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Stem Cell : Its Evolving role in Cancer Management and Research

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Summary : Hematopoietic stem cell transplantation has become established mode of treatment for various malignancies and hematological disorders. Continuous efforts are being made to improve the availability, applicability and survival by using different sources of stem cells (cord blood, fetal liver, partially matched/ mismatched donors), and reduced intensity conditioning regimens. During last few years there has been a growing interest in the area of stem cell research world wide. Apart from being implicated as a source of carcinogenesis, their self renewal, proliferative and differentiation potential is being utilized in treatment of various chronic ailments and congenital disorders (myocardial infarction, cardiomyopathy, cerebral palsy, muscular dystrophy and retinal degeneration). These are the areas of active research though the responses and underlying mechanism are still in their infancy and poorly understood.

Keywords : Hematopoietic stem cells, transplantation, fetal liver, stem cell therapy.

Introduction

The growing knowledge of biology has made it clear that stem cells have a key role not only in genesis and development of various organisms but also in tumorigenesis. Stem cells are present in almost every tissue of the body and they have the capability of self-

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renewal, trans-differentiation and extensive proliferation. These attributes if not regulated appropriately (genetic/ environmental factors) can result in development of malignant phenotype. These 'cancer stem cells' have been identified in various malignancies of hematopoietic system, breast and brain (1,2). Currently much of the research is being done to identify these stem cells and to target them (resistant to conventional treatment) to achieve complete cure.

In contrary to the cancer stem cell concept, the use of hematopoietic stem cells (HSC) in treating various malignant and non-malignant conditions is relatively well established. First successful transplant was done in 1968 in Minnesota (USA) in a child with severe combined immune deficiency (SCID) from an HLA matched sibling. Since then, over last 3-4 decades, HSC transplants have been used to treat cancers and hematological disorders such as aplastic anemia and hemoglobinopatheis. Hematopoietic stem cells are traditionally obtained from bone marrow. More recently they have been obtained from peripheral blood after mobilizing them from bone marrow with the help of growth factors and/or chemotherapy. Umbilical cord and fetal liver are other sources of hematopoietic stem cells.

In present article we will limit ourselves primarily to the HSC transplantation, and its current status and future prospects.

Fetal liver hematopoietic stem cells: Clinical and in vitro studies

Liver is the major site of hematopoiesis during second trimester of pregnancy (3). Due to paucity of lymphoid cells and weak expression of HLA antigens (3-5), fetal liver has the minimal potential to induce graft versus host disease (GVHD). Since GVHD is the major killer following bone marrow/ peripheral blood stem cell transplant, fetal liver appeared an attractive source for clinical use. More over, compared to adult marrow/peripheral blood, fetal liver hematopoietic stem cells have greater proliferative potential as revealed by the formation of more number of colonies, higher plating efficiency and larger colony size (6).

At AIIMS, in 1970's when we did not have the facilities for performing bone marrow or peripheral blood stem cell transplants, we treated aplastic anemia and acute myeloid leukemia (AML) patients following chemotherapy with the help of fetal liver infusion (FLI). Preconditioning was not utilized. Of the 41 patients of aplastic anemia treated with FLI, 40% responded in 10-30 days time. Median survival of the patients was better compared to that achieved with supportive care. However, since, preconditioning was not used; sustained engraftment could not be achieved. Engraftment demonstrable in some patients was temporary. It thus appeared that fetal liver infusion had induced

autologous hematologic recovery in 40% of the subjects (7-10).

Various in-vitro studies were carried out in our lab to find the mechanism of recovery. ELISA confirmed the presence of some colony simulating factors in fetal liver conditioned medium (FLCM). Stem cell factor (SCF) was found to be one of the candidate growth factor (11). Subsequent studies looked into the possibility - if additional factors like IL-6 and FLT-3 may also be contributing to autologous hematologic recovery. Indeed, apart from SCF which was present in about 55% of FLCM samples, IL-6 and Flt-3 was demonstrable in 80% or more of samples.

Bioneutralization assays with the use of antibodies revealed 40% suppression of Colony Forming Unit – Granulocyte Macrophage (CFU-GM) colonies with the addition of anti SCF antibodies, 42% suppression with anti IL-6 antibodies and 20% suppression with anti Flt-3 antibodies. This observation strongly supports the role of SCF, IL-6 and Flt-3 in hematopoiesis following FLI. Cytokine gene expression studies with the help of RT-PCR revealed the presence of genes in fetal liver for all the cytokines namely SCF, IL-6, GM-CSF, Flt-3 and EPO studied by us. It is noteworthy that the percentage of HSC is maximum in fetal liver compared to bone marrow, peripheral blood and cord blood (12).

