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MSC-Exosomes Carrying miRNA – Could they Enhance Tocilizumab Activity in Neuropathology of COVID-19?

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Introduction

It is known that the central nervous system (CNS) is affected by viral infections. The Coronavirus disease 2019 (COVID-19) is a systemic disease that compromises the primary site of infection, the lungs, and then spreads throughout the body, including the CNS [1, 2]. Like other organs, the CNS is affected by the overproduction of pro-inflammatory cytokines and chemokines [3] and not only severe patients suffer from neurologic disturbances. Milder cases have shown that these patients also manifest headache, anosmia, nausea, and vomiting, gray matter loss and abnormalities in the brain after COVID-19 recover [4, 5]. Recent findings suggest that the blood-brain and blood-cerebrospinal fluid (CSF) barriers are disrupted in severe COVID-19 patients and this allows the infiltration of infected leukocytes and IL-6 inside the brain interstitial system [6, 7]. Also, CSF lumbar punction samples indicated an increase in the CSF/serum ratio for albumin (QAlb), suggesting a dysfunction of the blood-cerebrospinal fluid barrier (BCB) in around of 50% collected samples [8].

Interleukin-6 has been suggested as a predictive biomarker of severity and progression of this disease [9–12]. Both detrimental and beneficial functions of this cytokine have been reported. When compared to other cytokines, IL-6 remains for a longer period in the blood circulation, causing organ damage but also becoming a potential target for new therapies [13]. Opinions diverge about the occurrence of the "cytokine storm" in COVID-19 and the presence of the high levels of IL-6 in comparison to other respiratory diseases [14]. Italian researchers reported that COVID-19 patients treated with one infusion of anti-IL-6 receptor Tocilizumab survived more than those patients who didn't and this was correlated with IL-6 serum levels [15]. Then, many clinical trials testing blockers against the IL-6R, such as Tocilizumab and Sarilumab, in COVID-19 patients, are currently completed or ongoing. Among them, the large and randomized clinical trials, REMAP-CAP, RECOVERY, and PROSPERO showed an improvement in survival rate and patients' clinical outcomes when they were treated with IL-6R blockers [16–18].

The mesenchymal stem cells (MSCs) can be isolated from different adult tissues and they have the capability of modulating other cells, like immune cells, to promote anti-inflammatory activity and tissue regeneration for clinical applications [19]. Several clinical trials evaluating the efficacy and safety of the MSCs, such as the START study in COVID-19, and other respiratory distress, were registered and interventional uses were reported [20–23]. In example, a double-blind, phase 1/2a, randomized, controlled trial was performed by Lanzoni et al., where patients suffering with acute respiratory distress syndrome (ARDS) induced by COVID-19 (n = 12) were treated with two infusions of umbilical cord MSCs (UC-MSCs). The endpoints of the study showed that the cell therapy infusions were safe and no adverse effects was registered. The inflammatory biomarkers, including the IL-6, decreased its levels after 6 days of the first cell infusion, leading to a positive prognosis for the patients [24]. In this line, a case report made by Senegaglia et al., showed an innovative combination, in alternate days, of these two potential therapies, Tocilizumab and UC-MSCs. They treated a severe COVID-19 patient admitted to the intensive care unit, showing an improvement of the patient's clinical outcome resulting in survival, faster recovery, and clinical discharge [25].

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Although it was only one patient that received the combination of treatments, the authors opened a new and promising investigation path.

Despite the mesenchymal stem cells being an alternative treatment for cell therapy, they have some disadvantages for the clinical use. Some of these are their size, their differentiation into other cell lines, tumorgenicity capacity, be trapped in the lungs vascularization, the incapacity to cross the blood brain barrier (BBB) and others [26]. Therefore, elements comprised in the secretome released by the MSCs such as the extracellular vesicles (EVs) could be an alternative. The EVs group are comprised by the exosomes, microvesicles and apoptotic bodies and they are released by the parental cells carrying specific cargos and they differ regarding their biogenesis. Here, we focus on the exosomes, the smallest type of EV, with 30-150 nm, formed in the endolysosomal pathway, and with similar activities as their parental cells. They encapsulate proteins, lipids, miRNAs, mRNA, and other important molecules used for communication between cells [27, 28]. In spite of most studies investigate the benefit of the MSCs and their exosomes in the lungs, due to their nanosized and lipophilic structure, the exosomes could also be used to reach and treat the symptoms in the CNS of COVID-19 patients, thanks to their ability to cross the BBB.

