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International Journal of Pharmaceutical and Phytopharmacological Research

ISSN (Online) 2249 – 6084

ISSN (Print) 2250 – 1029

Int.J.Pharm.Phytopharmacol.Res. 2011, 1(3): 134-139

Bone Marrow Derived Mesenchymal Stem Cells –A Boon for the Treatment of Complications in Diabetes Mellitus

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Received on: *04/12/2011* ***Accepted on:*** *31/12/2011*

ABSTRACT

Mammal Bone marrow is an invaluable source of Mesenchymal stem cells. Bone Marrow derived Mesenchymal Stem Cells (BM-MSCs) are multipotent, self-renewing cells found in almost all postnatal organs / tissues and are used in the treatment of various disease conditions. Diabetes mellitus is a metabolic syndrome characterized by increased levels of blood glucose leading to various complications like Diabetic Foot, Diabetic Neuropathy, Diabetic Retinopathy, Diabetic Cardiomyopathy and Diabetic Nephropathy. BM-MSCs are able to differentiate into many cell types, and to proliferate ex vivo. These attributes makes them a potential therapeutic tool for cell replacement therapy in diabetes and other diseases. The present review discusses the isolation and culturing of BM-MSCs along with their potential as new therapeutic agent in the treatment of Diabetes related complications and its limitations.

Key Words: Bone marrow, Mesenchymal stem cells, Diabetes Mellitus, Diabetic Foot Ulcer, Diabetic Poly Neuropathy, Diabetic Retinopathy

INTRODUCTION

Diabetes mellitus is a metabolic syndrome characterized by increased levels of blood glucose. Diabetes mellitus patient suffering from defective insulin secretion rely on lifelong substitution with exogenous administration of insulin. Whole pancreas and purified pancreatic islet transplantation have offered the potential for independence from insulin injections. Transplantation of islet is not possible every time because of lack of availability. Hence, the scientists have found out the renewable source of islet-replacement tissues. Mesenchymal stem cells, also known as multipotent Mesenchymal stromal cells showed tremendous potential for this therapy as they are self-renewing cells and are found in almost all postnatal organs and tissues[1]. BM-MSCs are responsible for conveying some chemical molecules like stromal antigen 1, erythrocytes (glycophorin A), CD44, CD90, CD166 (vascular cell adhesion molecule), CD54/CD102 (intracellular adhesion molecule), and CD49 (very late antigen), CD105 (SH2), CD73 (SH3/4) [2, 3.] Conversely MSCs lack the expression of surface markers characteristic for hematopoietic cells (CD14, CD45, and CD11a/lymphocyte function-associated antigen 1 (LFA-1)), and platelet and endothelial cell markers (CD31)[4]

The main functional characteristics of MSCs are their immunomodulatory ability, capacity for self-renewal, and differentiation into tissues of mesodermal origin [5, 6].Therapeutic effects and use of MSCs would be primarily based on their release of trophic and immunomodulatory factors[7, 8]. MSCs can alter the secretion profile of dendritic cells (DCs) resulting in increased production of anti-inflammatory cytokine interleukin (IL)-10 and decreased production of interferon-gamma (IFN-γ) and IL-12[5–7]. BM-MSCs can inhibit T-cell proliferation by engagement of the inhibitory molecule programmed death 1(PD-1) to its ligands PD-L1

and PD-L2, thereby producing soluble factors that suppress T-cell proliferation (such as TGF-β or IL-10) and through interacting with DCs [6, 7]. BM-MSCs can increase the number of $CD4^+CD25^+FoxP3^+$ T-regulatory cells that suppress the immune response. Susceptibility to diabetes induction and development may be related to the activity of T-regulatory cells and expansion of Th17 cells [8,9]. BM-MSCs are able to render T cells anergic by blocking differentiation of monocytes to DCs or by inhibiting DC maturation[5]. Through production of soluble factors, BM-MSCs can inhibit proliferation and IgG secretion of B cells[7].It has been reported that MSCs can be isolated in relatively high numbers from culture of bone marrow[10]. Previous studies have shown that BM-MSCs are able to differentiate into several cell types, including cardiomyocytes, vascular endothelial cells, neurons, hepatocytes, epithelial cells, and adipocytes, making them a potentially important source for the treatment of debilitating human diseases. Such multipotent differentiation characteristics coupled to their capacity for self-renewal and capability for the regulation of immune responses, described BM-MSCs as potentially new therapeutic agents for treatment of the complications of diabetes mellitus (DM)[11].

