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REVIEW ARTICLE

Mesenchymal stem cell-derived exosomes for clinical use

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Abstract

Mesenchymal stem/stromal cells (MSCs) are commonly used as a source of cellular therapy due to their strong immunosuppressive and regenerative effects. One of the key mechanisms of MSC efficacy appears to derive from their paracrine activity. Recently, it has been shown that the secretion of different factors through extracellular vesicles known as exosomes, orchestrate the principle mechanisms of action of MSCs after infusion. The use of MSC-derived exosomes may provide considerable advantages over their counterpart live cells, potentially reducing undesirable side effects including infusional toxicities. In this review, we examine clinical trials of MSC-derived exosomes currently in progress for gene delivery, regenerative medicine, and immunomodulation. In addition, we summarize the limitations and clinical potential of this cell-free therapeutic strategy.

Introduction

Mesenchymal stromal/stem cells (MSCs) are commonly used in cellular therapy trials for immunomodulation and regenerative medicine [1–3]. It was previously proposed that MSCs exert their therapeutic effect by migrating to sites of damage, engrafting, and interacting with other cells after infusion. More recently numerous in vivo studies demonstrated that the therapeutic benefit of MSCs is principally orchestrated by the paracrine secretion of a broad repertoire of growth factors, chemokines, and cytokines [4–6]. However, these mechanism(s) of action are not definitive and still under investigation. Despite their beneficial therapeutic effects, MSCs have several disadvantages including the difficulty generating a consistent source of cells with a stable phenotype, infusional toxicities caused by the large cells physically trapped in the lung microvasculature, cellular rejection by the host, ectopic tissue formation, and some reported concerns regarding the safety profile of the cells with respect to tumor formation [7–10].

Recently, it has been shown that MSCs release numerous extracellular vesicles (EVs), including microvesicles (MVs; 0.1–2 mm in diameter) and exosomes (30–150 nm in diameter), which may act as paracrine mediators between MSCs and target cells [11, 12]. Other studies show that MSC-derived exosomes can recapitulate the biological activity of MSCs, and may serve as an alternative to whole cell therapy [13, 14]. The use of exosomes may present considerable advantages over their cellular counterparts due to a higher safety profile, lower immunogenicity, and the inability to directly form tumors [15]. In contrast to the relatively large MSCs (30–60 µm in diameter), nanosized exosomes, have the potential to migrate efficiently to the target organ after infusion without getting trapped in the lung microvasculature [15, 16].

Similar to exosomes derived from other cells types, MSC-derived exosomes participate in intercellular communication and carry proteins, mRNA and microRNA (miRNA), into targeted cells [11]. To date, more than 850 unique gene products and 150 miRNAs have been identified in MSC-derived exosomes [17, 18]. Previous studies indicate that the phenotype and function of MSC-derived exosomes may vary depending upon the source of MSCs [16]. Comparative studies of RNA sequencing of MSC-derived exosomes isolated from human bone marrow and adipose tissue reveal marked differences in tRNA species defined by Sox2, POU5F1A/B, and Nanog expression, which seems to be associated with the differentiation status of MSCs [19]. In addition, the source of MSCs has been reported to influence the biological effects of MSC-derived exosomes [16]. A recent study compared the therapeutic benefits of human MSCs derived from endometrium, bone marrow,

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and adipose tissues in a rat model of myocardial infarction [20]. The data confirmed the superior cardioprotection by endometrial relative to bone marrow and adipose-derived MSCs [20]. These results suggest that innate differences of MSC-derived exosomes due to their original source may play a key role in their clinical efficacy.

In addition to their intrinsic properties, MSC-derived exosomes are ideal vehicles to carry and deliver molecules to targeted cells including therapeutic genes, drugs, enzymes, or RNA [13]. Several studies show that MSC-derived exosomes can protect their cargo against degradation and facilitate their intracellular uptake via endocytosis [13]. Exosomes have recently been shown to have intrinsic homing capabilities similar to their parental cell type [21]. Therefore, MSC-derived exosomes may represent an ideal delivery system to transiently modulate processes in specific target cells. Moreover, similar to their parental cells, the surface of exosomes could be modified to enhance cell type specific targeting [21], which turns MSC-derived exosomes into promising tools for cell-free based therapeutics.

MSC-derived exosomes in clinical trials

Currently, 93 clinical trials involving exosomes are listed in www.clinicaltrials.gov. The majority of these trials focus on the use of exosomes from several body fluids as early diagnostic tools to predict the outcome of various treatments. MSC-derived exosomes have been shown in preclinical studies to be safe and scalable to large, clinically relevant doses [13]. However, the clinical use of MSC-derived exosomes is limited. This is partly due to the fact that the translation of MSC-derived exosome-based therapies from the preclinical studies to the clinic requires the resolution of critical parameters. Among the major issues to be addressed are establishment of the optimal MSC culture conditions and protocols for exosome production, isolation, and storage that provide uniformity between batches, optimal dose, and schedule of exosomes administration, and the development of potency assays, which allow the evaluation of efficacy [2, 14, 16]. There are currently three clinical trials evaluating MSC-derived exosomes reported to Clinicaltrials.gov and a fourth trial that has been published.

