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General review

Mesenchymal stem cell therapy in Parkinson's disease animal models

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ABSTRACT

Parkinson's disease is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, and as a consequence, by decreased dopamine levels in the striatum. Currently available therapies are not able to stop or reverse the progression of the disease. A novel therapeutic approach is based on cell therapy with stem cells, in order to replace degenerated neurons. Among stem cells, mesenchymal stem cells seemed the most promising thanks to their capacities to differentiate toward dopaminergic neurons and to release neurotrophic factors. Indeed, mesenchymal stem cells are able to produce different molecules with immunomodulatory, neuroprotective, angiogenic, chemotactic effects and that stimulate differentiation of resident stem cells. Mesenchymal stem cells were isolated for the first time from bone marrow, but can be collected also from adipose tissue, umbilical cord and other tissues. In this review, we focused our attention on mesenchymal stem cells derived from different sources and their application in Parkinson's disease animal models.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder caused by the loss of dopaminergic (DAergic) neurons in the substantia nigra (SN), leading to decreased dopamine (DA) levels in the striatum. The presence of intracytoplasmic inclusions, known as Lewy bodies, is considered the pathological hallmark of the disease. The main PD symptoms, that appear after the loss of at least 60% of DAergic neurons, are tremor, muscle rigidity, bradykinesia and postural instability. When the disease proceeded also non-motor manifestation can be present such as anxiety, passivity, depression, psychosis, dementia, and sleep disturbance. The available therapeutic approach is based on L-3,4-dihydroxyphenylalanine (L-DOPA), a precursor of DA, that increases DA levels in the striatum, improving PD symptoms. However, L-DOPA treatment does not change the course of PD, and, at later disease stages, its efficacy decreases, while adverse effects appear, such as dyskinesia. Hence, there is the need for new therapies, able to reverse or stop the progression of the disease. Given that PD is characterized by the loss of DAergic neurons, a therapeutic approach could be the replacement of DAergic neurons in SN. Evidence showed that stem cells can differentiate into DAergic neurons *in vitro* [1–6] and are able to protect or promote regeneration of damaged DAergic neurons [7–11].

Stem cells are able to self-renewal and to differentiate into different specialized cell types. There is an increasing interest in their use in cell therapy that is the introduction of cells into a tissue to treat a disease. On the basis of the origin, it is possible to divide stem cells in different categories: embryonic stem cells, neuronal stem cells, induced pluripotent stem and mesenchymal stem cells (MSCs).

MSCs are non-haematopoietic, multipotent cells that have gained more attention in the last decades thanks to their advantages compared with other stem cells. Indeed, MSCs present low immunogenicity, no teratoma risk, and presented no ethical problems. Another advantage is their potential use for personalized medicine, given that MSCs could be collected from the same patients, with no risk of immune responses. Moreover, MSCs show a low probability of being tumorigenic after transplantation into patients or animals [12,13]. The "Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy" had established the minimal criteria to define MSCs that can be summarize as follow: MSCs are plastic-adherent cells in standard culture conditions, able to differentiate into osteoblasts, adipocytes and chondroblasts *in vitro* and express the surface markers CD105, CD73 and CD90, while CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR molecules must be absent [14].

MSCs were isolated for the first time from the bone marrow (BM), but they can also be isolated from various adult and neonatal tissues, the main are the adipose tissue [15] and the umbilical cord [16,17], but it was reported that MSCs could be derived *in vitro* from different organs and tissues [18].

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2. MSCs properties

MSCs possess the ability to trans-differentiate to non-mesenchymal cell types, such as epithelial, endothelial and neuronal cells [1,19–22].

Interestingly, MSCs express neural and DAergic neuron associated genes and proteins even when not exposed to differentiation stimuli [23]. Different studies reported that MSCs from various sources can differentiate into DAergic neurons, exposed to a mixture of growth factors and chemicals, such as sonic hedgehog (Shh), fibroblast growth factor (FGF) 2, FGF8 and basic FGF (bFGF), brain derived neurotrophic factor (BDNF), forskolin, retinoic acid (RA) [2,4,7,24–28]. Differentiated MSCs showed a neuronal morphology and expressed neuronal markers, in particular DA-specific genes, and secreted DA, but lacked of neuronal excitability [2,25]. However, some reports described the presence of currents in MSCs exposed to various neural differentiation protocols [29,30]. Enriched medium induced a down-regulation of stemness and mesenchymal markers and an up-regulation of neuronal markers in bone marrow-derived MSCs (BM-MSCs). However, either BM-MSCs cultured in standard and in enriched medium expressed genes encoding neurotrophic factors, such as BDNF, glial derived neurotrophic factor (GDNF), FGF2 and FGF8 [7]. It was reported that gene manipulation of MSCs through the lentiviral gene delivery of the homebox transcription factor LIM homebox transcription factor-1 alpha (LMX1a), that have a role in DAergic differentiation, could make the DAergic differentiation easier. However, no expression of the DA transporter (DAT) was found, indicating that the DAergic phenotype was still immature [31]. In periodontal ligament MSCs it was reported that the exposition to epidermal growth factor (EGF) and bFGF induced both neuronal and glial phenotypes, as indicated by the increased expression of neuron-specific β-tubulin III and the neural stem/progenitor cell marker nestin, as well as positive staining for glial fibrillary acidic protein (GFAP). Moreover, cells displayed inward currents conducted through voltage-gated sodium (Na^+) channels [32].

