Global Journal of Biotechnology & Biochemistry 6 (4): 162-170, 2011
ISSN 2078-466X
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Use of Umbilical Cord Blood for the next Generation Myocardial Tissue Regeneration

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Abstract: Cord blood stem cell based repair or regeneration of organs like heart, brain, liver etc. is under high priority research worldwide. Cord blood stem cells are being tried for the cure of hematological, oncological or other acute diseases. In the next generation, there is immense possibility of using cord blood stem cells (CBSCs) for myocardial regeneration. Cord blood (CB) stem cells have a high rate of engraftment, more tolerant of HLA mismatches, result in a reduced rate of graft-versus-host disease (GVHD), rarely contaminated with latent viruses and finally it is also easy to procure and restore. Results of several successful clinical trials with animal models indicate that CBSCs are even superior to the bone marrow or other sources derived stem cells for the usage in regenerative medicines. In the present review we have focused the present status and the future of CBSC research particularly in myocardial repair and regeneration.

Key words: Cord Blood · Masenchymal Stem Cells · Myocardial Repair · Regenerative Medicine · Tissue Engineering

INTRODUCTION

Congestive heart failure is the common cause of frequent death after myocardial infarction. Moreover, there are also many congenital heart defects or problems with the heart's structure present at birth. In children with heart valve abnormalities, the valves do not fully operate and obstruct the flow of blood. Surgeons can transplant new valves from donors or from artificial material, but such valves naturally do not grow along with child's natural growth. So it requires repeated operations to provide children with new and larger valves. Therefore, there is urgent need for alternative therapeutic measure. Modern surgical or interventional restoration of blood supply to ischemic myocardium effectively reduce myocardial infarction, treat angina, but viability and function of necrotic myocardium cannot be restored. This means, modern treatment cannot reverse the heart failure induced by loss of contractile heart tissue. Regeneration of the heart muscle appears to be a must.

The most important function of the heart muscle regeneration is to grow new contractile cells and their interaction with the surrounding cells. Replacement of heart valves made from the child's own cord blood stem cells might grow with the child's growth as needed. This will significantly reduce the number of surgeries necessary for those patients. Recent discovery of several types of cardiac muscle stem cells (SCs) enlightened the path of heart tissue regeneration. Their number as well as capability is not, however, sufficient in most of the patients. For cell-based regenerative medicine it is, therefore, a challenge to grow more number of functional muscle cells in the diseased heart. This review we shall discuss the importance of using CBSCs in therapeutics and focus the recent advances and possibilities of myocardial regeneration based on umbilical cord blood.

Diseases Which Can Be Treated with Cord Blood: Though treatment of diseases with CB is in the early stages, a number of diseases are being treated worldwide mostly with animal models. Some of the diseases where enormous success has been obtained being treated with CB stem cells are cancer, bone marrow abnormalities, blood abnormalities, metabolism disorders, immunodeficiency disorders etc. Using Cord Blood stem cell (CBSC), which is now commercially available, intensive researches are going on to repair and regeneration of different organ tissues like heart, neurons,

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Table 1: A survey of successful treatment of some disorder with cord blood

Diseases	Results	References
i) Hurler,s syndrome	85% alive	[25]
ii) Huntersyndrome	Above 55 % normal	[24]
iii) Behcet's disease	Almost cure	[26]
iv) Severe chronic active Epstein-Barr virus	Complete cure	[24]
v) Diamond Blackfan anemia	Successful recovery after 22-34 months	[24]
vi) Refractory anemia	Free from disease	[27]
vii) Spinal cord injury	Regeneration of SC	[28]
ix) Omenn syndrome	T cell reconstruction	[29]
x) Non-healing wounds	Accelerated healing	[30]
xi) Krabbe's disease	Successful improvement	[31]
xii) Malignant infantile osteopetrosis	Normalized	[32]
xiii) Rthmund-Thomson syndrome	Immune regeneration	[33]

liver etc. [1-23]. Table 1 shows a short past history of successful applications of CB in different diseases [23-33]. Many reputed clinical laboratories worldwide have made clinical trials with CB for the treatment of leukemia, lymphoma, myeloma, born errors of metabolism, diabetes, sickle cells anemia, autoimmune diseases etc. Experiments on several animal models have been made showing immense possibility of using CBSCs for myocardial regeneration [23, 34]. Though experimenting on animal model is quite promising, application in Humans is still underway.

