COVID-19

Mesenchymal Stem Cells: Potential Application in COVID-19 patients with Acute Respiratory Distress Syndrome (ARDS)

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ABSTRACT

Objective: **To highlight the potential of mesenchymal stem cells (MSCs) as an adjuvant therapy for severe COVID-19 patient.**

Materials and Methods: We searched Pubmed and Google Scholar, using keywords: 'MSC AND lung injury', 'MSC AND ARDS', 'MSC AND COVID-19', 'Stem cell AND Lung injury' and 'Stem cell AND ARDS', on 19 Mei and 19 July 2020.

Results and Discussions: **Severe manifestations in COVID-19 patients are due to immune system response. MSCs have immunomodulatory and anti-inflammatory effect and therefore may be beneficial to alleviate acute respiratory distress syndrome (ARDS). A small published study showed that MSCs had beneficial effect on COVID-19 patients, who showed clinical symptom improvements. Further, application of MSCs from several sources such as bone marrow, menstrual blood, and umbilical cord-derived MSCs, which were used in patients suffering from lung injury/ARDS due to conditions other than COVID-19, showed that a dose of up to 1.0 x 10^7 cells/kg body weight was well tolerated.**

Conclusion: **administration of MSCs to COVID-19 patients showed improvement in clinical symptoms, and a dose up to 1.0 x 10^7 cells/kg body weight showed tolerance in ARDS patients with moderate to severe conditions. However, the results came from studies with small number of patients, so the results need to be interpreted with caution, and more well design studies with a larger number of patients are needed.**

KEY WORDS

coronavirus, COVID-19, stem cells, Mesenchymal stem cell

INTRODUCTION

Coronavirus disease 2019 (COVID-19), which was detected in Wuhan, was first reported by China at the end of December 2019[1]. The disease spread rapidly, and caused a global pandemic that might result in serious respiratory morbidity and mortality. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the responsible agent for COVID-19. It is an enveloped single-stranded RNA virus of the Coronaviridae family[2]. Coronaviruses belong to a large family of viruses that several of them are better and previously known, such as Middle East respiratory syndrome (MERS-CoV) and (SARS-CoV)[3].

The time from exposure to COVID-19 symptoms is between 2 and 14 days (median 5 days)[1]. However, about 80-85% COVID 19 patients are asymptomatic[3] or have mild to moderate symptoms and are cured in 14-20 days of the first time they are infected[4]. Most patients present with mild respiratory tract infection, most commonly characterized by fever (82%) and cough (81%)[5], while part of them may have dyspnea due to pneumonia, and pulmonary oedema that may proceed into acute respiratory distress syndrome (ARDS)[3]. Patients with ARDS, who have a likelihood of mortality due to respiratory failure, may require mechanical ventilation and support in the intensive care unit (ICU)[6].

Worldometers site (*https://www.worldometers.info/coronavirus/*) on 25 May 2020 reported that more than 213 countries and territories around the world have been affected, with 5.512.752 cases, and 346.867 deaths. Until recently, effective treatment for severe COVID 19 has not been found; therefore various studies were conducted to obtain effective treatments to overcome this pandemic[7]. One of the options is the use of mesenchymal stem cells (MSCs) to prevent and mitigate severe form of COVID-19.

Therefore, this mini-review aimed to highlight the potential of MSCs as a therapy for a patient with severe COVID-19 and therefore discussed pathogenesis and immune system response in patients with COVID-19, the potential of MSCs to modulate cytokine storm and inflammation and to mitigate cytokine storm in severe COVID-19, and potentials of MSCs from various sources to cure ARDS.