Currently, we are studying the correlation of cytokine secretion and expression of genes with the fetal gestation period, if any and we are also studying the interaction of various cytokines. Due to limited availability of abortuses in mid gestation, we are also in the process of establishing fetal liver HSC lines so as to study further the potentials of fetal liver HSC.

Hematopoietic Stem Cell Transplantation (HSCT)

In 1950's Sir E. Donnal Thomas pioneered the application of early studies of transplantation in animals to the treatment of leukemia in humans (13). Since then over the years improvement in HLA typing, supportive care and graft versus host disease (GVHD) management has made the HSCT a relatively safer option with considerable cure rates. It is being done for various hematological and non-hematological malignancies. HSCT utilizes the self-renewal potential of stem cells after myeloablation (14). The myeloablative preparative regimens are aimed to eradicate the malignant cells, create space for the graft and to induce immunosuppression (in allogeneic transplantation). Various preparative regimens include alkylating agents, platinum with or without total body irradiation. Most of these regimens are too toxic (non hematological toxicity) and contribute toward high transplant related mortality (15). This has limited the use of conventional HSCT to the young patients with good performance status. Recently, the emerging concept of non-

myeloablative and reduced intensity transplantation has widened the application of HSCT to elderly and patients with poor performance status as the regimen related toxicity is less (16,17). It relies on the use of immunosuppressive drugs and graft versus malignancy effect where donor lymphocytes eradicate the malignant cells. Non-myeloablative transplants are also being used for solid tumors such as renal cell carcinoma (18).

HLA matching and haplo-identical stem cell transplantation- With the help of molecular techniques HLA matching has become more accurate. This has led to decrease in incidence of GVHD due to previously undetected mismatches (using serology and mixed lymphocyte culture techniques) (19). These advancements come at the cost of lesser availability of fully matched sibling or unrelated donors resulting in rise in the numbers of mismatched or haploidentical transplants. Various graft manipulation techniques such as T cell depletion (mechanically or with the help of monoclonal antibodies - Campath) are being used to decrease the high risk of GVHD in these settings. Still due to mismatches and graft manipulation these stem cell transplantation suffer the high incidence of graft failure (20).

Sources of stem cells - Conventionally stem cells are obtained from the bone marrow (donor or patient) under general anesthesia. More recently the use of peripheral blood stem cells has come in a big way. It involves mobilization of stem cells with the use of growth factors with or without chemotherapy. Apheresis is done with the use of cell separator over a period of 6-8 hours. The advantages over conventional bone marrow transplantation include early hematopoietic recovery for both platelets and neutrophils, safety for the donor and no requirement for general anesthesia (21).

Use of cord blood stem cells- As discussed above, due to the limited availability of suitable donors, umbilical cord blood is being used as a source of stem cells in various malignant and non-malignant disorders. It has the advantages of easy procurement, no risk to donors, acceptable partial mismatches and reduced risk of transmitting infections. It has certain limitations - limited cell dose leading to failure of engraftment, limited application in adults due to body size and lack of back up for stem cells in case of graft failure (22).

Graft versus host disease - The risk of severe acute and chronic GVHD has come down due to better HLA match, use of immunosuppressive drugs for the prophylaxis and treatment (23).

Cryopreservation of stem cells– Cryopreservation is required to preserve the viability of autologous stem cells, cord blood stem cell and as stem cell

back up (in allogeneic transplants). Depending on the temperature, the cells can be preserved for variable time periods. Cryopreservation can be done by dump freezing or rate controlled freezing.

Supportive care - In post transplant period the patient needs to be supported during the period of aplasia with packed red cells, platelets and antibiotics. Due to mucositis, patients may need parenteral nutrition and organ function should be monitored closely. Isolation nursing is required to decrease the chances of infection though in most cases it's the endogenous flora, which is responsible for infectious episodes.

Stem cell transplantation at IRCH

Two hundred and fifty two (252) transplants have been performed by us at IRCH; autologous - 170 and allogeneic-82. Autotransplants have been for multiple myeloma (95), lymphomas (35), acute leukemia (17), CML (5), and solid tumors (18). Allogeneic stem cell transplants have been performed for CML (40), CLL (1), severe aplastic anemia (19), acute leukemia (12), Hurler's syndrome (3), congenital erythropoietic porphyria (1) Beta thalassemia (2), Multiple myeloma (1), Myelodysplastic syndrome (2), and Hodgkin's disease (1).

