ABSTRACT: Since the first umbilical cord blood (UCB) transplant, performed 25 years ago, UCB banks have been established worldwide for the collection and cryopreservation of UCB for autologous and allogeneic transplants.

CONTENT: Much has been learned in a relatively short time on the properties of UCB hematopoietic progenitors and their clinical application. More interestingly, non-hematopoietic stem cells have been isolated from UCB. These cells can be grown and differentiated into various tissues including bone, cartilage, liver, pancreas, nerve, muscle and so on. The non-hematopoietic stem cells have an advantage over other sources of stem cells, such as embryonic stem cells or induced pluripotent stem cells, because their supply is unlimited, they can be used in autologous or allogeneic situations, they need minimal manipulation and they raise no ethical concerns. Future studies will test the potential of UCB cells for the treatment of several diseases including, among other possibilities, diabetes, arthritis, burns, neurological disorder and myocardial infarction.

SUMMARY: In addition to hematopoietic stem cells, UCB contain a large number of non-hematopoietic stem cells. In the absence of ethical concern, the unlimited supply of UCB cells explains the increasing interest of using UCB for developing regenerative medicine.

KEYWORDS: UCB, transplantation, UCB bank, HSC, MSC, CD34, CD133, VSEL

Abstrak

LATAR BELAKANG: Sejak transplantasi darah tali pusat (DTP) pertama yang dilakukan 25 tahun yang lalu, telah banyak didirikan bank DTP di seluruh dunia untuk menyimpan DTP, untuk dipakai pada transplantiasi autologus maupun alogenik.

ISI: Banyak yang telah dipelajari dalam waktu yang relatif singkat tentang sifat progenitor hematopoietik DTP dan aplikasi klinisnya. Yang lebih menarik, sel punca non-hematopoietik telah diisolasi dari DTP. Sel ini dapat berkembang dan berdiferensiasi menjadi berbagai jaringan seperti tulang, tulang rawan, hati, pankreas, saraf, otot dan sebagainya. Sel punca non-hematopoietik ini memiliki kelebihan dibanding sel punca sumber lainnya seperti sel punca embrionik atau sel punca pluripoten hasil induksi, karena sumbernya tidak terbatas, dapat digunakan untuk situasi autologus maupun alogenik, membutuhkan manipulasi minimal dan tidak menimbulkan masalah etika. Penelitian di masa mendatang akan menguji potensi sel DTP untuk terapi berbagai penyakit, diantaranya adalah diabetes, artritis, luka bakar, gangguan saraf, dan infark miokard.

RINGKASAN: Selain sel punca hematopoietik, DTP mengandung banyak sel punca non-hematopoietik. Dengan tidak adanya masalah etika, ketersediaan sel DTP yang tidak terbatas ini meningkatkan keinginan dalam penggunaan DTP untuk pengembangan kedokteran regeneratif.

KATA KUNCI: DTP, transplantasi, bank DTP, HSC, MSC, CD34, CD133, VSEL
Introduction

The first umbilical cord blood (UCB) transplant (UCBT) by Gluckman et al. was performed in 1988 in a patient with Fanconi’s anemia. (1) This patient had a healthy human leukocyte antigen (HLA)-identical sibling who was shown by prenatal testing to be unaffected by the disorder, to have a normal karyotype and to be HLA identical to the patient. Her cord blood was collected at birth, cryopreserved and used after thawing for transplantation. Since the first UCBT, more than 20,000 UCBTs have been reported worldwide and more than 620,000 UCB units have been stored in more than 100 UCB banks. (2) The main practical advantages of using UCB as an alternative source of stem cells are the relative ease of procurement, the absence of risks for mothers and donors, the reduced likelihood of transmitting infections, particularly cytomegalovirus (CMV) and the ability to store fully tested and HLA-typed transplants in the frozen state, available for immediate use (3).

