Biochimie 95 (2013) 2257–2270

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Review

The role of mesenchymal stromal cells in spinal cord injury, regenerative medicine and possible clinical applications

Serhiy Forostyak [a,b], Pavla Jendelova [a,b], Eva Sykova [a,b,*]

[a] Institute of Experimental Medicine, Academy of Science of the Czech Republic, Prague, Czech Republic
[b] Department of Neuroscience, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic

ARTICLE INFO

Article history:
Received 10 March 2013
Accepted 5 August 2013
Available online 27 August 2013

Keywords:
Stem cells
Regeneration
Neuroprotection
Cell-based therapy
Clinical trials

ABSTRACT

Diseases of the central nervous system still remain among the most challenging pathologies known to mankind, having no or limited therapeutic possibilities and a very pessimistic prognosis. Advances in stem cell biology in the last decade have shown that stem cells might provide an inexhaustible source of neurons and glia as well as exerting a neuroprotective effect on the host tissue, thus opening new horizons for tissue engineering and regenerative medicine. Here, we discuss the progress made in the cell-based therapy of spinal cord injury. An emphasis has been placed on the application of adult mesenchymal stromal cells (MSCs). We then review the latest and most significant results from *in vitro* and *in vivo* research focusing on the regenerative/neuroprotective properties of MSCs. We also attempt to correlate the effect of MSCs with the pathological events that are taking place in the nervous tissue after SCI. Finally, we discuss the results from preclinical and clinical trials involving different routes of MSC application into patients with neurological disorders of the spinal cord.

1. Introduction

An increasing number of people are affected by neurological diseases such as traumatic spinal cord (SCI) and brain injury, neurodegenerative diseases, stroke and central nervous system (CNS) tumors. In this list, spinal cord injuries are among the most devastating disorders, since the affected patients and their families are often deprived of qualities that change their lives forever [1]. According to the National Spinal Cord Injury Statistical Center (NSCISC), it is estimated that new spinal cord injury cases occur worldwide with almost the same frequency, around 40 cases per million of population, excluding those who died at the scene of an accident [2]. Of those, most SCI cases are caused by traffic accidents, followed by violent assaults, falls, sport and industrial traumas.

Generally, human SCIs are very heterogenous, and the therapeutic approach differs depending on the location, extent, stage and time after the SCI. Traumatic SCI can be divided into three phases: acute, subacute and chronic. The acute phase starts after the injury of the spinal cord (SC), when mechanical deformation of the SC and shear forces lead to the rupture of neuronal cell membranes with the subsequent release of their intracellular contents and glutamate from intracellular stores, leading to excitotoxicity, vasospasm, localized edema, the breakdown of the blood–brain barrier, a cascade of biochemical and cellular processes resulting in massive necrotic cell death and a shift of metabolism toward anaerobic glycolysis [3,4]. The acute phase persists for hours up to days and resolves into the subacute phase.

The subacute phase is characterized by processes that lead to secondary damage of the nervous tissue after the initial traumatic shock. These processes trigger a chain of events that are accompanied by an inflammatory reaction, the activation of macroglial and oligodendroglial cells, ongoing demyelination, vascular defects with related hypoxia, a depletion of ATP regeneration, the production of free radicals with subsequent lipid peroxidation [5],

Abbreviations: AMSCs, adipose-derived MSCs; ALS, amyotrophic lateral sclerosis; BDNF, brain-derived neurotrophic factor; BMSC, bone marrow MSC; CNS, central nervous system; CST, corticospinal tracts; ESCs, embryonic stem cells; GRP, glial restricted precursors; GDNF, glia derived neurotrophic factor; GVHD, graft-versus-host disease; hNSC, human neural stem/progenitor cells; hUCB, human umbilical cord blood; iPSCs, induced pluripotent cells; IGF-1, insulin growth factor-1; MRI, magnetic resonance imaging; MSC, mesenchymal stromal cells; MN, motoneurones; MV, microvesicles; NGF, neural growth factor; NF, neurofilament; NMJ, neuromuscular junction; NPCs, neural progenitor cells; SC, spinal cord; SCI, spinal cord injury; NTF, neurotrophic factors; OMgp, oligodendrocytemyelin glycoprotein; PNN, perineuronal nets; SOD1, superoxide dismutase 1 gene; VEGF, vascular endothelial growth factor.

* Corresponding author. Department of Neuroscience, Institute of Experimental Medicine AS CR, Videnska 1083, 142 20 Prague 4, Czech Republic. Tel.: +420 241 062 230; fax: +420 241 062 706.

E-mail addresses: serhiy.forostyak@biomed.cas.cz (S. Forostyak), jendel@biomed.cas.cz (P. Jendelova), sykova@biomed.cas.cz (E. Sykova).

0300-9084/$ – see front matter © 2013 Published by Elsevier Masson SAS.
http://dx.doi.org/10.1016/j.biochi.2013.08.004

local inflammation [6], secondary necrotic cell death at the core of the injury site and apoptotic cell death in the surrounding areas, reaching its highest levels at about 1 week after injury [7–12]. The ongoing demyelination [13,14] and degeneration of the fiber tracts leads to neuronal death not only in the immediate proximity of the primary lesion site, but also in more remote locations, such as the motor cortex in the brain [15,16]. At this stage a number of oligodendrocytes and astrocytes die in the core of the injury [10]; meanwhile, there is an activation of astrocytes at the edge of the primary injury site. These astrocytes display an increased metabolism and start to form long neurites, aiming to prevent the spread of an aggressive environment further in both directions [17,18]. This infiltration subsequently acts to block regeneration after SCI due to the formation of a barrier to axonal sprouting across the lesion [19]. The activation of oligodendrocytes is another important mechanism leading to the synthesis of oligodendrocyte-myelin glycoprotein (OMG) and myelin-associated glycoprotein (MAG), both of which have neurite growth inhibitory activity [20–22].

The chronic phase of SCI can last for years and is characterized by ongoing demyelination [14,23,24], local inflammation and apoptosis [25], a decrease in the number of activated macrophages, and the formation of a glial scar and pseudocysts (also called syringomyelia) [26–29]. This phase of SCI presents a major challenge to doctors and scientists and attracts the greatest research interest, as most SCI patients remain in this phase, to a greater or lesser extent, for the rest of their lives.

Regeneration of the adult CNS is limited due to weak neuronal plasticity, an umbrella term referring to a variety of compensatory processes (spontaneous regeneration of affected axons, dendritic remodeling, changes in neuronal and synaptic strength) that are taking place inside the spinal cord after the trauma in order to overcome a number of neurites growth-inhibitory molecules and to restore lost structures and functions [30,31]. On one hand, these powerful intrinsic inhibitory substances and processes that prevent axonal growth are vital for the normal functioning of the adult mammalian spinal cord (SC). On the other hand, these same factors create a major obstacle for functional recovery after SCI, as well as limit the therapeutic effects of drugs that are currently used in the treatment of patients after SCI. Therefore, novel therapeutic strategies, by confronting the above obstacles, including the glial scar components, providing neuroprotective support for the remaining host cells and/or acting as an anti-inflammatory treatment, should stimulate the regeneration of the adult CNS and improve neurological functions, thus providing an effective therapy and improving the quality of the patient's life.

