LITERATURE REVIEW

Mesenchymal Stem Cells and Clinical Application in Chronic Lung Diseases

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ABSTRACT

Mesenchymal stem cells are a multipotent mature non hematopoietic stem cells, with characteristics such as ability to self-renew and differentiate in mesodermal, ectodermal, and endodermal pathway. Mesenchymal stem cells also secrete cytokine and immunoreceptor which regulate micro environment in host tissues and angiogenic mediators which are able to improve damaged tissues. Mesenchymal stem cells are obtained from the human body by isolation, culture, proliferation, characterization, and/or differentiation originating from fat cells (adipose), periosteum tissue, and other tissues from the body. Mesenchymal stem cells can be obtained by autologous and allogenic way. Stem cell processing includes isolation, proliferation, differentiation, and temporary storage for clinical application adhering to good drug manufacturing practice. Approach to cell therapy and bioengineering in lung disease is rapidly developing in the last 10 years. In the current era of cell therapy and transplantation, a lot of research has been done to understand and develop mesenchymal stem cells as a therapeutic alternative, particularly in respiratory area.

INTRODUCTION

Stem cell is a cell from the human body with special capability to self-regenerate or self-renewal and differentiate into other cells. An important trait possessed by the stem cell which is not available in other cells is the absence of functional specialization, but it can self-renew by cell division even after being inactivated in a long time. On certain situation, stem cell can be induced into cells with certain function, such as tissue cell or even organ cell with its own role. In bone marrow and umbilical cord blood, stem cells are continuously dividing and repairing damaged tissues. When stem cells divide, the new cell has the potential to stay as stem cell or becomes cell from other types with specialized function, such as muscle cell, blood cell, or brain cell.[1–3]

Differentiation process of stem cell is triggered by signals from inside and outside of the cell. Signal from inside of the cell which influences differentiation process originates from the gene in the DNA which contains codes for cell's structure and function, while signals from outside of the cell are chemical substances secreted by other cells, physical contact with neighboring cell, and certain molecules in micro environment. The signals cause change of expression in the DNA and lead to differentiation as certain cells which are inherited by cell division.[1]

The role of stem cells in lung repair and cell therapy approaches for chronic lung disease is still an open area of research. Many studies in stem cells used animal model, such as acute and fibrotic lung injury. Recently, it has been extended to human lung model. However, any succeed in animal study is not always

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followed by the fruitfulness of human study. In this review, we explored mesenchymal stem cells and its applications in chronic lung diseases such idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), and asthma.

Sources of Stem Cells

Based on the source, stem cells are classified as embryonic and non-embryonic cells. Embryonic stem cell can be obtained from multiple embryonic phases, such as embryonic stem cell, germinal stem cell, and fetal stem cell. Embryonic stem cell is a progenitor cell obtained from the embryo in the blastocyte phase (5-7 days after fertilization). Embryonic stem cell can be directed into all types of cells found in mature organism, such as blood cell, muscle cell, liver cell, kidney cell, and other cells. Embryonic germ cell is germinal cell/primordial germ cell, such as sperm and ovum cell, and diploid germinal cell precursor which exists momentarily in embryo before being associated with somatic gond cell and becomes germinal cell. Human embryonic germinal cell includes progenitor cell which originates from fetal primordial germinal cell aged 5-9 weeks. Fetal stem cell is a primitive cell found in fetal organs, such as fetal hematopoietic stem cell and pancreatic cell progenitor.[1]

Adult stem cells, also called somatic cells, refer to various cells from the body which are not originated from germinal cell. This cell plays a role in managing and repairing tissue where the stem cells are found. Currently, almost all mature body tissues and organs have been proven to contain adult stem cells. Categorization of adult stem cells is based on the organ or cell group that will be the pathway of differentiation, such as hematopoietic stem cell, heart stem cell, nerve stem cell, mesenchymal stem cell, skin stem cell, and so on.[2–4]

