THE USE OF HUMAN MESENCHYMAL STEM CELLS FROM THE UMBILICAL CORD IN REGENERATIVE MEDICINE - A NOVEL THERAPY?

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S u m m a r y. Cellular therapies and cell biopharmaceuticals open an important therapeutic option for many human diseases that cannot be cured at all. Stem cells seem to represent the greatest potential for the future of molecular and regenerative medicine There has been increasing interest in mesenchymal stem cells (MSC) recently. This is mainly due to their properties, including prolonged *ex vivo* proliferation, potential of multiline transformation, and immunomodulatory properties. Currently, numerous studies on the isolation of MSCs from alternative sources are underway. MSCs obtained from Wharton's jelly (WJ) and umbilical cord (UC) have gained much attention in recent years, because they can be easily isolated without any ethical problems from the tissue rejected after birth. In addition, MSCs from WJ represent a more primitive population than their adult counterparts, which opens new perspectives for cell therapies. Studies of MSC cells isolated from human Wharton's jelly have shown that MSC cells demonstrate low immunogenicity and immune regulation. Stem cells are a useful tool for the treatment of many diseases associated with inflammation, tissue damage and subsequent regeneration and repair. Considering the experiments in which the regenerative properties of MSC cells were used, it can be predicted that the clinical application of MSCs could change the course of treatment of many diseases. Doctors would have a 'new cell therapy'. MSCs seem to offer new potential in the regenerative medicine of many disorders and diseases, more effective than existing pharmaceutical protocols. Promising and impressive early results have been achieved on the basis of several clinical trials, although the exact repair mechanisms of MSC activities remain unknown and require more research.
K e y w o r d s: human mesenchymal stem cells, umbilical cord, regenerative medicine.

INTRODUCTION

The interest in mesenchymal stem cells (MSC) has increased recently. This is mainly due to their properties, including prolonged *ex vivo* proliferation, potential of multiline transformation, and immunomodulatory properties. However, their use is limited due to painful procedure of isolation and MSC characteristics decreasing with the age of the donor. Currently, numerous studies on isolation of MSCs from alternative sources are underway. MSCs obtained from Wharton's jelly (WJ) and umbilical cord (UC) have gained much attention in recent years, because they can be easily isolated without any ethical problems from the tissue rejected after birth. In addition, MSCs from WJ represent a more primitive population than their adult counterparts, which opens new perspectives for cell therapies (Batsali et al., 2013).

Studies of MSC cells isolated from human Wharton's jelly have shown that MSC cells demonstrate low immunogenicity and immune regulation. Human umbilical cord Wharton's jelly (hUCWJ) provides a new source of MSCs that are highly proliferative and have the potential to multidifferentiation. To study the immunomodulatory effects of human gelatinous Wharton cells (WJC) on lymphocytes, MSCs from human Wharton's jelly are being successfully isolated. WJCs showed the presence of MSC markers, but low levels of human leukocyte antigen (HLA) -ABC, and lack of HLA-DR. These results indicate that WJCs have low immunogenicity. Both WJCs and their culture supernatant can inhibit the proliferation of phytochloagglutinin-stimulated human peripheral blood lymphocytes and splenocytes in mice. In addition, WJCs inhibited the secretion of transforming growth factor-β1 and interferon-γ by human peripheral blood lymphocytes (Zhou et al., 2011).

Researchers conclude that the WJC immunomodulatory effect may be associated with direct cellular contact and inhibition of cytokine secretion by human peripheral blood lymphocytes.

