Human umbilical cord blood mononuclear cells decrease fibrosis and increase cardiac function in cardiomyopathy

Aims: We investigated whether human umbilical cord blood mononuclear cells (HUCBC) can limit progressive cardiomyopathy in TO2 hamsters. Materials & methods: A total of 22 TO2 1-month-old hamsters were treated with intramyocardial HUCBC, $4 \times 10^6$ in Isolyte®, and 23 TO2 1-month-old hamsters were treated with intramyocardial Isolyte. A total of 16 1-month-old F1B hamsters served as controls and received intramyocardial Isolyte. Echocardiograms were performed on all hamsters prior to and monthly after treatment for 6 months. Heart tissues were then stained with hematoxylin and eosin, Masson’s Trichrome and human leukocyte antibody. Results: In F1B hamsters, left ventricular fractional shortening (FS) and ejection fractions (EF) did not significantly decrease over 6 months. By contrast, in Isolyte-treated TO2 hamsters, FS decreased from 56.2 ± 1.0% to 19.7 ± 3.2% and EF decreased from 89.5 ± 1.4% to 44.9 ± 5.9% at 6 months (both $p < 0.0001$). The FS and EF in HUCBC-treated TO2 hamsters also progressively decreased over 6 months but the changes were more gradual, especially during the first month after HUCBC treatment when FS was 52.0 ± 1.5% and EF was 89.5 ± 1.4%, which was not significantly different from the FS and EF in the F1B hamsters. Moreover, in the HUCBC-treated hamsters, the FS and EF were 20–30% greater than FS and EF in Isolyte TO2 hamsters at 3 and 5 months ($p < 0.01$). In Isolyte-treated TO2 hamsters at 6–7 months, fibrosis involved 30.0 ± 5.0% of left ventricle and 35.0 ± 5.0% of septum. By contrast, in HUCBC-treated hamsters, fibrosis involved only 6.5 ± 2.3% of the left ventricle and 6.3 ± 1.8% of septum ($p < 0.05$). The average number of blood vessels per myocardial microscopic field in HUCBC-treated hearts was 53.5 ± 0.8 versus 46.2 ± 3.0 in Isolyte-treated TO2 hearts ($p < 0.05$). Conclusion: HUCBC, when given as a single intramyocardial injection, can limit fibrosis and increase heart function over the short term in TO2 hamsters with cardiomyopathy.

KEYWORDS: cardiomyopathy  left ventricular ejection fraction  umbilical cord blood stem cells  ventricular fibrosis

Recently, investigators have found that human embryonic stem cells and bone marrow stem cells can reduce myocardial damage and fibrosis in research animals and in patients with infarcted hearts [1,2]. Although human embryonic stem cells can acquire a cardiomyocyte phenotype with the expression of muscle actin and myosin heavy and light chain proteins and can induce angiogenesis, the procurement and therapeutic use of human embryonic stem cells has generated considerable debate and intense controversy, which has significantly limited the use of these stem cells for the treatment of cardiovascular diseases. For this reason, medical researchers have investigated autologous and allogeneic bone marrow stem cells for the treatment of damaged hearts. Bone marrow stem cells can express troponin and myosin cardiac muscle proteins, induce angiogenesis, limit myocardial infarction size and improve left ventricular (LV) ejection fraction. However, the number and viability of stem cells in donor bone marrow decreases with the increasing age of the donor and/or the presence of associated diseases such as diabetes mellitus and atherosclerotic vascular disease [3,4]. Moreover, the administration of allogeneic bone marrow stem cells often requires the use of immunosuppressive therapy, which can contribute to a persistently immature transplant stem cell phenotype and subject the recipient to possible side effects such as infection.

For these reasons, we have investigated the use of mononuclear cells isolated from human umbilical cord blood, which contain hematopoietic, mesenchymal and endothelial stem cells, for the treatment of damaged hearts [5–8]. Because approximately 134 million births occur each year in the world, there is a tremendous resource of human umbilical cord blood mononuclear cells (HUCBC) potentially available for transplantation. Cord blood stem cells have longer telomeres than adult bone marrow stem cells, which indicates that they are more immature and have undergone less cell division than adult bone marrow stem cells [9]. In addition,
HUCBC T lymphocytes are phenotypically and functionally naive and have experienced little or no antigen exposure [9].