Results following allogeneic as well as autologous transplants have been similar to those achieved at other Indian centers engaged in hematopoietic stem cell transplant programme. For instance, in CML-chronic phase, reported survival from CMC Vellore is 47% at a median follow-up of 30 months, 47% by Tata Memorial Hospital at a median follow up of 48 months and 65% at IRCH at a median follow up of 27 months. Similarly, in severe aplastic anemia, survival has been 16% at TMH, 32% at CMC and 21% at IRCH. Multiple myeloma has been gratifying to manage with autologous hematopoietic stem cell transplants as the survival at 5 years has been 52% which is much superior to that achieved with chemotherapy alone (24, 25, 26).

Stem Cell Therapy at AIIMS

More recently stem cells have achieved wider and even greater recognition due to their capacity to differentiate into variety of cell types namely, cardiac, neural, hepatic and muscular. This has opened up newer potentials for the use of stem cells to treat myocardial, neural, pancreatic and muscular diseases (27, 28).

AIIMS has taken a lead in the use of autologous stem cells in various cardiac, muscular, neurological & ocular degenerative disorders. The special advantage is that there are no rejection reactions, because the cells are from the same body i.e. autologous transplantation.

Stem cell therapy in myocardial infarction (MI):

Forty two patients of MI, underwent stem cell therapy at AIIMS during

coronary artery bypass graft (CABG). Control group had 10 patients who did not receive stem cell therapy. MI patients underwent stem cell therapy procedure in addition to routine CABG. None of the patients suffered any mortality and morbidity as a result of this therapy. The preliminary results showed no arrhythmia. There was improved ventricular function. There was improvement in New York Heart Association functional class (from 2.9 ± 0.7 to 1.25 ± 0.6) and left ventricular ejection fraction (from $32 \pm 12\%$ to $41 \pm 9\%$ $p = 0.04$). All Patients of MI had Transthoracic echocardiography (16 segment analysis); stress thallium (20 segment analysis), ventricular angiography (5 segment analysis) before surgery and on follow-up. Left ventricular dimensions remained stable with no progression to aneurysm formation in the stem cell group Vs control group. Not only the scar size reduced but also there was viability at the center of the scar in two patients. This will be confirmed once we get more information from PET scan, which has been installed, at our institute recently. The 10 control patients showed no change in left ventricular function and no change in the number of scarred segments. (30)

Stem cell therapy in Dilated cardiomyopathy (DCM):

We studied the effects of intracoronary autologous bone marrow stem cells (BMSC) implantation in patients with dilated cardiomyopathy (DCM). Twenty four patients with DCM with normal coronaries formed the study group while 20 patients who refused the stem cell therapy formed the control group. Injection of BMSC was made into the coronary arteries with percutaneous occlusion of the coronary sinus for 3 minutes. The patients were reevaluated at 3 months with echocardiography and endomyocardial biopsy.

There was improvement in the New York Heart Association (NYHA) Class (3.3 ± 0.5 to 2.4 ± 0.7, $p < 0.05$) in the treatment group. Left ventricular ejection fraction improved from $20 \pm 8.2\%$ to $27 \pm 13\%$ ($p < 0.05$) in the NYHA III patients while class IV patients showed no improvement in LV function. Out of 7 patients who were NYHA class IV, 4 expired. Endomyocardial biopsy showed evidence of increased vascularity with no evidence of any immature cells or any evidence of any adverse pathology (inflammation, infarction). There was evidence of cell proliferation (binucleate cells in 2 and Ki-67 positive cells in one). This is the first study to show the potential safety and efficacy of intracoronary transplantation of autologous bone marrow stem cells in patients of dilated cardiomyopathy. It demonstrates clinical and echocardiographic improvements in class III patients. Preliminary histopathological evidence points to a possible paracrine effect (30).

Although various studies have shown remarkable improvement in myocardium regeneration following stem cell therapy; the mechanism of this potential benefit is not clear. The various mechanisms postulated are generation of new myocytes, endothelial cells, and smooth muscle cells by transdifferentiation, cell fusion or paracrine effect. However there is a risk of tumor formation using these stem cells as suggested by some researchers. (31)

Stem cell biology still remains one of the most intriguing fields of scientific inquiry and holds long-term potential. There is still some unanswered questions regarding the optimum cell type, cell dose, in vivo delivery, efficiency of grafting, tracking of stem cells. There is a need to conduct studies with large number of patients, double-blind, randomized-controlled clinical trials to establish the effect of stem cell therapy. Our goal is to understand the mechanism/ basis of this potential therapy. (30)

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