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Case Series

Exosomes as a Preventive for COVID-19: Case Series

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Abstract

Coronavirus disease 2019 (COVID-19) infects human lung epithelium, initiating an inflammatory immune cascade that can cause pneumonitis and pneumonia. Progressive endotheliitis in multiple organs can lead to the often lethal systemic acute respiratory syndrome. This case series report demonstrates short term safety of administering exosomes as a potential preventive therapy to both healthy and at risk patients.

Keywords: *Regenerative Medicine; Preventative Medicine; Covid-19; SARS-CoV-2; Exosomes*

Abbreviations

SARS-CoV-2: Coronavirus Causing Systemic Acute Respiratory Syndrome; COVID-19: Coronavirus Disease 2019, FGF2: Fibroblast Growth Factor 2; GCSF: Granulocyte-Colony Stimulating Factor; GMCSF: Granulocyte-Macrophage Colony-Stimulating Factor; IFNγ: Interferon Gamma; IP10: IFNγ-Inducible Protein; MCP1: Monocyte Chemoattractant Protein-1; MIP1α or CCL3 and MIP1β or CCL4: Macrophage Inflammatory Proteins; PDGFB: Platelet Derived Growth Factor Subunit B; TNFα: Tumor Necrosis Factor Alpha; VEGFA: Vascular Endothelial Growth Factor A; MSCs: Mesenchymal Stem Cells; EV: Extracellular Vesicles; FDA: Food and Drug Administration; ASCs: Antibody-Secreting Cells; Ig: Immunoglobulin

Introduction

The first reports of a novel pneumonia in Wuhan, Hubei province, China in December 2019 were caused by a coronavirus causing systemic acute respiratory syndrome (SARS), SARS-CoV-2 [1,2]. The subsequent outbreaks were declared the COVID-19 pandemic by the World Health Organization on March 11, 2020 [1,2].

COVID-19 has been anticipated to be highly transmissible with the onset of symptoms within 14 days from exposure. Symptoms may include, but are not limited to, sore throat, dyspnea, body aches, anosmia, viral pneumonia and SARS [2]. Activated T cells appear to be critical for viral clearance and they may not be produced and circulating until 7 days into the course of the illness [3,4]. Some risk factors that are associated with more severe disease include older age, diabetes, malignancy, chronic lung conditions, and surgery [2].

SARS-CoV-2 infects through the host ACE2 receptors in the epithelial alveolar lining and other tissues including the vascular endothelial lining, disrupting the vascular beds of several organs. The subsequent inflammatory immune cascade causes pneumonitis and

pneumonia as well as progressive endotheliitis in other organ systems [5]. The pathophysiology of severe COVID-19 exhibits significantly high levels of inflammation on laboratory studies. Pro-inflammatory cytokines and chemokines included interleukins, basic FGF2, GCSF, GMCSF, IFNγ, IP10, MCP1, MIP1α, MIP1β, PDGFB, TNFα, and VEGFA; C-reactive protein, erythrocyte sedimentation rate**,** D-dimer; ground-glass opacities on lung CT [3]. SARS-CoV-2 RNAemia was detectable in a fraction of COVID-19 cases [6]. Cytokine storm and chemokines are predictive of severe clinical outcomes [4]. People who have chronic inflammatory conditions or cannot meet the metabolic needs for managing the inflammation found in COVID-19 may be more disposed to SARS. No preventative therapies have been established.

Mesenchymal stem cells (MSCs) possess strong immunomodulatory ability [7]. Prior studies found that MSCs produce extracellular vesicles (EV) containing immunomodulatory molecules, including indoleamine 2,3-dioxygenase, nitric oxide, prostaglandin E2, transforming growth factor, haem oxygenase 1, leukemia inhibitory factor, programmed death-ligand 1, hepatocyte growth factor, and galectins [7]. These function as paracrine effectors. They regulate T and B cells and have strong anti-inflammatory potential. Exosomes, a type of EV, also contain lipid-based molecules and nucleic acids. The nucleic acid content is largely mRNAs and miRNAs. The vesicular contents, including the RNAs, enter the recipient cells by endocytosis, ligand-receptor binding, or direct binding. The RNAs have been found to affect protein expression in recipient cells [7]. The anti-inflammatory potential included reducing levels of pro-inflammatory cytokines and promoting mechanisms of anti-oxidation and anti-apoptosis [7]. The source and preparation of MSCs and EV varies. One preparation method includes a conditioning process to induce expression of and/or allow for selection of molecular defenses to a particular virus.