Human umbilical cord blood is an extremely rich source of hematopoietic, endothelial and mesenchymal stem cells [9]. The total content of hematopoietic stem cells in human cord blood equals or exceeds that found in human bone marrow [9]. In our experience, the percentage of endothelial CD34 stem cells in umbilical cord blood ranges from 6 to 3% from gestations of 17–32 weeks to 0.5 to 1.5% for gestations of 37–41 weeks [6–8]. The functional properties of mesenchymal stem cells in human cord blood closely resemble the characteristics of bone marrow-derived mesenchymal stem cells [10]. In addition, cord blood mesenchymal and hematopoietic stem cells can be expanded in culture by as much as 77–95% [9]. For all these reasons human umbilical cord blood is being used as a source of marrow-repopulating stem cells in patients treated for leukemia, myelodysplastic syndromes, neuroblastoma, Fanconi’s anemia and aplastic anemia [9,11]. To date more than 4000 human cord blood transplants have been performed in patients with these disorders [11].

Human umbilical cord stem cells also produce beneficial effects in animal models of ischemic vascular diseases such as myocardial infarction, cerebral infarction and limb ischemia. In our studies, HUCBC, when injected into ischemic/infarcted myocardium, reduce the size of myocardial infarctions by as much as 75% and enhance LV ejection fraction, fractional shortening and the maximal rate of change of LV pressure per unit time (dP/dt) without the requirements for immunosuppressive therapy [5–8]. Similarly, HUCBC given to research animals with cerebral infarction (i.e., stroke) or limb ischemia significantly reduce brain infarct size and can enhance limb vascular density and limb blood flow [12–14].

Based on the beneficial results of HUCBC with ischemic vascular disease, we investigated whether HUCBC can be helpful in the treatment of progressive cardiomyopathy. Progressive cardiomyopathy is characterized by myocardial fibrosis with reductions in the number and function of sarcolemmal and cytoskeletal proteins, which cause progressive heart failure. TO2 hamsters have a congenital deficiency in sarcoglycan muscle protein, a component of the dystrophin–glycoprotein complex in cardiac muscle, and therefore develop progressive myocardial fibrosis that significantly decreases LV fractional shortening, ejection fraction and cardiac output [85]. As a consequence, these hamsters develop, over approximately 10–12 months, left then right ventricular failure with pulmonary and peripheral edema. The present report demonstrates that HUCBC, when given as a single injection, can limit heart myocardial fibrosis and increase heart function in TO2 cardiomyopathic hamsters over the short term.

### Materials & methods

#### Human umbilical cord stem cells

Human umbilical cord mononuclear blood cells or human cord blood were obtained from human cord cell blood banks (Cambrex Inc, Saneron Therapeutics and CordUse) and stored at -196°C in liquid nitrogen. The cord blood was rejected if the maternal blood was positive for HIV, human T-lymphotropic virus, hepatitis, syphilis or cytomegalovirus. The mononuclear fraction of cord blood was obtained by Ficoll density gradient separation and cryopreserved at -196°C in liquid nitrogen. The cryopreserved HUCBC were thawed at 37°C and transferred into centrifuge tubes containing Isolyte® S, pH 7.4 (B. Braun Medical). The cells were washed three times, centrifuged at 1500 rpm for 7 min, the supernatant discarded and the HUCBC viability determined by Trypan Blue dye exclusion technique. The HUCBC viability was greater than 90%. Between 6 and 9% of the HUCBC took up Trypan Blue and were considered to be nonviable cells. The HUCBC were not propagated in culture flasks. Each dose of HUCBC administered was adjusted to deliver a total of 4 × 10^6 Trypan Blue-negative HUCBC by a precisely calibrated syringe. The HUCBC contained 1.0% CD34+ cells and approximately 1% SH2 (CD105) cells as determined by fluorescent antibodies to CD34 and SH2 cell antigens that were obtained from Invitrogen and Osiris, respectively, and fluorescent activated cell sorting cytometry (Becton Dickinson) in our facility. A Mac OS9 G-3 computer (Apple Computer) was used for initial cell analysis. Cell Quest, version 3.3 (Becton Dickinson), was used for cell acquisition. The data was then analyzed with FlowJo, version 6.4.7 (Tree Star) on a Mac OS10 G-5 (Apple Computer). The use of HUCBC in animal studies has been approved by our Institutional Review Board.