ISOLATION OF HUMAN BONE MARROW CELLS

The Bone marrow is collected first from human and then mechanically disrupted to obtain a single cell suspension .The marrow is then diluted with α-MEM (Minimum Essential Medium eagle and subsequently the diluted samples are overlaid with density gradient solution and centrifuged . Following centrifugation, cells are removed from the plasma/Ficoll-Hypaque interface, and suspended in 5 ml of α-MEM (GIBCO) supplemented with 10% human umbilical cord blood serum (UCBS), 100 U/ml penicillin, and 100 U/ml streptomycin (GIBCO). Cells are then plated at a density of 2 x $10^5/cm^2$ in 6 well culture plates, and incubated at 37°C in a humidified atmosphere containing 5% CO_2. After 72 hours, the non-adherent cells are discarded and the adherent cells are cultured for approximately 10 days. Fresh medium is replaced twice a week and the cultures are maintained for 18-20 days. Upon reaching near confluence (90%), cells are detached with TPVG (0.25% trypsin, 1 mM/l EDTA) for 3-5 minutes at 37°C. After centrifugation, cells are re-suspended with fresh medium and replated. The resulting cultures are morphologically heterogeneous, containing cells ranging from narrow spindle-shaped cells to large polygonal cells and, in confluent cultures, some slightly cuboidal cells[12].

CULTURING

The majority of modern culture techniques still take a CFU-f (Fibroblast colony-forming units) approach, where raw unpurified bone marrow or ficoll-purified bone marrow Mononuclear cell are plated directly into cell culture plates or flasks. Mesenchymal stem cells, but not red blood cells or haematopoetic progenitors, are adherent to tissue culture plastic within 24 to 48 hours. However, it has been reported that Nonadherent cell population of human marrow culture is also complementary source of mesenchymal stem cells (MSCs)[13]. Other flow cytometry-based methods allow the sorting of bone marrow cells for specific surface markers, such as STRO-1[14]. STRO-1+ cells are generally more homogenous, and have higher rates of adherence and higher rates of proliferation, but the exact differences between STRO-1 + cells and MSCs are not clear[15]

ROLE OF BM-MSCs IN VARIOUS DIABETIC COMPLICATIONS

BM-MSCs in Diabetic Foot Ulcer

Diabetic foot ulcers (DFU), chronic, non-healing wounds on the feet of diabetic patients, present a serious challenge to global health. DFUs have a huge impact on our health care system, not only in terms of economic cost, but also from a psychosocial perspective, associated with significant morbidities, decrease in quality of life, prolonged hospitalization and importantly, often result in the amputation loss of lower extremity. MSCs derived from bone marrow or adipose tissue, pre-conditioned to optimize reparative properties, will promote vascularization of the wound and improve healing[16].

Autologous skin fibroblasts on biodegradable collagen membrane combine with BM-MSCs used for the treatment of Diabetic ulcer. The bone marrow aspirate of the patient with diabetic foot was applied directly to the wound and injected into the edges of the wound, finally covered with prepared autologous biograft. The patient received two additional treatments with cultured MSC on day 7 and 17.The wound showed a steady overall decrease in wound size and an increase in the vascularity of the dermis and in the dermal thickness of the wound bed after 29 days of combined treatment. After treatment we can conclude that Closing and healing of the non-healing diabetic ulcer was achieved by using the above therapy[17] .

BM-MSCs in Diabetic Polyneuropathy and Retinopathy

Diabetic polyneuropathy (DPN) is one of the most frequent and troublesome complications of diabetes mellitus as it are responsible for damage to nerve fibers[18]. It also produces Spontaneous pain, hyperalgesia, and diminished sensation. The reasons behind spreading of DPN are neural cell degeneration and decreased nerve blood flow[18]. Studies have shown that angiogenic cytokines like basic fibroblast growth factor (bFGF) and VEGF could be useful for the treatment of DPN[29,19,20]. It was shown in diabetic rats that MSCs, because of their ability to secrete bFGF and VEGF[29], could be used as a new and effective therapeutic agent for the treatment of DPN[19, 20]. MSCs were isolated from bone marrow of adult rats and transplanted into hind limb skeletal muscles of rats with an 8-week duration of streptozotocin (STZ)-induced diabetes or age-matched normal rats by unilateral intramuscular injection. Four weeks after transplantation, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) productions in transplanted sites occur; it leads to increase in neovascularization responsible for neural cell regeneration which finally shows improvisation in diabetic polyneuropathy (Fig. 2) [20]. However, by releasing paracrine factors and through differentiation into photoreceptor and glial-like cells in the retina, transplanted MSCs improved the integrity of the blood-retinal barrier, thus ameliorating diabetic retinopathy in STZ diabetic rats [21]

