Brief Communication

Fetal Stem Cell: From Research to Clinical Use

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The discovery of new sources of stem cells over the past few years has raised the expectation that stem cells may in the future provide new biological therapies for a number of diseases, the incredible potential for substituting damaged or lacking cells, tissues, and even organs. A number of stem cell types have been identified, including bone marrow stem cells, embryonal stem cells, and fetal stem cell including umbilical cord blood (UCB) stem cells. The UCB contains a rich source of hematopoietic stem cells that can be used to reconstitute the blood system and can easily be extracted and cryopreserved, thus allowing for the establishment of HLA-typed stem cell banks. UCB have also the potential to give rise to non-hematopoietic cells, such as bone, neural and endothelial cells.

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Fetal stem cells are described as multipotent, meaning that they exist within specific fetal tissues and give rise to differentiated cells of that tissue only. Fetal stem cells have several advantages over adult cells because of their greater differentiation potential , better intrinsic homing and engraftment, greater multipotentiality, more rapid proliferation and lower immunogenicity. Fetal tissue transplantation was introduced as a research therapy for chronic degenerative diseases such as Parkinson's disease and insulin-dependent diabetes[1], although their development potential is more restricted than pluripotent embryonic stem (ES) cells[2]. Ethical issues were raised with the use of Human embryonic stem (ES) cell from the human embryo source, the human blastocyst. The use of therapeutic fetal tissue transplants, which were obtained following elective pregnancy terminations will also be a source of ethical concern. Stem cells from less controversial sources, UCB cells, have been used extensively investigated and widely utilised over the last 10 to 20 years for years to reconstitute the bone marrow of recipients suffering from a number of hematological and non-hematological disorders[3].

Umbilical Cord Blood Stem Cells

Stem cells remain in the placenta and umbilical cord post-delivery and on average 50-150 ml of blood can be collected with no risk to either the mother or the baby, this rich source of stem cells is usually discarded. Blood or cells from the umbilical cord can be stored and used for later transplantation[4,5] UCB contains more hematopoietic stem cells per volume than peripheral blood or bone marrow[6]. These cells are characterized by an expression of the CD34 antigen, and are also called CD34+ cells.

History and Current Practices

UCB was first used for a successful bone marrow transplant in 1988, when a child with Fanconi's anemia received an allogeneic transplant using the cryopreserved collected from his human major histocompatibility complex (HLA) identical sibling[7]. The transplanted child remains alive and well 16 years later. After the success of this report, UCB has now became an established source of HSCs for transplantation, particularly for children with a variety of malignant and non-malignant disorders, such as acute and chronic myeloid and lymphocytic leukaemias, myelodysplastic syndrome, myeloma, lymphoma, solid tumours (e.g. neuroblastoma, retinoblastoma), osteopetrosis, liposarcoma, bone marrow failure syndromes, hemoglobinopathies, severe combined immunodeficiencies, inborn errors of metabolism and autoimmune diseases[6,8-9]. Restoration of hematopoiesis in recipients may be successful even when unrelated UCB donors are used, and despite several human leukocyte antigen (HLA)

mismatches between donors and recipients[10].

The UCB HSCs have a particular advantage over other HSC sources because

- Engraftment potential of UCB haemopoietic cells in preclinical studies showed higher proliferative capacity primitive UCB haemopoietic cells compared with adult bone marrow.

- UCB is readily available and non-controversial. It may be the only source of allogeneic HSCs available to patients with rare HLA types and hence to ethnic minorities, to siblings suffering from diagnosed hematological disorders and for urgent unrelated donor transplants[10].

- No donor morbidity : they can be easily collected from autologous, or related and unrelated donors, tested, HLA typed and banked for immediate use[11].

- Low CMV transmission (<0.1% of healthy neonates), compared with 10–60% of adult volunteer donors[11].