Human studies have used amniotic fluid, umbilical cord, muscle, blood, and urine-derived MSCs and non-nucleated EV such as exosomes. Exosome vesicles are 30 - 150 nm in diameter. They circulate in body fluids to mediate cellular responses without inducing foreign-body-immune responses. MSC non-cellular contents have been used to treat ischemic and toxic injuries to organs, promote connective tissue and bone regeneration, enhance myogenesis and angiogenesis, and reduce inflammation [7,8]. Full-term amniotic fluid-derived nonnucleated EV appear to also maintain other factors that promote host defenses against infection [8]. The potential for amniotic fluid-derived exosomes to prevent COVID-19 has not been studied in humans, however, prior studies have shown that exosomes relieved hyperoxia-associated inflammation, bronchopulmonary dysplasia, pulmonary hypertension, fibrosis, and pulmonary vascular remodeling in rodent lung [7]. Recent pilot studies found that human elderly, critically ill COVID-19 patients with pneumonia benefitted by intravenous infusion of non-conditioned human umbilical cord MSCs [9,10].

Advantages of using EV, or exosomes, rather than whole cell therapy are non-invasive isolation from donor patients, decreased allergic reactions, and less pre-treatment laboratory testing. Minimizing processing of the exosomes provides the widest array of molecular content [8]. Preparations that use sterile filtration without diluents appear to maintain more natural molecular machinery than those using radiation or synthetic cryoprotectant [8]. The objective of this report was to demonstrate short term safety of exosomal therapy in the prevention of COVID-19 morbidity and mortality.

Cases

In this case series, patients who expressed a desire for preventive treatment to avoid SARS and reduce mortality received treatment. Some had chronic conditions and risk factors for poor recovery from respiratory infections or potential sick contacts. There were 22 patients, ages 18 - 81 years (mean 50 ± 18 yrs.) without signs of acute COVID-19 illness at the time of treatment (Table 1). Their medical histories between November 1, 2019 and exosome treatment, March 19 to April 21, 2020, were reviewed. Nine patients had had a moderate to severe upper respiratory infection without hospitalizations. Nine patients were healthcare workers. Five had international travel. Four had been to New York City. Past medical histories included pulmonary conditions (fibrosis 1, pneumonia 4, asthma 2, neuromuscular restrictive airway 1, smoke exposure 1), heme/immune conditions (other severe or persistent infections 3, chronic fatigue syndrome 2, porphyria cutaneous 1), other conditions including prostate cancer 1, gallstones and pancreatitis 1, and Ehlers Danlos Syndrome 1. One patient was seven months pregnant. None of the patients had any vaccinations within the prior 48 months.

		% (#patients)
Sex	Male	36% (8)
	Female	64% (14)
Race/ethnicity	White	86% (19)
	White and Hispanic	9% (2)
	West Asian and Hispanic	5% (1)
Social History	Smoked Tobacco	18% (4)
	Works in Healthcare	41% (9)

Table 1: *Patient demographics.*

Materials and Methods

De-identified patient information was collected back to November 1, 2019 for this case series. While retrospective accounts of possible SARS-CoV-2 infections are still being discovered, the first case in China was traced back to November 17, 2019 [1]. Currently, voluntarily donated amniotic fluid prepared according to the US Food and Drug Administration (FDA) is available for homologous use. Organicell™ Flow (Organicell Regenerative Medicine, inc. Miami, FL) is procured and processed in the United States according to standards and regulations established by the FDA. It contains approximately 400 billion extracellular, nonnucleated vesicles per milliliter obtained from amniotic fluid voluntarily donated after Cesarean section delivery [11]. Donors were tested for infectious disease by laboratories certified by Clinical Laboratory Improvement Amendment of 1988 and American Association of Blood Banks. Minimal manipulation was used during preparation under cGMP standards. Preparation included sterile filtration without radiation or synthetic cryoprotectant or dilutants. There was endotoxin and USP <71> testing on all lots. The acellular, nonnucleated, exosomal fluid was not expanded, cultured or preconditioned. Each milliliter contains cytokines, chemokines, growth factors, extracellular vesicles and hyaluronic acid [11].