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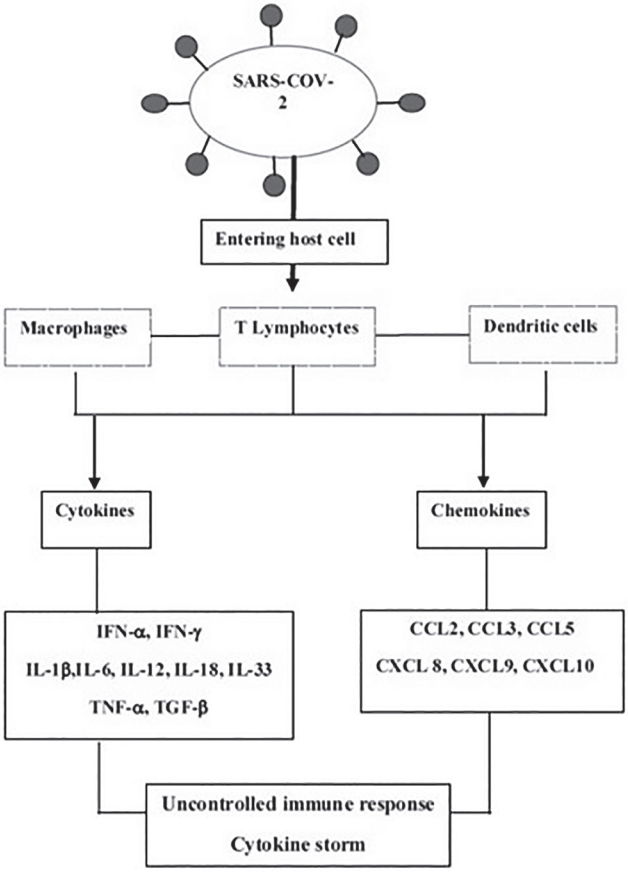

Figure 1: Schematic representation of cytokine storm in patients with COVID-19

MATERIALS AND METHODS

We searched Pubmed and Google Scholar, using keywords: 'MSC AND lung injury', 'MSC AND ARDS', 'MSC AND COVID-19', 'Stem cell AND Lung injury' and 'Stem cell ARDS', on 19 Mei and 19 July 2020. In addition, relevant existing articles in authors collections were added. The articles were grouped according to their content to fill required information on pathogenesis and immune system response in patients with COVID-19, the potential of MSCs to modulate cytokine storm and inflammation and to mitigate cytokine storm in severe COVID-19, and potentials of MSCs from various sources to cure ARDS.

RESULTS AND DISCUSSIONS

Pathogenesis and immune system response in patients with COVID-19

A study showed that at the initial stage of COVID-19 pathogenesis, SARS-CoV-2 spike protein (S protein), which has strong interaction with angiotensin enzyme 2 (ACE-2), acts as a receptor for cell entry[8]. In addition, a serine protease (TMPRSS2) is an essential protein that is also important for entry into host cells[9]. After the virus enters the host cell, viral genome transcription and translation occur, followed by viral replication at cytoplasmic membrane[10]. The ability of S-protein to bind to ACE2 and enter host cells is due to its two functional units, namely S1 and S2. The S1 unit contains N terminal and C domain, and both domains have receptor binding domains (RBDs) to bind the ACE2, while S2 functions to integrate the viral and host membrane[8,11]. ACE2 can be found in cells of various organs, such as type 2 alveolar cells, pulmonary capillary endothelium, cells in gastrointestinal tract, liver, kidneys and cardiomyocytes[12]. However, ACE2 is absent in cells of the immune system (macrophages, T and B lymphocytes), and some reticular tissue containing organs, such as lymph nodes, spleen, thymus, and bone marrow[13].

Viral infections will cause innate immune system response that appears quickly at the beginning of the infection. In the early stages of viral infection, the natural immune system will produce interferon (IFN) I or IFN -α/β, which is a key molecule that act as an antiviral. In COVID-19, there are increases in various cytokine serum levels, such as interleukin (IL)-10, and IL-4, which are anti-inflammatory cytokines, as well as pro-inflammatory cytokines, IL-2R and IL-6. Further, severity of the disease is correlated with IL-6 and IL-2R serum levels[14]. Moreover, observations on 99 COVID-19 patients with moderate-severe conditions with cytokine storms in Wuhan showed an increase in total neutrophils (38%), while total lymphocyte was decreased (35%). There was also an increase in serum IL-6 (52%), and C-reactive protein (84%)[15].

Another study on patients with severe conditions showed a very significant reduction in CD8+ T lymphocytes and NK cells when compared to patients with moderate conditions and healthy controls[16]. Several studies showed that COVID-19 patients, who required ICU care, showed higher levels of TNFα, CXCL10, CCL2, granulocyte colony stimulating factor (GCSF), IP10, MCP1, MIP1A, IL-2, IL 7 and IL-10 compared to COVID-19 patients who did not require ICU care[17,18]. The combination of various highly elevated cytokines, such as TNF-α, interferons, chemokines, interleukins, and GCSF will cause a cytokine storm effect, which may cause ARDS and/or damage to multiple organs that may cause death[17]. Moreover, GCSF is a protein that is associated with inflammatory conditions and an amplification cascade to increase cytokine production; thereby increasing inflammatory reactions[17].