The Legality of Stem Cell Service in Indonesia

The implementation of stem cells service in Indonesia is stated in the Regulation of the Ministry of Health Republic of Indonesia No. 32/2018 regarding arrangement of stem cells service and/or cells, which regulates the definition, committee, service, cell source and type, activity of service (involving extraction, storage, processing, and/or clinical application of stem cells and/or cells), use, therapy-based research, organizer, quality audit, funding, note-taking and reporting, and also guidance and supervision. In this condition, stem cell and/or cell service can only be performed for disease-healing purposes (degenerative and non-degenerative). It is forbidden to use stem cells for reproductive purpose or creating new individual. The regulation also intends to provide guideline, protection, and legal certainty to patients, donors, and healthcare service facilities as administrator of stem cells and/or cells service.[2]

Mesenchymal Stem Cells

Mesenchymal stem cells are multipotent adult non hematopoietic stem cells with characteristics such as ability to self-renew and differentiate in mesodermal pathway (osteocyte, adipocyte, and chondrocyte), ectodermal (neurocyte), and endodermal (hepatocyte). Moreover, mesenchymal stem cells have immunomodulatory ability which is very useful for repairing heart, improving result of bone marrow transplant, healing degenerative diseases, and regenerating connective tissue. Mesenchymal stem cells are obtained from the human body by isolation, culture, proliferation, characterization, and/or differentiation from fat cells (adipose), periosteal tissue, and other body tissues, as illustrated in Figure 1.[1,2,5,6] International Society for Cellular Therapy decided to make terminology definition for mesenchymal stem cells as multipotent mesenchymal stromal cells in 2006. Recently, mesenchymal stromal cells became the most investigated adult stem cells for the treatment of lung disease.[5]

Latest research shows that mesenchymal stem cell can be isolated from lung tissue. Martin, *et al*., demonstrated mesenchymal stem cell isolation from neonatal tracheal aspirate and adult broncho-alveolar lavage. Gong, *et al.*, were able to isolate mesenchymal stem cell and proved its capability to form alveolar type II (AT-II) cell. Mesenchymal stem cell which is isolated from tissues other than the lungs can also differentiate into alveolar epithelium.[7,8]

International Society for Cellular Therapy has released the minimum criteria to determine mesenchymal stem cells, such as adhering to plastic in culture condition, having specific marker (CD 73, CD90, CD105), few or absence of marker (CD14, CD34, CD45 or CD11b, CD79a or CD19) and Human Leucocyte

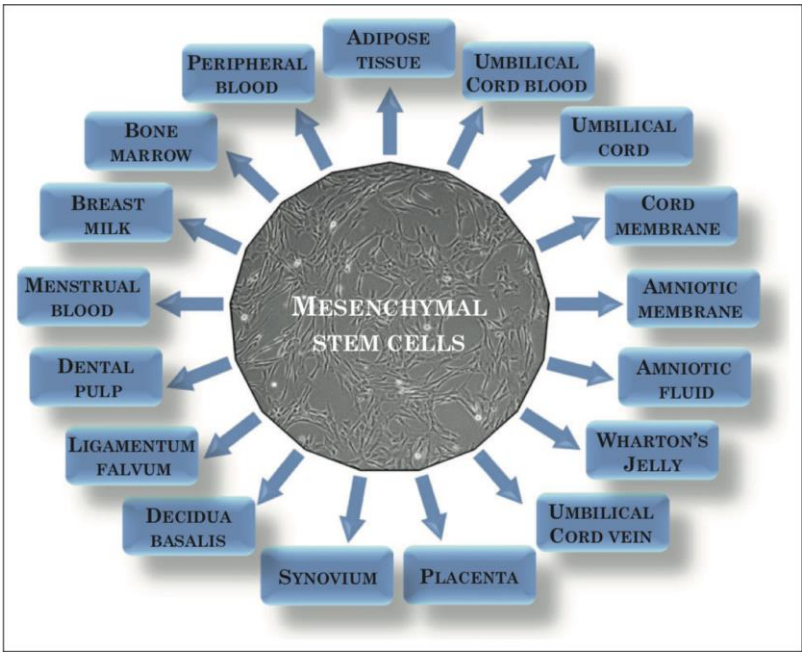

Figure 1. Sources of stem cells[6]

