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Review Article

Potential Use of Umbilical Cord Mesenchymal Stem Cells for Improving Patients with COVID-19: A Review

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Abstract

The SARS-CoV-2 virus is a member of the coronavirus family that caused the COVID-19 respiratory disease epidemic in China before the global pandemic of the disease in late 2019. The virus's genome is of 79% similarity to that of the SARS-CoV virus, using the ACE2 receptor to enter its target cells. The most common symptoms of this disease include fever, cough, pulmonary involvement, and sometimes gastrointestinal symptoms. A decline in both the number and function of lymphocytes and a severe increase in leukocyte inflammatory activity are among the most obvious immunological complications of this disease. If the immune system response to the virus is inadequate, the disease can become acute. Immune cells activity leads to a sharp increase in the number of blood cytokines, causing "cytokine storm," which in turn can cause systematic damages to the heart, lungs, and kidneys, and ultimately may lead to death. Mesenchymal stem cell therapy offers a promising approach to reducing the destructive impacts of infection in patients with COVID-19. Mesenchymal stem cells can secrete immune-modulating factors that suppress cytokine storms. Furthermore, the role of mesenchymal stem cells in preventing cell death and inhibiting tissue fibrosis has been well demonstrated. This review shows available clinical trials that have tapped into the therapeutic potential of the umbilical cord mesenchymal stem cells in patients with COVID-19.

Keywords: Umbilical Cord; Mesenchymal Stem Cell; COVID-19; SARS-CoV-2; Immunomodulatory Effects; Clinical Trial.

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Introduction

In late December 2019, a case of unidentified pneumonia was reported in Wuhan City (Hubei Province, China) whose clinical manifestations were similar to those of viral pneumonia. While the World Health Organization (WHO) named the virus "COVID-19", the International Committee on Taxonomy of Viruses (ICTV) called it "SARS-CoV-2". This virus belongs to the β coronavirus family, which is prevalent in the environment and, like other viruses, has many potential natural hosts that can act as either intermediate or ultimate hosts (1, 2).

Coated with a single-stranded ribonucleic acid (RNA) genome, this virus uses a cellular receptor called angiotensin-converting enzyme (ACE2) to enter the target cells. In addition to multiplying in the upper respiratory tract efficiently, this coronavirus also tends to multiply in the cells located in the lower respiratory tract. As a result, lesions in the lower respiratory tract are bound to occur. Approximately 81% of the patients diagnosed with Covid-19 show

mild symptoms, and in 14% of the cases, the patient manifests severe symptoms, including pneumonia and shortness of breath. Moreover, in 5% of the cases, the patient condition worsens, which is associated with respiratory failure, Septic shock, and failure in other body organs (3). Due to the novelty of this virus amid human populations, new reports are being published daily regarding various aspects of its pathogenicity. Symptoms such as fever, cough, fatigue, muscle aches, joint pain, and shortness of breath have been reported as common clinically confirmed symptoms of the virus (3–6).

Extensive lung damage detectable in patients with MERS, Covid-19, and SARS infections leads to Acute Respiratory Distress Syndrome (ARDS) in some patients. Alterations in the balance of immune cells and uncontrolled production of various cytokines are the main causes of ARDS (7). Immunopathological studies have proved that the role of the acute inflammatory responses in cases of severe infections with respiratory viruses is associated with a high mortality rate. The migration of various types of innate immune cells into the respiratory tract triggers the cytokine storm process_ which is referred to as life-threatening, maximizing the severe immune system responses in the lungs (8). Recent studies have shown that macrophages, neutrophils, dendritic cells, innate immune receptors, and cytokines have a key role in lung tissue damage. Macrophages accumulate in lung tissue, producing proinflammatory cytokines that reduce the virus titers in the lung tissue. However, various studies have shown that following the infection with respiratory viruses, CCR2-expressing monocytes can play a destructive role and cause lung tissue damage (9,10). These cells are the principal source of TNF-α production in the infected lungs and therefore considered one of the major factors in the immunopathogenesis of disease and thus mortality rate. Even at low virus titers, their products hinder the recovery of pneumonia and can lead to death (11). Thus, what is vital in the pathogenesis of this disease is the immune system's response against the virus.