UCB bank (UCBB) established criteria for standardization of UCB collection, banking, processing and cryopreservation for unrelated donor transplants in patients with various hematological malignant and non-malignant diseases. (3) Recently, the use of UCB stem cells (UCB-SCs) in several regenerative medicine applications has expanded its clinical utility.

UCB-SCs contains a mixture of multipotent stem cells capable of giving rise to cells derived from the endodermal, mesodermal and ectodermal lineages. It has been shown that UCB-SCs have the ability to regenerate numerous tissue types, and when transplanted into animals and humans, produce measurable functional improvements. (4,5) Generally, tissue-derived stem cells have been described for neural (6), retinal (7), pancreas (8), skin (9) and liver tissues (10). UCB-SCs appear to be unique in their ability to undergo pluripotential differentiation. Thus, UCB-SCs appear to be a practical substitute for embryonic stem cells and are readily available for use in tissue engineering and regenerative medicine. Recently clinical trials have begun using UCB-SCs to treat type I diabetes mellitus (T1DM), cerebral palsy and peripheral vascular disease among others. (4,5) UCB-SCs has transitioned from the laboratory to the clinic and numerous patients are currently being treated in clinical trials. Other trials will surely rapidly follow, including therapies for eyes, joints, wound and spinal cord. The key to these advances lies in the pluripotency of UCB-SCs and their ability to be used in many instances under the practice of medicine, as it appears in many instances that it is possible to merely infuse the stem cells directly without timely and costly in vitro culture and differentiation. (11)

UCB-SCs

Hematopoietic stem cell (HSC) transplantation (HSCT) can be performed using stem cells derived from three sources: bone marrow (BM), peripheral blood (PB) and UCB. Only about 30% of patients in need of HSCT have a matched-related donor and, although there are currently around 10 million adult volunteer donors registered worldwide, still about 60% of patients will not find a suitably HLA-matched unrelated adult donor, and thus cannot access this potentially curative therapy. (12)

As UCB products are HLA-typed, tested for lack of infectious agents and stored, they are immediately available upon request and can be shipped to any transplant center in the world with relative ease and without delay. (13,14) UCB has become such a popular adult stem cell source for many reasons, not least because over 130 million births worldwide per annum represents the largest, easily available stem cell source. It also allows for storage of units from ethnic minorities not easily possible within BM registries. (15) This potentially allows for an increase in the rate of matched unrelated donor allogeneic transplants. (16) It has also been found that there is a lower risk of graft versus host disease (GvHD) when transplanting UCB compared to BM. (17) This could be due to the fact that the cells transplanted from UCB are more naive and have lower HLA protein expression. (16) UCB has been shown to contain a higher frequency of early progenitor cells than PB or BM. (18) Further findings show that term and pre-term UCB contain significantly higher number of early and committed progenitor cells, and that they are better able to form colony-forming-unit granulocyte-macrophage (CFU-GM) when compared to adult PB. (19)

UCB also contains non-haematopoietic stem or progenitor cells including mesenchymal and endothelial precursor. (20) Even more recently UCB is becoming a real player in the regenerative medicine field. There are many groups looking to develop tissues for either transplantation or drug testing with many successes. (21) It has become a real alternative to BM and PB as a source of adult stem cells to treat multiple diseases. More than 85 conditions can currently be treated using this stem cell source, such as the previously mentioned Fanconi’s anemia, a BM failure disorder (1), metabolic disorders like Krabbe’s disease (22) and immune defects like severe combined immune deficiency (SCID) (23). Well over 1 million UCB have been
stored globally in the last 10 years. Already, over 20,000 transplants have been performed using UCB for hematopoietic reconstitution alone, now this potential is joined in the regenerative medicine.(21)

UCBB has gained significant interest in recent years because of the success of UCBT and its more recent applications within regenerative medicine. Both public and private banking facilities have been established over the past 20 years to facilitate these endeavors.