Furthermore, Tocilizumab, a large neutralizing monoclonal antibody (mAb), has a minimal crossing capacity through the natural barriers in hemostatic conditions [29]. In pathological conditions, the patients have a disrupted barrier that could allow the anti-IL-6R to cross it and reach the CNS microenvironment. However, as aforementioned, it is not all barriers that are disrupted in COVID-19, therefore, not every patient would beneficiate of this therapy in the brain [8]. Likewise, it is known that after the first infusion of Tocilizumab, there is an elevation of circulating IL-6 levels due to saturation of the IL-6 receptors by the mAb [30]. This transient elevation of IL-6 has been associated in Chimeric Antigen Receptor (CAR) neuro-toxicity, which can occurs independently from Cytokine Release Syndrome (CRS) [31]. Thus, a second infusion of Tocilizumab might be hazardous for the CNS, although in ARDS patients this therapeutic scheme proved to be safe [24]. Consequently, due to their nanosized diameter and their lipophilic bilayer structure, the exosomes can easily cross the natural barriers and attenuate neurologic symptoms, by acting in resident immune cells, such as astrocytes and microglial cells, which are activated by the IL-6. Meaning another advantage of using the MSC-exosomes in COVID-19 patients with neurologic symptoms. Yet, it was suggested that the IL-6 could be used as a biomarker of long-term neuropsychiatric symptoms, such as fatigue, depression, and anxiety in COVID-19 survivors, usually classified with long COVID-19 or post-COVID-19 syndrome [32, 33]. Therefore, the use of IL-6/IL-6R blockers in association with MSCs-exosomes, on these patients could also attenuate these late symptoms and sequelae [34].

MicroRNAs (miRNAs) are non-coding RNAs and are associated with post-transcriptional gene repression or degradation [35] and it is well known that IL-6 is modulated by miRNAs at multiple levels. Previously, we performed the prediction of the miRNAs targeting the cytokines involved in the PANoptosis pathway [36] and genes of coagulation cascades, present in severe COVID-19 patients, with ARDS only for a 3′ untranslated region (3'UTR) binding site [37]. For that, we analyzed 4 available datasets of MSCs-derived extracellular vesicles data from different tissues and found an overlay of 58 miRNAs, which indicate that MSC-derived EVs from different tissues share a common cargo. Considering that the IL-6 has been used as a biomarker of severity and mortality in COVID-19, we can infer that targeting the IL-6 complex (*IL6, IL6R,* and *IL6ST)*, not only in the 3'UTR, but also in the coding sequence (CDS) and 5′ untranslated region (5'UTR), would increase its effectivity. Targeting these three regions could increase the efficacy of degradation and stop the translating actions of proteins through a perfect or imperfect base-pairing in the regions 5'UTR, CDS, or 3'UTR of the mRNA [37]. In this line, our intention is to reinforce the potential use of the MSC-exosomes containing miRNAs for COVID-19 as monotherapy, or in combination with other treatments, like Tocilizumab, enhancing their effects against COVID-19 as proposed in Fig. 1.

There are a few successful case reports of patients treated with Tocilizumab [38], MSCs [39], or as performed by Senegaglia et al., a combination of both [25]. Despite of we have the evaluation of these approaches as monotherapy, there is no study evaluating the combination of these therapies in the CNS deeply. Notwithstanding the intravenous administration (IV) of the MSC-derived exosomes allow their distribution systemically, the intranasal administration (IN) of the MSC-exosomes may be an alternative route to be considered. This is a shorter route to the CNS, and the exosomes would arrive faster to the primary site of infection, the respiratory system [40]. Accordingly, clinical trials are evaluating this administration method and are registered on clinicaltrials.gov and positive results are available.

As mentioned above, the MSC-exosomes carry several molecules in addition to miRNAs, which characterize their cargo as a heterogeneous material. Thus, the miRNAs and other elements present in their inner core can modulate multiple targets besides the *IL6* complex. The diversity of modulators presented inside this biological product can be an advantage in comparison to therapies focusing on a single target anti-IL-6R. Moreover, besides the benefits of the MSC-exosomes promoting immunomodulatory effects to the lungs and the heart [41], leading to attenuation of fibrosis and tissue damage, this therapy could also be useful