Stem cell-based therapies, particularly mesenchymal stem cells (MSCs), have been shown to have excellent potential to treat many diseases (12,13). MSCs have the function of regulating the immune system, known as immunomodulatory function, and affect both the innate and acquired immune system. A unique feature of these cells is their ability to suppress and moderate immune responses. MSCs have an inhibitory impact on various immune system cells such as T lymphocytes, B lymphocytes, natural killer cells (NK), and dendritic cells, thereby reducing and regulating immune responses (14). Furthermore, MSCs reduce the expression of MHC-II, CD11c, CD83, and co-stimulation molecules on monocytes (15), thereby decreasing pro-inflammatory cytokines_ TNF-α and IL-12 (16,17). In contrast, they increase the production of anti-inflammatory cytokines such as IL-10 on monocytes, which indicates the ability of these cells to inhibit dendritic cells. Besides, MSCs have a major impact on the innate immune system, particularly inhibiting natural killer cells. They exert their inhibitory function on NK cells via reducing the expression of NKG2D, NKP44, and NKP30 receptors on the surface of these cells. They also inhibit IFN-Y production. Consequently, the proliferation of NK cells is ultimately hindered (18,19).

The principal source of mesenchymal stem cells in the bone marrow, from which the cells are isolated and cultured. Yet, mesenchymal cells have recently been isolated from other sub-sources_ adipose tissue, placental tissue, peripheral blood, connective tissue, and skeletal muscle. These sources are far more accessible than bone marrow, and thus, mesenchymal cells are isolated with ease (20).

Therefore, due to the ability of MSCs to secrete anti-inflammatory, anti-fibrosis, and anti-apoptotic cytokines, these cells have been used to treat ARDS, which ultimately reduces the cytokine storm (21,22). This review article demonstrates the therapeutic potential of mesenchymal stem cells to modulate the immune system and available clinical trials that have used the therapeutic potential of umbilical cord mesenchymal stem cells in Patients with COVID-19.

COVID-19 diagnosis and immunopathology

Immunopathology

COVID-19 is a positive-strand RNA virus that can target both humans and animals (23). Patients with chronic respiratory and cardiac diseases, diabetes,

and malignancies are deemed high-risk groups (24). Fever, cough, fatigue, and myalgia are the most common symptoms in confirmed COVID-19 cases (25). SARS-COV-2 enters cells through the interaction of its spike (S) protein with angiotensin-converting enzyme-2 (ACE-2) after its activation by TMPRSS2 or furin proteases. ACE-2 and TMPRSS2 are markedly expressed on the heart, brain, and kidney (26).

COVID-19 has two major clinical phases. The first phase identifies with infecting target cells, causing severe clinical symptoms like pulmonary damage, and the so-called "cytokine storm" is the second phase identified by overexpression of several genes such as NF-kB, STAT-3, IL-6, IL-8, G-CSF (27). When the virus commences replication, even with a very low viral load, the inflammatory response rapidly expands, leading to organ failure (Fig1). Increased levels of IL1B, IL6, IFN$_{\gamma}$, and MCP1 in the blood of patients with severe lung damages highly suggest that cytokine storm has pertained to the severity of the disease (26). Whereas it appears that young patients have a balanced concentration of proinflammatory and anti-inflammatory cytokines, it is not the case for elderly patients. A balanced immune system can restrict the progression of the disease (28).

RT-PCR

Up to now, Real-time reverse transcription-polymerase chain reaction (RT-PCR) is the most sensitive and specific method for the diagnosis of covid-19 (29). Nonetheless, this method is costly, time-consuming, and has a 20% false-negative rate (30). The study of Wenling Wang et al. defined the most sensitive sample as Bronchoalveolar lavage (93% sensitivity), and in the case of SARS-Cov-2 detection, by RT-PCR, the most common targets are the ORF1ab/RdRp, E, N, and S genes (29).

CT scan

A diagnosis and treatment protocol published by the National Health Commission of China shows there are four severity levels for COVID-19 based on clinical manifestation: mild, moderate, severe, and critical disease. Respiratory factors, including oxygen saturation and progression in pulmonary imaging, are some criteria for classification (31). A study on acute lung injury of COVID-19 cases reported three diagnostic hallmarks_ persistent viral infection, dysfunction of endothelial, and presence of abnormal pneumocystis and syncytial giant cells. Moreover, some researchers have reported atypical bilateral ground-glass opacities in chest CT scans (26).

Serological tests

Serological tests measure host humoral immune response and can detect both waning and previous infection, thus they are a monitoring system for COVID-19 pandemic. these tests, however, cannot determine whether individuals are resistant or not (25). Tests based on antibodies (IgM and IgG) are of low sensitivity and specificity, but they are better means of screening than CT scans in the sense that the radiation is detrimental to health, and the costs are comparatively high (30). Rapid antigen detection (RAD) tests (the select method of this study) can detect COVID-19 antigen by immobilized coated SARS-COV-2 antibody (32).

