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Review article

Stem cell therapy in amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a rare and lethal neurodegenerative disease for which there is no effective medical treatment. Although riluzole, an *N*-methyl-D-aspartate receptor antagonist, has been shown to be reasonably safe for patients with ALS, the drug has been demonstrated to prolong median survival by only 2–5 months. There is mounting evidence that stem cell-based gene therapy is a promising treatment modality for patients with ALS. In this review, we focus on the types, sources, and doses of stem cells that have been shown to be effective for ALS patients, the differences in cytokines or chemokines secreted from these various stem cells, and the immune-modulation activity of stem cells as treatment for ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an incurable, degenerative neurological disease. The clinical characteristics of ALS include muscle weakness and atrophy, spasticity, and eventual paralysis due to the progressive loss of spinal and brainstem motor neurons. Death typically occurs 3–5 years after symptoms begin [1,2]. The disease currently affects an estimated 350,000 people worldwide, and there are no effective treatments. Although riluzole, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, is the only drug approved by the US Food and Drug Administration for ALS, it has been shown to offer only a modest improvement in symptoms and to prolong median survival by a maximum of 3–5 months. Therefore, an effective treatment for ALS is urgently needed. This review article focuses on the emergence of stem cell-based gene therapy in ALS.

2. Molecular mechanisms mediating the development of ALS

It is still not fully understood why specific neuronal populations are selectively vulnerable in ALS. Mutations in several genes have been shown to be related to the development of the disease, including mutations in the *SOD1*, TARDBP

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(*TDP-43*), *FUS/TLS*, *FIG4*, and chromosome 9 open reading frame 72 (*C9orf72*) genes. A hexanucleotide repeat expansion of the *C9orf72* gene has been identified as the underlying genetic cause of chromosome 9p21-linked frontotemporal lobar degeneration and ALS [3–5].

In addition, about 20% of cases of inherited ALS are caused by mutations in the superoxide dismutase-1 (*SOD1*) gene, particularly mutations that cause misfolding of the protein product [6]. Studies have shown that mutant *SOD1* transgenic mice with loss of *SOD1* function show phenotypic characteristics of motor neuron disease, including progressive deterioration of the brainstem and a functional loss of spinal motor neurons, resulting in weakness, loss of muscle function, and premature death. Interestingly, studies have shown that epigenetic factors, such as aging, are possible causes of ALS in more than 90% of patients with the disease [7].

3. Superoxide dismutases

In vivo, superoxide dismutases are responsible for peroxidation reactions in cells. These enzymes are divided into several species based on their intracellular locations. SOD1 (Cu/ZnSOD) is located in the mitochondrial intermembrane space and cytosol, while SOD2 (MnSOD) is found in the mitochondrial matrix. Although these dismutases are located in different intramitochondrial locations, these enzymes have the same catalytic functions.

4. Other molecular mechanisms of ALS

Excitotoxicity of motor neurons has also been implicated in the pathogenesis of ALS. Most patients with sporadic ALS express reduced levels of synaptosomal high-affinity glutamate uptake and glutamate transporters such as excitatory amino acid transporter 2 (EAAT2 or GLT1) in the motor cortex and spinal cord, resulting in apoptosis of motor neurons due to elevated extracellular glutamate concentrations [8,9]. The NMDA receptor antagonist riluzole effectively minimizes the overexcitation of motor neurons caused by elevated levels of extracellular glutamate and has been shown to have a good safety profile in patients with ALS; however, the drug only extends the lifespan of ALS patients by several months [10–14].

5. Pathology of mutant SOD1 transgenic mice

In 1994, Gurney et al established strains of transgenic mice that express mutant human SOD1 (mSOD1) in order to study the impact of overproduction of mutant SOD1 protein and its accumulation on motor neuron function in ALS [15,16]. Of these mSOD1 transgenic mice, a strain of hemizygous mice harboring human SOD1 with the G93A mutation in high copy number has been shown to be an appropriate model for studying ALS in mice with a short lifespan because these mice become completely paralyzed and die within 16–18 weeks of age. On the other hand, G93A-mSOD1 mice with a low transgene copy number are used to study ALS in mice with longer lifespans. These mice demonstrate much slower disease progression and die within 8–9 months of age [16]. The results of pathological studies of these mutant SOD1 mice have revealed accumulation of mutant SOD1 in the brainstem and spinal motor neurons, marked inflammation around the dying neurons, and overexpression of cytokines such as tumor necrosis factor alpha and interferon gamma in spinal lesions [17,18].