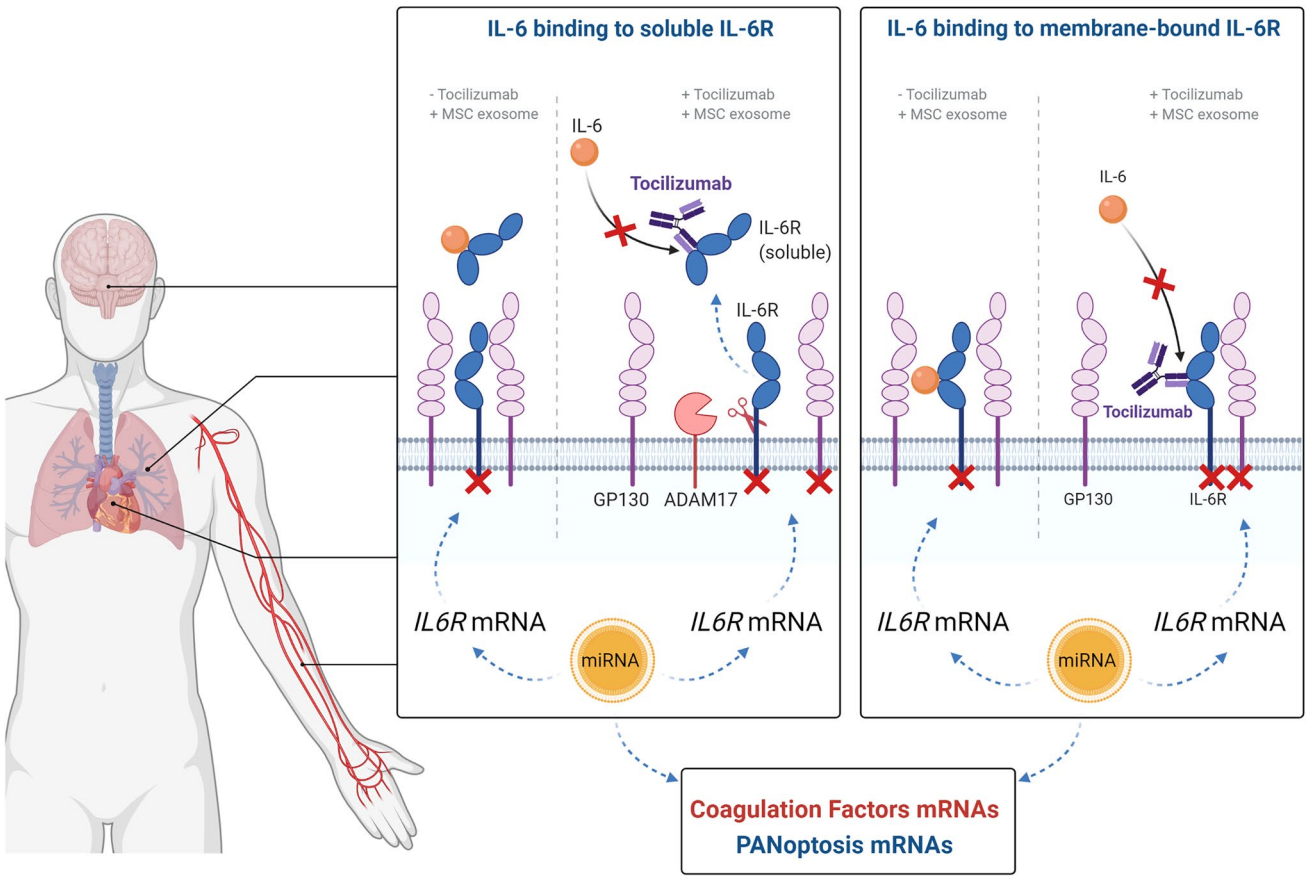

Fig. 1 Mechanism of action of the monoclonal antibody anti-IL-6R (Tocilizumab) and the mesenchymal stem cells derived exosomes carrying microRNAs (miRNAs). The neutralizing antibody Tocilizumab attach and inhibit the activation of the signaling pathway started by the IL-6 reducing the patient inflammatory state. Due to their nano-sized diameter, the exosomes can cross the central nervous system natural barriers (BBB and CSF) and release the small and non-coding miRNAs inside the target cell cytoplasm. The miRNAs perform a post-transcriptional activity by binding to the IL-6 complex mRNAs (*IL6, IL6R and IL6ST*) through perfect and imperfect base pairing stopping protein translation and attenuate the inflammation and consequently blood coagulation inside microvasculation of the CNS

to neuro COVID-19 management. When associated with Tocilizumab, the exosomes could improve the action of this monoclonal antibody by targeting the *IL6*, *IL6R,* and *IL6ST.* The combination of these therapies can act systemically and target other genes from PANoptosis and coagulation pathways throughout the body. Before going further into clinical trials, a pre-clinical analysis may prove this theory's potential. The use of transgenic mice expressing human angiotensin-converting enzyme 2 receptor has been well accepted to study COVID-19 development and treatments [42, 43]. Therefore, infecting animals with SARS-CoV-2 and then treating them with the combination of Tocilizumab and the MSCs-derived exosomes could be a smart choice for an initial step to evaluate the impact of treatment on the inflammation and blood coagulation.

Even so the debate around IL-6 blockage continues, the rationale of investigating the use of the MSC-derived exosomes against COVID-19 and especially in the CNS has great potential and deserves deeper investigations. However, some questions remain open: could miRNAs of the MSCs-exosomes prevent the IL-6 complex formation and prevent the transient elevation after Tocilizumab infusion in COVID-19 patients? Can we count with a disrupted BBB during COVID-19 for Tocilizumab to perform its crossing activity and decrease the IL-6 levels and consequently damage inside the CNS? Or the use of MSC-exosomes in combination with the IL-6R blocker would be a more guaranteed way of effectiveness?

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Declarations

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