Laboratory findings

The complete blood count (CBC) is a quick, inexpensive test that provides beneficial information_ count of WBCs, platelets, lymphocytes, as well as NLR (Neutrophil-to-Lymphocyte ratio), and PLR (Platelet-to-lymphocyte ratio) (33). In addition to decreased amounts of FDP, a significant decline in fibrinogen and antithrombin levels has recently been reported in non-survivor hospitalized patients. C-reactive protein is an acute-phase protein that increases in 75-93% of patients with severe COVID-19. Elevation of LDH enzyme is common in Patients with COVID-19, particularly patients in the ICU setting, indicating an unfavorable outcome. Lymphopenia (defined as an absolute count <10 × ten 9/L) is deemed an informative index in Patients with COVID-19. However, it is less prevalent in children (34). Several Interleukins, including IL-1β, IL-6, IL-2, IL-10, and TNFα have been shown to increase in the patients with severe COVID-19. Furthermore, some studies have shown that the increased D-dimer levels, as

well as decreased PLT counts, are highly linked with severe conditions and high mortality rates.
(31).

Treatment of COVID-19

Since, at this time, there are no approved treatments for COVID-19, prevention is considered crucial. However, some attributes of this virus such as non-specific features of the disease, the infectivity, transmission from even asymptomatic individuals, and long incubation period, make prevention somewhat arduous (35).

Drugs

Of all drugs, treatments are somewhat confined to Hydroxychloroquine. Hydroxychloroquine is an antimalarial drug whose antiviral impacts against COVID-19 have been shown. Its direct antiviral activity lies in increasing intracellular pH. Other mechanisms are the immune-modulating effect through inhibition of toll-like receptors signaling, decreasing inflammatory cytokines, and anti-thrombotic effects (36). It also functions as a novel class of autophagy inhibitors (37). Some drugs are available to control the proinflammatory cytokines, including "Inhibitor of Janus kinases" (i.e., Ruxolitinib), and "Monoclonal antibodies" (i.e., Tocilizumab). Tocilizumab is an anti-IL-6 monoclonal antibody binding to both soluble and membrane receptors of IL-6 (38). One of the possible anti-inflammatory tactics is "microRNA targeting" since microRNAs are deeply involved in the expression of cytokines (27).

CCP

Due to the prior success of convalescent plasma (CP) against RNA viruses, it appears sensible to utilize it against COVID-19. In addition to neutralizing the pathogen, CP can create passive immunomodulatory attributes. Thus, the World Health Organization (WHO) and the United States Food and Drug Administration (FDA) published informative guidelines as to how COVID CP (CCP) could be efficiently used and who the appropriate donors are. A new study demonstrated that CCP significantly improved oxygen saturation, elevated lymphocyte counts, and CRP levels within 14 days after treatment (39).

Vaccine

There are various vaccine platforms: protein-based, live attenuated, inactivated virus, and novel gene-based vaccines (GBVs) tactics such as nucleic acid and viral vector (40). The conserved regions are considered appropriate targets for neutralizing antibodies in the sense that they do not alter significantly over time, and thus this strategy could be useful for the development of a universal vaccine (38).

Mesenchymal stem cell-based therapies for inflammatory mediated disorders

MSCs are being studied as a promising and attractive cell-based treatment alternative to inflammatory disorders due to their immunomodulatory functions. Several clinical trials on MSC-based products are currently being conducted. The immunosuppressive properties of MSCs lie in the release of immunoregulatory molecules and cell-to-cell contact. MSCs can prevent the proliferation as well as the function of T cells, dendritic cells, B cells, and natural killer cells. They can also increase the proliferation of Treg cells. Furthermore, it has been shown that the immunomodulatory activities and viabilities of MSCs may increase by genetic modifications, preconditioning, and 3D-cultured systems. Nevertheless, due to the different problems clinical applications are somewhat restricted (41).
Considerable advances have been made in understanding the anti-inflammatory and immunomodulatory basic mechanisms of MSCs. In Vitro, studies have shown that MSCs may respond in opposite directions depending on the intensity of environmental signals. For example, they appear to promote inflammation when the immune system is suppressed and vice versa (42). In the primary stages of inflammation, MSCs activate pro-inflammatory signals through IL-1β and Toll-like receptors, tumor necrosis factor (TNF-α), and interferon (IFN-γ); hence, inflammation. These stem cells also boost T-cell activation by secreting some chemokines, including macrophage inflammatory protein-1, C-X-C motif ligand (CXCL9), CCL5, and CXCL10,

thereby recruiting more lymphocytes. In this stage, low levels of inflammatory signals such as TNF-α and IFN-γ, are enough to upregulate chemokine secretion, but not to induce a remarkable expression of immunomodulatory mediators (43).