Advantages of Using Umbilical Cord Blood Derive Stem Cells: Several stem cell populations (e.g., CD34, CD133, mesenchymal stem cells, VSELs) have been isolated from cord blood which can differentiate *in vitro* into several lineages, including cardiac and vascular lineages [24]. For clinical uses, MSCs are generally derived from bone marrow. But there are immense possibilities of immune rejection, while in clinical uses, due to decline in MSC numbers, differentiation potentials [24, 35], viral infection etc. Interestingly, UCB is found to be an alternative source of hematopoietic stem cells (HSC) and MSCs [26] which can be used for transplantation and in regenerative medicine [36-39]. As UCB are younger SC, there is little or no scope of variation of number of UCB-MSCs with age as in the case of BM SCs. Umbilical cord blood is a promising alternative source for regeneration therapy in humans. The UCB-MSCs could differentiate into a number of cells types of mesenchymal lineage, such as cardiomyocytes (CMs), osteocytes, chondrocytes and fat cells [40]. In fact, there are many other advantages of using UCB stem cells. CBSC are available in large numbers. No need of donor as in bone marrow derived SCs. There is lower risk of virus infection and low rejection rate after the transplantation. Probably we can use more HLA mismatches with CB transplantation [41]. UCB-MSCs are younger than adult BM-MSCs, so more active and less immune resistive [42].More potent in application in allograft (same patient) transplantation. CB-SC, compared to BM-SC or peripheral blood, has a bigger telomerase length and demonstrates higher proliferation potential. The human placenta derived MSCs can even be combined with HSCs from UCB to reduce the potential graft-versus-host disease (GVHD) in recipients [40]. Magro *et al*. and other researchers [41-43] reported marvelous co-transplantation of CB together with limited amount of HSC from hapliodentical donor. The HSC from the CB provided long term stable engraftment while the haploidential cells removed completely. Transient contribution of haploidential HSC also provides necessary protection from infections in the early stage of engraftment. UCB-MSCs can be well cryo-preserved at liquid nitrogen temperature for long time and can be used when required.

Cord Blood Stem Cell and Myocardial Regeneration: Human CB contains enough mononuclear Cells (MNC) to be used in myocardial regeneration. Figure 1 schematically describes the use of CB SC for heart or other tissue regeneration process. UCB containing pluripotent stem cells have clinical advantages over that of patient adult-derived stem cells in potential applications for therapeutic angiogenesis [44]. Availability of diverse HLA genotypes, lower T-lymphocyte immunoreactivity and lower inherent pathogen transmission are important advantages of using CB. Recently embryonic-like stem cells have been identified in UCB [45] and UCB is the only tissue used for successful transplantation across HLA barriers [46]. In transplantation, single UCB units can reconstitute the entire lymphohematopoietic systems in both pediatric and adult patients [47-49]. UCB has higher proliferation capacity and longer telomeres (indicating more primitive cells) than equivalent aliquots of adult. Current attributes for the selection of UCB for malignant transplantation involves combining both cell dose and HLA matching.

The overall scenario is that if UCB-MSCs are properly expanded in culture, they would be most important practical units for cardiac or other muscle regeneration. Moreover, usage of CB overcomes considerable problems encountered with other sources of MSCs, such as allergenic and tumorigenic issues. Furthermore, as CB containing both hematopoietic stem cells (HSCs) and