2. Current treatment of spinal cord injury

Therapeutic approaches toward patients with SCI fall into three separate time frames, which target the featured molecular events at the particular injury phase. The first could be described as management of vital functions, immobilization, and transportation to the emergency unit. It is directed at stabilizing vital functions and interrupting the cascade of reactions leading to secondary injury. The aim of the second phase is neuroprotection immediately after the injury. This is the most critical period after the injury, therefore most phase I–III human clinical trials have been organized during this period. The following groups of drugs have been tested: steroids (methylprednisolone, Tirilazard) [32], opiate receptor antagonists (naloxone) [33], gangliosides (GM-1, Sygen®) [34], the potassium channel blocker 4-aminopyridine (fampridine, Acorda Therapeutics) [35], autologous cellular therapy (stimulated homologous macrophages, Proneuron) etc. [36,37]. Of these, methylprednisolone (MP) has been the only drug that has resulted in the significant improvement of motor and sensory functions not just in animal studies, but also in patients after SCI in the NASCIS-3 human trial [32,33,38]. However, an ongoing debate is in progress regarding the mechanism, efficacy and clinical impact of MP's action [39]. Nevertheless, at the present time, the only standard method to treat patients with SCI is surgical intervention, high doses of MP and symptomatic therapy (control or management of urinary and cutaneous infections, pain, spasticity, bladder and bowel management, sexual and reproductive function) followed by rehabilitation. The third therapeutic phase deals with the consequences of SCI. Rehabilitative efforts aim to stabilize the current status and to train the reflexes and residual circuits to achieve optimal living conditions for the patient who has a given deficit.

New neuroregenerative strategies are focused on the neuroprotection or even the replacement of the injured neurons and glial cells by the application of various types of stem cells or their progenitors [40]; however, without a permissive environment only little progress in regeneration can be achieved. In the future, treatment of SCI will be directed toward the enhancement of axonal regeneration (also called rewiring) by inhibiting astroglial scar formation and the synthesis of inhibitory proteoglycans, netrins, semaphorines and ephrines [41–44]; modulation of inflammatory and immune responses [45]; stimulating endogenous stem cells [46,47]; filling the post-injury cavity by biomaterials [48,49]; or blocking myelin-associated glycoproteins and anti-Nogo-A therapy [50].

3. Stem cell therapy in the treatment of SCI

Stem cells are pluripotent or multipotent cells with unlimited self-renewal capacities. In addition, they are able to differentiate into diverse specialized cell types, including neuronal and glial cell lineages [51,52]. It is expected that after their application into the pathological environment within the subacute phase after SCI, the grafted stem cells will be able to stimulate regeneration by: i) the release of neurotrophic factors, modification of extracellular matrix and even downregulation of some inhibitory molecules that will promote and facilitate axonal sprouting [53]; ii) the regeneration of damaged nervous tissue through differentiation or transdifferentiation into mature neural cells (neurons or oligodendrocytes), thus promoting the remyelination of the surviving axons and the restoration of specific connections [51,52,54–56]; iii) the filling of small cavities, thus acting as a scaffold that will support axonal outgrowth between the rostral and caudal stumps and stimulating the revascularization of the damaged nervous tissue etc. [57,58]; iv) the stimulation of endogenous neurogenesis and angiogenesis, the secretion of exosomes, and the activation of endogenous stem cell proliferation, migration and differentiation toward neural cells in certain parts of the adult CNS such as the subventricular zone (SVZ) [59,60]. Interestingly, only the subacute transplantation of stem/precursor cells enhances the recovery of locomotor functions, whereas during the chronic phase of SCI monotherapy with stem cells is not enough [61]. A solution to the above concern might be provided by the implantation of stem cells in combination with biomaterials. Biomaterials have become increasingly important in the development of drug delivery systems and tissue engineering approaches, and can play key roles in overcoming the inherently insufficient protection, repair and regeneration of the nervous tissue [62]. The creation of a mechanical scaffold from natural or artificially synthesized materials and its transplantation into the transected SC or during the chronic phase of SCI could provide a platform for the growth of host cells and guide axons through the glial scar and post-traumatic cysts to form new connections [63]. The implantation of a hydrogel seeded with MSCs into a chronic lesion of the SC stimulates the regeneration of lost sensorimotor functions, promotes axonal and vessel

ingrowth, is well-tolerated in animal experiments and currently is undergoing evaluation in pre-clinical trials (Fig. 1) [48,64].

4. Pluripotent and neural stem cells

Different types of stem cells have been used to promote regeneration after experimental SCI. Embryonic stem cells (ESCs) derived from blastocysts have the greatest differentiation potential, therefore they can become an important source of oligodendrocyte precursors, spinal precursor cells, neural stem cells (NSCs) and motoneurons for the therapy of SCI [51,52,65–68]. One of the major questions of cell-based therapy is to find the proper stage when the cell can mature into the desired phenotype without causing potential danger in terms of tumor formation or hyperproliferation. The implantation of hESC-derived oligodendrocyte progenitor cells (OPCs) into adult rat spinal cord injuries enhanced remyelination and promoted the improvement of motor function at early time points after SCI, and the OPCs even differentiated toward mature neurons showing electrophysiological activity *in vivo* [69,70]. If transplanted 7 days after injury, OPCs were able to remyelinate the spared axons as well as improve the locomotor activity of the injured rats [71]. Past *in vitro* and *in vivo* studies have generated neurons from animal and human ESCs that maintained a typical motoneuronal (MN) phenotype and showed functional incorporation after intraspinal transplantation [72]. The transplantation of NSCs has also been shown to result in functional integration of the graft into the recipient spinal cord resulting in improved locomotor function during the early stage of chronic SCI [73]. However, successful neuronal replacement would necessitate the formation of not only local functional connections, but also of long tracts of axonal outgrowth and the formation of newly formed synapses by the grafted cells. So far, only a few studies after acute injury of the peripheral nerves have demonstrated newly formed functional connections between grafted ESCs and the host muscles after transplantation [55,74]. Nevertheless, Geron Corporation (Menlo Park, CA, USA) announced in 2011 the enrollment of 10 patients with complete, subacute thoracic SCI into a clinical trial involving the administration of human ESC-derived oligodendrocyte progenitor cells. [75]. This event prompted great interest and some concerns among the scientific community, as the preclinical data achieved from spinal cord injured rodents that underwent the same approach of cellular therapy as the human involved in the trial had far less severe SCI compared to the human patients; however, due to a lack of funding the trial has been placed on clinical hold, but is still ongoing with 5 patients recruited (http://www.fiercebiotech.com).

