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Human umbilical cord blood stem cells, myocardial infarction and stroke

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ABSTRACT – Myocardial infarction (MI) and stroke are the first and third leading causes of death in the USA accounting for more than 1 in 3 deaths per annum. Despite interventional and pharmaceutical advances, the number of people diagnosed with heart disease is on the rise. Therefore, new clinical strategies are needed. Cell-based therapy holds great promise for treatment of these diseases and is currently under extensive preclinical as well as clinical trials. The source and types of stem cells for these clinical applications are questions of great interest. Human umbilical cord blood (hUCB) appears to be a logical candidate as a source of cells. hUCB is readily available, and presents little ethical challenges. Stem cells derived from hUCB are multipotent and immunologically naive. Here is a critical literature review of the beneficial effects of hUCB cell therapy in preclinical trials.

KEY WORDS: animal models, cerebral infarction, myocardial infarction, stem cells, umbilical cord blood

Introduction

The study of stem cell therapies to address some of the most daunting medical challenges, including heart disease and stroke, has advanced steadily over the last three years. The majority of preclinical studies of stem cells as a potential therapy for either myocardial or cerebral ischaemia were positive on average. Small clinical trials, however, show either no or modest improvement in cardiac function after myocardial infarction (MI). Currently, there are two major types of autologous cells that are clinically used for MI and stroke. The first is skeletal myoblasts, harvested from skeletal muscle. These cells can be expanded in culture. Positive outcomes were recently reported in a phase 1 clinical trial using catheter-based injection of myoblasts to the endocardium (CAUSMIC, American Heart Association (AHA) Scientific Sessions 2007). The second is bone marrow cells (BMCs). Intracoronary injection of BMCs improve global left ventricular function (IC-BMC, AHA Scientific Sessions 2007). However, direct injection of BMC administration into scarred myocardium does not alter cardiac contractility of the injured area (IC/IM-BMC, AHA Scientific Sessions 2007). The effects of stem cell therapy can only be addressed using clinical trials that:

- are randomised, blinded, placebo controlled and adequately sized
- use standardisation of autologous stem cell processing protocols
- use robust endpoints of efficacy and safety
- ensure that follow-up is complete and of adequate duration.

It is becoming clear that realisation of the full potential of the therapeutic benefit of stem cells will require understanding the biology of these undifferentiated cells. A successful therapy will require a source with plentiful supply of multipotent stem cells with minimal or no immune rejection. Several sources of stem cells were explored such as adipose tissue,[1–3] cardiac tissue,[4] skeletal muscle biopsies,[5,6] and hUCB. Whether these subpopulations of cells are best suited to treat a disease is still unanswered.

Currently, the only confirmed source for totipotential cells is embryonic. However, there are ethical and scientific obstacles to unbridled use of such cells. For clinical application, autologous adult stem cells are the obvious choice. To date, only adult stem cells derived from a patient's own bone marrow are being used in clinical trials.

Autologous BMC therapy is not without problems. The majority of instances of MI and cerebral ischaemia (CI) occur in the elderly. Since the quantity and function of BMCs decrease with age, an allogeneic younger donor may be used to source BMCs. This may hinder the efficiency of such a treatment and suffer rejection, therefore another source of stem cells is needed.

Cryopreserved stem cells derived from human leukocyte antigen (HLA)-matched and unmatched unrelated donor hUCB were realised as a sufficient source of transplantable haematopoietic stem cells with high donor-derived engraftment and low risk of refractory acute graft-versus-host disease. However, the use of hUCB cells as treatment for either MI or CI has only been recently investigated in preclinical models.

There are several outstanding review articles on stem cells derived from cord blood in MI[7–11] and stroke.[12–17] This article adds depth to the debate by providing an updated review as well as presenting an integrated overview of studies involving MI and CI cell-based therapy. In the preparation of this review, every effort was made to include all relevant publications since 2005. Due to space limitations, the number of articles cited has been limited.