To mitigate cytokine storm effects, administration of cytokine storm suppressing agent may reduce lung inflammation. One of the cytokine storm suppressing agents is mesenvhymal stem cell (MSC)[19].

The potential of MSCs to modulate cytokine storm and inflammation

MSCs secrete various beneficial factors against cytokine storm, i.e. immune modulatory and anti-inflammatory secretomes, which mitigate the cytokine storm effects. In addition, they secrete pro angiogenesis and regenerative growth factors to repair damaged tissues[19].

Immunomodulatory effect of MSCs

Besides secreting immune modulatory secretomes[19], MSCs play an important role in modulating hyperactive immune response by affecting dendritic cells that subsequently regulate lymphocyte subsets toward anti inflammatory state[20]. In addition, MSCs have immune modulatory effects on neutrophils, lymphocytes, and macrophages, which play a role in inflammatory reaction, thus reducing inflammation and finally facilitate epithelial and endothelial repair. These beneficial effects are supposed to be mediated by keratinocyte growth factor (KGF) for epithelial, and angiopoietin (ANG-1) for endothelial repair[21].

Anti-inflammatory effect of MSCs

Harmful stimuli, such as toxic compound, bacteria or viruses will cause innate immune response that trigger inflammation[22]. In general, mechanism of inflammation begins with recognition of harmful stimuli by cell surface receptors, which activate the inflammatory pathway, to release inflammatory cytokines and chemokines that recruit inflammatory cells to the site of inflammation[23]. Therefore, the inflammatory pathway consists of inducers (harmful stimuli), sensors (cell surface receptors), mediators, and target tissue (site of inflammation). In innate immune response, the sensors are Toll-like receptors (TLRs), which are expressed on various sentinel cells such as dendritic cells, tissue macrophages, and mast cells. Upon binding of their TLRs, the sentinel cells produce various pro-inflammatory mediators such as cytokines and chemokines as well as bioactive amines, bradikinin and eicosanoids that cause detrimental inflammatory responses[22].

MSCs have anti-inflammatory effect that is supposed to be mediated by supressing pro-inflammatory cytokine-producing immune system cells[20], and by secreting anti-inflammatory cytokines[19].

The potential of MSCs to mitigate cytokine storm in severe COVID-19

A study on MSCs for COVID-19 patients was conducted by Leng at al on ten COVID-19 patients, whose pneumonia severity varied from moderate to very severe, in Beijing You An Hospital China. These patients were divided into two groups, three patients with severe conditions were entered in placebo group, while seven patients with variable conditions from moderate to very severe were given clinical-grade human MSCs. The MSCs, which were supplied by Shanghai University and were devoid of ACE2 and TMPRSS, were suspended in 100 ml saline. The dose given was $1x10^6$ cells per kilogram body weight, and was administered intravenously with a drip speed of 40 drops per minute. No adverse event occurred in treatment group during a 14 day-assessment after MSC injection. Two days after infusion of MSCs, pulmonary functions and symptoms were significantly improved[20].

Moreover, in treatment group there was a decrease in C-reactive protein, which is an inflammation marker, a substantial decrease in serum pro-inflammatory cytokines and chemokines, and an increase in peripheral lymphocytes. Decrease in pro-inflamatory cytokines might be due to disappearance of pro-inflammatory cytokine-secreting lymphocyte subsets, namely $CXCR3^+$ CD8, $CXCR3^+$ CD4 , and $CXCR3^+$ NK cells in 3-6 days. In addition, in the treatment group there was a significant increase in the anti-inflammatory IL-10, as well as vascular endothelial growth factor (VEGF) that might promote lung repair. However, though the results seems promising, it should be interpreted with caution, due to the limited number of cases, and the unequal base line characteristics between control and treatment[20].