A new category of pluripotent cells are induced pluripotent stem cells (iPSCs). Using reprogramming technology, we can generate patient-specific pluripotent cells that are derived from adult somatic cells. These cells can be generated from different adult somatic cells by the overexpression of several different defined factors [76]. So far, only a few reports have been published using human iPS cell-derived neural precursors for the treatment of SCI and TBI [77]. It has been shown that these cells restored motor function and participated in the reconstruction of the corticospinal tract by forming synaptic connections and integrating into neuronal circuits [78,79]. iPS cells are a promising source for the future; however, current human medical practice relies more on stem cells generated from adult or fetal tissues.

The transplantation of region-specific stem cell lines generated from fetal tissues is also a very promising strategy. A clonal neural stem cell line from human fetal spinal cord (designated SPC-01), conditionally immortalized by means of 4-hydroxy tamoxifen (4-OHT)-inducible cMyc (cMycERTAM), has been shown to be karyotypically stable after prolonged passages, to differentiate toward V2a inter- and motoneurons expressing functional T-, L-, N-, and P/Q-type Ca2+ channels, and to display spontaneous calcium oscillations in some cells, which are typically observed in dissociated embryonic rat motoneuron cultures [80]. Transplantation of SPC-01 cells into a rat model of spinal cord injury did not lead to tumor formation or hyperproliferative activity, but showed long term cell survival, the ability to fill the lesion cavity, and specific neural differentiation 4 months after engraftment. After transplantation these cells also showed better proliferation, region-specific migration, the promotion of host tissue regeneration and axonal sprouting into the lesion site, as well as improvements in locomotor and sensory functions [81–83]. Human neuroepithelium stem cells derived from fetal cortical brain tissue have been reported to be a safe, non-tumorigenic strategy, that promotes the recovery of lost function after transplantation into a rat model of stroke and is safe to progress to clinical trials [84]. Probably the most significant effect after the transplantation of human fetal stem cells has been reported using a rat model of Parkinson's disease, in which grafted fetal dopamine neurons incorporated into the host brain and improved motor function in a rodent model of the disease; the same effects were also observed in patients [85–87]. Perrin et al. reported an antipathic effect after an intraspinal graft of human neural progenitor cells (NPCs), naïve or engineered to express Neurogenin-2: rats grafted with Neurogenin-2-engineered NPCs showed significantly faster recovery as early as 7–10 days after implantation [88]. Due to an increasing number of publications involving cellular therapy with different types of SC, 27 SCI researchers who are actively involved in either preclinical and/or clinical research of cellular interventions for SCI discussed and summarized the current state of experimental preclinical data as well as the future perspectives in terms of the level of evidence required in experimental studies of cellular therapies before proceeding with clinical trials in humans [89]. A quick summary of the cells and animal models used to treat SCI is presented in Table 1.

5. Mesenchymal stromal cells (MSCs)

5.1. Characterization and origin

MSCs are multipotent, tissue-specific stem cells that are very attractive for regenerative medicine in general as well as for neuroprotective and neurorestorative therapy in particular. It has also been demonstrated that the plasticity (the ability of a cell to change its default fate) and tissue regenerative potential of MSCs may far exceed the primary use of bone marrow cells in the treatment of hematopoietic diseases. MSCs represent a small fraction (0.001–0.01 percent) of the bone marrow population. They are adherent cells with a fibroblast-like morphology that can be isolated *ex vivo*, readily differentiate into mesodermal cell derivatives and are characterized by specific surface markers [90]. Due to the large number of markers expressed by MSCs, the International Society for Cellular Therapy recommended the following minimal criteria to identify the multipotent properties of human bone marrow MSCs (BMSCs): they should be able to adhere to a plastic surface and express certain surface antigens (CD29, CD73, CD90 and CD105) while remaining negative for CD19, CD34, CD45 or CD79a and HLA class II during *in vitro* cultivation [91]. Despite the tissue of origin, all MSCs can differentiate *in vitro* into chondrocytes, osteocytes, muscle cells and adipocytes [92–94]. MSCs can be isolated from a patient's bone marrow by a relatively simple procedure, and after harvesting they can be easily expanded in culture. They can also be isolated from alternative organs such as Wharton's jelly, umbilical cord blood (UCB), the placenta, dental pulp and fat tissue, of which the most promising are adipose-derived MSCs (AMSCs).