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Cardiovascular disease

Since 2005, several studies have explored the use of various subpopulations of hUCB stem cells for regenerative therapy. Five types of UCB-derived stem cells were investigated: umbilical cord derived stem (UCDS), unrestricted somatic stem cells (USSC), mononuclear progenitor cells (MNCs), CD133+ and CD34+ subpopulations. The experimental parameters of the studies varied. The majority of studies, however, were performed using the rat animal model and utilising the left anterolateral descending (LAD) coronary artery ligation model of MI with intramyocardial injection of the stem cells. The laboratory used a similar model to determine the efficacy of stem cell derived from hUCB to improve cardiac function after ischaemia and reperfusion. The data indicated that intracoronary administration of mononuclear or CD34+ cells derived from hUCB improved cardiac function after MI by inducing neovascularisation and retarding left ventricular (LV) remodelling.[37]

The majority of reported studies using hUCB cells showed improvement in the outcomes.[18–25] Cardiac functional improvements were almost universally reported as evaluated by: increased ejection fraction; improved wall motion; lowered LV end-diastolic pressure; and increased cardiac contraction as determined by the maximum slope of LV pressure.[18–21,23–25] There were conflicting reports on the effects of stem cells on LV fractional shorting. One study reported improved shortening while another reported that BM but not UCB cells produced improved shortening.[22,23] Improvements in myocardial perfusion, evaluated by increased capillary density, were repeatedly demonstrated as were reductions in infarct size and the number of apoptotic cells.[18–25] Retardation or reduction in LV remodelling were also reported.[18,21,22] Although the vast majority of studies showed positive outcomes, HLA matching and further study are still needed before UCB stem cell therapies can become safe and effective treatments in humans. A prime example of the need for further elucidation of these emerging therapies can be illustrated by the findings in a study by Moelker.[26] This study used intracoronary administration of unrestricted somatic stem cells (USSCs) in a balloon left circumflex artery (LCX) occlusion ischaemia-reperfusion porcine model of MI. They found that treatment did not improve outcome and actually increased infarct size. Their histological analysis revealed that the injected cells worsened the infarct by obstructing vessels downstream.

Furthermore, the mechanisms of the observed benefits of UCB stem cell therapy in MI are under investigation: improved myocardial perfusion, attenuation of cardiac remodelling, reduction of inflammatory responses by limiting expression of TNF-α, MCP-1, MIP and INF-γ, and cardiac regeneration.[18–5] Tissue regeneration may be mediated by incorporation of delivered cells in the target tissue.[18–21,23] An *in vitro* study confirmed that mononuclear cells were migrated toward homogenised infarcted myocardium and that the greatest migration occurred at two and 24 hours post-MI.[20] Paracrine effect, ie the delivered cells release factors that promote neovascularisation, was also reported. Indeed, the study laboratory has shown that hUCB cells release angiogenic factors *in vitro* under hypoxic conditions. The data are consistent with a previous report that showed increased expression of VEGF 164 and 188 accompanied by angiogenesis and improved remodelling after administration of hUCB mononuclear cells into the myocardium.[21]

Identifying subpopulations of progenitor cells with the highest potential for tissue repair is another unanswered question prior to widespread application of this therapy in clinical settings. Previous studies showed that UCB-derived endothelial progenitor cells (EPC) to be a promising subset of stem cells for treatment of MI; however their number may be insufficient to treat adult patients. This problem can be addressed by expanding these cells in culture prior to transplant. Techniques are being developed to culture clinically significant quantities (60 population doublings) of EPCs from UBC CD.[25] Transplantation of these expanded cells improved ejection fraction (EF) and vascular density *in vivo*, demonstrating that such a culture method may be a viable option to produce EPCs for future use in humans. Another study evaluated the use of gene therapies in conjunction with UCB stem cell therapy.[24] CD34+ cells were transfected with AAV-Ang1 and/or AAV-VEGF 165. The gene-modified stem cells resulted in greater increases in capillary density and cardiac performance along with larger reduction in infarct size compared to CD34+ cell therapy alone.

Stroke/neurological injury

In contrast to cardiac disease, which in its own right has an extraordinarily complex aetiology, finding effective treatment for neurological damage will probably be one of the most challenging problems of the next century. The simplest reason for this is that the brain remains the least understood system in the body in terms of mechanistic functionality and pathology. Unfortunately, cerebrovascular diseases remain the third leading cause of death in the USA, with scores of more individuals who survive to suffer debilitating lifelong injuries. Cerebral ischaemia (CI) is by far the most prevalent cause of stroke (87%, American Heart Association 2007) and about 700,000 people in the USA are affected by stroke annually; 1 in 16 Americans who suffer a stroke will die from it. Surgical interventions and hypothermia have advanced greatly in the last decade. However, as with the heart, the brain is extremely sensitive to hypoxic assault and even in the best interventional outcomes some degree of tissue death is likely.

