UMBILICAL CORD BLOOD STEM CELLS AS A SOURCE OF NON-HEMATOPOIETIC CELLS: ROLE IN REGENERATIVE MEDICINE

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1.0 Introduction

In recent years, there has been much biomedical research in regenerative medicine as well as much debate on the presence of non-hematopoietic stem cells in human umbilical cord blood. Some researchers have suggested that UCB is not only a potential source of hematopoietic progenitor/stem cells, but also a source of other stem cells for use in human regenerative medicine [1-4]. The multipotent stem cells derived from the UCB can differentiate into a limited number of cell types and there is some suggestion that these cells could be useful for regenerative medicine purposes with the following characteristics: 1) survive in the recipient after transplant, 2) repopulate and differentiate to certain cell types in the host, 3) proliferate extensively, and also 4) generate sufficient cells of tissue(s) in the organ.

Table 1 shows a summary comparison of potential stem cells from the regenerative point of view. Although embryonic stem (ES) cells have pluripotent characteristics and can differentiate to various tissue types, ethical issues such as tumor formation and allogenecity could be a hurdle to clinical application [5].

Generation of patient-specific induced pluripotent stem (iPS) cells to circumvent allogenecity is time-consuming and expensive. Cord blood cells have some degree of pluripotentiality and can differentiate into various tissue types like the ES cells. Both in vitro and in vivo experiments proved that cord blood stem cells have the capacity to produce neural,
epithelial, endothelial and hematopoietic tissues [6]. It has been used for around 20 years to treat hematological disorders but no malignant transformation has been seen, except for instances where an abnormal gene is already present at the time of cord blood collection. BM cells also have a very good track record of safety but it has been postulated that BM cells have less ability to differentiate to other cell types.

Therefore, the pluripotency, ethical clarity and proven safety of cord blood stem cells raised some optimism that it could be useful in treating diseases such as cerebral palsy, traumatic brain injury, spinal injury, keratopathy and other diseases when there are no other treatment options.

### 2.0 Preclinical studies on novel indications for UCBT

For the last 10 years, stem cells derived from the human UCB have been extensively investigated in laboratory experiments and by pre-clinical animal models to treat various non-hematopoietic human diseases. Table 2 summarizes some of the ongoing clinical trials along with some of the animal studies.

#### 2.1 Neurological disease

UCB is able to produce primitive neuroepoetic progenitors [7] and intravenous administration of human UCB in a mouse model of amyotrophic lateral sclerosis appears to repair damaged neurons in mutant SOD1 (superoxide dismutase-1) mice [8,9]. Thus, it has been suggested that UCB cells could have neurotropic, neuroprotective and anti-inflammatory properties [10-12]. Saporta et al showed that infusion of human UCB stem cells in rats with spinal cord injury improved neurological impairments. [13] Infusion of UCB stem cells into rats which resemble stroke, induced by occlusion on the middle cerebral artery resulted in reduction of behavioral deficits associated with the injury [14]. Other studies using various animal models of stroke have also showed that infusion and intracerebral transplantation of UCB derived stem cells resulted in significant healing of damaged neurons [15,16]. However, there is growing evidence that the main benefit of transplanted UCB stem cells is not from trans-differentiation to target tissue, but from the secretion of neurotropic or neuroprotective factors that can counteract the degeneration or promote regeneration of neural tissue [17].

Infusion of UCB stem cells has also been shown to lead to neurological improvement in animal models of amyotrophic lateral sclerosis, Parkinson’s disease, cerebral plasticity, traumatic brain injury and spinal cord trauma [18-20]. Recently, Kogler et al suggested that the route of UCB stem cell administration, number or amount of cells and also the mode of action are needed to be addressed before the stem cells from UCB can be applicable to clinical studies [21].

#### 2.2 Diabetes Mellitus

In recent years, human UCB has emerged as an alternative tool for the cell-based therapy for the cure of diabetes mellitus. In the diabetic mouse model, Ende et al showed that transplantation of human UCB cells resulted to an improvement of blood glucose levels with prolongation of animal survival [22]. In another study, UCB cells transplanted into the type 1 diabetic mice delayed the onset of autoimmunity, insulitis and elevations of blood glucose in

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**Table 1: Comparison between the various types of stem cells**

<table>
<thead>
<tr>
<th>Stem Cells</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Embryonic stem cells</td>
<td>Higher development &amp; proliferative potential, Minimal risk of viral contamination</td>
<td>Supply is limited, Ethical concerns, Clinical data- not available, Biological limitations- possible allo-reactivity, and carcinogenesis</td>
</tr>
<tr>
<td>Cord blood stem cells</td>
<td>Excellent proliferation and differentiation capabilities, Immediate availability, Probably less allo-reaction, Lower risk of infections, Possible autologous transplants</td>
<td>Just one-time supply. Fresh sample and thawed sample could have different outcome</td>
</tr>
<tr>
<td>Bone marrow stem cells</td>
<td>Clinical studies showing good outcome in case of vascular diseases and cardiac diseases, Higher concentration of stem cells, Faster engraftment, Possible autologous transplants</td>
<td>Complex harvesting procedure, Ability to differentiate other cell types is highly controversial might not differentiate to other cells, probably works through paracrine mechanism.</td>
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mice [23]. Interestingly, insulin-producing cells of apparent human origin were identified in non-obese diabetic severe combined immunodeficiency (NOD-SCID) mice following the intravenous transplantation of UCB cells [24].