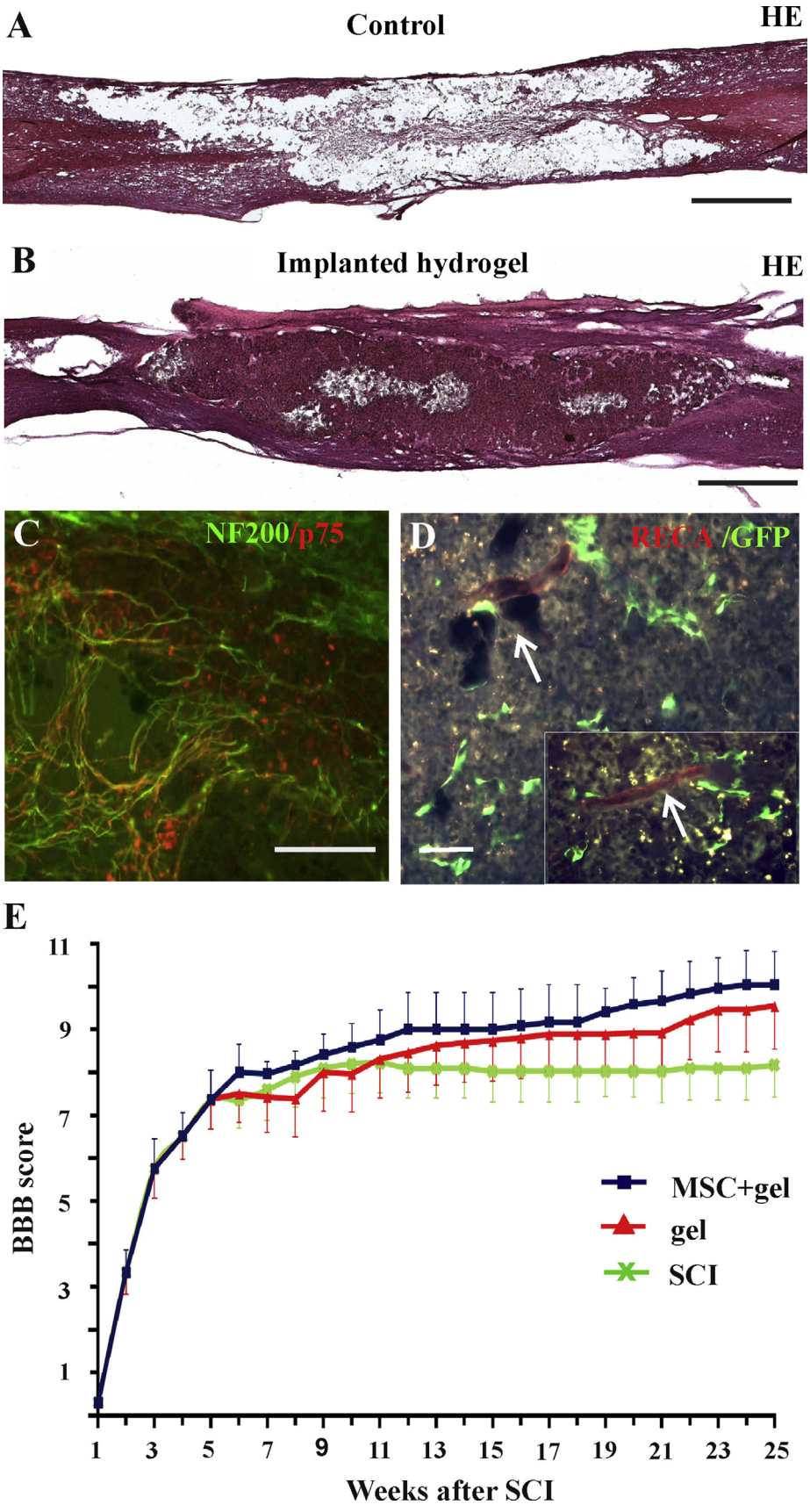


Fig. 1. Transplantation of a hydrogel seeded with BMSCs during the chronic stage of SCI fills the lesion cavity and reconstitutes the spinal cord tissue (B), forming a scaffold for the migration of host neurites (NF200) and Schwann cells (P75) (C) as well as the ingrowth of vessels (RECA) (D). GFP-positive BMSCs survive inside the hydrogel for at least 20 weeks after grafting (D). The combination of BMSCs with a hydrogel stimulates a significant improvement of motor function when compared with the non-treated group; however, the results did not significantly differ from those found in the gel-treated group (E) evaluated using the BBB motor scale. Scale bars: A, B 500 μm, C, D 50 μm. Error bars indicate s.e.m.; significance was set at $p \leq 0.05$. Adapted from Hejcl et al. [48].

Table 1
Overview of preclinical trials involving different types of stem cells and different models of SCI.

Cell type	Animal/model of injury	Stage of SCI	References
Human embryonic stem cells (ESCs)	Rat/weight-drop SCI; transection; contusion lesion; compression lesion;	Acute/subacute, chronic	[67,70,71,88]
Induced pluripotent stem cells (iPSCs)	Mouse/contusion lesion	Acute/subacute	[78]
Fetal stem cells	Rat/contusion lesion; compression lesion	Acute/subacute	[68,83]
Human bone marrow mesenchymal stromal cells (BMSCs)	Rat/compression; contusion; compression; compression; compression; compression lesion	Acute/subacute, chronic	[48,65,114,128,138,146]
Adipose-derived MSCs (AMSCs)	Rat/compression lesion; dorsal transection	Acute/subacute	[97,99]
Neural stem/progenitor cells	Rat, mouse/contusion; contusion; contusion; compression lesion	Acute/subacute, early chronic, chronic	[61,69,73,157]

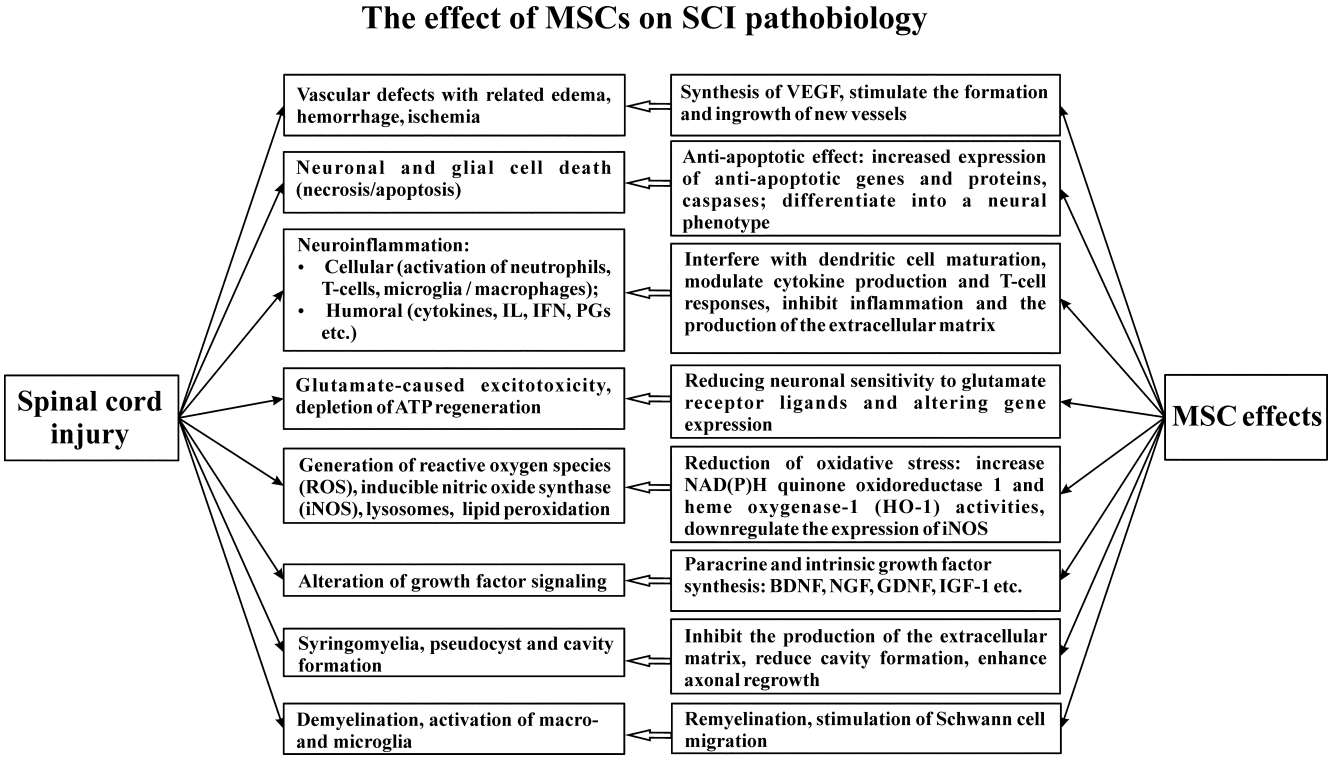
5.2. Gene expression

Either bone marrow or adipose MSC populations have been shown to express a variety of neural genes such as NCAM, NG2, S100 and p75, as well as transcription factors, suggesting a wide differentiation potential, including neural; however, these cells did not show specific neuronal electrophysiological properties [95–99]. Lattanzi et al. showed that the neurotrophic features of AMSCs reside in their specific capability of expressing not only secreted neurotrophins/neuroprotective molecules, but also structural protein-coding genes, mimicking astrocytic function in sustaining neuronal metabolism and function in the central nervous system and being able to differentiate into astrocytes [100].