Brain tissue is extraordinarily complex and diverse in its organisation and function. However, in younger children the brain is plastic in its organisation and very large portions can be removed (such as removal of tumors or hemispherectomy for severe seizures) with relatively low to no noticeable long-term neurological damage. At a relatively young age, the brain loses most of its plasticity of functional organisation so any significant tissue death can be profoundly devastating.

Since 2005 a large number of studies explored the use of UCB-derived stem cell therapy for treatment of a wide variety of neurological injuries. The most common injury studied was CI. However there are studies using models of heat stroke, inner cranial haemorrhage (ICH) and cerebral palsy (CP). Most studies used the middle cerebral artery occlusion (MCAO) model of CI in rats. Cord blood cells were typically administered intravenously. Mainly, four types of UCB-derived stem cells were used: mononuclear cells (MNCs), CD34+ cells, hUCB MNCs and a cell line termed non-haematopoietic UCB stem cells (nh-UCBSC). The nh-UCBSC progeny was reported to express transcription factors: Oct-4, Rex-1 and Sox-2.[27] The majority of reported studies showed that cord blood cell administration in stroke, resulted in some degree of therapeutic benefit with no adverse effects.[27–34] Few studies reported non-effective neuroprotective effects.[33] Neuroprotective effects as well as functional/behavioural improvements of UCB cell therapies on CI were widely reported.[27,28,31–35] These improvements were accompanied by a number of factors including decreased inflammatory cytokines (CD 45/CD11b-, CD45/B220+, NF-kappaB binding and mRNA protein expression),[19] neuron rescue/reduced ischaemic volume, reduced splenic CD8+ T-cell via lowered IFN-γ and increased IL-10, as well as lowered parenchyma levels of granulocyte and monocyte infiltration and astrocytic and microglial activation.[27,28,31,34]

Several other mechanisms for the observed neuroprotection afforded by UCB cell therapies were proposed including: prevention of splenic mass loss; apoptotic protection; and a combination of trophic actions and nerve fibre reorganisation.[27,28,34] This later thesis is particularly encouraging if it holds true, as it demonstrates that UCB cell therapy can mediate both direct restorative effects to the brain as well as tropic neuroprotection. Many of the studies lend support to this tropic role, in that several reported some degree of neural protection with little to no detection of UCB cells engrafted in the brain.[27,31,33] One study did find, however, minimal cell engraftments in the brain as well as no significant therapeutic benefit.[5] The level to which cell migration to the brain occurred appears to be a function of route of administration. All of the studies which administered cells intravenously and examined migration found little to no migration to the brain[12,31,33,35]; while a study which used both intravenous and intraperitoneal (IP) administration only found evidence of neural restorative effects in the IP cases.[12]

Furthermore, two studies investigated the optimal delivery time of cells. One found that the potential therapeutic window for mononuclear cell attraction is 24–72 hours post-CI, while another study determined the optimal therapeutic administration of HUCBC in MCAO rats is 48 hours post-CI.[34,36]

Several neurological injury pathologies other than CI were also investigated. MNC treatment of ICH was reported to mitigate neurologic and motor defects. Pre-treatment with CB-derived MNCs effectively prevent heat stroke with significant reductions in atrial hypotension, inducible nitric oxide synthase-dependant nitric oxide levels in the striatum, cerebral ischaemia and hypoxia.[30] However, one of the most encouraging non-CI studies examined the use of MNC therapy for an animal model of CP.[29] This IP administration, CB-MNC in an animal model of CP produced alleviation of the neurologic affects of CP. They reported reduction of spastic paresis and normal walking behaviour.

Two clinical trials are currently in progress: one at Duke University treating newborns with CP and another at University of Texas–Houston treating children with traumatic brain injury. Both trials are treating the children with autologous cord blood stem cell infusions. This is an example of the promise UCB holds for supplying stem cells for emerging therapies in a functional as well as logistic sense. Future research and clinical trials in this area will undoubtedly produce preliminary treatment regiment for some of today's most untreatable ailments as well as a broader physiological understanding of the function and pathologies of the brain.

Conclusion

Experimental models and clinical trials over the past three years have repeatedly demonstrated the therapeutic benefits of UCB-derived stem cell therapies. Such therapies have shown utility in attenuating the effects of MI and neurologic injuries. Further, unlike most other sources of stem cells, UCB cells are readily available, multipotent, immunologically naive and present minimal ethical issues. The potential of such treatments to impact millions of lives each year is tremendous. However, the realisation of this potential will require continued commitment to research and expanded clinical trials.

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