#### Hamster surgery

All hamsters received care in compliance with the principles of laboratory animal care and in accordance with our Institutional Animal Care and Use Committee (IACUC). The study conforms
to the Guide for Care and Use of Laboratory Animals of the National Institutes of Health. A total of 16 1-month-old normal F1B hamsters and 45 1-month-old TO2 cardiomyopathic hamsters (Bio-Breeders), weighing between 50 and 100 g, were anesthetized with 4% Isoflurane by inhalation, intubated and placed on mechanical ventilator support with continuous 2.5–4% Isoflurane anesthesia and oxygen. The TO2 hamsters were randomized to treatment with either 0.3 ml intramyocardial Isolyte, which was given to 23 TO2 hamsters, or 4 × 10^6 Trypan Blue-negative HUCBC in 0.3 ml intramyocardial Isolyte, which was given to 22 TO2 hamsters. The F1B hamsters received 0.3 ml intramyocardial Isolyte and served as normal controls in order to account for myocardial changes due to surgery and aging. The heart rate and arterial oxygen saturation were continuously monitored (Imed) in all hamsters during the surgery. A thoracotomy was performed through the left fifth intercostal space. The pericardium was opened and the hamsters were given either 0.3 ml Isolyte or HUCBC in 0.3 ml Isolyte by direct injection into the LV myocardium. One or more myocardial wheals were created with each injection in order to insure that each injection was directly into the LV myocardium and not into the LV cavity or pericardial cavity. The accuracy of this technique was confirmed by the fact that HUCBC were identified in the myocardium of three TO2 hamsters that died due to respiratory infections within 1 week of the HUCBC injection. The dosage of HUCBC was based on our previous experiments [7] and all the hamsters tolerated the injection procedure. The chest of each hamster was then closed in three layers with 3–0 Vicryl and 6–0 Prolene suture, and each hamster was allowed to recover. Buprenorphine (0.05–0.5 mg/kg intramuscularly) was given for postoperative analgesia every 6–8 h for 24–48 h. Immunosuppressive therapy was not given to any hamster. The hamsters were then followed until age 6–8 months, when they were euthanatized.

### Myocardial histology

Two pathologists who were totally unaware of the treatments examined the heart tissues from each group of hamsters. Each heart was sectioned into three parasagittal slices in order to evaluate the left ventricle, the interventricular septum and the right ventricle. The sections of the heart were fixed with 10% buffered formalin, embedded in paraffin, sectioned and 5 micron myocardial tissue sections were stained with hematoxylin and eosin stain (Abbey), Masson’s trichrome stain (Biogenex), or with a primary antibody to human leukocyte antigen (HLA; Santa Cruz) and a secondary biotinylated antibody followed by ABC reagent (Santa Cruz) to identify HUCBC. The tissue slides were examined with Zeiss and Olympus microscopes. Representative tissue sections were photographed with a digital camera (Nikon). Percentage fibrosis was quantitated using 40× objectives from each heart section stained with Masson’s trichrome. Each heart section was scanned at 40x and each objective field was divided in halves, quarters and eighths. The percentage fibrosis in each field was summed and divided by the total number of tissue fields examined to determine the average percent fibrosis in each parasagittal slice of the heart. The amount of fibrosis involving each of the sections of the left ventricle, the interventricular septum and the right ventricle were then averaged in order to determine the average amount of fibrosis present in each of these structures. In
addition, the number of arteries, veins and capillaries in five different random microscopic myocardial fields in each heart were counted and averaged from the Isolyte and the HUCBC-treated hamsters. Each pathologist also scanned each tissue slide for HLA-positive cells. Each heart tissue slide was read twice by two different pathologists in the same manner. If major differences occurred on a single heart, the heart was re-evaluated by each of the pathologists and the results averaged.