Generally, UCB samples are red cell- and volume-reduced prior to cryopreservation, in order to facilitate banking and minimize costs. Samples may be frozen using a variety of media, and can be stored in either cryovials or cryobags, generally in a limited number of frozen aliquots. The storage containers may also be overwrapped with a plastic protective sheath for protection from contact with the liquid nitrogen (LN$_2$) itself, as well as a barrier to sample contamination.(24) Two of the most critical steps in the banking process are the cryopreservation and long-term LN$_2$ storage of the sample in such a fashion that the sample will be viable and usable decades into the future.(24)

Finally, great concern was raised in the mid-1990s over potential cross-sample contamination during liquid-phase LN$_2$ storage, after the demonstration that pathogenic viruses and other microbial pathogens could survive long-term direct contact with LN$_2$ and could infect other samples stored in the same dewar(25,26).

While donor-specific factors probably impact on the potency of a UCB unit (27), the collection, processing, distribution and infusion stages may also affect the potency of this stem cell product, whatever processing technology a laboratory chooses, the goal is to achieve maximal recovery of total nucleated cells (TNC), mononuclear cells (MNCs), CD34$^+$ cells, CFU, progenitor cells and stem cells.(28) While the overall cellular recovery declines as a result of the freezing process, the overall potency may be preserved for more than 15 years if the UCB is maintained below -150°C. (29)

Currently the United States Food and Drug Administration (US FDA) recommends evaluation of potency through assessment of the following in vitro assays: TNC count, cell viability and CD34 analysis. TNC are measured using automated hematology analyzers, the CD34 assay is performed using flow cytometry, and the CFU assay is accomplished using a methylcellulose-based media impregnated with cytokines.(28) There is increasing evidence that UCB unit potency should be assessed post-cryopreservation because events associated with freezing and storage can affect subsequent cellular viability and overall UCB potency.(28)

Although there are many benefits to using UCB for transplantation there is one major limitation: the TNC count and cell number recoverable from a single unit. This is affected by unit size, maternal factors such as number of previous pregnancies and age of mother (30), limited volumes available from each sample, but not least the processing method used. Together, these factors highlight the need to make processing as efficient as possible (31) to make UCBB a real option. Many methods are currently available. Techniques were varied; from density gradient separation, like Lymphoprep or Ficoll-Paque (32), rouleaux formation using Hetastarch (HES), a starch based method causing red cells to clump (33), plasma depletion; a simple volume reduction method which avoids the addition of any chemicals and simply removes the plasma (34), a novel closed separation kit, known as PrepaCyte-CB which offers rapid and specific cell separation (35), and the only fully automated system, provided by Biosafe, known as the ‘Sepax’ machine (36). PrepaCyte-CB and an automated centrifugal machine, Sepax gives the highest recovery of nucleated cells, an average of 78.8% (SD±21.36).(21)

Fifteen to 24% of patients receiving UCBT do not engraft. One potential cause of engraftment failure is a loss of reconstituting HSC potency that may have occurred at some stage during UCB collection, processing, storage, shipment and infusion.(28) The National Marrow Donor Program (NMDP) Cord Blood Advisory Group can assist in the development of assays by providing resources in terms of UCB, expertise and access to a network for testing assays, once developed, in the field.(28)

UCB-SCs for Hematopoietic Disorders

HSC transplantation is a curative approach for a variety of malignant and non-malignant diseases.(37-40) Mobilized PB, BM and UCB are the main sources of HSC. UCB from related and unrelated donors has emerged as a promising source of stem cells for a variety of hematological disorders and as a supportive therapy for malignant diseases.(41-44) In addition to its widespread availability, UCB contains HSCs and hematopoietic progenitor cells (HPCs) with higher immunological tolerance and higher proliferation ability than adult-derived BM (44), however, the low yield of HSCs and HPCs in UCB grafts limits their applications in clinics. (45) For optimum engraftment, current transplantation
requires at least 2,500,000 CD34+ cells per kilogram of patient body weight. An optimally collected UCB donation generates approximately 10,000,000 CD34+ cells, which is just adequate for pediatric patients right now.