5.3. Growth factors

Despite the wide use of autologous MSC transplantation in attempts to regenerate CNS disorders the exact mechanisms of MSC action is still unclear. Based on numerous reports, Table 2 correlates the known effects of MSCs on the nervous tissue after transplantation with the events that take place after SCI. It is generally accepted that the efficacy of MSCs is based on the secretion of a wide range of substances, either by host cells or by the MSCs themselves (paracrine function). MSCs are known to secrete several growth factors, such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), neural growth factor (NGF), glia cell-line derived neurotrophic factor (GDNF) and insulin-like growth factor 1 (IGF-1), that play a crucial role in nourishing and protecting neurons [101–105]. After transplantation, MSCs are able to mediate direct neuroprotection by reducing neuronal sensitivity to glutamate receptor ligands and altering gene expression, suggesting a link between the therapeutic effects of MSCs and the activation of cell plasticity in the damaged CNS [106]. Some studies, after comparing the *in vitro* properties of AMSCs and BMSCs, have suggested that AMSCs produce a significantly larger amount of cytokines and growth factors compared to BMSCs; however, they have a lower differentiation potential than BMSCs [99,107,108]. Previous publications from our group suggest that AMSCs could be used as an alternative to BMSCs for the cellular therapy of SCI.

Table 2
The properties of mesenchymal stromal cells and their therapeutic effect on SCI pathobiology that have been described in the literature (see the text for description).



In vitro and *in vivo* experiments using conditioned media have shown that the MSC secretome promotes axonal growth and neuroprotection [109,110]. Interestingly, it has been shown *in vitro* (hypoxic preconditioning of MSCs) and *in vivo* (injection of secretome from hypoxic-treated MSCs) that harvesting MSCs under hypoxic conditions stimulates greater secretion of growth factors, better stem cell survival and increased neurogenesis [111,112]. These phenomena might be explained by the natural location of the MSCs in sites that have reduced blood circulation, such as the bone marrow. This makes MSCs even more attractive for transplantation purposes, as often there is a mild edema, decreased circulation and hypoxia in the region where stem cells are grafted (e.g., in stroke, TBI and SCI).

5.4. Anti-inflammatory effect

An anti-inflammatory effect is another beneficial factor that is connected with the application of MSCs via different routes. An anti-inflammatory effect occurs mainly due to the upregulation of the anti-inflammatory factor TGF-β1, which is dominant over the upregulated proinflammatory chemokines/cytokines, such as IL-1β, IL-6 and TNF-α [113–115]. The migration capacity of transplanted MSCs and their subsequent homing to injured tissues also depends on the state of both local and systemic inflammation and is under the control of a large range of receptors, tyrosine kinase growth factors and chemokines [116]. Several reports have shown the ability of MSCs to reduce oxidative stress and even to secrete superoxide dismutase [117–119]. The grafting of MSCs 3 days after SCI modified the inflammatory environment by shifting the macrophage phenotype from M1 (pro-inflammatory) to M2 (anti-inflammatory), sparing axons and myelin [120]. The activation of M2 macrophages has been suggested as one of the mechanisms improving cardiac function after acute myocardial infarction, as well as one of the mechanisms underlying the regeneration of the central and peripheral nervous systems [121].

5.5. Exosomes

Another emerging mechanism of MSC therapy is the formation of microvesicles (MVs) that are formed in the soma of MSCs and then released into the extracellular space. MVs (exosomes) contain a great variety of biologically active molecules, lipids, proteins, growth factor receptors, messenger and microRNA etc [122–124]. It has been demonstrated that the therapeutic benefit of MSC treatment of stroke is mediated by exosome-enriched extracellular particles contacting the miRNA that is transferred to adjacent neural cells (astrocytes and neurons), which evokes neurite remodeling and brain plasticity and subsequently leads to functional recovery [125].

5.6. Immunomodulation

Additionally, MSCs have been shown to have an immunosuppressive effect on B lymphocytes (decreased proliferation and differentiation) [126]. MSCs also provide a permissive environment for axonal ingrowth, stimulate functional recovery and angiogenesis [127,128], are immunopotent, do not stimulate alloreactivity, increase the expression of anti-apoptotic proteins, escape lysis and inhibit the proliferation of cytotoxic T-cells and natural killer cells [129–131]. Maggini et al. showed that MSCs constitutively produce prostaglandin E2 at levels sufficient to inhibit the production of TNF-α and IL-6 by activated macrophages and also inhibit the upregulation of CD86 and MHC class II in LPS-stimulated macrophages, thus impairing their ability to activate antigen-specific CD4+ T cells [132]. Spaggiari et al. showed that MSCs can exert a profound inhibitory effect on NK-cell function, because they can suppress not only IL-2–induced cell proliferation, but also the generation of cytolytic activity and the production of cytokines; the authors also showed that indoleamine 2,3-dioxygenase and prostaglandin E2 represent key mediators of the MSC-induced inhibition of NK cells [133].

5.7. Experimental studies on MSCs and acute SCI

Transplanted MSCs can differentiate into myelinating cells that remyelinate demyelinated axons. These remyelinated axons, coated with myelin and surrounded by a basement membrane, display improved conduction velocity [134,135].

The above properties enable the allogeneic transplantation of MSCs. A comparison between autologous and allogenic transplantation into a balloon-induced SCI in dogs showed that autologous MSCs yielded a better therapeutic effect and better survival of the cells compared to allogenic MSCs; however, despite the smaller improvement of neurological function, allogenic MSC implantation has some advantages from a practical aspect such as the ability to store the cells in a cell bank, the decreased time and cost of preparation, and the possibility to use much larger numbers of cells [136]. *In vivo* experiments employing different SCI models and various routes of MSC administration revealed significant functional recovery, i.e. increased motor activity and sensation in the paralyzed limbs, reduced cavity formation in the spinal cord, and the formation of bundles that bridge the lesion and enhance axonal sprouting through the glial scar [48,57,65,137–143]. The tissue matrix formed by MSC grafts supports longitudinally directed axonal growth and orientation, with their long axis parallel to that of the spinal cord. MSCs were also oriented longitudinally in the close vicinity of the host neurites [144,145]. It was also reported that the intravenous delivery of BMSCs enhances remyelination throughout a demyelinated spinal cord lesion [54]. Intraspinal grafting of BMSCs into the injured spinal cord was shown to promote axonal regrowth and to reduce the lesion volume [140]. The repetitive intrathecal delivery of BMSCs has been shown to improve behavioral functions in a rat model of contusive spinal cord injury as well as to augment the survival of the cells and their migration to the lesion site [146]. It is important to note that after acute SCI, there is a therapeutic time window within which the application of stem cells can ameliorate the consequences of secondary injury by preserving rather than replacing the host nervous tissue [147].