However, MSCs differentiation seems not to be the main beneficial property of MSCs. Indeed, evidence *in vitro* and *in vivo* suggest that MSCs do not need cell-cell contact or to be differentiated to neuronal-like cells to exert their functions, but conditioned medium alone is able to show clinical effects. It was reported that the conditioned medium derived from BM-MSC cultures prevented DAergic neurons death in *in vitro* serum deprivation model and in cells exposed to the neurotoxin 6-hydroxydopamine (6-OHDA) [10]. Moreover, DAergic neurons pre-treated with MSCs conditioned medium, transplanted in a 6-OHDA-induced PD model, showed increased survival, suggesting that BM-MSCs could secrete different factors that protect DAergic neurons against neuronal damage [10]. Indeed, BM-MSCs expressed and released BDNF, FGF2 and GDNF into the culture medium [10]. It is known that MSCs secrete different growth factors, chemokines and cytokines with autocrine or paracrine actions, such as BDNF, nerve growth factor (NGF), GDNF, vascular endothelial growth factor (VEGF), but also extracellular matrix proteins and neuro-regulatory factors [33–37]. Through the release of different molecules, MSCs can exert pro-angiogenic, anti-apoptotic, neurogenic, immnomodulatory, neuroprotective, mitotic or chemotactic effects and stimulate the differentiation of resident stem cells [38–40]. Moreover, MSCs are able not only to release anti-inflammatory factors but also to inhibit cells involved in the immune response by cell-cell-contact-dependent mechanism [41]. In *in vitro* models, it was reported that BM-MSCs administration decreased lipopolysaccharide (LPS)-induced microglial activation, tumor necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS) mRNA expression, and NO and TNF-α production, while interleukin (IL)-6, IL-10 and transforming growth factor (TGF) β expression increased. Moreover, MSC treatment was able to decrease the loss of tyrosine hydroxylase (TH)+ cells in co-cultures of microglia and mesencephalic DAergic neurons [42].

An important property of MSCs is "homing", that is the tendency of cells to migrate to damaged tissue sites. The homing process involves chemokines, adhesion molecules, and matrix metalloproteinases. It was observed that MSCs administered to humans and animals are localized in tissues with inflammation or brain damage [43–47]. Interestingly, transplanted BM-MSCs in 6-OHDA-lesioned animals, migrated and survived better in the injured hemisphere [46]. MSCs, recruited at the damaged site by chemotaxis, could release different molecules in order to repair the lesioned tissue.

3. Differences and similarities of MSCs obtained from different sources and their application in PD models

It is known that MSCs derived from various sources presented similarities but also different features. It is important to compare them in order to identify which type is more suitable for cell therapy. Characterization, advantages and disadvantages of each MSC type were reported in Table 1.

MSCs derived from different sources showed similar surface markers [16,48,49]. Moreover, BM-MSCs and adipose tissue-derived MSCs (AD-MSCs) showed a very similar transcriptional profile for stemness-related genes [50]. Umbilical cord-derived MSCs (UC-MSCs), BM-MSCs and AD-MSCs had similar surface antigen expression, immunosuppressive activity, and differentiation ability, but UC-MSCs showed the highest proliferation rate and lower expression of senescence markers. On the contrary AD-MSCs showed the lowest culture time and proliferation rate [51]. However, other reports indicated a higher proliferation ability of AD-MSCs compared to BM-MSCs, associated with a higher neural markers expression in differentiated AD-MSCs [52,53]. BM-MSCs secretion levels of some factors such as VEGF and TGFβ1 were significantly higher compared to AD-MSCs [54]. Moreover, UC-MSCs showed a higher proliferation rate compared with dental pulp derived MSCs (DP-MSCs), but DP-MSCs presented a low rate of apoptosis and senescence, and maintained cell morphology after subculture [55]. Moreover, DP-MSCs and human exfoliated deciduous teeth (SHEDs) showed a higher differentiation potential for neurogenesis compared to BM-MSCs [56].

3.1. Bone marrow-derived MSCs application in PD animal models

MSCs were at first isolated from BM, so it is not surprisingly that BM-MSCs have been the gold standard in MSCs experiments. A summary of the PD experimental models evaluating BM-MSC effects is shown in Table 2.

Li and co-workers [57] showed that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned mice transplanted with BM-MSCs a week after lesion induction exhibited improvements on the rotarod test 35 days after MPTP injection. Moreover, MSCs survived in the transplanted areas at least 4 weeks after administration and expressed TH [57].

Given that MSCs are able to differentiate into neuronal-like cells, some experimental studies tried to evaluate if differentiated BM-MSCs possessed better neuroprotective potential. Interestingly, undifferentiated BM-MSCs transplanted in a PD model survived and differentiated into DAergic neurons, leading to behavioral improvements [24]. Moreover, examination 4 and 8 weeks after BM-MSCs transplantation into the SN of PD rats showed that BM-MSCs survived, migrated in the brain and differentiate into

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