Antigen-DR (HLA-DR), and having the ability to differentiate by in vitro into adipose tissue, chondroblast, and osteoblast. Some recent research showed limitless amount of marker, such as Stage-Specific Embryonic Antigen 4 (SSEA-4), CD146, and Stromal Precursor Antigen-1 (Stro-1). Until now, bone marrow stays as the best source to find mesenchymal stem cell and sets the standard when comparing mesenchymal stem cell from other sources. Another source of mesenchymal stem cells is umbilical cord which has been used widely for pre-clinical and clinical studies.[7–9]

Isolation and Initial Culture

The procedure of stem cell retrieval must obtain written agreement from patient and/or donor, and performed by medical staff with competence and rights, while adhering to profession ethics and also the patient's and the donor's safety. Obtained stem cells which are not immediately used in clinical application service will be stored in cell bank or other standardized storage location to ensure stem cell quality. Stem cell processing involves isolation, proliferation, differentiation, and storage, while for clinical application is adhering to good drug manufacturing practice.[2]

Mesenchymal Stem Cell Interaction with Immune System

Studies about interaction between mesenchymal stem cell with immune system have rapidly developed in the last 10 years. Scientists found interactions between mesenchymal stem cell and immune system, with some of them having advantageous feedback to the mesenchymal stem cell. The interaction is seen between mesenchymal stem cell towards natural killer cells, macrophage, T cell, dendritic cell, B cell, monocyte, neutrophil, and T-reg cells. Some studies show that stem cell regenerating effect is not mediated by the stem cell's differentiation ability to repair damaged tissue, rather by its ability to secrete bioactive metabolites through paracrine mechanism.[2,10]

Mesenchymal stem cells have immune-modulatory effect by suppressing T cell proliferation, inhibiting B cell proliferation, suppressing migration, maturation, and dendritic cell presentation. Mesenchymal stem cells can secrete bioactive molecules such as prostaglandin E2, IL-10 with anti-inflammatory properties, TGFβ1, and hepatocyte growth factor (HGF) which suppresses T cells-dependent inflammation. Total number of effector of T cells will be reduced by transplanted mesenchymal stem cells and finally

attenuates Th1-, Th2, or Th17-driven inflammation in the lungs. Another mechanism is through induction of dendritic cells (DCs) which promote polarization from macrophages of inflammatory M1 to immune-suppressive M2 macrophages. Therefore, the production of inflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha), IL-1beta, and IL-12 can be reduced. On the other hand, it could induce the production of anti-inflammatory cytokine IL-10 and TGFβ which will enhance tissue reparation and regeneration of the lungs.[10]

Clinical Application in Chronic Lung Disease

Up until 2019, 30 years after mesenchymal stem cell was discovered, there are a total of 55,000 publications about mesenchymal stem cell and 950 clinical trials in humans registered in Food and Drug Administration (FDA). More than 10,000 patients have undergone therapy under clinical trials, with 88 completed phase 1 and 2 trials, and 10 studies in phase 3. A lot of progress has been made, becoming the basis for research and clinical application, but there are still many unanswered questions.[11]

Approach of cell therapy and bioengineering in lung disease has rapidly developed in the past 10 years. In the current era of cell therapy and transplantation, intravenous mesenchymal stem cells administration and host compatibility become the main focus. Homing ability, secretion of anti-inflammatory molecules, and immunoregulatory effect make mesenchymal stem cells as a promising cell source for therapy of autoimmune, inflammatory, and degenerative disease. In the last 5 years, many research have been performed to find and develop mesenchymal stem cells as alternative therapy, especially in respirology division. Some of the clinical studies are being conducted in the field of asthma, COPD, and idiopathic pulmonary fibrosis (IPF) as shown in Table 1. However, some of them are not recruited yet or being suspended due to COVID-19 pandemic. A keen awareness is also advised since trials which are listed on Table 12 can become an advertisement for private clinics.[12]

Idiopathic Pulmonary Fibrosis (IPF)

IPF patients suffer dyspnea, dry cough, and fatigue resulting from destruction of lung structure which leads to progressive fibrosis and finally reduction in lung function. The architecture of the lungs in IPF patients predominantly consist of fibroblast proliferation, depositing huge amount of extracellular matrix. In animal study using irradiation-induced lung fibrosis model, after the administration of mesenchymal stem cells, it will diminish inflammatory injury as result attenuation of the production of inflammatory cytokines and reducting accumulation of collagen and impaired fibroblasts proliferation.[10]