5.8. Experimental studies on MSCs and chronic SCI

Despite success in the use of MSCs to treat acute SCI, there are few studies evaluating the efficacy of MSC administration in chronic SCI repair. Zurita has shown that the intravenous administration of MSCs three months after SCI achieved some degree of functional recovery when compared to controls [148]. Cellular therapy during the chronic phase of SCI aims to reconstruct the spinal cord via cellular replacement, glial scar modification, axonal guidance and the filling of formed syringomyelia, thus leading to functional regeneration [48,149,150]. However, considering the complex pathways and interactions within the spinal cord and in the CNS, a combination of stem cells with other strategies might bring even better results. Different methods of chondroitinase ABC application after SCI have been shown to facilitate neuroanatomical and functional recovery of sensorimotor functions, as well as to stimulate the migration of host oligodendrocyte progenitors to the lesion [151–154]. Combined therapy of chondroitinase ABC with NPCs and growth factors led to enhanced neuroanatomical plasticity in the chronically injured spinal cord, significantly improved neurobehavioral recovery and axonal integrity, promoted the

plasticity of the corticospinal tract, enhanced the plasticity of descending serotonergic pathways, and was accompanied by the better integration, extensive migration and differentiation of NPCs within the recipient spinal cord [155–157]. Another promising strategy for chronic treatment of SCI is a combination of MSCs with biomaterials. Recent advances in the application of synthetic materials, alone or in combination with stem cells/growth factors, to treat SCI have been reviewed by Kubinova and Sykova [158,159].

6. Clinical trials of mesenchymal stem cell therapy

The above properties of MSCs, preclinical trials, along with long experience with the transplantation of MSCs in the treatment of hematological malignancies, led to the first clinical trials, initially to treat myocardial infarction and later to treat stroke, SCI, ALS, PD and other diseases of the CNS [160,161]. These and other trials utilizing different methods of BMSC application showed that the grafting of such cells is a safe procedure that can bring benefits for patients [137,162–164]. Experimental studies suggest that the therapeutic effect of grafted cells starts before the establishment of a tissue bridge suitable for the passage of axons, therefore the recovery of neurological functions at the early post-transplantation stage could be explained by the activation of different regenerative processes [149]. Based on preclinical experiments in rats with SCI that showed significant improvement in behavioral scores after the intravenous implantation of BMSCs labeled with iron oxide nanoparticles 7–21 days post-injury, followed by *in vivo* magnetic resonance imaging (MRI), a nonrandomized phase I/II clinical study

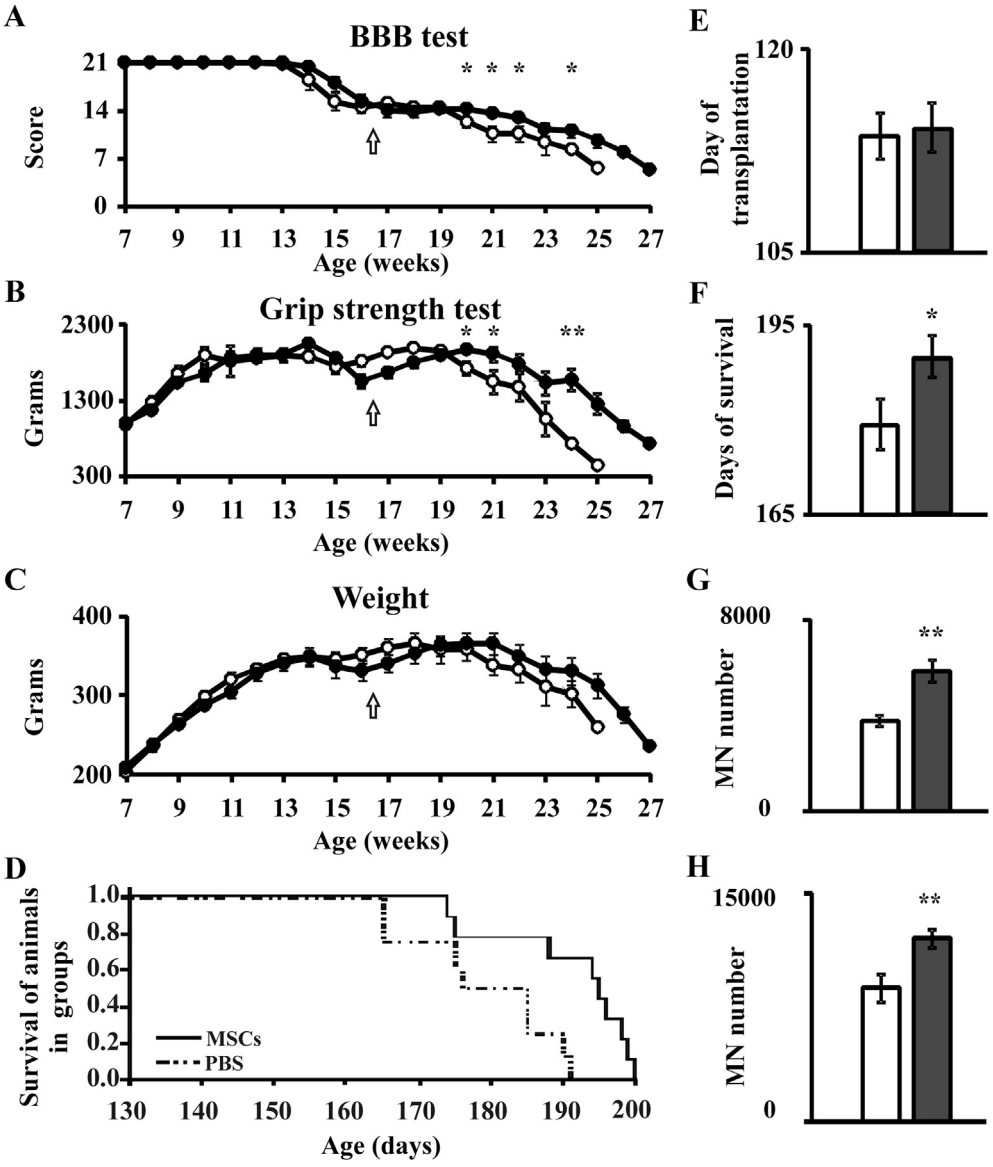


Fig. 2. Characteristics of disease progression in groups of SOD1^{G93A} rats treated with BMSCs (filled circles) or PBS (empty circles). The rats treated with BMSCs showed significantly higher scores on the BBB (A) and grip strength (B) tests. BMSC treatment did not significantly delay the loss of body weight but showed a tendency to slow it down (C). There were no differences between the mean time of BMSC (114 ± 5.5 days) transplantation and PBS (113.75 ± 5 days) injection in the two groups of animals (E). The lifespan of animals was significantly prolonged by BMSC treatment by 11 days as a group mean ($p \leq 0.05$). Compared to an injection of PBS, BMSC transplantation significantly increased overall survival, 179 ± 3.6 days versus 190 ± 3.3 days respectively (D, F). BMSCs significantly increased the number of motor neurons counted using an unbiased stereological method in serially sectioned spinal cords from both the left and right ventral horns in the upper thoracic (G) and lumbar (H) levels of the spinal cord in the treated group of animals ($p \leq 0.01$ in both levels; gray columns) compared to controls (white columns). Arrows in A–C show the time of transplantation. Error bars indicate s.e.m.; significance was set at: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$ (Forostyak et al. [145]).