The umbilical cord is a rich source of MSCs that can be used to develop allogenic cell therapy for the treatment of incurable diseases. Immunomodulatory properties of umbilical cord mesenchymal stem cells (UCMSC) are likely to facilitate their survival in an allogenic environment. UCMSCs do not show human leukocyte antigen (HLA) -DR and CD80, and CD86 costimulatory molecules required for T cell activation. More importantly, UCMSCs are constitutively expressing a negative regulator of T-cell activation, B7-H1, and its expression increases after interferon-treatment. Studies have shown that IFN-γ treatment induced 2,3-indolamine 2,3-dioxygenase (IDO) and HLA-DR expression in UCMSCs. Neither control nor IFN-γ UCMSCs stimulated allogenic T-cell proliferation, and both cell populations inhibited the stimulated activity of cells initiated by the third dendritic cell (DC). The authors report that the addition of a B7-H1-specific blocking or IDO inhibitor 1-methyltryptophan (1-MT) abolished immunosuppressive activity of T cells. Furthermore, UCMSCs prevented differentiation and maturation of DCs derived from the peripheral blood monocytes, and increased regulatory production of T cells (Treg) in culture. The immunosuppressive effects of UCMSCs are largely dependent on cell contact, although some inhibitory activity has been observed in the cell-free supernatant. The results suggest that these immunomodulatory properties of UCMSCs can potentially improve the result of allogenic stem cell therapy (Tipnis et al., 2010).

Koh et al. (2008) investigated the neuroprotective effects and mechanisms of implantable human mesenchymal stem cells derived from human umbilical cord (hUC-MSC) in ischemic stroke. HUC-MSCs were isolated from the endothelium / sub-epithelium of human umbilical cord and were cultured. Twenty days after induction of neuronal differentiation *in vitro*, about 77.4% of vaccinated hUC-MSCs showed morphological characteristics of neurons. Before, during, or one day after *in vitro* neuronal differentiation, hUC-MSCs produced granulocyte colony stimulating factor, vascular endothelial growth factor, a neurotrophic factor derived from a glial cell line, and a neurotrophic factor from the brain. In an *in vivo* study, the implantation of hUC-MSCs into the injured hemisphere of immunosuppressive ischemic stroke rats improved neurobehavioral function and decreased infarct volume compared to control rats. Three weeks after the implantation, the majority of the implanted hUC-MSCs were detected in the damaged hemisphere; some of these cells expressed detectable levels of neuron-specific markers. Nestin expression in the hippocampus was increased in the group that received hUC-MSCs in relation to the control group. Because hUC-MSCs were morphologically differentiated into neuronal cells and capable of producing neurotrophic factors but did not become functionally active neuronal cells, the authors suggested improvements in neurobehavioral function and decreased infarct volume may be associated with the neuroprotective effect of hUC-MSCs instead of creating a new network between host neurons and implanted hUC-MSC cells.

Mesenchymal stem cells were investigated in the treatment of many autoimmune diseases. For example, Liu et al. (2010) conducted research into the use of UC-MSCs in the treatment of rheumatoid arthritis (RA), a systemic autoimmune disease mediated by T cells. RA is characterized by inflammation of the synovium and destruction to joints. The potential of immunosuppressive effects of human UC-MSCs in RA was assessed. RA studies have shown that human UC-MSCs suppressed different inflammatory effects of fibroblast-like synoviocytes (FLS) and T cells *in vitro,* and weakened the development of collagen-induced arthritis (CIA) *in vivo,* strongly suggesting that UC-MSC may be a therapeutic strategy in RA.

Maintaining stable bone mass in adulthood after rapid skeletal growth in childhood is the result of a carefully controlled balance between osteogenic osteoblastic cells and bone resorption (osteoclasts). Although bone turnover lasts throughout adulthood, bone mass begins to decline with age, because bone resorption exceeds its formation, especially in postmenopausal women, leading to an increased risk of fracture. Although tremendous progress has been made in the knowledge of bone biology, only few studies investigated the role of MSC *in vivo* in maintaining skeletal integrity or fracture repair. The studies found that MSCs also play an important role in repairing the fractures in addition to regulating normal skeletal homeostasis. Bone fracture or injury initiates a series of cellular and molecular pathways that begin with the formation of hematoma and an inflammatory cascade that regulate MSC activity leading to fracture healing and restoring skeletal integrity. In recent years, the

key role of anabolic therapies in establishing osteoporosis, in which bone mass is significantly greater than what can be obtained with bisphosphonate drugs, has introduced the MSC to the research program into regenerative potential of MSCs (Bielby R. et al., 2007).