In the final stages, high levels of pro-inflammatory factors_ IL-1β, IFN-γ, and TNF-α_ stimulates MSCs to reduce inflammation and prevents autoimmune reactions by releasing TGF-β, IL-10, IDO, or iNOS. Eventually, it leads to puberty, antigen presentation to DC and T cells, proliferation, and also inhibition of migration (44,45). Thus, the switch between the anti-inflammatory and pro-inflammatory states occurs by IDO or iNOS interventions (Fig2) (46).

Furthermore, it has been shown that, in in vitro conditions, MSCs can initiate immunosuppressive activities on activated B cells. They reduce B cell proliferation by stopping the cell cycle, affecting B cell differentiation. In addition, MSCs decrease the production of immunoglobulin IgM, IgA, and IgG (47).

Many studies have proved that MSCs have a major role in inducing regulatory B cells. Inflammation is eliminated by regulatory CD23+/CD43+ B cells treated with MSCs, for example (48,49). More recent evidence shows that human umbilical cord-derived MSCs, via CD5+ regulatory B cells, creates a defensive mechanism against colitis (50). In addition, MSCs suppress inflammation processes in various ways_ the down-regulation of pro-inflammatory factors, the up-regulation of anti-inflammatory factors, and perhaps direct cell contact suppression through immune reactions (51).

During lung injury, an unsuitable immune response or inappropriate repair process often results in irreversible lung tissue damage, causing fibrosis development followed by lung tissue malfunction. Anti-inflammatory drugs and inhaled corticosteroids appear highly efficient in patients with inflammatory lung disorders, but their long-term use is associated with severe side effects. Therefore, novel therapies that promote the regeneration of damaged alveolar epithelial cells as well as reduce inflammation are vital. MSCs can modulate the proliferation, activation, and effector function of each immune cell that plays a pivotal role in the pathogenesis of acute and chronic inflammatory lung diseases. Thus, to suppress lung-infiltrated immune cells, MSCs have the potential to change into alveolar epithelial cells in vitro and, therefore, represent new players in cell-based therapy for inflammatory lung disorders (52).

Clinical applications of umbilical cord mesenchymal stem cells for COVID-19 treatment

A guideline recently published by The Italian College of Anesthesia, Analgesia, Resuscitation and Intensive Care demonstrates the promising role of stem cells in treatment and early discharge of coronavirus patients, with a decreased number of hospitalized patients in intensive care units as a result of faster recovery (53). Thus, pharmacologic and supportive therapies aside, UC-MSCs seem to be the most potent strategy for COVID-19 treatment.

A good number of studies have been conducted into investigating the effectiveness of umbilical cord mesenchymal stem cells in COVID-19 cases. By way of illustration, findings of the research have demonstrated that Immunity-and-matrix-regulatory cells extracted from human embryonic stem cells have therapeutic impacts on mice with lung injury and fibrosis_ without any side effects. Because Immunity- and matrix-regulatory cells (IMRCs) have shown promising effectiveness without any side effects, they seem more beneficial, for the treatment of mice and monkeys, than both UCMSCs and the FDA-approved “pirfenidone” (54). Studies that have evaluated the efficacy of umbilical cord mesenchymal stem cells in Patients with COVID-19 are summarized in table 1.

In an experimental study (55), researchers randomly divided 41 severe Patients with COVID-19 into two groups: the standard treatment group and the standard treatment plus hUC-MSC infusion group. To assess the effectiveness of UC-MSCs for the treatment of severe COVID-19, researchers measured several items in both groups _ the incidence of progression from severe to a critical stage, mortality within 28 days, clinical symptoms improvement, the required time for clinical symptoms improvement, imaging, hematologic indicators, including C-reactive protein, lymphocyte count, and interleukin 6. The results showed that in the treatment group, no one neither turned into critical condition nor died, while in the control

group, the condition for 4 participants aggravated to the critical one so that they received invasive ventilation, 3 of whom passed away, and the 28-day mortality rate was 10.34%. Moreover, it took a long time for clinical manifestations to improve in the control group in comparison with the hUC-MSC treatment group. The improvement of some indices such as weakness and fatigue, shortness of breath, and low oxygen saturation was remarkable on the third and 7th days of stem cell infusion. Besides, from day 3 of infusion, CRP and IL-6 levels were significantly lower, the time for the lymphocyte count to return to normal was noticeably faster, and the uptake of lung inflammation, based on CT imaging, was considerably shorter in the hUC-MSC group than in the control group. Altogether, intravenous transplantation of hUC-MSCs seemed to be a safe and effective choice for treatment and saving the lives of Patients with COVID-19.