- Less acute and chronic graft versus host disease associated with the transplantation of human major histocompatibility complex (HLA) mismatch than adult donor stem cell because of the "naïve" immunophenotype of UCB lymphocytes and, alloantigen primed UCB T cells are relatively unresponsive to the original stimulator in secondary mixed lymphocyte reactions[12,13].

- A better tolerance of 1-2 HLA mismatches compared with other sources of allogeneic.

HSCs, such as bone marrow and mobilized peripheral blood (MPB)

- A lack of risk to and attrition of donors compared with bone marrow donors (the annual donor loss in bone marrow registries being around 7%)[14].

The Disadvantage of UCB Transplantation

- The availability of single donations from a specific donor (i.e. no possibility of going back to the donor for a repeat stem cell donation).

- A limit to the number of HSCs within the UCB unit. At present UCB transplantation should only be considered if a suitably matched donation contains at least 2×10^7 nucleated cells/ kg and CD34+ cell dose ($\sim 0.3–0.4 \times 10^6$/kg) (approximately one tenth of given in bone marrow grafts)[14]. The number of cells infused correlates with initial peripheral blood platelet and neutrophil recovery and post transplant survival and is usually sufficient to engraft only children and small adults. This means that most adults and larger children over 50 kg. in weight are not suitable recipients. Despite this, with the development of new collection approaches to the use of UCB for transplanting adults with hematological disorders[15].

- There is often delayed hematological and immune reconstitution[16]. The median time to neutrophil and platelet recovery after UCB transplantation is longer than that expected after marrow or MPB transplants and in adults UCB has often been used as a last resort for transplants with advanced disease, generally undergoing myeloablative therapies.

-The difference in leukaemic relapse seen after cord blood compared with bone marrow transplantation is also unknown[17].

Tentative recommendations for the use of UCB for transplantation are as follows

- Collection is indicated from healthy newborn siblings when urgent transplantation is required for an older child in a family.

-The hematologist responsible for the older child, with the approval of the family and the obstetric team, should contact the National Blood Centre to discuss arrangements for the UCB to be collected and HLA typed.

- UCB should be collected without influencing the management of mother or child during birth. UCB can be collected both at vaginal delivery and in connection with Caesarean section. Variety collection methods (open and closed systems) have been proposed. To optimize the collection volume and minimize microbial contamination rate, the open systems have been replaced by the closed system[4,15].

-The success of the transplant depends on many considerations which include the degree of HLA matching between the donor and the recipient, the numbers of stem cells required, the use of accessory cells or factors, the extent and type of disease, the time lapse expected between injury of disaese and transplant and the required rate of repair in order to successfully repair the injury. The time element is an important consideration because the length of the hospital stay and medication or accessory therapies the patient required and might die as a result of secondary factors (infection or bleeding) related to the lack of a functional hematopoietic system[18-19].

- In addition to the hematological disease indications that normally require a bone marrow transplant, the availability of HLA-matched cells make it feasible to use hematopoietic stem cells derived from

UCB for indirect indications such as allowing more aggressive treatments of non-hematological cancers or to prevent or cure metastases[20].

UCB Banking

There has been considerable UCB banking activity worldwide over the past decade. Over 175,000 cord blood donations are banked and available for patients worldwide[21]. Units can be entered onto worldwide registries (e.g. Bone Marrow Donors Worldwide and Netcord) and easily resourced[22]. More than 5000-6000 transplants are estimated to have been carried out with UCB worldwide[21]. These UCB banks have a particular importance for patients with rare HLA types and hence for ethnic minorities, for the donors of siblings suffering from diagnosed hematological disorders and for urgent unrelated donor transplants. Although there are potentially over 9 million registered bone marrow donors worldwide, the proportion of matched unrelated donors that cannot be found in these registries may be, for some racial groups, of the order of 20-50%[11,16,23]. Such patients therefore have the opportunity for UCB transplants when an autologous, related or unrelated bone marrow donor cannot be sourced. Accessing banked cord blood units takes on an average about 2 weeks, whereas the work-up for an unrelated bone marrow donor from a registry may require up to 4 months[22]. A collection of 18,000 cord blood samples stored in The New York Blood Center has provided a suitable donor for 85% of requests[26]. The necessary size of UCB banks depends on how exact the match between donor and recipient needs to be, and also on how large a proportion of a given population it should be able to provide matched cells for[6]. Important points for consideration include financing of such banks, and whether information about the donor should be linked to the stored cord blood cells[25]. In some countries there has been massive central financial support, whereas in others, including Thailand, banking activity to date has been financed through research and charity funds raised by individual cord blood banks. The National Blood centre manages and coordinates the Thai Bone Marrow Registry, which searches for and provides donors nationally, and also maintains the Cord Blood Bank, which currently has over 230 UCB units banked, HLA-typed, microbiologically screened and ready for transplantation[4].

