Basics of Adult Stem Cells, Mesenchymal and Hematopoietic Stem Cells for Future Clinical Application

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After the discovery of adult stem cells, these cells became the subject of intense experimental and clinical investigation for tissue regeneration. Bone marrow–derived adult stem cells consist of hematopoietic and mesenchymal stem cells. In 1997, Asahara’s group found vascular stem cells named endothelial progenitor cells (EPCs) within the hematopoietic stem cell population. After the identification of EPCs, investigators focused on their involvement in the treatment of ischemia and tissue regeneration. After injury or ulceration, bone marrow–derived EPCs are mobilized into peripheral blood and recruited to the foci of pathophysiological neovascularization and reendothelialization, thereby contributing to vascular regeneration and wound healing. Topical application of EPCs and their injection in tissue regeneration have shown success in preclinical studies and human clinical trials and are now under investigation by various facilities. However, in certain diseases, EPCs are reported to have impaired mobilization, proliferation, adhesion, differentiation and tubular formation. In this review, we focus on the biology of EPCs and the future possibilities of EPC therapy for therapeutic vasculogenesis.

Key words: regenerative therapy, adult stem cells, endothelial progenitor cells, vasculogenesis, wound healing

Introduction

An adult stem cell is a population of cells with the potential of self-renewal and multi-potent differentiation capacity found among differentiated cells in a tissue or organ. The bone marrow contains at least two kinds of stem cells. One population is called the hematopoietic stem cells and other is called the mesenchymal stem cells. Hematopoietic cells mainly give rise to red bloods cells and white blood cells and also includes vascular stem cells called endothelial progenitor cells (EPCs). The non–hematopoietic stem cells make up a small proportion of the stromal cell population in the bone marrow, and can generate bone, cartilage, fat, cells that support the formation of blood, and fibrous connective tissue. After the discovery of adult stem cells, these stem cells became the subject of intense experimental and clinical investigation for tissue regeneration. The aim of my talk was to familiarize the students with this upcoming therapeutic modality for tissue regeneration based on adult stem cells especially on endothelial progenitor cells where my research is focused on.

Discovery of endothelial progenitor cell

In the adult, blood vessel formation was thought to only occur through angiogenesis, endothelial cells sprouting from pre-existing vasculature through migration and proliferation. After Asahara’s discovery of endothelial progenitor cells (EPCs) in 1997, Vasculogenesis, an embryonic neovascularization was found to contribute to adult vascular formation (Figure-1). EPC were first identified as CD34 antigen–positive mononuclear cells (MNCs). EPCs can be isolated from peripheral blood which are mobilized from the bone marrow in response to...
cytokines released after ischemic stimuli. EPCs isolated from adult peripheral blood were shown to proliferate and differentiate into endothelial cells in vitro, and bone marrow transplantation experiments have demonstrated the incorporation of BM-derived EPCs in the site of ischemia or neovascularization in vivo. Factors that influence the recruitment and mobilization of EPCs into the circulation was further investigated. Cytokine and growth factor releases post ischemia, vascular trauma, and hypoxia have been reported to increase the levels in circulating EPCs. Among growth factors known, vascular endothelial growth factor (VEGF), granulocyte-colony stimulating factor (G-CSF) and stroma-derived factor (SDF-1) are the important factors for vasculogenesis and EPC kinetics. Having demonstrated the potential for endogenous mobilization of BM-derived EPCs, many researchers have investigated the iatrogenic expansion and mobilization of EPC population that might represent an effective means to augment postnatal neovascularization. Further animal studies have shown the potential of cell based therapy, rather than growth factors alone, achieving enhanced neovascularization for various clinical conditions. First study demonstrating the efficacy of EPC cell therapy was in the hindlimb ischemia model of immunodeficient mouse, using human volunteer donor cells. Results showed EPC transplantation improve neovascularization and blood flow recover following reduction in limb necrosis and auto amputation by 50% in comparison with control mice receiving no therapy. The discovery of EPC as a new therapeutic option has now lead to great deal of research for various diseases.

**Biology of diabetic endothelial progenitor cells**

Many researches to date have targeted on diabetic endothelial cell dysfunction as one of the cause of poor outcomes following ischemic events. However, investigators now speculate that EPC dysfunction may also account for impaired new blood formation in diabetic patients. First study on relation between EPC and diabetic was done by
Tepper et al. demonstrating that diabetic patients exhibit significant decrease in number of peripheral blood EPCs and impaired EPCs function in proliferation, adhesion and tubulization. Capla et al. demonstrated that diabetic animals with ischemic flap exhibit threefold fewer endothelial progenitor cells within the flap, impaired endothelial progenitor cells within the flap, impaired EPC incorporation into existing vasculature, and diminished overall neovascularization. Supporting this Tanaka et al. demonstrated that EPCs dysfunction is seen not only in the peripheral blood but also in the bone marrow in response to flap injury and that diabetic EPC dysfunction lies mainly on differentiation.