Murohara et al showed that UCB cells could promote revascularization when CD34 (hematopoietic marker) cells isolated from the UCB were transplanted into an immunosuppressed nude rat model of ischemic hind limb.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical study</th>
<th>Animal study</th>
</tr>
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<tbody>
<tr>
<td><strong>1. Neurological</strong></td>
<td>a. Has been used to treat more than 50 children.</td>
<td>Animal studies showed that cord blood stem cells could be collected at injured site or infarcted areas of spinal cord or brain and have regenerative and neuroprotective effect.</td>
</tr>
<tr>
<td>a. Cerebral palsy, anoxic and traumatic brain injury</td>
<td>Preliminary observation is encouraging. Younger patients seem to derive greater benefit.</td>
<td></td>
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<tr>
<td>b. Spinal injury</td>
<td>b. Case report of a patient showed that cord blood transplantation improved sensory perception and mobility of hip and thigh joint. CT and MRI could find evidences of regeneration of spinal cord.</td>
<td></td>
</tr>
<tr>
<td>c. Cerebro-vascular disease (stroke)</td>
<td>c. Based on the animal studies and early clinical case reports, studies have begun on autologous stem cell infusion in cases of traumatic brain injury.</td>
<td></td>
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<tr>
<td><strong>2. Juvenile Diabetes</strong></td>
<td>Clinical trial ongoing using autologous cord blood. Among 23 treated children significant improvement in glucose control and much longer production of insulin could be seen. Increased retention of endogenous insulin could be assessed by increased c-peptide secretion in clinical subjects</td>
<td>In vitro, CB cells can be driven to become insulin secreting beta cells. Animal model showed in vivo differentiation into insulin secreting islet cells and mediate an immune tolerance to newly derived islet cells.</td>
</tr>
<tr>
<td><strong>3. Epithelial tissue applications</strong></td>
<td>Case report- use of allogenic cord blood admixed with autologous fibrin matrix injected around the wound, in two patients showed significant wound healing at 3-7 months</td>
<td>Animal studies done in white rabbit showed cord blood stem cells could express corneal epithelium- specific cytokeratin forming an optically clear surface.</td>
</tr>
<tr>
<td>a. Wound repair</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Corneal epithelium</td>
<td></td>
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Furthermore, Sharma et al showed that human UCB cells transplanted into NOD-SCID mice also contribute to the formation of hepatocyte-like cells [25].

These experimental studies appear to suggest that UCB stem cells have the potential to differentiate and function as pancreatic and liver cells. Although these studies are still in early stages of development, the available studies suggest some optimism that future research could result in cure of diabetes mellitus using stem cell therapy.

2.3 Cardiovascular Disease

Acute myocardial infarction is a growing worldwide problem and cardiovascular disease is one of the leading causes of death for both men and women [26]. Stem cells isolated from the UCB have shown some promise in the area of cardiovascular research [27]. Recent experimental studies have demonstrated cardiac regeneration using human UCB stem cells in various experimental models.

The neovascularization response were seen by increased blood flow with the increase in capillary density distal to the femoral artery ligation [28]. In myocardium, Hirata et al showed that the transplanted human UCB stem cells survived and improved the cardiac function following myocardial infarction induced in rats by ligation of the left coronary artery [29]. Ma et al shows new evidence concerning an alternative cellular source for cardiac repair after myocardial infarction in NOD-SCID mice. Their research suggested that the injected human UCB cells from the tail vein migrated to the infarcted area of the heart, participated in neoangiogenesis and remodeling of the damage heart [30].

The mechanism by which these UCB cells contribute to cardiac regeneration has also been closely studied. Endoglin (an accessory receptor for transforming growth factor-β in vascular endothelial cells) was postulated to be essential for the cord blood cell mediated vascular repair in the mice myocardial infarction studies [31], while combined delivery of basic fibroblast growth factor (bFGF) appeared to enhance the efficacy of UCB cells in the treatment of myocardial infarction in rats [32]. Differentiation of UCB stem cells
within the infarcted myocardium, increased capillary and arteriole density, while secretion of angiogenic factors, and prevention of apoptotic cell death were demonstrated in the rat myocardial infarction model [33].