UCB HSCs have numerous phenotypic and functional characteristics that distinguish them from their adult counterparts. (48-52) UCB CD34+ cells are thought to be more primitive due to their extensive proliferative capacity, their increased ability to generate hematopoietic colonies in vitro, their capacity to produce erythroid cells, which contain fetal hemoglobins, and the ability of smaller numbers of such cells to reconstitute a myeloablated allogeneic recipient. (48,53)

While UCB represents a readily available source of HSC for transplantation, the low cell dose available in a UCB graft correlates with a significant delay in hematopoietic recovery and a higher risk of primary graft failure. (54,55) In order to provide higher absolute numbers of HSC for transplantation, the low cell dose available in a UCB unit is used increasingly in clinical applications involving ex vivo expansion. (67)

Several innovative therapies with human UCB-SCs (hUCB-SCs) are currently developing to treat central nervous system (CNS) diseases. It has been shown that UCB contains multipotent lineage-negative (LinNEG) SCs capable of neuronal differentiation. Clinically useful UCB samples are stored in different biobanks worldwide, but the content and neurogenic properties of LinNEG cells are unknown. Jurga et al. showed that Sepax-processed blood units contained 10-fold higher number of LinNEG cells after cryopreservation in comparison to all other methods. (71)

Neonatal hypoxic ischemic (HI) encephalopathy due to perinatal asphyxia is an important cause of death in the neonatal period, in both developing and developed countries. Among the surviving infants, up to 25% will have a permanent neurologic deficit in the form of cerebral palsy, epilepsy, learning disability, or mental retardation. (73) Treatment is currently limited to supportive intensive care. Given the severity of the problem, it is necessary to find new approaches that could reduce the neurologic sequelae of HI newborns. (74)

In the last few years, preclinical studies have shown that UCB-SCs injected systemically in the acute phase of animal models of stroke, have a therapeutic effect. These
cells can reduce the area of brain infarction (75) and the inflammation, and increase the regenerative capacity of the brain, improving behavioral recovery (76). The potential of UCB-SCs to reduce the neurologic deficits associated with neonatal hypoxia-ischemia was shown in rats in a study that assessed the functional benefits of these cells, using a walking-pattern analysis test. The UCB-SCs, when injected intraperitoneally 24 hours after the insult, migrated to areas of brain damage in large numbers, and alleviated the spastic paresis of the animals. (77) Thus, UCB-SCs transplantation might rescue striatal neurons from cell death after a neonatal HI injury resulting in better functional recovery. (74)

Cerebral palsy (CP) is the most frequent neurological disorder associated with perinatal injury of the developing brain. Major brain lesions associated with CP are white matter damage (WMD) in preterm infants and cortico-subcortical lesions in term newborns. Cell therapy is considered promising for the repair of brain damage. The hUCB-MNCs are a rich source of various stem cells that could be of interest in repairing perinatal brain damage (78).

Spinal cord injury (SCI) is often characterized by immediate and irreversible loss of sensory and motor function below the level of injury. Cell transplantation is a potential method for certain neurological diseases as well as a viable treatment for acute SCI. (79-83) The present results indicated CD34+ UCB-SCs accelerated function recovery after SCI by governing blood vessel formation and restoration. Also, early administration of CD34+ UCB-SCs was able to increase tissue vitality and the blood vessel density, as well as improve behavioral deficits after SCI obviously. (84)

Traumatic brain injury (TBI) is a major public health problem associated with death and permanent disability worldwide. (85) Both acute and chronic symptoms accompany TBI, with survivors suffering from progressive post-TBI pathological manifestations such as neuroinflammation coupled with behavioral dysfunctions including sensory motor deficits, learning and memory impairments and a range of neuropsychiatric symptoms including anxiety, depression and aggression. (86, 87) At present, there is significant unmet need for clinically efficacious therapies for TBI. (88)