- **Statistical analyses**
  All results are expressed as the mean ± SEM. The differences between the Isolyte and HUCBC groups were tested by Student’s t-test or Dunnnett’s test. When multiple comparisons among groups were performed, repeated measures analyses of variance were performed then the Bonferroni modification of the t-test was used for planned comparisons and Tukey’s procedure was used for post-hoc comparisons. A value of p ≤ 0.05 was considered significant.

**Results**
- **Echocardiographic evaluations**
  
  **LV fractional shortening**
  Serial echocardiograms were performed on the control F1B, the Isolyte-treated TO2 and the HUCBC-treated TO2 hamsters at monthly intervals between the baseline prior to treatment at 1 month of age and 6 months of age. In the F1B hamsters, the LV fractional shortening decreased by approximately 2% from 56.2 ± 1.0 to 55.0 ± 2.8% as the hamsters aged over 6 months (p = NS). By contrast, in the Isolyte-treated TO2 hamsters the LV fractional shortening decreased substantially over the 6 months by 65% from 56.2 ± 1.0 at the baseline to 19.7 ± 3.2 (p < 0.0001) at 6 months of age (Figure 1). The fractional shortening measurements in the Isolyte-treated TO2 hamsters were 12–72% lower than the fractional shortening measurements in the F1B hamsters between 2 and 6 months of age (p < 0.001) (Figure 1). The decrease in LV fractional shortening in these hamsters was associated with pulmonary fluid accumulation.

  The LV fractional shortening in the HUCBC-treated TO2 hamsters also decreased over the 6 months of observation. However, the changes were more gradual, especially during the first month after the injection of the HUCBC, when the fractional shortening decreased by only 7% from 56.2 ± 0.8 to 52.0 ± 1.5 (p = NS), which was not significantly different from the F1B control hamsters (Figure 1). Moreover, in the HUCBC-treated hamsters the LV fractional shortening was 20–30% greater than the fractional shortening in the Isolyte-treated TO2 hamsters at 3 and 5 months of age (p < 0.05) (Figure 1).

  The injection of HUCBC in a subset of Isolyte-treated TO2 hamsters at 4 months of age increased the LV fractional shortening at 5 months of age by 49% to 25.9 ± 2.0% in comparison with the fractional shortening of 17.4 ± 1.5% (p < 0.01) in the TO2 hamsters treated with only Isolyte at 5 months of age.

  **LV ejection fraction**
  The LV ejection fraction in the F1B hamsters decreased slightly but not significantly with age from 92.3 ± 0.5% to 89.5 ± 1.8% between 1 and 6 months. By contrast, in the Isolyte-treated TO2 hamsters the LV ejection fraction decreased by 50% from 89.5 ± 1.4% at 1 month of age to 44.9 ± 5.9% at 6 months of age (p < 0.0001) (Figure 2). The LV ejection fractions in these TO2 hamsters were as much as 50% lower than the ejection fractions in the F1B hamsters at 6 months of age (p < 0.002) and were associated with pulmonary congestion (Figure 2).

  The LV ejection fractions in the HUCBC-treated TO2 hamsters also decreased over the 5 months of observation but the changes were

![Figure 1. Fractional shortening changes.](image-url)
less than the changes in the Isolyte-treated TO2 hamsters. The LV ejection fraction at 2 months was 87.5 ± 1.3%, which was not significantly different from the ejection fractions in the F1B hamsters at 2 months. Moreover, in the HUCBC-treated hamsters the LV ejection fractions were 14 and 25% greater than the ejection fractions in the Isolyte-treated TO2 hamsters at 3 and 5 months of age (p < 0.01) (Figure 2). The LV ejection fractions at 3–5 months were 76.0 ± 2.4% and 53.5 ± 1.7%, respectively (p < 0.01) and were associated with little edema and bodyweights at 5–6 months of age that were 13–15% lower than the bodyweights of the Isolyte-treated hamsters at the same age.