6. Stem cell therapy as treatment for ALS

Stem cells have the ability to continuously divide and differentiate into a number of different types of cell. Stem cells also secrete various cytokines, chemokines, and trophic factors that are known to modulate inflammation, attract other stem cells to sites of injury, enhance cell survival, and participate in angiogenesis and neurogenesis [19,20]. Tables 1 and 2 provide a review of clinical trials in ALS patients and animals.

Table 1 – Clinical trials of stem cell therapy as treatment for amyotrophic lateral sclerosis (ALS).

Humans	Stem cell source	Conditioning regimen	Delivery method	Dose	Outcome
sALS patients	$CD34^+$ HSCs	Total body irradiation (450 cGY); tacrolimus (0.3 mg/kg/d IV) and methotrexate (5 mg/m^2 IV)	IV injection	Absolute neutrophil count $>5 \times 10^8$/L	No clinical benefit
ALS patients	Autologous MSCs	None reported	Multiple intraspinal thoracic subcutaneous injections	Approximately 5.7×10^7 cells total	Decelerated linear decline of forced vital capacity
ALS patients	Autologous $CD133^+$ cells	None reported	Bilateral injection into frontal motor cortex	$2.5–7.5 \times 10^5$ cells/site	Survived more than 47 months

IV = intravenous; HSC = hematopoietic stem cells; MSC = mesenchymal stem cells; sALS = sporadic amyotrophic lateral sclerosis.

Table 2 – Current clinical trials in amyotrophic lateral sclerosis (ALS).

Trial institutions	Stem cell source	Delivery method	Dose	Outcome
Neuralstem Inc.	Human spinal cord-derived neural stem cells	Surgical implantation	None reported	Phase I Safe. Delay in symptoms of disease
Fundacion para a Formacion e Investigacion Sanitarias de la Region de Murcia	Autologous bone marrow stem cells	Intraspinal transplantation and intrathecal infusion of autologous bone marrow stem cells	None reported	Phase II
Corestem, Inc	Autologous bone marrow-derived stem cells	HYNR-CS intrathecal injection with 1 mL/10 kg body weight at an interval of 26 days	None reported	Phase II
Mayo Clinic	Mesenchymal stem cells	Single intrathecal lumbar puncture	10×6 cells	Phase I
TCA Cellular Therapy	Autologous bone marrow-derived stem cells	Infusion of autologous bone marrow-derived stem cells	None reported	Phase I
Hadassah Medical Organization	Autologous cultured mesenchymal bone marrow stromal cells secreting neurotrophic factors	IM in patients with early ALS IT in patients with progressive ALS	IM: patients were injected at 24 sites with a total of 24 million cells IT: intrathecally via a standard lumbar puncture, with a total of 60 million cells	Phase II

HYNR-CS = autologous bone marrow-derived stem cells; IM = intramuscular; IT = spinal cord injection. muscle injection.
Data source: http://clinicaltrials.gov/ct2/home.

7. Hematopoietic stem cell therapy in ALS

$CD34^+$ hematopoietic stem cells (HSTCs) were first used to treat patients with leukemia because these cells are easily isolated from bone marrow and peripheral blood. In 2008, however, Appel et al reported that $CD34^+$ stem cell transplantation therapy could only be performed in patients who received peripheral blood HSTC from an identically matched human leukocyte antigen donor [21]. Transplantation by intravenous injection of HSTCs to ALS patients with an absolute neutrophil count $> 0.5 \times 10^9$/L revealed that inflammatory cells (macrophages and monocytes) proliferated in the spinal cord. The authors then used immunohistochemical staining to show that HSTCs accumulated in the spinal cord and released chemokines. Although the results showed that administration of HSTCs induced a strong immune response, the authors found that the use of $CD34^+$ stem cells did not lead to any marked improvement in symptoms of ALS [21] (Table 3).