As COVID-19 is a new emerging disease, various clinical trials on MSC for COVID-19 are registered and on-going, but there is only one published study on MSC use for COVID-19. However, there is a safety study on the use of MSC secretome on COVID-19, that was conducted by Sengupta et al in New York, USA. In the study, 24 severe COVID-19 patients with moderate to severe ARDS were given a single dose of exosome (ExoFlo) intravenously over 60 minutes. The exosome was derived from bone marrow mesenchymal stem cells, and 15 mL exosome was suspended in 100 mL normal saline. There was no treatment related adverse event, and during the 14 days of evaluation, there were improvements in clinical outcomes and laboratory tests, which showed an improvement in neutrophil and lymphocyte counts, namely a statistically significant decrease in neutrophyl, and increases in CD3, CD4 and CD 8 counts. In addition, there was a significant reduction in inflammation marker C-reactive protein that might downregulate the cytokine storm. As MSCs may work via secretions of secretomes, the results of this study support the prospect of MSCs to mitigate cytokine storm in severe COVID-19[24].

The potensials of MSCs from various sources to cure ARDS

As studies on the use of MSCs for COVID-19 are very limited, while severe COVID-19 are very likely to develop ARDS, it is of high importance to explore studies on the potential of MSCs from various sources to alleviate ARDS.

Mensenchymal stem cells can be obtained from various tissues, namely bone marrow, adipose tissue, umbilical cord Wharton's jelly, umbilical cord blood, amniotic fluid, fetal membrane, placenta, endometrium, menstrual blood, dental pulp etc.[25]. Application of MSCs from several sources such as allogeneic bone marrow, menstrual blood, and allogeneic umbilical cord-derived mesenchymal stem cells have been used in patients suffering from ARDS[26-29].

Allogeneic bone marrow MSC application in ARDS

A phase-1 study used allogeneic bone marrow-derived MSCs with escalating doses to see the safety profile in nine patients with moderate to severe ARDS, who were in the intensive care units. The MSCs were obtained from healthy donors and given as a single dose intravenously. The doses were three escalating doses, which each was given to three patients, i.e. 1 million (low dose), 5 million (intermediate dose), and 10 million cells/kg body weight (high dose), respectively. The study showed that the low dose of 1 million cells/kg body weight showed no treatment related adverse event and was well-tolerated[26].

In a further phase 2a multi-center study, Matthay et al assessed the safety of the single high dose intravenous MSCs in patients with moderate to severe ARDS. Patients were randomized to receive either MSCs (40 patients) or placebo (20 patients). The MSC dose was 10 million cells/ kg body weight, and suspended in a volume of 100 mL. The MSCs were derived from bone marrow of three donors aged 18-45 years old. This study showed that there was no MSC related adverse events, and thus one dose of 10 million MSCs/ kg body weight was regarded safe[27].

Allogeneic menstrual blood MSC application in ARDS

A single center open-label study by Chen *et al*. used menstrual blood-derived MSCs for patients with ARDS due to H7N9 infection. In this study, the menstrual blood was obtained from a healthy female donor. The MSC dose per infusion was 1 million per kilogram body weight, which was suspended in 100 mL of vehicle. Patients were divided into three treatment and control groups. The first group consisted of three early stage, while the second and third groups consisted of six and eight late state H7N9 infection. The first and second treatment groups received three infusions, the third treatment group received four infusions, and control groups received standard treatment. This study showed that allogeneic menstrual blood-derived MSCs significantly reduced mortality compared to the control groups. In addition, there was no treatment related adverse event during 5 years of observation[28].

Allogeneic umbilical cord-derived MSC application in ARDS

A phase 1 study, which used umbilical cord derived MSCs (UC-MSCs) on moderate-severe ARDS patients, was conducted at a medical center in Kaohsiung, Taiwan. This study enrolled nine patients that were allocated into three groups of three patients. The three groups were given intravenous escalating doses of 1, 5 and 10 million UC-MSCs/kg body weight, respectively. The study indicates that up to a dose of ten million cells/kg showed excellent tolerance in ARDS patients with a medium to severe conditions[29].

CONCLUSIONS

Administration of MSCs to COVID-19 patients showed improvement in clinical symptoms, and a dose up to 10 million cells/ kg body weight showed excellent tolerance in moderate to severe ARDS patients. However, for efficacy, the current published study is limited to a small number of patients and unequality between treatment and controls, so the results need to be interpreted with caution, and more well-designed studies with larger number of patients are needed to get a more robust proof.

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