BM-MSCs in Cardiomyopathy

Ventricular dysfunction in patients with Diabetes mellitus in the absence of coronary artery disease, valvular heart disease, or hypertension is defined as diabetic cardiomyopathy (DCM)[22]. BM-MSCs have the capacity to differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells. In brief, BM-MSCs showing tendency to reduce cardiac fibroblast proliferation and expression of collagen I and III and they are able to promote matrix metalloproteinase secretion by cardiac fibroblasts, leading to reduced cardiac ventricular fibrosis[23,24]. These effects may at least partially be mediated via the release of antifibrotic factors such as hepatocyte growth factor [25]. Chronic hyperglycemia is responsible for myocardial remodeling and is a central feature in the progression of DCM. An additional feature that contributes to the pathogenesis of DCM is the activity of matrix metalloproteinase (MMP)-2 and MMP-9[26,27]. The diabetic myocardium is characterized by decreased activity of MMP-2, leading to increased collagen accumulation, and increased activity of the proapoptotic factor MMP-9, which is responsible for apoptosis of endothelial cells, reduction of capillary density, and poor myocardial perfusion[26,27]. Microcirculatory defects, necrosis and apoptosis of cardiomyocytes, and interstitial fibrosis are the main pathological characteristics of DCM [23, 27].

BM-MSCs can also induce myogenesis and angiogenesis by releasing different angiogenic, mitogenic, and antiapoptotic factors including vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), adrenomedullin (AM), and hepatocyte growth factor (HGF). This was proved with the help of rat model of DCM in which intra venous administration of BM-MSCs improved cardiac function of treated animals. MSC that was transplanted get differentiated into cardiomyocytes and improved myogenesis and angiogenesis. This phenomenon increases myocardial arteriolar density and decreases collagen volume resulting in attenuation of cardiac remodeling and improved myocardial function[28].

BM-MSCs improve myocardial perfusion and myocardium regeneration..Improvement in cardiac function following MSC therapy may also be attributed to the release of MSC-derived paracrine factors capable of cardioprotection. These factors include secreted frizzled-related protein 2, Bcl-2, heat shock protein 20, hypoxia-regulated heme- oxygenase-1, hypoxic Akt-regulated stem cell factor, VEGF, HGF, AM, and stromal-derived factor[29]. A growing body of evidence strongly suggests that these factors affect remodeling, regeneration, and neovascularization leading to the improvement of myocardium contractility and viability, ameliorating consequences of infarction[29-32]. Double-blind, placebo-controlled trials showed that i.v. autologous MSCs transplantation increased left ventricular ejection fraction, reduced episodes of ventricular tachycardia, and led to reverse remodeling in postinfarction patients reducing the mortality rate in patients with ischemic stroke [30,31].

BM-MSCs in Diabetic Nephropathy

Diabetic Nephropathy is a comprehensive disease with metabolic disturbance which is caused by long-term unstable blood sugar levels in patients body.BM- MSCs administration can prevent and treat diabetic nephropathy, which is the most common complication of Diabetes mellitus, and is defined as progressive kidney disease caused by angiopathy of the capillaries supplying the kidney glomeruli[33]. BM-MSCs have been used for the treatment of diabetic nephropathy in nonobese diabetic/severely compromised immunodeficient (NOD/SCID) and C57 black 6 (C57/BL6) mice, which succumb to DM after application