Mesenchymal stem cells are also promising tools for treating diseases such as myocardial infarction and stroke due to their ability to promote endogenous angiogenesis and neurogenesis through various secreted factors. MSCs found in human Wharton's jelly are easily obtained and are capable of transplantation without rejection (Hsiech et al., 2013). Researchers isolated MSCs from Wharton's jelly and bone marrow (WJ-MSC and BM-MSC respectively) and compared their secretomes. They found that WJ-MSC expressed more genes, especially secreted factors involved in angiogenesis and neurogenesis. Functional analysis showed that WJ-MSCs better induce differentiation of neurons and migration of nerve cells *via* the paracrine mechanism. Furthermore, WJ-MSC provided better neuroprotective efficacy because they preferentially increased neuronal growth and decreased the apoptotic death of primary cortical cells in an oxygen free and glucose culture model that mimics acute ischemic stroke in humans. In terms of angiogenesis, WJ-MSC induced better formation of small blood vessels and cell migration on co-cultured endothelial cells. The results obtained suggest that WJ-MSC cells due to unique secretome may be a better source of MSCs to promote nerve regeneration *in vivo* and endothelial repair. Researchers suggest that this study is the basis for the development of cell therapy and complementary research related to MSC biology.

Research has been carried out on the treatment of MSC cells in Buerger's disease. Buerger's disease, also known as obliterative thrombosis, is a rare disease characterized by a combination of acute inflammation and thrombosis of the arteries and veins in the hands and feet. Obstruction of the blood vessels in the hands and feet reduces the availability of blood to the tissues, causes pain, and eventually damages or destroys the tissue. It often leads to ulcers and gangrene of the fingers and toes. Rarely, in advanced stages of the disease, it can affect the vessels in other parts of the body.

In four men with Buerger's disease who had already received treatment and surgical therapies, human mesenchymal stem cells with human leukocytic matched umbilical cord blood antigen (UCB) were transplanted. After the transplantation of MSCs researchers observed sudden loss of pain in the affected limbs. Necrotic skin lesions were cured within 4 weeks. In addition, the vascular resistance in the affected limbs compared to the preoperative examination was significantly reduced due to improved peripheral circulation. Because the animal model of Buerger's disease is absent, and to understand human results, human MSCs derived from UCB have been transplanted into a nude athymic mouse with hind limb ischemia by means of ligation of the femoral artery. The results showed that 60% of the hind limbs were saved in animals with ligated femoral arteries. As a result of *in situ* hybridization, human MSCs derived from UCB were detected in the ischemic posterior limb arteries of the treated group. Therefore, it is suggested that the transplantation of MSCs from human UCB may be a new and useful therapeutic alternative in the case of Buerger's disease and similar ischemic diseases (Kim et al., 2009).

Recently, research has been carried out on a new strategy, i.e. gene therapy for the treatment of malignant brain tumors using stem cells as delivery vehicles for therapeutic agents. In this study, UCB-MSC cells were used as delivery vehicles and modified interleukin-12 (IL-12p40N220Q; IL-12M) as a new therapeutic gene for the treatment of glioma. Animal studies have confirmed the ability of UCB-MSC-IL12M migration to mouse GL26 glioma cells by using an *in vitro* migration test and *in vivo* UCB-MSC-IL12M injection into the ipsilateral hemisphere of implanted gliomas in 6 mice. *In vivo* efficacy studies showed that UCB-MSC-IL12M tumor injection significantly inhibited tumor growth and prolonged survival of mice with glioblastoma compared to control mice. Anti-cancer effects were associated with the increase of local IL-12M levels, followed by the secretion of interferon-γ and infiltration of T cells in intracranial gliomas, as well as against angiogenesis. Interestingly, tumor-free mice after treatment with UCB-MSC-IL12M were resistant to re-emergence of the tumor on the ipsilateral and contralateral side. Thus, the results provide justification for the development of new experimental protocols to induce long-term anti-tumor immunity in intracranial gliomas using UCB-MSC as an efficient delivery medium for therapeutic cytokines, including IL-12M (Ryu et al., 2010).