Full-term amniotic fluid, including its exosomes, contains anti-inflammatory and anti-infection cytokines and chemokines, proteins involved in regulating apoptosis, and other factors to promote host defenses against infection [8]. Other proteins commonly found in exosomes include tetraspanin family (scaffolding membrane) proteins, membrane transporters and fusion proteins, heat shock proteins, multivesicular body biogenesis proteins, lipid-related proteins, and phospholipases [7]. The lipid bilayer membranes and contents may contain large amounts of cholesterol, sphingomyelin, and ceramide as well as phosphatidylserine [7]. In addition, full-term amniotic fluid has growth factors and structural components for healing and growing tissue [8]. Data repositories and databases of exosomal contents are available for further information (ExoCarta, EVpedia) [7].

All patients were without difficulty breathing, clubbing, cyanosis, fever, or edema. Three 0.17 ml intravenous infusions of exosomes (Organicell™Flow) via antecubital intravenous catheter followed by a 5 ml flush of normal saline were administered 2 minutes apart. Patients were monitored for side effects for 2 hours post injection. Patients were instructed to call and report any reactions at 2 and 4 weeks. Out-patient monitoring for side effects, adverse effects, and development of respiratory infections since infusion continued for eight months.

Results and Discussion

The only complaint reported following treatment was fatigue in six patients, two of which had a history of chronic fatigue syndrome. No infusion-site reactions occurred. There were no adverse events. There was one 18 year old white female patient who tested positive for COVID-19 while living on her college campus six months after infusion and developed mild symptoms of sore throat for three days and fatigue. She was not hospitalized. There was one 20 year old white male patient who tested positive for COVID-19 while living on his col-

lege campus seven months after infusion and developed mild symptoms of upper respiratory infection and anosmia for two days. There were no other new illnesses.

The potential role for amniotic fluid exosomes in early adaptive immune responses and recovery from infectious injury could be investigated as a preventative measure during SARS outbreaks. There are three strains of coronavirus that can cause severe disease (SARS-CoV-2, SARS-CoV, and MERSCoV) and other strains that typically only cause mild disease (HKU1, NL63, OC43, and 229E) in humans [1]. Coronaviruses are most susceptible to genomic mutations, insertions and deletions, near the S1-S2 junction leading to a natural evolution. The s region of viral RNA encodes the spike glycoprotein, or S protein, which plays a major role in determining if the virus can infect a host, the host response and the susceptible species [1]. With rapidly evolving strains of coronavirus, vaccines developed on a yearly basis will likely have low effectiveness for the immediate infections. Preventive treatments such as exosomes could prove useful in addressing the regular mutations that vaccination programs miss. As well, exosomes can attenuate the immune system cascade, thereby resisting the progression of infection into SARS.

The human immune response to COVID-19 demonstrated increased blood levels of antibody-secreting cells (ASCs), activated follicular helper T cells, activated CD4+ T cells and CD8+ T cells and immunoglobulin M (IgM) and IgG antibodies that bound to SARS-CoV-2 [4]. The ASCs and activated T cells critical for viral clearance produced greater amounts of granzymes and perforin than non-activated or healthy controls [4]. Perforin assists granzymes in inducing apoptosis of virus-infected and transformed cells. Studies have found marked increases in pro-inflammatory cytokines and chemokines predictive of severe clinical outcomes in SARS [3,4,6].