UCB can be stored according to two fundamentally different principles. One possibility is general storage banks similar in principle to traditional blood banks. In such banks the cord blood is not stored for later use in the donor, but for use in a matching recipient unrelated to the donor[23-24]. Another approach to cord blood banking is storage for possible future use as autotransplantation, in case the individual requires a cord blood transplant later in life[23-26]. A number of private cord blood banks have emerged, offering this form of storage. The probability that an individual will ever need an autologous cord blood transplant has been estimated to be in the order of 1:1000 to 1:200000[23-26]. Consequently obstetricians may now or in the near future be involved in informing patients about the possibilities for cord blood collection, either for individual-based storage in privately organized banks, or for donation to presumably public cord blood banks. It should also be considered that the use of autologous cells for transplantation in cases of malignant diseases may carry a risk that the transplanted cells harbour a genetic defect, resulting in an unacceptably high risk of again developing the same malignancy[24,26]. Future research may, however, result in the development of techniques for differentiating cord blood cells in directions other than hematopoiesis (multipotential mesenchymal cells) , and this could result in many more indications for the use of cord blood stem cells in the replacement of damaged cells and tissues. The multipotential mesenchymal cells are currently showing a strong propensity for proliferation and multi-lineage differentiation[27–33].

Increasing haemopoietic cell dose by transplantation of multiple UCB donations

Investigators have explored the possibility of using multiple donations to enhance early engraftment in larger recipients receiving UCB transplants. Using multiple UCB donations to increase cell dose in clinical UCB transplantation. Later, one of the donations predominated[34].

Hematopoietic Stem Cell Expansion

Expansion is dependent on triggering proliferation without inducing the internal differentiation programme. The main obstacle to overcome in stem cell expansion is the maintenance of the self-renewing stem cell population during induced proliferation thus reducing the loss of self-renewing stem cells that occurs. Attempts to stimulate hematopoietic stem cells to proliferate generally leads to differentiation into mature cells since growth factors have both mitogenic and differentiation properties[35].

Although many laboratories have reported hematopoietic stem cell expansion, most of the reported

successes are based on in vitro results, which do not realistically measure engraftment potential of the cells[36]. The mean level of engraftment with cultured cells was lower (1%) compared with fresh cells (7.4%) when the same number of expanded or fresh CD34+ cells was transplanted. This suggests that although engraftment of expanded cells occurs, there is less efficient proliferation and differentiation in vivo.

Conclusion

Fetal stem cells including UCB stem cells which is an acceptable alternative to bone marrow, were believed to represent powerful tools for exploring many aspects of cell biology and hold considerable promise as therapeutic tools for cell transplantation especially in children. Family-directed UCB donations are acceptable for transplantation of siblings with lifethreatening diseases. UCB HSC transplants are associated with less severe acute graft-versus-host disease, but will delay hematological reconstitution. Transplant-related mortality and limited HSC dosages remain a major obstacle to UCB transplantation in adults. UCB is also a source of non-hematopoietic stem cells that have exhibited properties previously attributed to embryonic stem cells such as the necessary plasticity (capable of building tissue) to repopulate and restore organ function in a variety of animal models and, more recently, in human clinical settings.

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