Preclinical studies were also carried out using MCS cells in the treatment of Autism spectrum disorders (ASD) (Siniscalco et al., 2014). A non-randomized, open, controlled, single-center, I / II phase clinical trial was conducted to investigate

the safety of the treatment and efficiency of human umbilical cord blood (CBMNC) and / or human mesenchymal umbilical cord transplantation (UCMSC) in children with autism (Lv et al., 2014). MSCs were administered by intravenous infusion. Autistic children were followed up for 24 weeks after the transplantation. According to the authors, cell treatment was safe, well tolerated without immediate or long-term side effects, no allergic or other serious adverse events were observed at the time of injection or during the entire follow-up period. The researchers noted improvement in visual, emotional and intellectual responses, adaptation to changes, fear or nervousness reactions, non-verbal communication and activity level measured by the scale of infantile autism evaluation scales as well as in the social, stereotypical behavior. Moreover, they noticed that a group of patients receiving CBMNC and UCMNC simultaneously showed a more robust therapeutic effect than the group receiving mono-cell therapy. They concluded it must have been attributed to the synergistic effect of CBMNC and UCMSC, and suggested that the synergistic mechanism is associated with increased cellular perfusion in the brain areas and / or regulation of immune dysfunctions.

Cells isolated from the Wharton's jelly adhere to a plastic substrate in tissue culture, exhibit mesenchymal surface-cell markers, self-renewal and are multipotent (are able to differentiate into bone, fat and cartilage) *in vitro*. Those properties confirm that UCMS cells belong to the MSC family. Researchers believe that UCMS cells have immune properties that enable allograft. UCMS cells express mRNA for pan-HLA-G and express vascular endothelial growth factor, IL-6, molecules involved in the modulation of immune response, produce cytokines and other factors that may promote hematopoiesis. Together, these results support previous observations that after xenotransplantation, for example, there was no evidence of immune rejection of undifferentiated UCMS cells. The results suggest that human UCMS can be well tolerated in allogenic transplantation (Weiss et al., 2009). The same authors report that they isolated a population of CD45-, CD34- and HLA-DR-negative cells as well as CD73-, CD105-, CD90- and CD29-positive cells derived from human Wharton's jelly and the umbilical cord. Human UCMS cells can be rapidly isolated in large quantities from over 90% of human umbilical cord cells. Thus, UCMS cells can be a rich source of MSC-like cells that can be used for therapeutic purposes because they can be frozen / thawed, cloned, engineered to express exogenous proteins, and cultured. It can therefore be assumed that UCMS cells have therapeutic potential because they are reminiscent of MSC in many respects, including surface phenotype, plastic adhesion and multipotency - so they can be bred *in vitro* to obtain bone, cartilage, and fat cells.

CONCLUSIONS

Currently, cellular therapies and cell biopharmaceuticals open an important therapeutic option for many human diseases that cannot be cured at all. Stem cells seem to represent the greatest potential for the future of molecular and regenerative medicine (Siniscalco et al., 2012). Stem cells are a useful tool for the treatment of many diseases associated with inflammation, tissue damage and subsequent regeneration and repair (Dimarino et al., 2013). Considering the experiments in which the regenerative properties of MSC cells were used, it can be predicted that the clinical application of MSC could change the course of treatment of many diseases. Doctors would have a 'new cell therapy'. MSCs seem to offer new potential in the regenerative medicine of many disorders and diseases, more effective than existing pharmaceutical protocols. Promising and impressive early results have been achieved on the basis of several clinical trials, although the exact repair mechanisms of MSC activities remain unknown and require more research.

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