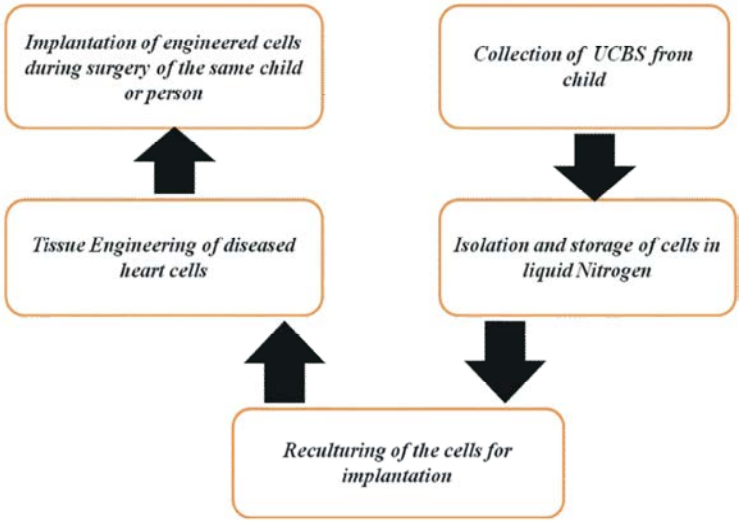

Fig. 1: UCB collection after child's birth, isolation of mononuclear cells and differentiation of mesenchymal stem cells, storage in cryo-refrigerator and use when required.

MSCs [50-52] provides special advantages for clinical use. It is believed that both proliferation rate and functional capacity of UCB-HSC are higher for such younger cells compared to the BM-HSCs. There is exciting recent development elucidating the potentiality of using CB Cells in repairing myocardial hepatocytes, muscles and neural tissues [21, 53,54].

However, one of the main drawbacks is the prolonged time to cell recovery and the early mortality associated with CB transplant. Intensive research is going on to overcome this problem. Although a number of preclinical studies have focused on using UCB stem cells to treat damaged myocardium [55-58] more clinical trials using UCB-derived stem cells are needed to assess the overall effectiveness. In the use of CB to treat hematological malignancies, several enhancing mechanisms are being tested to increase the homing of stem cells to the bone marrow and to the heart to repopulate the hematopoietic system. However, there are limited therapeutic options for patients with chronic ischemia secondary to chronic total occlusion (CTO).

Though UCB with greater cellular yields better outcomes, a better identification of contributing cells is still necessary. While using CB in the treatment of hematological malignancies, substantial morbidity and mortality are associated with pretransplant ablation of the recipient hematopoietic system [22]. Because of the unique immunological properties of CB, it is a possibility to utilize allergenic cells for regenerative applications without the need to fully compromise the recipient immune system by ablation [60].

Besides the expansion of limited stem cells, changes in the expression of key homing receptors (e.g., CXCR4) may lead to increase deposition into myocardial tissue in a synonymous manner to increased engraftment in the bone marrow. It is, however, unclear as to the effective dose of cord blood stem cells that may be required to treat ischemic myocardium [21,22]. Insufficient endogenous homing mechanisms and survival of transplanted cells into the ischemic environment limit the full potential of cell-based cardiac repair. A complete understanding of the involved molecular mechanisms of critical steps in injury and in cell-based repair will definitely facilitate to enhance functional recovery after myocardial infarction. Clinical trials are going on to evaluate the safety of these populations and their ability to home to the proper sites in treating cardiovascular diseases.

Ma and his group [43, 50] used CB-MNC injecting in mice that had undergone coronary artery ligation shortly before and observed the flow of such cells to the heart. Their study indicated that human CB-MNC responded to forming factors like SDF-1 and other chemokines expressed during acute myocardial ischaemia. The presence of human CB cells in the mouse heart provides important information about the functioning of CB cells in heart cell repair. Henning and his group [46] also carried out similar *in vitro* experiment and confirmed that CB MNCs are attracted to ischaemic myocardium. It was also found that direct injection of CB MNC in the infarct border zone also reduces the infarct size on histology. This group also showed that these effects are functionally relevant *in vivo*. Interestingly, Hu and his group [47] also

