Peripheral Blood Progenitor Cell Transplant : A Way Forward

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Tahir S. Shamsi (Dr. Ziauddin Postgraduate Institute of Medical Sciences, Karachi.)

Allogeneic bone marrow transplantation (BMT) has improved the outlook for acute myeloid leukaemia while autologous BMT has given encouraging results in relapsed non Hodgkin\'s lymphoma and Hodgkin's disease[1,2]. However, majority of patients do not have a suitable fully matched sibling donor and the use of alternative donors, either a fully matched unrelated or a partially matched sibling donor increases the risk of GvHD and its associated complications resulting in a procedure related mortality of upto 40%. GvHD incidence has been reported between 42-90% as the HLA dispanty increases[3]. Autologous BMT is also associated with a procedure related morbidity and mortality, most studies reporting a mortality rate between 8-15%[4]. The potential of circulating peripheral blood progenitor cells (PBPC) to reduce morbidity and mortality by shortening the period of cy topenia following transplantation and lower relapse rates by reducing the contamination of blood mononuclear cells with clonogenic tumour cells has focused attention on this approach[5].

Concept of circulating progenitor cells in peripheral blood

Haemopoictic stern cells are defined by their ability to self renew under the control of the cytokinc network and produce progenitorrs[6]. Because of the high degree of numerical amplification that accompanies haemopoiesis, the proportion of stem cells in the bone marrow is small[7]. In a steady state, they are not in cell cycle, they are heterogenous in size, density and function[6,7]. Stein cells are described phenotypically as CD 34± and lineage specific differentiation antigens e.g. (CD 38, CD 33, CD 13, CD 71 and CD 45RA) negative[6,7]. In long term cultures these cells am capable of providing mature cells and demonstrate resistance to chemical purging with cell cycle specific agents like 4-hydro-peroxy-cyclophosphamide[6,9,10]. The existence of progenitor cells in the peripheral circulation has been known for more than two decades[5,11]. Although assays for haemopoietic cells are imperfect, it is known that the mononuclear cell (MNC) fraction of peripheral blood when given in sufficient number can restore haemopoiesis in immunosuppressed, lethally irradiated mice[8]. The number of PBPC in the peripheral blood rises in response to different physiological stimuli including stress, exemise and infections[12,13]. PBPC also increases in the cimulation during the recovery phase from chemotherapy which could be further enhanced by using a variety of cytokines[14-16] e.g., (G-CSF, GM-CSF, IL-3 and SCF). In a steady state, progenitor cells constitute approximately 1% of all the nucleated cells of the bone marrow and 10 times less in the peripheral blood[7].

Progenitor cell mobilization

The adequacy of progenitor cell mobilization depends upon a number of variables. Individual variation, type and duration of prior chemotherapy, priming strategy i.e., the type and the dose of chemotherapy used alone or in combination with colony stimulating factors (CSF) and the dose of CSF. Many different progenitor cell mobilization strategies are currently in use including chemotherapy alone, CSF alone or a combination of both. The chemotherapy schedules whichare currently being evaluated include cyclophosphamide, high dose meiphalan and drug combinations incorporating cyclophosphamide, etoposide and cisplatin but the optimum dose and combination has not been defined[14,17,18]. The prinung strategies which incorporate chemotherapeutic agents have associated morbidity and risk of mortality. Chemotherapy induced mobilization increases the circulating CFU-GM but it is difficult to predict the peak levels in the blood and thus the optimum time to begin stem cell collection. This unpredictable and variable “window” of opportunity of haemopoietic recovery means that resources such as cell separators and personnel may not be used optimally.

he colony stimulating factors either as single agents or in combination sequentially or concurrently have been used effectively as mobilizing agents[17,18]. In addition, priming chemotherapy can be combined with colony stimulating factors including G-CSF, GM-CSF stem cell factor (c-kit ligand) and IL-3[17].

Harvesting

PBPC are harvested during the recovery phase by cell separators which collect MNC fraction either in a semi-continuous (Haemonctics V50) or a continuous flow principle (Fenwal Baxter CS 3000 and Cobe Spectra) and return the red cells and platelets to the patient[19].. These instruments differ in their ability to handle the volume of blood they can process, MNC collecting efficiency, volume of the final product and involvement of the operator[19]. Using the Fenwal CS 3000 or the Cobe Spectra, 2-3 sessions of apheresis, each of which lasts for 3-4 hours usually provide sufficient numbers ofMNC in an optimally primed patient.