of multiple low doses of STZ. About 30–60 days after STZ injection, kidneys of treated mice showed the presence of abnormal glomeruli characterized by increased deposits of ECM protein in the mesangium, hyalinosis, and increased number of macrophages in the glomeruli [33,34].Result obtained from above mice transplanted with human MSCs (hMSCs) and C57Bl/6 mice that received murine MSCs indicate that injected MSCs was engrafted in damaged kidneys was get differentiate into renal cells so, we can conclude that above treatment is effective in diabetic nephropathy[33,34]. Additionally, the small percentage of hMSCs in the transplanted kidneys differentiated into endothelial cells as evidenced by de novo expression of CD31[34]. The result of systemic administration of MSCs in diabetic mice was improvement of kidney function and regeneration of glomerular structure[33,34] as MSCs are able to reconstitute necrotic segments of diabetic kidneys[35]. However, it is not clear whether MSCs can propagate after engraftment in the kidney. One month after MSC treatment, only a few hMSCs were detected in kidneys, suggesting that they were unable to proliferate[34] so an alternative scenario for improvement of kidney function could be the ability of MSCs to scavenge cytotoxic molecules or to promote neovascularization[29-32]. In addition, successful MSC treatment of diabetic nephropathy could be explained by MSCs competence to differentiate into insulin-producing beta cells followed by decrease of glycemia and glycosuria, factors important for damaging renal cells[33]. This indicate that MSC transplantation prevents the pathological changes in the glomeruli and enhances their regeneration resulting in improved kidney function in diabetic animals. For "in vivo" tracking of BrdU-marked MSCs, immunostaining for BrdU (Bromodeoxyuridine) was performed in the heart, liver, spleen, pancreas, lung and kidney of recipient rats. BrdU positive cells were detected in the heart (into which the MSCs were infused), pancreas and kidney of recipient rats, while no positive cells were found in other organs. In the pancreas, BrdU positive cells were mainly located in the interstitium. And BrdU-marked MSC were mainly located in renal interstitium instead of glomerulus and renal tubles [36].

DRAWBACK OF BM- MSCs THERAPY

Spontaneous differentiation frequency of MSCs in the host tissue is extremely rare and hence, the therapeutic efficacy of MSCs depends on its ability to control in-vivo differentiation into target cells. poor engraftment and limited differentiation under in vivo conditions is another major drawback of this therapy [37].The potential of MSCs to differentiate into unwanted Mesenchymal lineages [38], leads to impair their therapeutic activity. Apart from this, few limitations like malignant transformation and cytogenetic aberrations of MSCs.

CONCLUSION

BM-MSCs is the most trusted stem cells because BM-MSCs shows differentiation capacity, pluripotency, immunomodulatory ability and self-renewability. These functional properties makes them suitable for treatment of complications of Diabetes mellitus like Diabetic cardiomyopathy, Diabetic nephropathy, Diabetic neuropathy and retinopathy and foot ulcer. In case of embryonic stem cells ethical issue arises but BM-MSCs are free such issue. These cells also showing immunosuppressive effect. A disadvantage of this therapy includes unwanted Mesenchymal lineages differentiation, risk of malignant formation, uncontrolled differentiation, the need of pure culture of BM-MSCs. If we overcome these problems then definitely this therapy would be boon for the treatment of many life threatening diseases in human being.

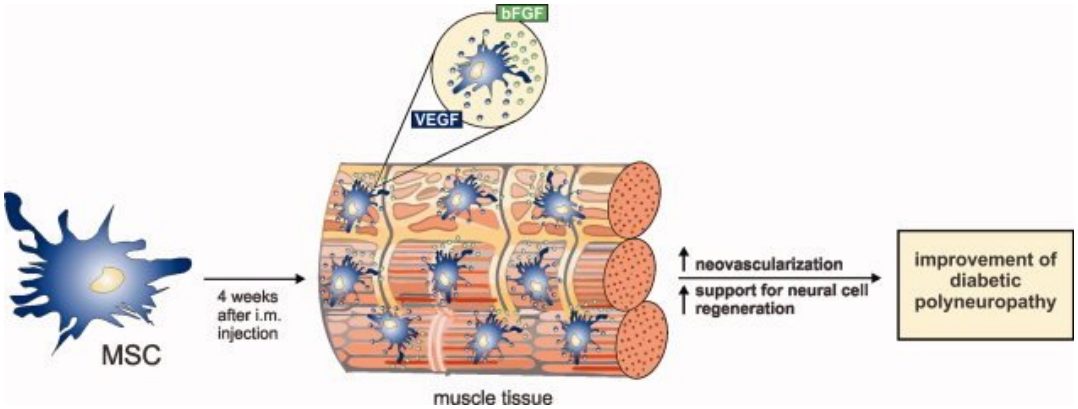

Figure 1: Effects of MSCs treatment on diabetic polyneuropathy.

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