obtained very similar results with respect to myocardial contractility, angiogenesis and remodeling processes in infracted rat hearts after CB-MNC injection. It, therefore, appears that human CB cells have some regenerative effects on infracted myocardium showing real possibility of using CB in heart cell regeneration. Though, the most appropriate cell type for restoration of damaged myocardial tissue has not yet been defined, variable degrees of improvement in cardiac function in animal models have been observed with transplantation of stem cells from different sources viz. (a) Embryonic stem cells, (b) Umbilical Cord Blood cells, (c). Resident Cardiac stem cells, (d) Skeletal Myoblasts, (e) Adult Bone Marrow stem cells (Heamtopoieitc stem cells, Mesenchymal stem cells, Endothelial progenitor cells, Umbilical Cord Blood Stem Cells). Comparatively, umbilical cord blood (UCB) stem cells contain higher numbers of SC than in adult human blood or bone marrow. UCB containing both HSC and MSCs can be used for cardiac repair. Ma *et al.* [51] injected human mononuclear UCB cells, a small fraction (˜1%) of which was CD34+, intravenously 1 day after myocardial infarction (MI) in mice. The cells homed to the infracted hearts, reduced infarct size and enhanced neovascularization with capillary endothelial cells of both human and mouse origin. Interestingly, they found no evidence of myocytes of human origin, arguing against cardiomyogenic differentiation. In a rat model of MI [23,60], UCB CD34+ improved cardiac function when injected into the peril-infarct rim immediately after MI compared with control animals that received injection of medium. Apart from these, Kogler and colleagues [52] have described a population of cells from human UCB called unrestricted somatic stem cells. These cells, which are fibroblast like in appearance, adhere to culture dishes are negative for c-kit, CD34 and CD45 and are capable of differentiating, both *in vitro* and *in vivo*, into a variety of tissues, including cardiomyocytes. These stem cells [52,61-63] when delivered by direct injection at thoracotomy in immunosuppressed pigs after MI, improved perfusion and wall motion, reduced infarct scar size and enhanced global cardiac function.

Cardiomyocytes Cooperates with Cord Blood: *In vitro* Studies in Humans: Permanent loss of cardiomyocytes (heart muscle cells) and the formation of scar tissue following a heart attack result in an irreversible damage to cardiac function. It has already been mentioned that Human cord blood contains several different types of stem cells including hematopoietic, endothelial and mesenchymal stem cells to repair this damage. Although still in early stages, several *in-vitro* studies have shown that under certain treatment conditions, cord blood mesenchymal stem cells differentiate into cardiomyocyte-like cells [55-60] and were able to induce regeneration of healthy cells from damaged cardiomyocytes [58-61]. Umbilical cord blood (CB) is a promising source for regeneration therapy in humans [1-19]. It was further demonstrated that brown adipose tissue derived cells (BATDCs) differentiated into CMs and these CMs could adapt functionally to repair regions of myocardial infarction. It was also examined [58] whether CB mononuclear cells (CBMNCs) could effectively differentiate into CMs by co-culturing them with BATDCs and determined which population among CBMNCs differentiated into CMs. The results show that BATDCs effectively induced CBMNCs that were non-hematopoietic stem cells (HSCs) (e-CBCs) into CMs *in vitro*. E-CBCs reconstituted infarcted myocardium more effectively than non-educated CBMNCs or CD34-positive HSCs. Moreover, it was noticed [58] that E-CBCs after 3 days co-culturing with BATDCs induced the most effective regeneration for impaired CMs. This suggests that e-CBCs have a high potential to differentiate into CMs and that adequate timing of transplantation supports a high efficiency for CM regeneration.

This strategy might be a promising therapy for human cardiac disease and also suggests that cord blood stem cells have a high potential to differentiate into cardiomyocytes and aid the regeneration of cardiomyocytes lost due to heart damage. Moreover, the ability of CB stem cells to become vascular endothelial-like cells and thus, blood vessels, indicates they will likely have potential applications beyond the heart regeneration.

Peripheral vascular disease is a restriction of blood flow outside of the heart usually occurring in the legs and arms. Restricted blood flow is caused by blood vessel narrowing from fatty plaque formation on vessel walls (atherosclerosis) or blockage due to blood clots. If the blockage is severe enough, tissue death can occur. In animal models, cord blood stem cells have been able to significantly reverse the effects of ischemia, or loss of blood flow in the blood vessels. In models of hind limb ischemia, transplantation of cord blood stem cells appeared to reverse surgery-induced ischemia resulting in limb salvage [61-63]. These observations may lead to future human clinical trials using cord blood stem cells to treat patients with peripheral vascular disease. At present there is a wealth of pre-clinical and early clinical data [62-63] showing that the treatment of cardiovascular disease

has benefited from advances in pharmacologic and intravascular intervention reducing the morbidity and mortality associated with this disease. To address the need in managing clinically complex vascular disease with limited therapeutic options, studies have focused on cellular therapy as a means to augment compensatory mechanisms and to potentially prevent escalation and advancement of disease. Umbilical cord blood with rich source of hematopoietic stem cells appears to be a potential source of cells for this type of therapy. UCB has a wider availability of HLA phenotypes with a possible lower immune reactivity. Moreover, stem cells isolated from patients with chronic disease have problems of their reparative abilities limiting their therapeutic activity. The potential of UCB HSC in augmenting this process has been studied extensively both *in vitro* and *in vivo* and has shown a benefit in acute and chronic vascular ischemia. However, the mechanism for this therapeutic effect is not yet realized.