Recently, Das et al reported the ability of expansion of UCB stem cells with nano-fibers in vitro [34]. These expanded nano-fiber stem cells showed an impact on the improvement of rat myocardial function with the up-regulation of tissue connexin 43 (a gap junction protein) and pro-angiogenic molecules after infarction. These animal studies strongly suggest that stem cells from UCB could be a promising cell source for myocardial repair and regeneration.

2.4 Non umbilical cord derived sources

2.4.1 Orthopedic applications

Cord blood contains both ES cell-like cells and mesenchymal stem cells which can generate bone [35]. In animal studies, implantation of UCB cells in bone fracture resulted in significant bone healing [36]. Compared to MSC and adipose tissue-derived stem cells, UCB-derived cells showed encouraging results for the generation of cartilage [6].

2.4.2 Hearing loss

Human CB stem cells were intravenously injected into immunodeficient mice with hearing loss. Well-repaired cochlea with dramatic hearing cell regrowth could be seen [37].

Besides the umbilical cord, other sources of stem cells being studied for regenerative medicine purposes are adult bone marrow, skeletal muscle, skin, brain, fat and liver. These stem cells have potential for multilineage differentiation and functional engraftment into the recipient system. Currently, the most widely used source is autologous bone marrow because of its easy availability. Long term outcome analysis among clinical subjects with critical limb ischemia receiving autologous bone marrow stem cells has revealed significantly increased major amputation free survival [38,39] and currently this therapy is performed under advanced insurance program in Japan. Whether UCB or other umbilical cord-derived sources will turn out to be better than bone marrow or other sources for regenerative medicine purposes remains to be elucidated.

3.0 Current clinical trials

Inspired by pre-clinical experiments, several clinical trials were undertaken to assess the regenerative efficacy of UCB stem cells (Table 2). Autologous cord blood has been used to treat juvenile type I diabetes in a current clinical trial listed by the US NIH [40]. Significant improvement in glucose control and sustainable insulin production was reported among the 23 patients treated with autologous cord blood transplantation. There were signs of retention of endogenous insulin production as evaluated by stimulated c-peptide secretion. [41] Although insulin levels still fell after transplant, it is suggested that the rate of fall may have been slowed down. [42,43] However, no comparative studies have yet been done. On the other hand, autologous peripheral blood stem cells transplantation (PBSCT) have been carried out and promising results have been seen with reduction in insulin requirements for the patients. The effect from the transplants was probably due to the immunosuppressive effects of cyclophosphamide and other drugs used in the pre-transplant stem cell mobilization and conditioning. In any case, it suggests that autologous PBSCT can be as good as a source of cells as UCB for this potential treatment of type I diabetes.

A clinical trial is being carried out in Duke University in North Carolina where infants with pediatric cerebral palsy, anoxic and traumatic brain injury are treated with autologous cord blood stem cells [6, 44]. Preliminary results are encouraging and benefit was greater if the UCB cells were infused early and similar results have been found in treated patients of Europe and Asia with those disorders [6,44]. The University of Texas in the US has also submitted a clinical trial application to FDA for the treatment of traumatic brain injury in children. In a patient with spinal injury injection of UCB cells resulted in signs of regeneration of spinal cord as evidenced by CT and MRI [45].

For the treatment of wound healing, researchers have used fibrin-platelet glue combined with HLA compatible (2 mismatches accepted) buffy coats containing CD 34+ cord blood cells. Two patients showed faster healing time after injection around the margins of their wounds [46].

Patients with Alzheimer’s disease, autism and broncho-pulmonary dysplasia are other areas where utility of UCB derived MSC is under exploration by clinical trials. [47]

4.0 Conclusion

The possibility of using UCB-based therapies for people suffering various diseases created a high expectation in both medical and public communities and some parents store their baby’s UCB in a stem cell bank, in the hope that it will be a potential source for future therapies to repair or replace the damaged tissues/organs. However, there is questionable utility on the use of autologous UCB cells to treat genetic diseases or malignancies for which the predisposing gene is already present at birth. Furthermore, the functionality and potential therapeutic efficacy of these UCB stem cells for regenerative medicine purposes have not yet been clearly defined.
Although reports from animal studies are promising, there is still lacking of clinical data.

It is imperative to understand the mechanisms involved in the UCB migration, proliferation and differentiation through preclinical research using both in vitro and in vivo models before successful clinical trials can be carried out. It is also important to remember that while many of the earlier preclinical experiments were carried out using fresh UCB, future clinical application will often require frozen and thawed samples. The isolation and expansion of mesenchymal stromal cells and other non-hematopoietic cells from frozen UCB is particularly challenging and this hurdle needs to be resolved.

In summary, the role for UCB in hematopoietic stem cells transplantation is clearly established and is likely to continue to grow in importance over the next decades. The utility of UCB in the treatment of non-hematological disorders is still being explored and requires much study. While the significance of publicly banked or sibling-banked UCB has been validated by over 13,000 unrelated donor UCBTs and over 2,000 more each year, the role of private banking of autologous UCB remains controversial.

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References