In a pilot study, 16 severe and critically severe COVID-19 pneumonia patients were recruited in a single-arm trial to receive transplantation of UC-MSCs during four rounds. The intervention lasts for four weeks, and at the end of the first, second, and fourth week following data were obtained: oxygenation index, inflammatory biomarkers, radiological presentations of the disease, and lymphocyte subsets count. In addition, any adverse event was recorded from the inception of the study. The findings indicated that oxygenation index, the radiological presentations (ground-glass opacity), as well as lymphocyte count and lymphocyte subsets (CD4+ T cells, CD8+ T cells, and NK cells), count ameliorated after the transplantation. No infusion-related or allergic reactions were reported during the study. The mortality rate of participants was considerably lower than historical mortality rates (6.25% and 45.4%, respectively). The above-mentioned findings showed that patients with severe and critically severe COVID-19 pneumonia might benefit from intravenous transplantation of UC-MSCs as a safe and feasible intervention (56).

At Taikang Tongji Hospital in Wuhan, China, physicians treated 31 severe Patients with COVID-19 with pneumonia using UC-MSCs. They found out UC-MSC can revitalize oxygenation and inhibit cytokine storm via down-regulatory effect (57), which was consistent with previous studies. Similar to UC-MSCs, bone marrow MSC can successfully inhibit cytokine storms and result in tissue repair and regeneration as well as hypoxia improvement, immune reconstitution, and cytokine storm inhibition (46,47).

In a phase 1/2a double-blind, randomized controlled trial, 24 COVID-19 ARDS patients were enrolled. ARDS severity was the basis of randomization, and the study was performed with the intent of designing a treat. In the UC-MSC treatment group, participants received $100 \pm 20 \times 10$ UC-MSCs on days 0 and 3. In the control group with equal samples, two infusions of vehicle solution were administrated. Results offered those infusions of UC-MSC might benefit Patients with COVID-19 suffering from ARDS. Significant reduction in Inflammatory cytokines on day 6, considerable improvement in patient survival (91% vs 42%, $P = .015$), SAE-free survival ($P = .008$), and time to recovery ($P = .03$) were observed in UC-MSC treatment group. Therefore, researchers concluded UC-MSC infusion is a safe and beneficial treatment for ARDS consequent to COVID-19.

In another study, researchers infused allogeneic human umbilical cord mesenchymal stem cells (UC-MSCs) to a critically ill male patient and monitored the treatment process as well as outcomes to decide whether UC-MSCs is an adjuvant treatment for COVID-19 or not. The patient was referred to the hospital, complaining of fever, cough, sputum, dyspnea, poor appetite, poor mental state, and fatigue. In the first days of admission, he was not diagnosed with COVID-19, and thus routine treatment for bacterial pneumonia was administrated. On February 3, the result of the nucleic acid test indicated that he had been infected with SARS-nCov-2019. The medical team decided to commence UC-MSCs transfusion. They monitored the patient for 14 days. On February 9, the SpO2 raised and reached 95%. 4 days later, the patient could independently breathe without a ventilator, and his O2 saturation remained within the normal range. On February 4, severe fibrosis in both lungs was observed via a CT scan. One week after UC-MSCs transfusion, infiltration decreased considerably and the absolute lymphocyte count was

unfavorable. Absolute Lymphocyte Count markedly increased following adjuvant UC-MSC therapy. It is hypothesized that UC-MSCs may have changed the immune system function, enhancing inflammatory reaction, the lung, and multiple organ functions, and as a result, critically ill patient's condition was improved. Based on these results, UC-MSC transfusion appears to be a novel treatment for patients suffering from COVID-19. However, more evidence-based practices are required to support the effectiveness of this method (58).

Laiang et al. monitored a patient complaining of fatigue, white foaming sputum, and fever with a body temperature of 38.2° (59). Being a passenger on a flight from Wuhan_ on January 21, 2020_ she was instantly referred to the nearby hospital, and the result of the real-time PCR showed she was infected with SARS-CoV-2. On day 11 (February 9), following severe injuries observed in the body organs, secondary to the inflammatory response and side effects, the medical team decided to begin hUCMSCs adoptive transfer therapy. After the first transfusion of hUCMSCs, the patient tolerated the treatment so well that no adverse effect was reported. Serum bilirubin, CRP, ALT, and AST concentrations steadily decreased_ inconsistent with improvement in several other vital signs. On the 17th day, the patient could breathe without a trachea cannula, and was able to stand by herself. The second transfusion returned the white blood cell and neutrophil counts to the normal range. After the transfusion of UCMSCs, clinical manifestations and indices like CD3+ T cell, CD4+ T cell, and CD8+ T cell ameliorated (60). Because MSCs can modulate immune responses, repair tissues, down-regulate pro-inflammatory cytokines and inhibit inflammation, it has the potential to be a beneficial treatment for Patients with COVID-19.