The injection of HUCBC in a subset of Isolyte-treated TO2 hamsters at 4 months of age increased the LV ejection fraction measurement at 5 months of age by 38% to 57.5 ± 2.3% in comparison with the ejection fraction of 41.6 ± 3.1% (p < 0.01) in the TO2 hamsters at the same age treated only with Isolyte (Figure 2). The LV ejection fraction then decreased slightly but not significantly at 6 months.

LV posterior wall thickness

The LV posterior wall thickness in the F1B hamsters did not change significantly between 1 and 6 months of age (Figure 3). In the Isolyte-treated hamsters the LV posterior wall thickness was as much as 26–40% less than the LV posterior wall thickness of the F1B hamsters (p < 0.002). Moreover, in the Isolyte-treated hamsters the posterior wall decreased in thickness by 21% from 0.135 ± 0.003 cm at 1 month of age to 0.105 ± 0.002 cm at 6 months of age (p < 0.001). In contrast to the Isolyte-treated hamsters, the posterior wall thickness in the HUCBC-treated TO2 hamsters was 11–26% greater between 2 and 4 months of age (p < 0.005 at 3 months). The posterior wall then decreased in thickness at 5 and 6 months of age (Figure 3). Injection of HUCBC into Isolyte-treated TO2 hamsters at 4 months of age increased the posterior wall thickness by as much as 24% to 0.130 ± 0.003 cm between 5 and 6 months in comparison with the Isolyte-treated hamsters of the same age (p < 0.005) (Figure 3).

Ventricular histology

Fibrosis in the left and right ventricles of the 6-month-old hamsters was determined with Masson’s trichrome stain. Less than 5% of the left ventricle of the F1B hamsters was fibrotic at 6–7 months of age. By contrast, the LV walls and the interventricular septums of the Isolyte-treated TO2 hamsters were substantially fibrotic, with areas of calcification. The fibrosis in the Isolyte-treated hamsters significantly exceeded the fibrosis in the hearts of the HUCBC-treated TO2 hamsters (Figure 4). At 6–7 months of age in the Isolyte-treated TO2 hamsters, fibrosis involved 30.0 ± 5.0% of the LV walls and 35.0 ± 5.0% of the ventricular septum. By contrast, in the TO2 hamsters treated with HUCBC at 1 month, fibrosis involved only 6.5 ± 2.3% of the LV walls and 6.3 ± 1.8% of the ventricular septum at 6–7 months (p < 0.005) (Figure 4A & 4B). Fibrosis involved 10.0 ± 0.1% of the right ventricle in the Isolyte-treated hamsters and 3.5 ± 1.7% of the right ventricle in the HUCBC-treated hamsters (p < 0.01) (Figure 4C). Representative sections of the ventricles of F1B, Isolyte-treated TO2 and HUCBC-treated TO2 hamsters are shown in Figure 5.

The average number of blood vessels per microscopic field in the HUCBC-treated hamster hearts was 53.5 ± 0.8 versus 46.2 ± 3.0 in the Isolyte-treated hamster hearts (p < 0.05). HLA-positive cardiomyocytes or vascular cells were not identified at 6–7 months in the hamster myocardium treated with HUCBC.

The subset of Isolyte-treated TO2 hamsters that were treated with HUCBC at 4 months of age were examined for fibrosis at 8 months.
of age and compared with a similar number of 8-month-old TO2 hamsters previously treated with only Isolyte. In the Isolyte-treated TO2 hamsters at 8 months of age, the LV fibrosis involved 38.0 ± 5.0% of the LV wall and the interventricular septum, 15.0 ± 3.0% of the right ventricle and was associated with areas of myocardial calcification. By contrast, the fibrosis in the TO2 hamsters treated with HUCBC involved 20.0 ± 5.0% of the LV wall and the interventricular septum and 10.0 ± 2.0% of the right ventricle (p > 0.05).