A number of groups have focused on the potential of UCB-SCs as a graft source for various intractable neurological disorders (e.g., stroke, Parkinson’s disease and Huntington’s disease, among others). (89) Moreover, clinical trials have been performed to determine the efficacy of UCB-SCs in cerebral palsy, inborn metabolic disorders and stroke. (90) Combined therapy of UCB-SCs plus granulocyte colony stimulating factor (GCSF) synergistically dampened traumatic brain injury-induced neuroinflammation while significantly enhancing endogenous neurogenesis and reducing hippocampal cell loss. (91)

UCB-SCs holds great potential as a source for cellular therapy. Mesenchymal stromal cells (MSC) and unrestricted somatic stem cells (USSC) found in UCB have been reported to differentiate in vivo into osteoblasts, adipocytes and neural progenitor cells (92). UCB-SCs cells have been used in high doses to delay symptom progression in a mouse model of amyotrophic lateral sclerosis (ALS) (93) and have been identified within the central nervous system of a patient with Krabbe’s disease 10 months post-UCB-SC transplant (94), suggesting the persistence of these cells as a possible contributor to cellular repair in patients with neurodegenerative diseases (95). UCB-MSC could restore behavioral functions and attenuate the histopathological deficits of experimental autoimmune encephalomyelitis mice over the long term (i.e., 50 days) by suppression of perivascular immune cell infiltrations and reduction in both demyelination and axonal injury in the spinal cord. These findings suggest that transplantation of UCB-MSCs may be a potential therapy. (96)

### UCB-SCs Successes

SC Researchers at the Steenblock Research Institute in San Clemente, California, have been following the cases of patients treated with UCB-SCs, as well as tabulating results from pilot studies performed abroad involving stem cell therapy for specific conditions such as cerebral palsy in children and MS in adults. The following are but a few of the many responses documented as of the date of publications. (97)

A 65 years old man with progressive multiple sclerosis (MS) was treated with UCB-SCs in July 2003. Prior to this treatment, he could not swallow water normally. Within a week of receiving UCB-SCs, he was able to do so without a problem. He subsequently made noticeable gains in his ability to get around and could communicate more clearly. (97)

Ramirez hUCB-SCs therapy program in Mexico has treated more than forty children with cerebral palsy since March 2003. Eighty five percent of these children have experienced significant improvements in motor skills and cognitive functions. In one case, a four years old boy was cortically blind (a lack of visual functioning despite structurally intact eyes), could not speak well, and could not get around well prior to therapy with UCB-SCs. Within seven months of therapy, however, he was able to track
objects with his eyes, was beginning to speak, and could move around more ably. (97)

Jordan Logan, a four years old girl, with a terminal genetically based neurological disease called metachromatic leukodystrophy (MLD) was treated with 1.5 million UCB-SCs. MLD is caused partly by a genetic defect in which the Arylsulfatase A (ARS-A) is missing or not functioning properly. This enzyme makes it possible for a person’s body to deal with toxic molecule that we all generate called sulfatides children and adults who do not produce ARS-A or very little experience declines in their neurological function that culminate in disability and death. Within two months of her UCB-SCs injection, however, she could track objects with her eyes, and lift her arms and legs high in the air. Eventually, two of the three medications she was on were discontinued. Jordan’s story has appeared in numerous regional and national newspapers, and also has been the focus of TV coverage in Mississippi. (97)

Conclusion

In the last few years, pure UCB-SCs have been utilized by physicians to treat a multitude of intractable diseases such as progressive MS, ALS, macular degeneration, retinitis pigmentosa, stroke, diabetes, and various forms of heart disease. This body of patient responses indicates that UCB-SCs therapy does produce clinically significant improvements in many instances. While certainly no cure for all, UCB-SC therapy appears to be amassing a respectable track record in terms of both safety and clinical utility.

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