Therapeutic potential of UC-MSCs-derived exosomes in COVID-19

UC-MSCs-exosomes are an increasingly potential candidate for the treatment of ARDS and COVID-19. However, there have been few studies on the use of UC-MSCs-exosomes in the treatment of COVID-19. Preclinical studies have provided favorable therapeutic benefits for the exosomes derived from other sources of MSCs (61). Nevertheless, considering the source of MSCs, some controversial issues emerge as follow the donor and source; the dosage, the route of injection and the repetition number; the age of cells (young cells or cryopreserved cells); primary cells or expanded passages. In addition, the exosomes themselves are subject to controversy based on their bioactive constituents, capacity, mechanisms of interaction with the inflammatory immune cells and stromal cells in the damaged lung microenvironment.

Large-scale generation of UC-MSCs-exosomes needs many MSC cells and a massive culture medium, which is expensive. Therefore, further studies on biological facets of the UC-MSCs-exosomes would help alleviate the harmful effects of COVID-19 pandemic (62).

Challenges to use UC-MSCs

Clinical observations indicate the high potential of UC-MSCs in the treatment of disease COVID-19 disease. However, there are still some issues associated with the administration of MSCs. Methods of preparation, the number of MSCs, and the age of the cell population can surely lead to several functional differences. This variety might lie in the potential of cytokine liberation, cytokine content, and therapeutic ability. Even the difference in size, shape, and passage number of UC-MSCs or MSC-EVs may additionally lead to complex curative effects of this biological substrate. The expense and immediacy of sample preparation are also subjects that should be taken into consideration. Nevertheless, MSC-EVs seem to be much more affordable and more comfortable in large-scale production than their cells of origin (63).

Another principal challenge to using UC-MSCs therapy for COVID-19 cases is that COVID-19-infected patients are at the risk of hypercoagulation. In addition, MSCs have been shown to possess pro coagulation characteristics. Therefore, attention to nonintravascular administration routes for MSC or MSC-EV is encouraged to diminish the risk of COVID-19 cell therapy and increase the safety and efficacy of this type of therapy (64).

Table 1. Details of UC-MSC therapy and outcomes.

First Author	Country	Study Design	Details of UC-MSC Therapy	Outcome
Zhu et al. (58)	China	Case Report	Before the intravenous drip, UC-MSCs were suspended in 100 mL of normal saline, and the dosage was calculated by 1 × 106 cells per kilogram of wedight. The injection was performed within one hour, with an average speed of ~25 drops per minute. Antibiotic administration was terminated on the day UC-MSC was administered, and the rest of the treatment remained unchanged.	ALT, AST, BUN, CRP↓, the absolute number of lymphocytes, NK cells ↑
Feng et al. (56)	China	Pilot study	Before the intravenous drip, UC-MSCs were suspended in 50 mL of normal saline, and the total number of transplanted cells was 1×10^8 cells once. The patients were administered 4 times in total, with one-day intervals in between. The transplantation was performed within 1.5 hours with a speed of 30-60 drops per minute.	$CD4^+$ T cells, $CD8^+$ T cells, NK cells ↑, IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, CRP, PCT ↓
Shu et al. (55)	China	Clinical trial	Intravenous administration was used. Before the intravenous drip, the MSCs were suspended in 100 ml of normal saline and the total number of transplanted cells was calculated as 2×10^6 cells/kg. The infusion was into the patients' right cubital veins and lasted approximately 1 h (35 drops/min).	C-reactive Protein, IL-6 ↓, Lymphocyte number, oxygenation index ↑, Chest CT scans indicated that CT scores, the number of lobes involved, GGO, and consolidation was improved.
Lanzoni et al. (65)	the United States	Clinical trial	Subjects in the UC-MSC treatment group received two intravenous infusions of 100 ± 20 × 106 UC-MSCs each, in a 50 mL vehicle solution containing human serum albumin and heparin, infused over 10 ± 5 minutes, on days 0 and 3. Subjects in the control group (n = 12) received two infusions of 50 mL vehicle solution, on day 0 and day 3. Best standard care was provided for both groups regarding the current institutional COVID-19 guidelines.	IFNg, IL-6, and TNFa cytokines and RANTES chemokine. GM-CSF and PDGF-BB ↓ (only in the UC-MSC treatment group), SAE-free survival, time to recovery↓, patient survival↑
Guo et al. (57)	China	Clinical trial	UC-MSCs (1×10^6 cells per kilogram of weight) were suspended in 100 ml normal saline and transfused during three steps according to the patients' condition.	Lymphocyte count, PaO2/FiO2↑, Interleukin-6, Procalcitonin, C-reactive protein, White blood cell count, D-dimer ↓
Liang et al. (59)	China	Case report	The allogenic hUCMSCs were administrated intravenously 3 times ($5*10^7$ cells each time) on days 13, 16, and 19. During the treatment, antibiotics were given to prevent infection. thymosin α1 was also given.	counts of $CD3^+$T cell, $CD4^+$ T cell, and $CD8^+$ T cell ↑, neutrophil-to-lymphocyte ratio, D-dimer, serum bilirubin, white blood cell count, neutrophil count, CRP, ALT, and AST (after the second administration) ↓, pulmonary inflammatory reaction: ameliorated (based on chest CT images)