Assessment of progenitors

Once the leukapheresis is completed the cellular composition can be evaluated by total MNC count, CD 34+ content, S-phase fraction and CFU-GM culture. Most centres use MNC count and CD34+ cell content of the harvest to assess the adequacy of progenitor cells (CFU-GM) and to predict the time to engraftment.

Tumour relapse is the main cause of treatment failure following bone marrow transplantation. The relative contribution of tumour cells remaining after high dose induction therapy given to the patient and tumour cells contaminating the harvest product in contributing to relapse is untested. The reduced risk of contamination of peripheral blood progenitors cell harvestsby tumour cells orthe reduced ability ofsuchcells to re-establish tumour is one reason which has motivated peripheral blood progenitor cell transplant programmes. Ex vivo purging of progenitors by positive selection of CD 34 positive cells is an alternative approach to reduce the risk of tumour cell contamination further and is currently under investigation[20-22]. The advantages of this technique are to reduce the risks of relapse and reduce the volume of harvest product which minimises the volume of dimethyl sulplioxide (DMSO), a cell ciyo-protectant, being re-infused and reduced storage space and costs. Once hacmopoictic progenitors are isolated and purifled, it is possible to expand a selective population of cells by using a cocktail of cytokines[20]. Potential advantages of this approach include more rapid and selective haemopoietic reconstitution, expansion of small number of cells to be used to support multiple chemotherapy cycles and differential growth support for normal progenitors compared to malignant cells. Most exciting is the potential for expansion techniques to be used in conjunction with gene therapy programmes where clinical studies are in progress to assess this technology.

Storage of PBPC

Unmanipulated PBPC harvest may be stored unfrozen for upto 72 hours at 2-6 degree celsius, storage at this' temperature reduces leucocyte viability and may not be ideal for the majority of patients as many pre-transpiant conditioning regimen last longer than 72 hours[23], If haemopoietic progenitors are to be stored at -85 to -190 degree celsius, a ciyo-protective agent must be added to the final product to prevent intracellular ice crystal formation, cellular disruption and cell lysis from a raised external osmolality[24,25]. Dimethyl sulphoxide (DMSO) and hydroxy ethyl starch (HES) have both been used as a cryo-protectant. DMSO at a final concentration of 10% has become the reagent of choice forthis purpose in most institutions. DMSO is toxic to stem cells at room temperature and therefore, processing and manipulation must be done at 4 degrees celsius and freezing should begin as soon as possible after the addition of DMSO. Control rate freezers are employed in most laboratories to decrease the temperature of the final product at a rate of 1-3 degree celsius per minute.

Direct freezing of the final product into a mechanical freezer at -85 degrees celsius does not appear to have any adverse effects on stem cell viability, CFU-GM recovery after thawing and no significant

difference in haemopoietic recovery after transplant[25,26]. Recently, the need for control rate freezing has been questioned. Cryo-preserved PBPC should
celsius at the patient's bed side prior to infusion. Leaving thawed, DMSO containing PBPC at room temperature can cause toxic damage to progenitor cells.
The patient may develop tachycardia, transient hypertension, cough, fever, chills, facial flushing nausea and red urine due to DMSO toxicity and release of free haemoglobin and cellular stroma[27]. Intravenous hydration prior to and 6-8 hours after PBPC infusion and pre-medication with hydrocortisone and chiorphenemmine and if needed antiemetics will help to ameliorate these symptoms.

Engraftment

One problem encountered in comparing techniques arises because of difficulties in assaying the haemopoietic stem cells. Whilst it is clear that PBPC transplantation reduces the risk of cytopenia post-transplanatation, this effect is only seen using cells collected following recovery from mvelosuppressionorhaemopoietic growth factor radministration and not with cells collected in steady state. Most investigators have also defined a threshold of CFU-GM below which rapid engraftment does not occur but this number varies widely. Although CFU-GM shows a significant correlation with the time to cngraftment multiple variations in assay techniques make inter-laboratory comparison difficult. A correlation between days to engraftment and the number of CD 34+ cells infused varies from 1x10"6 CD 34+ cells/kg to 7.8x10"6 CD 34+/kg. This reflects the fact that standardisation of immunophenotyping techniques are not as uniform as once suspected.