Discussion
The present study demonstrates that HUCBC, when injected into the LV myocardium in 1-month-old TO2 hamsters with progressive cardiomyopathy, can limit LV fibrosis and enhance LV contractility, as demonstrated by an increase in the LV fractional shortening and ejection fraction over the ensuing 5 months, in comparison with cardiomyopathic TO2 hamsters of the same age treated with only Isolyte (p < 0.005). Moreover, HUCBC, when injected into the myocardium of 4-month-old TO2 hamsters with established cardiomyopathy, produce an increase in LV fractional shortening and ejection fraction and limit LV fibrosis during the ensuing 2 months in comparison with the fractional shortening and ejection fraction measurements in Isolyte-treated hamsters of the same age. The present experiments suggest that HUCBC, when given as a single injection, can be beneficial over the short term in the treatment of cardiomyopathy.

**Human umbilical cord blood stem cells**

The mechanism by which HUCBC and other stem cells can limit myocardial damage may involve stem cell transdifferentiation into heart muscle cells or vascular endothelial cells and vessels, or alternatively stem cell paracrine effects. HUCBC are reported to differentiate in vitro and in vivo into cardiac muscle [17]. HUCBC can also produce muscle dystrophin protein in vitro [18,19] and can express dystrophin and dysferlin in muscle fibers in myopathies due to deficiency of these proteins [20]. Similarly, skeletal muscle stem cells (myoblasts) are reported to form myocytes in the left ventricle and limit LV wall thinning in cardiomyopathic animals [21]. However, the possibility that stem cells actually transdifferentiate into significant numbers of myocytes has recently been questioned because the frequency of stem cell engraftment and the number of newly generated cardiomyocytes as a result of either transdifferentiation or cell fusion appear to be too low to adequately explain the cardiac improvement that occurs with stem cell transplantation [1,22]. Moreover, in the present investigation, HUCBC-induced HLA-positive cardiomyoblasts and myocytes were not identified in myocardium. Although we did not extract and amplify myocardial DNA with specific human primers in order to determine if human DNA was present in the hamster myocardium, we believe it is unlikely that HUCBC transdifferentiation to cardiomyocytes or vascular endothelial cells explains the decrease in myocardial fibrosis and the increase in fractional shortening and ejection fraction that was observed in the present investigation. Furthermore, we did not observe any inflammatory cellular infiltrate characteristic of an immune rejection response that might possibly have significantly decreased the cardiomyopathic process in the TO2 hamster hearts.

A second HUCBC mechanism that can limit myocardial damage involves neovascularization that may be due either to HUCBC incorporation into new blood vessels or HUCBC release of growth factors that stimulate angiogenesis. In this regard, HUCBC have been reported to increase the number of capillaries in ischemic/infarcted hearts, which is associated with a
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decrease in infarct size and an increase in LV hemodynamics [5,23–26]. Whether stem cells in general can actually incorporate into blood vessels in damaged tissues or form vascular supporting structures is currently debated [27]. HUCBC do, however, express the angiogenic growth factors angiopoietin-1, angiopoietin-2 and VEGF, which can stimulate angiogenesis [28]. In addition, HUCBC secretion of VEGF can promote migration of native endothelial stem cells from the bone marrow and blood into damaged myocardium for neovascularization [28]. In the present investigation HLA-positive endothelial cells were not identified in blood vessels but an increase in the blood vessels in the myocardium was identified in the cardiomyopathic hamsters treated with HUCBC. Consequently, growth factors secreted by HUCBC may have chemotactically native stem cells from the hamster bone marrow and blood into the myocardium, which ultimately contributed to neovascularization and myogenesis.