PCT: procalcitonin; AST: aspartate aminotransferase; ALT: alanine transaminase; BUN: blood urea nitrogen; NK: natural killer cells; GGO: Ground-glass opacity; GM-CSF: granulocyte-macrophage colony-stimulating factor; PDGF: platelet-derived growth factor; RANTES: regulated on activation, normal T cell expressed and secreted; IL: interleukin; SAE: serious adverse events; IFNg: Interferon gamma; UC-MSCs: umbilical cord mesenchymal stem cells; PaO2/FiO2 ratio: ratio of the partial pressure of arterial oxygen to the percentage of inspired oxygen.

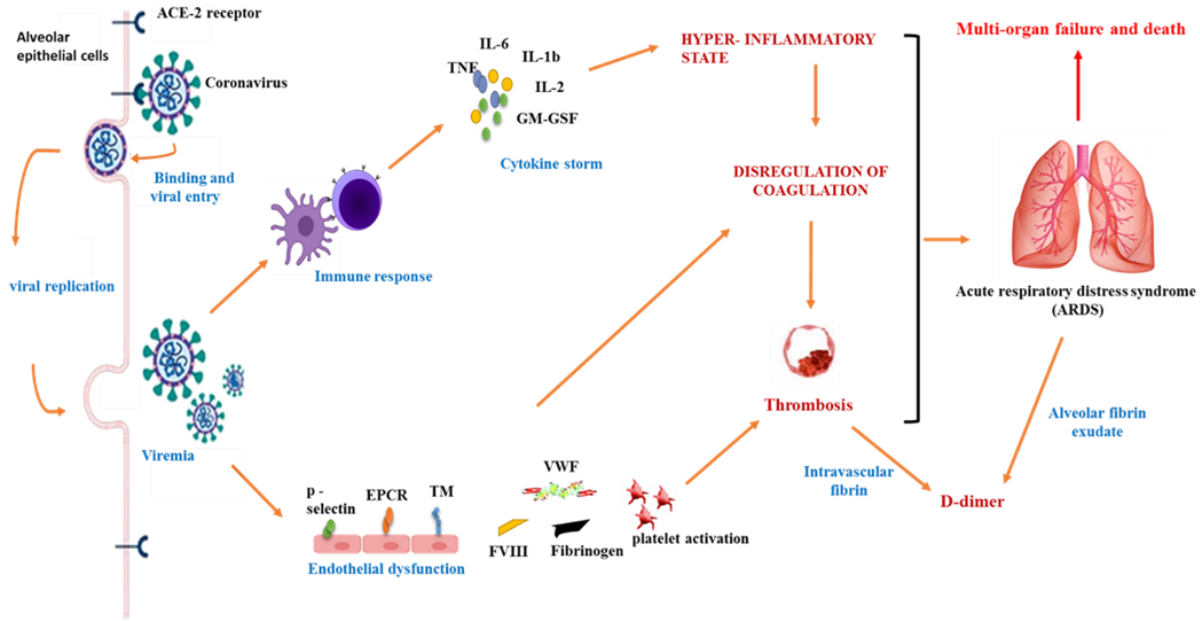

Figure 1. Angiotensin-converting enzyme 2 receptor is expressed on respiratory epithelial cells and endothelial cells. SARS-CoV-2 enters cells via binding to this enzyme. Viral replication disturbs inflammation and coagulation, eventually resulting in multiple organ failures.