Clinical

The PBPC transplantation is used increasingly in a variety of malignancies because of the faster post-transplant haemopoietic recovery, potential cost savings and the capacity to harvest stem cells in heavily pre-treated patients.Thc risk of boric marrow involvement in many tumours and the possibility of a "cleaner" product in the form of PBPC is another factor which has increased the impetus to use this approach in the place of autologous bone marrow transplantation.
Currently, trials using intensive chemotherapy with PBPC support trials are focusing on their role in advanced stages of chemosensitive malignancies. Hodgkin's lymphoma, Hodgkin's disease, myeloma, breast cancer, small cell lung cancer, teratoma and ovarian cancer are all being evaluated[30-37]. The value of PBPC transplantation needs to be compared against conventional chemotherapy and autologous

BMT

Low grade NHL is incurable with conventional treatment. In "clinical remission" tumour cells are detectable in bone marrow by polymerase chain reaction (PCR) and tumour colony assay[38,39]. The overall survival for relapsed and refractory high grade and intermediate grade NHL is 20%, while the survival for chernoresponsive disease is 35-50% with salvage therapy[40]. Hodgkin's disease is a curable disease in upto two third 01 patients but for those who relapse or have a refractroy disease, the outlook is poor[31]. Myeloma is an incurable disease on conventional chemotherapy with a median survival of 2 years[41]. Aggressive treatment with high dose meiphalan and allogenic BMT has been used in patients under the age of 45 years with a complete remission in one third of patients[40,41] .However, the procedure related mortality of up to 40% which is mainly due to the complications of GvHD associated with allogenic BMT has precluded its use as a first line treatment; moreover, less than 10% of the patients are under the age of 50 years and less than 3% under 40 years of age means that allogemc BMT cannot be offered to the majority of patients at present[42]. These group of patients emerge as the more promising indications for PBPC transplantation which offers the opportunity for long term disease free survival in these patients who might be considered incurable with other treatment. Funda-mental questions remain concerning the identification of prognostic factors for better patient selection, optimum use of priming agents, timing of transplant in relation to disease status, best conditioning

regimen, value of post-transplant consolidation and finally, whether PBPC is better than conventional treatment. In solid tumours, autologous BMT has been used with encouraging results. The use of dose intensification with PBPC support for the treatment of locally advanced breast cancer has been increasing in the USA and Europe. The preliminaty data of such an approach has been reassuring[35,36]. In acute leukaemia, the results of autologous BMT are comparable to combination chemotherapy but are significantly lower than allogenic BMT[43]. In CML, chronic phase cryo-prescrved buffy coat cells have been used in accelerated phase to attain a second chronic phase; whether this can translate into prolong survival is not clear[44-46]. However, in the absence of any effective treatment other than allogenic BMT to improve the survival in this disease, furtherdevelopment of PBPC transplantation will be interesting to observe, particularly if in vitro orinvivo purging of the graft can be improved.

Future direction

In a short period of time, PBPC transplantation has become a widely used procedure. Now, in many centres it has surpassed the use of autologous bone marrow transplantation for treatment of non-Hodgkin's lymphoma. Hodgkin's disease, multiple myeloma and breast cancer. Allogenic PBPC, human umbilical cord blood stem cells[47,48], selection and purification of stem cells and ex-vivo expansion of stem cells are the areas under intensive investigation. Such approaches may further reduce the procedure related morbidity and mortality, risk of tumour cell contamination, need of multiple apheresis and may improve the survival of poor risk patients. Lastly, gene therapy is emerging as a realistic approach for the
treatment of malignant and hereditary non-malignant disorders using peripheral haemopoietic progenitor cells. Large prospective randomized studies are required to compare PBPC transplantation versus autologous bone marrow transplantation and conventional high dose chemotherapy to show survival advantage, cost effectiveness and lower procedure related complications.

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