Study by Ortiz, *et al*. was the first to show protective effect of mesenchymal stem cells obtained from one marrow, using bleomycin model of pulmonary fibrosis. Furthermore, it discovered how the amount of stem cells in lung tissue significantly increases in the condition of injury. Nevertheless, there was a time limitation to administer stem cells, with no protective effect when performed 7 days after the injury.[13]

Study by Yan, *et al.* proved that mesenchymal stem cells can increase progressivity of lung disease by the process of fibrosis when given in later stage of chronic inflammation and fibrosis. They estimated the cause to be high levels of TGF-β, a cytokine that has important role in the development of fibrosis during later phase of lung tissue damage caused by irradiation. Therefore, the ideal time of mesenchymal stem cells administration has to be considered before its utilization for therapy.[8]

A clinical trial sponsored by The Prince Charles Hospital in 2013 proved the simplicity and safety of mesenchymal stem cells administration obtained from placenta into IPF patients. The study found minimal side effect and no proof of worsening in the condition of fibrosis. It was the first study to evaluate the safety of mesenchymal stem cells in IPF, and it became the foundation of further researches about the effectivity of mesenchymal stem cells.[8] Another study was performed by Glassberg, *et al.* in mild and moderate IPF patients using allogeneic human bone marrow-derived mesenchymal stem cells with primary end point to see the incidence of serious adverse events. They found no treatment-emergent serious adverse events were reported which supported the safety of single infusion hMSC in patients with IPF.[14]

Chronic Obstructive Pulmonary Disease (COPD)

COPD as one of the obstructive diseases per definition by Global Initiative for Chronic Obstructive Lung Disease (GOLD) is characterized by persistent

respiratory symptoms and airflow limitation caused by airway and/or alveolar abnormalities due to noxious particles or gases exposure. COPD patients may suffer symptoms such as dyspnea, cough, and/or sputum production and can be followed by periods of acute worsening symptoms (exacerbations). This chronic inflammation can cause structural damages, narrowing small airways, and destruction of the lung parenchyma which leads to reduction of the lung function.[15,16]

Mesenchymal stem cells mechanism that works in COPD through paracrine manner which downregulates the COX2/PGE2 pathway in inflammatory M1 macrophages via p38 mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinase (ERK) results in polarization towards anti-inflammatory M2 macrophages. Furthermore, mesenchymal stem cells will decrease the production of matrix metalloproteinase (MMP-2, MMP-9, and MMP-12) which mediates tissue remodeling. In turn, it will improve the structure of the lungs.[10]

Huh, *et al.* did the first research to prove reparative effect of stem cells in emphysematous model induced by cigarettes.[17] It is believed that the reparative effect is caused by the release of paracrine factors rather than the amount of mesenchymal stem cells due to the few amount of mesenchymal stem cells found in the lungs of female rats. Gu, *et al.* also did the same research using cigarette smoke-induced rat models.[18] This study showed that administration of mesenchymal stem cells could inhibit cyclooxygenase-2 (COX-2) expression and prostaglandin E2 (PGE2) production on alveolar macrophages in addition to paracrine factors, thereby improving airway inflammation and emphysema.

A study sponsored by Mesoblast International Sarl evaluated the effectivity and safety of PROCHYMAL™, an adult stem cells from humans, in 62 patients with moderate to severe COPD which were divided into two groups, receiving intravenous 100 x 10^6 mesenchymal stem cells or placebo. The patients were monitored for 2 years and the obtained result was no side effects and no increasing exacerbation frequency or changes in the lung function. Moreover, they found decreased CRP levels in sub population with increased CRP at the beginning of the study. Therefore, it was concluded that mesenchymal stem cells administration in moderate COPD is safe. However, there was no significant difference between two groups in lung function and quality of life indicators. Further research is needed to evaluate effective dose and therapy schedule for more accurate effectivity.[8,10,16]

Another study was performed by Le Thi Bich, *et al*. in Vietnam using umbilical cord-derived mesenchymal stem cells (UC-MSCs) which was intravenously infused with 1.5 x 10^6 UC-MSCs/kg in 20 COPD patients at stages C and D per GOLD classification.[19] There was improvement in most of the clinical parameters after 6 months of follow-up, including CRP, Modified Medical Research Council Score (mMRC), COPD Assessment Test (CAT), and number of exacerbations. Meanwhile, the six minute walk test (6MWT) score was slightly increased in stage D COPD patients.