A third mechanism by which HUCBC can limit muscle damage involves limiting the inflammatory changes that contribute to damaged muscle and cause fibrosis. Inflammatory cytokines, including cytokines released from damaged cardiomyocytes, can also cause vascular spasm and in this manner contribute to myocardial fibrosis in animal models of cardiomyopathy [29]. In our studies, HUCBC can significantly decrease the inflammatory cytokines TNF, monocyte chemoattractant protein, fractalkine, macrophage inflammatory protein, IFN-γ and IL-1β in damaged myocardium, in contrast to damaged myocardium treated with Isolyte, in which these inflammatory cytokines increase in cardiac muscle by two- to eightfold [8]. Moreover, the HUCBC-induced reductions in inflammatory cytokines in the myocardium are associated with a more than 65% decrease in the number of neutrophils and a more than 50% decrease in the number of lymphocytes in the myocardium after acute myocardial damage [8]. Similarly, HUCBC, administered intravenously to rats with acute strokes due to carotid occlusion, significantly reduce the brain concentration of the inflammatory cytokines TNF, IL-1, IL-2 and IL-6 by as much as 30–60% and decrease the number of inflammatory cells in the brain, with a consequent decrease in the amount of brain damage [30].

Human umbilical cord blood mononuclear cells can limit inflammatory cytokines in the myocardium, and limit muscle damage and fibrosis by expressing anti-inflammatory cytokines and growth factors. In this regard, HUCBC can express the anti-inflammatory cytokine IL-10 in vitro and in vivo [31,32]. HUCBC treatment in rats with damaged myocardium in our previous studies increased the myocardial concentration of IL-10 by more than twofold and HUCBC in cerebral infarction increased the production of IL-10 by nearly twofold [30,33]. IL-10 can directly inhibit nuclear factor-κB and suppress the production of the inflammatory cytokines TNF-α,
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Macrophage inflammatory protein, IL-1β, IL-6, IL-8 and IL-12 [8,30,33]. In addition, IL-10 induces the expression of suppressor of cytokine signaling 3, inhibits the expression of adhesion molecules and decreases lymphocyte activity by suppressing the secretion of IL-2 and IFN-γ [34]. Cord blood stem cells can also express FoxP3 and soluble nitric oxide that can inhibit inflammatory lymphocyte proliferation in damaged cardiac muscle [35,36]. Moreover, HUCBC expression of anti-inflammatory cytokines or growth factors might also limit the vascular spasm that can contribute to ventricular fibrosis and cardiomyopathy in sarcoglycan-deficient animals [29].

Human umbilical cord blood mononuclear cells and other stem cells can also express metalloproteinases and serine proteases that can inhibit myocardial fibrosis and can attenuate the proliferation of cardiac fibroblasts and the cardiac expression of collagen types I and III [37,38].

In the present investigation, the specific mechanisms that produced the decrease in LV fibrosis and improvement in LV function in the HUCBC-treated TO2 hamsters do not appear to be due to HUCBC transdifferentiation to cardiomyocytes or vascular endothelial cells in the ventricular myocardium. Growth factors and anti-inflammatory cytokines released by HUCBC most probably altered the cardiomyopathic process by limiting the amount of fibrosis in the HUCBC-treated hamsters at 1 month and the hamsters treated at 4 months. This HUCBC-induced paracrine process also caused some neovascularization and an increase in LV function in comparison with Isolyte-treated hamsters. Similarly, mesenchymal stem cells injected into skeletal muscle in TO2 hamsters have been recently reported to release growth factors and also activate the release of biologically active factors in the myocardium that limit myocardial fibrosis, mobilize native progenitor cells from the bone marrow to the heart and improve ventricular function [39]. Studies are in progress in our laboratory to determine the precise growth factors and anti-inflammatory cytokines whereby HUCBC can exert beneficial effects in animal models of cardiomyopathy.

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Figure 5. Representative left ventricular heart sections at 6 months.

(A) Shows a F1B hamster heart with 2% fibrosis. (B) Shows an Isolyte®-treated TO2 hamster heart with more than 35% fibrosis in the left ventricle. (C) Shows a human umbilical cord blood mononuclear cell-treated TO2 hamster with 11% fibrosis in the left ventricle.
Human umbilical cord blood mononuclear cells can increase neovascularization in progressive dilated cardiomyopathy.

The mechanism of action of stem cells can involves transdifferentiation, cell fusion, neovascularization and paracrine effects.

Bibliography