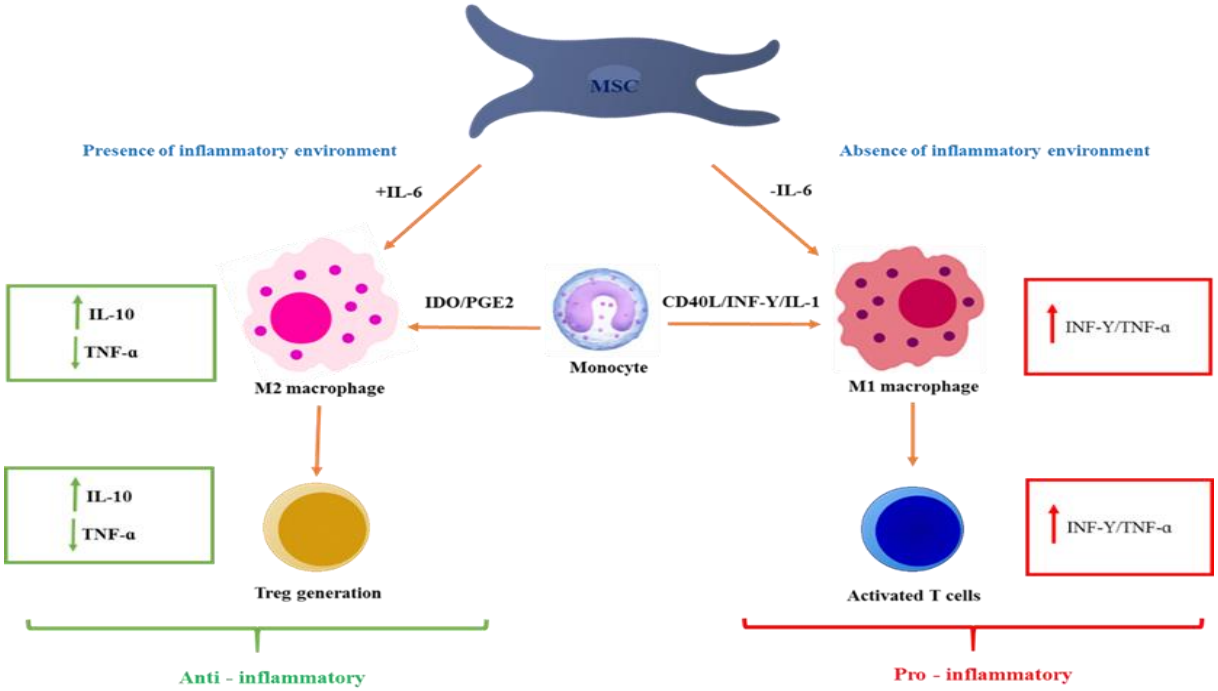

Figure 2. Histological evaluation of liver sections of normal rats treated with saline (control) or 700mg/kg of N-acetylcysteine (NAC), stained with Masson's Trichrome technique. 100x magnification of the liver sections showing the lobular central veins, the portal spaces, the normal sinusoidal radial branches, and the normal structure of the liver in both control and NAC groups. The portal space histology in control group indicated the presence of a small connective tissue and a very small number of mononuclear cells. Minimum connective tissue without any infiltration observed in the portal triad of NAC group. The lobular central vein of control and NAC groups indicating normal structure with normal sinusoidal space, without any signs of fibrosis.

Conclusion

COVID-19 disease is a global crisis, especially for healthcare workers, with a broad range of adverse effects which involve chronically ill patients who are non-responsive and unable to benefit from traditional therapies. Owing to the evidence obtained from preclinical and preliminary studies, UCS-MCS has anti-inflammatory and immunomodulatory functions, and thus, it can improve the recovery time as well as damaged tissues (53). Previous studies have suggested that the primary organ invaded by coronavirus is the lung, thus administering UCS-MCS into the lung may be beneficial to patients. In conclusion, it seems that UCS-MCS therapy, as an almost cost-effective and non-invasive intervention, might enhance the treatment of critically ill Patients with COVID-19.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

All authors have read the journal's policy on disclosure of potential conflicts of interest and a statement that all authors have disclosed any financial or personal relationship with organizations that could potentially be perceived as influencing the described research.

Abbreviations

ARDS: Acute respiratory distress syndrome
ACE2: Angiotensin-converting enzyme
CP: Convalescent plasma
CCP: COVID CP
CBC: Complete blood count
GBVs: Gene-based vaccines
ICTV: International Committee on Taxonomy of Viruses
NLR: Neutrophil-to-Lymphocyte ratio
PLR: Platelet-to-lymphocyte ratio
IFN: Interferon
IL: Interleukin
IV: Intravenous injection
MCP1: Monocyte chemotactic protein 1
MHC: Major histocompatibility complex
MSCs: Mesenchymal stem cells
UC-MSCs: Umbilical cord mesenchymal stem cells
hUCMSCs: Human umbilical cord mesenchymal stem cells
NK cell: Natural killer cell
NO: Nitric oxide
RT-PCR: Real-time reverse transcription-polymerase chain reaction
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
TGF-β: Transforming growth factor-β
TNF-α: Tumor necrosis factor-α
WHO: World Health Organization

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