The BNT162b2 vaccine, which was recently granted an emergency-use authorization and the candidate RNA vaccine of US clinical trial (NCT04368728), BNT162b1, are lipid nanoparticle formulated messenger RNAs [12,13]. BNT162b1 encodes the receptor-binding domain of the S protein [13]. The vaccine incorporates 1-methyl-pseudouridine to dampen innate immune sensing, increase stability, and increase translation. Studies of BNT162b1 and BNT162b2 demonstrated side effects of mild-to-moderate injection-site pain, redness or swelling reactions [12,13]. The systemic events in BNT162b1-recipients were mostly flu-like symptoms [13]. The systemic events in BNT162b2-recipients were most commonly fatigue and headache (approximately 35% and 27%, respectively, moreso in vaccine recipients than placebo controls) [12]. Transient fever was also reported. There were 15% more adverse events reported by vaccine recipients than placebo controls. Long-term effects and effects on the ACE2 receptor have not yet been shown. Prior to 2020, there was no vaccine demonstrating effectiveness in preventing any coronavirus infection despite decades of research. However, there is evidence suggesting a 36% increased risk for coronavirus infection following vaccination with inactivated influenza [14]. The report showed a significant association of coronavirus and metapneumovirus infections in individuals receiving the influenza vaccine rather than the less-specific immunity associated with natural infection [14]. The US FluVaxView reports that 38.8% - 45.3% adults, including 38.8% - 47.9% individuals 18 - 64 years old at high risk annually received at least one flu vaccine dose during 2010 to 2019 [15]. The CDC reports that flu vaccine effectiveness was 19 - 60% among vaccinated adults in the US [16]. Populations not vaccinated with influenza vaccine may have different risk levels for infection and complications from SARS-CoV-2. The vaccination status of the patients in this case series limits the generalizability of exosome use, though, it incorporates the natural immune response and the majority of US citizens that forego yearly flu vaccination.

Conclusion

The use of human exosome therapy offers a safe and effective preventive treatment approach to COVID-19 and sequelae. The ameliorative processes of exosomes would especially prove useful while, or if, a vaccine is properly and safely developed. In the population segment where a vaccine is not safe or declined after informed consent, exosome therapy could fill this treatment gap. Studies with larger sample sizes and longer monitoring are needed to further support human exosomes as preventive and corrective therapies.

Conflict of Interest

All data generated or analyzed during this study are included in this published article. One of the authors holds investments in pharmaceutical and other healthcare related companies both as individual stocks and within large funds, as well as, purchased BPSR stock after the case series was completed. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

Damon Whitfield, DO, MPH Study conception; Jayme D. Mancini, DO, Ph.D - Literature review; Damon Whitfield, DO, MPH and Jayme D. Mancini, DO, Ph.D - Manuscript preparation.

Bibliography

1. Andersen KG., *et al*. "The proximal origin of SARS-CoV-2". *Nature Medicine* 26 (2020): 450-452.
2. Beeching NJ., *et al*. "Coronavirus disease 2019 (COVID-19)". *BMJ Best Practice* (2020).
3. Rothana HA., *et al*. "The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak". *Journal of Autoimmunity* 109 (2020): 102433.
4. Thevarajan I., *et al*. "Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19". *Nature Medicine* 26 (2020): 453-455.
5. Varga Z., *et al*. "Endothelial cell infection and endotheliitis in COVID-19". *Lancet* 395 (2020): 1417-1418.
6. Chen X., *et al*. "Detectable serum SARS-CoV-2 viral load (RNAaemia) is closely associated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients". *Med Rxiv Posted* (2020).
7. Joo HS., *et al*. "Current knowledge and future perspectives on mesenchymal stem cell-derived exosomes as a new therapeutic agent". *International Journal of Molecular Sciences* 21 (2020): 727.
8. Pierce J., *et al*. "Collection and characterization of amniotic fluid from scheduled C-section deliveries". *Cell Tissue Bank* 17 (2016): 413-425.
9. Leng Z., *et al*. "Transplantation of ACE2- mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia". *Aging Disease* 11.2 (2020): 216-228.
10. Liang B., *et al*. "Clinical remission of a critically ill COVID-19 patient treated by human umbilical cord mesenchymal stem cells". *China Xiv* (2020).
11. https://organicell.com/organicell-flow/
12. Polack FP., *et al*. "Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine". *The New England Journal of Medicine* (2020).
13. Sahin U., *et al*. "Concurrent human antibody and 1 TH1 type T-cell responses elicited by a COVID-19 RNA vaccine". *Med Rxiv Preprint* (2020).
14. Wolff GG. "Influenza vaccination and respiratory virus interference among Department of Defense personnel during the 2017-2018 influenza season". *Vaccine* 38 (2020): 350-354.

15. CDC Flu Vax View.

16. CDC.

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