneficial in these patients than other cell-based therapies, especially those using bone marrow-derived CD34+ cells. In this context, the potential mechanisms underlying the effects of UCBMSCs are multifactorial and probably involve complex endothelial–myocardial interactions, leading to increased myocardial revascularization and subsequent improvement of cardiac muscle integrity and function.

Since the 1990s, the cardiac vasculature has been recognized as a key regulator of subjacent myocardial integrity and function [7]. Briefly, Brutsaert et al. showed that the endocardial vasculature is capable of modifying underlying myocardium contractility [82]. Using cardiac microvascular endothelial cells activated by inflammatory cytokines, Ungureanu-Longrois et al. reported reduced contractility of ventricular myocytes in a co-culture setting [83]. Taken together, the observations indicate that the endocardial and cardiac microvasculature modulate myocardial contractile responsiveness and could, therefore, contribute to the contractile dysfunction characteristic of some cardiomyopathies such as IDCM. Further studies have confirmed that endothelial injury accelerates the progression to myocardial dysfunction, whereas protection of the associated vasculature preserves myocardial function. For example, induction of damage to the cardiac microvasculature using intracoronary detergents triggered abnormal heart pumping in Langendorff perfused rat hearts [84], and protection against oxidative stress impaired LV hypertrophy in a guinea pig model in which pressure overload was induced by aortic banding [85]. Furthermore, in a post-infarct rat heart model, Qi et al. showed that chronic post-infarct endothelium-induced coronary dilatation was impaired and both endocardial and myocardial capillary vascular dysfunction contributed to myocardial depression [86].

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Fig. 2. Differentiation of human UCBMSCs into the endothelial lineage and formation of vascular structures in a mouse model of acute myocardial infarction. MSCs isolated and cultured from human UCB (A) were transduced with lentiviral vectors including the chimeric proteins Photinus pyralis luciferase – enhanced green fluorescent protein and Renilla reniformis luciferase – red fluorescent protein expressed under the control of the inducible promoter of the human gene encoding CD31 (a protein broadly used as an endothelial lineage marker) and the constitutively active CMV promoter (as a reporter of cell number), respectively. Double-transduced cells were then separated by fluorescence-activated cell sorting and locally implanted over the infarcted myocardium using a fibrin patch in mice (B). Subsequent macroscopic and microscopic analyses of treated animals and explanted hearts demonstrated the emission of light and green/red fluorescence (C) and self-organization of the implanted cells into new vascular structures expressing CD31 (D). UCBMSC = umbilical cord blood-derived mesenchymal stem cell, MI = myocardial infarction.
On the basis of the data reviewed here, UCBMSC-based therapy may restore the marked myocardial vascular derangements that contribute to the development and progression of IDCM [7]. Despite therapeutic changes not being perfectly linear, cardiac endothelial repair may increase the blood supply to damaged myocardium, improving the integrity and contractility of cardiac muscle and

Fig. 3. Current and potential benefits of UCBMSC-based therapies in the cardiovascular field. Increasing pre-clinical evidence indicates that UCB is a valuable source of multipotent MSCs that promote blood vessel growth and tissue repair, i.e., it is embedded in a fibrin-based scaffold and laid over post-infarcted myocardium. In line with this, UCBMSCs may also contribute to recovering from the profound cardiovascular deficits and progressive myocyte damage and loss in IDCM. UCBMSC = umbilical cord blood-derived mesenchymal stem cell, IDCM = idiopathic dilated cardiomyopathy. Designed and hand-drawn by C.G-M.
ultimately reversing ventricular remodeling and pump failure. Alternatively, UCBMSCs may exert their potential effects not only by the formation of new blood vessels, but also by secreting large amounts of angiogenic, anti-apoptotic, and anti-inflammatory factors [65, 69, 87, 88]. Moreover, UCBMSC transplantation may neutralize circulating autoantibodies present in IDCM via similar mechanisms that are thought to be responsible for the effects of CD34+ cell infusion in the treatment of therapy-resistant rheumatoid arthritis and multiple sclerosis [89]. These proposed mechanisms are collectively summarized in Fig. 4.

6. Conclusions and future perspectives

This review focuses on the growing interest in designing effective cell-based therapies for IDCM. In this context, the use of UCBMSCs may aid in the recovery of these patients from characteristic cardiac muscle and vascular alterations.

Although progress in genetics and molecular biology has ruled biomedical science in the past century [90], differences between animal models and human disease have hampered the translation of results from the bench to the bedside in diverse human diseases, including...
IDCM. Nevertheless, major advances have been made over the last decade in our understanding of the causes and progression of this extremely complex heart disorder. Accordingly, both cardiac muscle and vascular alterations are now recognized as invariant features of the myocardium in IDCM [7].

Endothelial recovery is a basic challenge for cardiovascular regenerative medicine [91]. Beyond pharmacological agents or surgical intervention, cell-based therapies show promise for regenerating injured human tissues by inducing new vascular growth or repair. These approaches have been assessed extensively for ischemic cardiac diseases, including the post-infarction heart [92], in both preclinical and clinical contexts. Benefits arise, in part, from the revascularization of damaged tissue induced by administered cells. However, the potential contribution to IDCM patients is not yet known, though in 2008, Ichim et al. reported positive clinical improvement following the delivery of allogeneic placental MSC and UCB-derived CD34+ cells [93]. Although the application of stem cells is seen as feasible and generally safe (no increased mortality has been reported after cell delivery), and positive outcomes in LVEF and NYHA functional class have been shown, the clinical implementation for IDCM has been poorly investigated. We can learn from these preliminary studies, which have laid the basis of the use of cell therapy in these patients, but key conclusions include low methodological quality and great heterogeneity in the inclusion criteria and measurement procedures. Thus, more adequate and rigorous randomized placebo-controlled trials are needed.

Recent findings highlight some MSC populations such as UCBMSCs that greatly contribute to vascular growth in vivo. Together with harnessing the secretome and paracrine effects of MSCs, this vascular potential may add value to these cells as beneficial therapeutic agents. For example, UCBMSCs previously embedded in a fibrin-based scaffold and laid over post-infarcted myocardium reduce the infarct size and increase myocardial vascularization. The use of valuable technical procedures also allows accurate monitoring of implanted cell survival and differentiation (BLI), as well as improvements in cell retention and benefit (cardiac tissue engineering). Therefore, with these new therapeutic weapons and pending of resolution of limitations and prejudices of cell reprogramming and embryonic stem cell research, we have the opportunity to further explore and possibly increase tissue repair in disorders with vascular deficits, including IDCM.

Conflict of interest
The authors report no relationships that could be construed as a conflict of interest.

Disclosures
None.

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