using patients with SCI was started in Prague [165–167]. In this study a freshly isolated mononuclear fraction of autologous bone marrow cells was grafted intraarterially (via *arteria vertebralis*) or intravenously into 42 patients with SCI at the cervical or thoracic level, and the effect of the treatment was evaluated by the ASIA protocol, the Frenkel score system and electrophysiological measurements of motor and somatosensory evoked potentials (MEPs and SEPs) 3, 6 and 12 months after cell administration [162]. The results of the trial showed that the transplantation of the cells is a safe procedure. The most significant regenerative effect was observed in 10 patients who received cells during a therapeutic window of 10–30 days after SCI. These results correlate well with those from clinical trials reported by Park et al. and Cristante et al., in which stem cells isolated from bone marrow or peripheral blood, combined either with granulocyte macrophage-colony stimulating factor (GM-CSF) or after cryopreservation in 6 and 39 patients respectively, were used in the treatment of complete SCI followed by neurologic evaluation [168,169]. One of the most recent clinical trials delivered autologous BMSCs via lumbar puncture into the cerebrospinal fluid of patients with subacute SCI. As a result, almost half of the patients (45.5%) showed marked recovery (two-grade improvement from baseline using the ASIA score impairment scale) within 6 months after cell delivery, and no side effects related to the procedure were reported during the whole trial [170]. Apart from the improvement of the ASIA score in SCI patients after BMSC application (intraspinal plus intradural; additionally, through lumbar tapping 4–8 weeks after the first procedure), Park et al. also reported electrophysiological improvements as well as a decrease in cavity size and the appearance of fiber-like low signal intensity streaks on MRI [171]. The intravenous injection of autologous AMSCs into patients with SCI also did not show any adverse effects connected with the procedure [172].

Autologous MSC transplantation has also been shown to have a positive effect and to be a safe procedure after application into

Table 3
An overview of clinical trials involving cell based therapy to treat diseases of the CNS (modified from www.clinicaltrials.gov). Abbreviations: ? – unknown; na – not applicable; w.o. – without; Tx – transplantation.

Cell type (additional intervention)/dose	SCI/ASIA score (level of SCI)	Time after SCI/place of cell delivery	Evaluation time after Tx
Umbilical cord mesenchymal stem cells	Acute stage (20 cases); chronic stage (20 cases)/na	?/Intrathecal	3, 6, and 12 Months post-transplantation
Umbilical cord blood cells alone or in the combination with lithium	Acute and subacute/A/C5/T11	Within 4 weeks/I.S. injection into upper and lower edges	Week 2, 6, 14, 24 and 48
Human spinal cord-derived neural stem cells (HSSC)	Chronic/A/T2–T12	Between 1 and 2 years after SCI/I.S.	Over a 60 month period
Bone marrow derived autologous cells/10^{10} cells per dose	?/Complete and/or incomplete/ below C4	?/3× Intrathecal injections (10 day interval)	Every 6 months for 36 months
Cultured autologous bone marrow stromal cells/10^7–10^8 cells per dose	Acute/A, B, C (complete tetraplegia)/cervical	Within 72 h after SCI/1× intrathecal	6 Months
Bone marrow derived mesenchymal stem cells/?	Chronic/?/?	10 Months – 3 years/?	18 Months
Umbilical cord blood mononuclear cells alone, in combination with lithium carbonate and/or methylprednisolone/1.6–6.4 × 10^6	Chronic/A/C5–T11	>12 Months post SCI/intraspinal	Week 0, 2, 6, 14, 24 and 48
Autologous bone marrow stem cells with/w.o. glial scar resection/?	Acute, subacute and chronic/ A, B, C, D or E/C4–T12	≥6 Months after SCI (chronic phase)/ I.S. < 2 weeks (acute phase) and 2–8 weeks (subacute phase)/intrathecal	18 Months
Autologous bone marrow progenitor cells (BMPC)/5 mL/kg of bone marrow	Chronic/A, B, C, D or E/C4–T12	≥6 Months after SCI < 4 years/ intra-venous infusion	1 Day, 30 and 180 days; 1 and 2 years
Autologous mesenchymal stem cells/?	Chronic/A, B and C/below C4	≥3 Months/intralesional	1 Year
Bone marrow derived mononucleated stem cells/10^8	Chronic/A, B and C/below C5	6 Months < 8 years/intrathecal	18 Months
Autologous bone marrow stem cells/?	?/Frenkel A/?	?/Intralesional	6 Months
Human central nervous system stem cells (HuCNS-SC)/?	Sub-acute/A, B and C/T2–T11	≥6 Weeks/intraspinal	1 Year + 4 years
Human central nervous system stem cells (HuCNS-SC)/?	Chronic/na/T2–T11	Observation	4 Years
Autologous bone marrow derived mesenchymal stem cells/?	Subacute-chronic/A and B/T1–L5	≥2 Weeks < 1 year/intravenous combined with intrathecal	1, 3, 6 and 12 Months
Bone marrow-derived mesenchymal stem cells/intramedullary (1.6 × 10^7) and intrathecally (3.2 × 10^7)	Chronic/B/cervical	≥12 Months/intramedullary and intrathecally	6 and 12 Months
Adipose tissue derived mesenchymal stem cells/intravenous (2 × 10^8 cells/20 mL) Intrathecal (5 × 10^7 cells/2 mL) Intraspinal (2 × 10^7 cells/1 mL)	Chronic/A or B or C/?	>3 Months/intravenous, intrathecal, intraspinal	3, 6 and 8 Months
Autologous adipose derived mesenchymal stem cells/4 × 10^8	Subacute/A or B or C/?	>2 Months/intravenously	12 Months
Autologous adipose tissue derived mesenchymal stem cells/9 × 10^7 cells in 3 mL Day 1 and month 1 & 2	Subacute/?/?	≥4 Weeks/intrathecal	6 Months
Human ESC-derived oligodendrocyte progenitor cells (GRNOPC1)/2 × 10^6 cells	Complete, subacute/A/thoracic	7–14 Days post injury/intraspinal	12 Months
Autologous, *ex vivo* expanded bone marrow-derived mesenchymal stem cells/?	Subacute/A/below C5	≥2 Weeks/intrathecal	Short-term (1–30 days), long-term (2–12 months)