Asthma

Asthma is an obstructive disease with chronic airway inflammation. Asthma patients have variable symptoms of wheeze, shortness of breath, chest tightness, and/or cough. The symptoms and airflow limitation usually vary over time and are often triggered by factors such as allergen, irritant exposure, or viral respiratory infection.[20] Mesenchymal stem cells-based therapy for asthma is targeted to attenuate airway inflammation and remodeling, thus it finally can improve lung function. Study from animal model of asthma using intravenously injected mesenchymal stem cells showed the mechanism of mesenchymal stem cells via extracellular vesicles (EVs) derived from mesenchymal stem cells which altered phenotype of antigen-specific CD4 T cells which resulted in decreasing cytokines such as IL-4, IL-5, and IL-13. It also reduced eosinophil infiltration and mucus production in the lung and it downregulated Th2 cytokines in bronchial lavage. Through phagocytosis process of transplanted mesenchymal stem cells, M1 macrophages changed towarda anti-inflammatory and immunosuppressive M2 macrophages. Therefore, immunosuppressive factors can suppress lung inflammation and induce repair and regeneration in the asthmatic lung.[10]

Castro, *et al*. found that adipose tissue-derived mesenchymal stromal cells (MSCs) reduced lung inflammation using mice model of experimental house dust mite (HDM)-induced allergic asthma.[21] They used

MSCs that were isolated and cultured from human adipose tissue obtained from healthy women who undergone abdominal plastic surgery at Clementino Fraga Filho Hospital, Rio de Janeiro. After giving MSCs into 3 groups of HDM group of mice randomly in different doses (2 or 3 doses of MSCs/10^5 cells per day or saline) intravenously, parameters of inflammation were measured. Levels of IL-4, IL-13, and eotaxin in lung tissue were reduced, although protein levels of IL-10 remain unaltered. Although mechanisms of MSCs in this study were not explored entirely, this MSCs showed its capability to promote immunomodulation.

Not many trials of stem cells in asthma patients have been performed in the past decade. Most of the evidences were based on animal studies and very few in human subjects. Study by Cruz, *et al*. showed that mesenchymal stem cells changed the phenotype of antigen-specific CD4 T cell in an inflammatory model of airway allergy by exosome derived from mesenchymal stem cells. They used extracellular vesicles released by MSCs into immunocompetent mouse model of allergic airway inflammation. Du, *et al*. discovered similar thing with Cruz by the increase of proliferation and immunosuppressive properties of Treg, and increased production of anti-inflammatory cytokines (IL-10 and TGF-β) in asthma patient's mononuclear cell from peripheral blood after being given mesenchymal stem cells exosomes.[10,22–24]

Current ongoing research in asthma patients to evaluate safety and efficacy of mesenchymal stem cells was performed in Miami Miller School of Medicine by administering mesenchymal stem cells from bone marrow in mild asthma patient. The randomized experimental research was performed towards 6 asthma patients who received 20 million or 100 million mesenchymal stem cells from bone marrow. Lung function monitoring and dyspnea questionnaire are assessed every 4 weeks and side effects are continually monitored until the end of the research.[10]

SUMMARY

Mesenchymal stem cells are able to reduce inflammation, halt the progressivity of lung fibrosis, and activate tissue repair. Migratory and immunomodulatory properties of mesenchymal stem cells make it a strong modality for chronic lung diseases therapy. Further research is still needed to understand not only the uses but also the therapeutic dose, time, and route of mesenchymal stem cells administration. Many clinical trials which used mesenchymal stem cells have been done and the safety and ease of administration has been proven generally. Nevertheless, clinical trials of cells therapy in lung diseases must be proceeded with a keen awareness. Hopefully, the ultimate goal to develop cells-based therapy to aid repair and regeneration of the lungs of the patients with chronic lung diseases will be reached.

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