The biological mechanisms investigating how diabetes impairs the function of EPC has also been studied. Hyperglycemia and oxidative stress is known as a key factor affecting EPC survival and function. Culturing EPC in non hyperglycemic environment and addition of anti-oxidative stress agents is shown to restore the function of EPCs dysfunction. Further investigation is still undergoing to understand the etiology of diabetic EPC dysfunction and related clinical conditions.

EPC therapy for diabetic wound: preclinical studies

Since diabetes is characterized by poor wound healing and EPC dysfunction, many investigators have researched the efficacy of EPC therapy for diabetes. Schattenmaier and colleagues showed the efficacy of EPC transplantation to wounded diabetic mice by demonstrating increased wound healing and vascularity post EPC transplant compared to non transplanted group. Topical application of EPC has also been shown to accelerate wound healing with increased angiogenesis. Animal studies on EPC therapy for diabetic wound have shown promising results and have lead to clinical trials at various institutes.

Clinical studies of EPC therapy

Therapeutic angiogenesis by EPCs uses the following cell types: (i) Non-selective EPC therapy uses bone marrow mononuclear cells (BM-MNCs) in general (ii) Selective EPC therapy which uses purified and isolated EPCs from peripheral blood or
bone marrow mononuclear cells after stimulation with granulocyte stimulating factor (GCS-F) (Figure 2). Since the number of EPCs in the peripheral blood is very low, GCS-F is used to mobilize EPCs to the peripheral blood. Selection of EPCs or CD34 positive cells is performed by magnetic cell separator. Tateishi-Yuyama et al. was the first to report the efficacy of autologous transplantation of BM-MNCs for patients with ischemic limbs including diabetic patients and reported the improvement of ABI and TcO2 in patients receiving the therapy.\(^\text{15}\) Later, more than 20 investigators have reported BM-derived MNC EPC therapy for peripheral arterial disease with similar results without any serious side effects.\(^\text{16,17}\) Kawamoto et al. was the first to report the efficacy of autologus and purified CD34 positive cell transplant in patients with chronic ischemia in the lower extremities.\(^\text{18}\) Although some studies are limited to small number of study subjects, lack of a control groups and by differing outcome parameters, the outcome of the therapy on perfusion parameters (ABI, TcO2) and clinical course (wound healing, walking distance, number of amputations) are positive throughout different studies.\(^\text{19}\) Large endpoint studies are still underway to further consolidate this evidence.

EPC therapy for chronic non healing diabetic wound: clinical study

We have previously reported the efficacy of G-CSF mobilized peripheral blood CD34 positive cell therapy for diabetic non healing wounds.\(^\text{20}\) To the best of our knowledge, our study is the first clinical trial of transplantation of autologous and purified CD34 positive cells in patients with chronic non healing diabetic wounds. The inclusion criteria for the trial are 1) diabetic patients with non healing chronic wound for at least three months, 2) diabetic patients with HbA1C less than 6.5%, and 3) patient age over 20 and less than 70 years old. All patients enrolled in this study received subcutaneous administration of G-CSF (10 μg/kg per day for 5 days) to mobilize EPCs from BM. On the fifth day of G-CSF administration, leukapheresis is performed to harvest peripheral blood MNCs. CD34 positive cells were isolated using magnetic separator, CliniMACS Instrument, CD34 reagent, phosphate-buffered saline/EDTA buffer, and tubing set (Miltenyi Biotec, Bergisch Gladbach, Germany). Cell transplantation is performed under general anesthesia. Total of \(2 \times 10^7\) CD34 positive cells were injected within 20 cm surrounding the wound. Since this is a Phase 1 trial, the primary endpoint of this trial is safety. The secondary endpoints are the wound closure 12 weeks post treatment and perfusion evaluation with ABI, TCO2, SPP (Skin Perfusion Pressure). Five patients are enrolled in this study so far. Two patients received minor amputations of the toe, but there was no major amputation and all patients are ambulatory with complete wound closure. ABI did not show any difference after treatment but TCO2 and SPP showed significant increase. No major adverse event was seen throughout the trial. Overall, the results seen in this clinical trial encourage the future application of autologous EPC therapy for diabetic foot.\(^\text{20}\)

Problems of present EPC therapy for diabetic patients

From the above study that we have reported, we have learned that diabetic patients have fewer EPCs and have lower EPC potential than non-diabetic patients. In addition, the efficacy of EPC therapy was co-related to the function of transplanted EPCs. Therefore we believe that applying dysfunctional diabetic EPCs to diabetic patients for autologous EPC therapy is not going to be effective compared to non-diabetic patients. We believe that technique to restore EPC dysfunction is needed to establish effective EPC therapy. Furthermore, the isolation technique to obtain EPCs from peripheral blood or bone marrow is at patient’s burden. Establishing non-invasive isolation technique with a procedure to enhance the vasculogenic potential of EPCs is the key for an ideal EPC therapy for effective tissue regeneration.

References