patients with an affected motor system (e.g., ALS or Huntington's disease). The long term outcome after nearly 9 years of monitoring 19 ALS patients, enrolled in two phase I clinical trials, showed no clear clinical benefits in these patients. The collected data show support for the implantation of autologous bone marrow MSCs into the dorsal spinal cord, as no structural changes (including tumor formation) or deterioration in psychosocial status were found, and all patients coped well with the procedure [164,173,174]. The transplantation of mononuclear CD133+ autologous stem cells from the peripheral blood into the frontal motor cortex of ALS patients significantly prolonged the survival of the treated patients and the maintenance of their lifestyle compared with untreated control patients [175]. Deda et al. reported the results of a one year follow-up after the implantation of bone marrow-derived hematopoietic progenitor stem cells into the anterior part of the spinal cord of 13 patients with a bulbar form of ALS: nine patients became much better compared with their pre-operative status, one patient was stable without any decline or improvement in his status [176]. Our preclinical data (Fig. 2) showed that the implantation of BMSCs into symptomatic SOD1^{G93A} rats decreases apoptosis in the host MN, significantly improves motor function and prolongs the survival of cell-treated compared with vehicle-injected rats [145]. This study formed a platform for a three year prospective, non-randomized, open label clinical trial that was launched in Prague (Czech Republic) in March 2012, aiming to assess the safety and efficacy of autologous multipotent mesenchymal stromal cells applied to patients with a confirmed diagnosis of ALS (http://www.sukl.eu). So far, 12 patients with a confirmed diagnosis have been recruited for the trial and injected with autologous BMSCs via lumbar puncture without any adverse effects.

A large number of preclinical trials using various types of stem cells have shown that cellular therapy has a beneficial effect in diseases of the cardiovascular, nervous (including neurodegenerative), cytoskeletal and endocrine systems. Of these, diseases of the CNS are of special interest considering their limited therapy and devastating character. The current achievements and future perspectives of *in vivo* preclinical studies involving cellular treatment

Country/company/identifier	Phase of the trial/stage/estimated trial end	Estimated number of patients	Results	Side effects	References
China/General Hospital of Chinese Armed Police Forces/NCT01393977	Phase 2/recruiting/2011	60	na	na	na
China/Chinese PLA Chengdu Army Kunming General Hospital/NCT01471613	Phase 1 and 2/active no recruiting/2013	60	na	na	na
US/Neuralstem Inc./NCT01772810	Phase 1/not started/2014	8	na	na	na
India/Chaitanya Hospital/Pune/NCT01833975	Phase 1 and 2/recruiting/2014	50	na	na	na
Japan/Translational Research Informatics Center, Kobe, Hyogo/NCT00695149	Phase 1 and 2/terminated/2010	5	ASIA B and/or C: improvement. ASIA A: limited/ no effect	None	[179]
Egypt/Cairo University/NCT00816803	Phase 1 and 2/Completed/2008	80	na	na	na
China/China Spinal Cord Injury Network/ NCT01354483	Phase 1 and 2/active not recruiting/2013	20	na	na	na
India/International Stemcell Services Limited/NCT01186679	Phase 1and 2/completed/2010	12	na	na	na
US/Memorial Hermann Healthcare Systém/ NCT01328860	Phase 1/recruiting/2014	10	na	na	na
Chile/Catalina Larrain M.D./NCT01694927	Phase 2/active, not recruiting/2014	30	na	na	na
India/Max Institute of Neurosciences/ NCT01730183	Phase 1 and 2/recruiting/2014	15	na	na	na
Brasil/Hospital Sao Rafael/NCT01325103	Phase 1/active, not recruiting/2013	20	na	na	na
Switzerland/StemCells, Inc./NCT01321333	Phase 1 and 2/recruting/2016	12	na	na	na
Switzerland/StemCells, Inc./NCT01725880	Post phase 1 and 2 follow up/enrolling by invitation/2018	12	na	na	na
China/Guangzhou General Hospital of Guangzhou Military Command/NCT01446640	Phase 1 and 2/recruiting/2014	20	na	na	na
Korea/Pharmicell Co., Ltd./NCT01676441	Phase 1 and 2/recruiting/2014	32	na	na	na
Korea/RNL Bio Company Ltd./NCT01769872	Phase 1 and 2/recruiting/2014	15	na	na	na
Korea/RNL Bio Company Ltd./NCT01274975	Phase 1/completed/2010	8	Unknown	Unknown	Unknown
Korea/Bukwang Pharmaceutical/NCT01624779	Phase 1/recruiting/2013	15	na	na	na
US/Geron Corporation/NCT01217008	Phase 1/ongoing, not recruiting/2013	10	na	na	na
US/TCA Cellular Therapy/NCT01162915	Phase 1/completed/2012	10	Unknown	Unknown	na

of SCI with different types of stem cells have been summarized and reviewed by a group of leading researchers in the field, in order to summarize the level of evidence required in experimental studies of cellular therapies before proceeding with clinical trials in human SCI patients [89]. We have overviewed and summarized completed and still ongoing clinical trials from the data available at the *www.clinicaltrials.gov* database, as well as trials that have just begun recruiting patients with SCI for treatment with MSCs (Table 3). As one can see, despite the fact that overall hundreds of patients were recruited into trials involving various cellular therapies, only a few groups have published the results of their studies, whereas the results from the others remain unavailable to the broad scientific community. Nevertheless, the number of new clinical trials increases from year to year. The explanation for this phenomenon might be the increasing number of results from *in vivo* experiments, as well as from phase I and II trials, which suggest that stem cell delivery is a safe procedure that leads to improvements in motosensory functions and tissue regeneration in up to 45% of patients, thus enhancing their quality of life.

7. Conclusions

Though mesenchymal stromal cells were described more than 20 years ago by Caplan, recently their rediscovered multiple effects and properties have brought new hope to the treatment of neurological diseases [177]. The use of different methods and routes of MSC administration into patients with traumatic, neurodegenerative and other diseases has shown that MSC grafting is a safe procedure that can bring benefits for patients in the form of improved motor and sensory function after transplantation. Several reports have shown that MSCs can modify the host microenvironment following CNS injury; however, most human trials have reported that the therapeutic effect of MSCs starts at the early post-transplantation stage, before the establishment of connections suitable for their incorporation into the host tissue, and that neurological improvement occurs within six months after the grafting of MSCs. Logically, this raises the question whether a recurrent graft of MSCs will further facilitate neuroregeneration? We also need a better understanding of the mechanisms of action and the behavior of stem cells in the pathological environment after transplantation, in order to determine what is the best time frame and what are the most efficient routes for cell delivery after the injury? Another important question is whether it is necessary to transplant a cell-containing suspension or whether the delivery of conditioned media will have a similar effect? These and many other questions remain to be answered before MSCs or other cell types can be translated into routine clinical practice. Other national or international multi-center collaborative clinical trials with larger and more homogenous groups of patients might be needed, to enable better understanding and comparison with control treatments, as well as to speed up the translation of the results into practical applications for the benefit of patients [178].

Acknowledgments

This study was supported by the grants CZ.1.07/2.3.00/30.0018, GA CR: 13-00939S, 304/10/0320, 304/11/0189 and P304/12/G069.

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