MSC-exosomes for diabetes

The first trial, led by Nassar et al. at the Sahel Teaching Hospital and Cairo University, opened in April 2014 (NCT02138331) and assesses the effect of allogeneic cord tissue MSC-derived MVs on B-cell mass in type 1 diabetes mellitus patients. In the preclinical studies leading to this trial, MSC-derived exosomes were shown to effectively suppress autoimmunity and prevent the onset of the disease in established mouse models of type 1 diabetes and experimental autoimmune uveoretinitis [22, 23]. Results indicated that MSC-derived extravesicles suppress development of T helper 1 (Th1) and Th17 cells restoring the balance between Th1 and Th2 immunological responses [22]. The study is evaluating the administration of consecutive doses of MSC-derived exosomes and MSC-derived MVs in 20 patients with type 1 diabetes. Patients receive the first dose of MSC-derived exosomes intravenously, that were isolated from the supernatant produced from $1.22–1.51 \times 10^6$ MSCs/kg. Seven days after the first dose, the patients receive a second dose containing MVs isolated from the supernatant produced from the same dose of MSCs used in the first infusion. Exosomes and MVs were characterized by the expression of exosome markers (CD63, CD9, Alix, TSG101, and HSP 70) and MV markers (Annexin V, Flotilin-2, selectin, integrin, and CD40 metalloproteinase), respectively. At the end of study (3 months) the following parameters are being evaluated: liver functions tests, kidney functions tests, HbA1c, glucose tolerance test, fasting and 2 h postprandial blood glucose levels, C-peptide chain level, and calculated total daily insulin dose. The clinical results from this trial have not yet been published.

MSC-exosomes for chronic kidney disease

In addition to conducting the study described above, Nassar et al. published their results of a phase II/III clinical trial using cord tissue MSC-derived EVs to ameliorate the progression of chronic kidney disease (CKD) [24]. In this study, 20 patients who have been diagnosed for more than 6 months with chronic kidney disease (eGFR 15–60 mg/ml) were treated with two doses (1 week apart) of MSC-derived EVs (100 µg/kg/dose). The first dose was administered intravenously and the second dose infused into the renal artery [24]. The primary endpoint was the safety and the secondary endpoint was the efficacy of treatment assessed by improvements in eGFR levels and/or reduction in serum creatinine. Patients treated with MSC-derived EVs exhibited improved eGFRs and urinary albumin creatinine ratio, as well as significant decreases in BUN and creatinine at 1 year [24]. In addition, the patients showed a significant increase in plasma levels of TGF-B and IL-10 with persistent significant decreases in TNF-α [24].

MSC exosomes for macular degeneration

The second ongoing trial (NCT03437759), led by Zhang et al. at the Tianjin Medical University Eye Hospital, was

initiated in March 2017. This study is evaluating the safety and efficacy of exosomes isolated from cord tissue-derived MSCs to promote healing of large and refractory macular holes in the eye. Preceding preclinical studies showed that systemic transplantation of MSCs reduced the inflammatory response and limited the damage in a model of laser-injured retina by regulation of the intraocular microenvironment in a paracrine manner [25]. A comparative animal study revealed that transplantation of both MSCs and/or their corresponding exosomes reduced retinal damage and inhibited apoptosis induced by laser injury partially by the downregulation of MCP-1 [26]. Together, these results led to their hypothesis that MSCs and MSC-derived exosomes may improve the visual outcomes of surgery for refractory macular holes. On this trial, cord tissue MSC-derived exosomes are isolated via sequential ultracentrifugation and resuspended in PBS. Forty-four patients with a confirmed diagnosis of macular holes will receive a single dose of 20–50 mg of MSC-derived exosomes in 10 ml of PBS injected directly around macular hole area. The participants will be followed up for at least 6 months via Best corrected visual acuity measurement, fundoscopy, optical coherence tomography, and physical examination. Results are predicted to be ready by December 2018.

MSC exosomes for ischemic stroke

The most recent clinical trial (NCT03384433) will begin accrual in October 2018. Led by Zali et al. at the Shahid Beheshti University of Medical Sciences, the study aims to determine the safety and efficacy of bone marrow MSC-derived exosomes genetically manipulated to contain miR-124 in patients with acute ischemic stroke. A recent preclinical study showed that MSC-derived exosomes loaded with miR-124 ameliorated brain injure, promoted neurovascular recovery after stroke, and prevented postischemic immunosuppression in mice [27]. The Phase 1/2 clinical trial aims to determine the beneficial effect of MSC-derived exosomes transfected with miR-124 administrated 1 month after the stroke, via stereotactic guidance into the ischemic area. Five patients will receive a single dosage of 200 mg total protein of allogenic MSC-derived exosomes loaded with miR-124. The primary endpoint is safety in the first 12 months following therapy with documentation of adverse events including progressive or recurrent stroke, brain edema, seizures, and ischemic to hemorrhagic transformation. The secondary outcome, efficacy will be measured by the improvement in the modified Rankin Scale during the first year posttreatment.

Future directions of MSC-derived exosome therapies

MSC-derived exosome therapy is emerging as a promising strategy for the treatment of several diseases, in particular those with an inflammatory component [12]. Their innate ability to transport genetic material, protecting it from extracelluar degradation and delivering it in a highly selective manner to recipient cells suggests that MSC-derived exosomes are an ideal delivery system for small molecules as well as gene therapies for cancer and potentially regenerative medicine [12, 28]. In addition, encouraging preclinical data demonstrated that MSC-derived exosome therapeutics might could be superior to cell-based therapy in terms of safety and versatility [12, 29–31]. However, critical technological considerations and determination of possible side effects need to be addressed in order to optimize their clinical use [31]. Also, with the rapid progress of bioengineering and cellular modification techniques, the next step in the exosome field will be the engineering or modification of the exosome surface and content, which may allow superior specificity, expanding their use to more complex areas of medicine.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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