Recently Samuel *et al.* [63] worked on Human umbilical cord blood stem cells infusion in spinal cord injury. The results suggest that cord blood stem cells are beneficial in reversing the behavioral effects of spinal cord injury, even when infused 5 days after injury. Human cord blood-derived cells were observed in injured areas, but not in non-injured areas, of rat spinal cords and it was also not seen in the corresponding areas of spinal cord of non-injured animals. These results are also consistent with the hypothesis that cord blood-derived stem cells migrate to and participate in the healing of neurological defects caused by traumatic assault.

Davies *et al.* [64] also observed that Human cord blood stem cells enhance neonatal right ventricular function in an ovine model. They demonstrate that in the presence of increased work load, cord blood stem cells engraft and augment right ventricular function. Transplanted cells adopt hematopoietic fates in the myocardium, bone marrow and spleen.

Thus far, in animal models of myocardial infarction, cord blood stem cells have shown the ability to selectively migrate to injured cardiac tissue, improve vascular function and blood flow at the site of injury and improve overall heart function [65-68]. Reports of recovery after MI in animal models are highly promising [46, 51, 54, 67]. Several pre-clinical studies of induced myocardial infarction in rats have shown that cord blood stem cells can migrate and engraft to damaged heart tissue can help to form new blood vessels decrease the size of infarction [47, 68-71] and also improve left ventricular remodeling, structural damage and function. From various experiments with animal it may, therefore, be well argued that cord blood stem cells have the ability to produce vascular endothelial-like cells, which are capable to repair heart tissue damage due to myocardial infarction. Infusion of human umbilical cord blood cells protect against cerebral ischemia and damage during heatstroke in the rat [67-72].

These observations may lead to future human clinical trials using cord blood stem cells to treat patients with peripheral vascular diseases. Moreover, these findings will stimulate many parents to take care to preserving their children's cord blood, since it might be used to save their or some other children's lives.

SUMMERY AND CONCLUSION

Though it is still mainly confined in the animal models, cord blood for Myocardial and other tissue regenerations have shown great promise in recent years. Recovery after MI in animal model is quite successful. Researchers are in a stage to implant the heart valves made from cord blood into the hearts of young lambs to observe their ability to grow and function over a period of time. The growing successful research reports on cardiac repair suggest that a child's own cord blood will be valuable for the treatment of not only the congenital heart defect, but also for the treatment of serious heart attack and other diseases. Clinical studies demonstrate that stem cells transplants are more successful (more than 60%) when the stem cells come from a family member rather than from a non-related donor. So setting up of individual family blood bank might be important in near future for the growth and prospect of regenerative medicine using cord blood stem cells. Several unique properties of cord blood have been discovered. It contains fetal hemoglobin, which is much more effective at transporting oxygen than adult hemoglobin. Human cord blood has been used without suppression of the immune system in animal studies for conditions such as type I diabetes, ALS and Parkinson's disease, myocardial infarction etc. The apparent ability of cord blood to induce therapeutic effects suggests that the cells were not rejected. Scientists have already used cord blood derived cells in treatment of heart failure. Some research group has also describes the use of cord blood cells in creation of new blood vessels. From the creation of new blood vessels, diseases like ischemic heart disease or peripheral artery disease where new blood vessels are required in order to compensate for occlusion in the existing blood vessels. Human body cannot make enough new blood vessels to keep up with demand. If cord blood

stem cells could be used to make new blood vessels, this treatment would have abundant applications. One of the major arteries that feed the leg, called the femoral artery, was blocked in order to mimic conditions of decreased blood flow. Usually this results decreased function of the leg and death of muscle tissue. Administration of CD133 cells was shown to stimulate new blood vessel formation, preserve leg function and decrease the amount of cell death in the digits of the mouse limbs. Activity of CD133 cells derived from cord blood seemed to be higher than that of bone marrow derived cells.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Biotechnology, Government of India, New Delhi for financial support to carry out the present work.

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