8. Effectiveness of mononuclear cells from umbilical cord blood in ALS

Recently, Garbuzova-Davis et al treated pre-symptomatic G93A-mSOD1 mice with an intravenous injection of a single low dose (10×10^6 cells), a moderate dose (25×10^6 cells), or a high dose (50×10^6 cells) of umbilical cord blood-derived mononuclear cells (MNC-hUCBs) and found that the moderate dose (25×10^6 cells) significantly increased lifespan by 20–25% and delayed disease progression by 15% [22]. The most beneficial effect on decreasing proinflammatory cytokines in the brain and spinal cord was found in mice that received moderate-dose therapy. In addition, results of hematological assays showed that the number of lymphocytes was significantly higher and the number of neutrophils significantly lower in the peripheral blood of mice that received a dose of 25×10^6 cells than in the peripheral blood of mice that received low-dose or high-dose therapy. Moderate-dose therapy was also shown to result in a marked reduction in microglial density in the cervical and the lumbar spinal cord, indicating that MNC-hUCB cells transplanted via intravenous injection can move into cervical and lumbar tissue. The findings demonstrate that transplantation via intravenous injection of a moderate dose (25×10^6 cells) of MNC-hUCB cells may provide a neuroprotective effect for motor neurons and prolong the survival rate of mice with ALS [20].

9. Mesenchymal stem cell therapy in ALS

Bone marrow-derived mesenchymal stem cells are widely used for the treatment of many human diseases [23]. Mazzini et al found that bone marrow-derived mesenchymal stem cells that had been cultured for two or three generations and then transplanted via multiple intraspinal thoracic injections at a dose of 57×10^6 cells did not produce a strong

Table 3 – Use of cell therapy for the treatment of amyotrophic lateral sclerosis in animal models.

Animal model	Stem cell source	Conditioning regimen	Delivery method	Dose	Outcome
$SOD1^{G93A}$ rats	Human NPCs	FK-506 (1 mg /kg daily)	Bilateral lumbar SC injections	2×10^4 cells/site, eight sites	No NMJs with host muscle
$SOD1^{G93A}$ rats	Rat GRPs	Ciclosporin A (10 mg/kg daily)	Bilateral cervical SC injections	1×10^5 cells/site, six sites	Prevented MN loss; increased lifespan
$SOD1^{G93A}$ rats	Human NPCs	Ciclosporin A (10 mg/kg daily)	Unilateral lumbar SC injections	$1.2–1.8 \times 10^5$ cells/site, four sites	Prevented MN loss; did not innervate muscle end plates
$SOD1^{G93A}$ rats	Human MSCs	Ciclosporin A (10 mg/kg daily); focal muscular injury with bupivacaine hydrochloride (0.35 mg)	Bilateral muscle injections	1.2×10^5 cells/site	Increased number of NMJs and MN cell bodies; prevented loss of proximal MNs
$SOD1^{G93A}$ mice	Human MSCs	None	Unilateral lumbar SC injection	1×10^6 cells/site	Delayed MN loss; improved motor performance
$SOD1^{G93A}$ mice	Human umbilical cord blood cells	Ciclosporin A (10 mg/kg daily)	IV injection	10×10^6 cells, 25×10^6 cells, or 50×10^6 cells per mouse	25×10^6 cells was the most effective dose; increased lifespan (20–25%) and delayed disease progression (15%)
$SOD1^{G93A}$/*PU.1*, $SOD1^{G93A}$/*RAG2* mice	Mouse BM	Gamma-irradiation (400 rads)	IP injection, $SOD1^{G93A}$/*PU.1*/ mice; IV injection, $SOD1^{G93A}$/RAG2/ mice	1×10^7 cells per $SOD1^{G93A}$/*PU.1* mouse; 3×10^7 cells per $SOD1^{G93A}$/*RAG2* mouse	Prolonged survival

BM; GRP; IP = intraperitoneal; IV = intravenous; MN = motor neuron; MSC = mesenchymal stem cells; NMJ = neuromuscular junction; NPC; SC = spinal cord.

inflammatory response and did not result in abnormal cell proliferation in the spinal cord in ALS patients during 4 years of post-transplant follow-up. In addition, 50% of patients showed evidence of a significant slowing down of the linear decline of forced vital capacity [24]. Cell transplantation by spinal injection is a high-risk procedure, and the long-term safety profiles of administering stem cells via that approach need to be established in clinical trials involving larger numbers of patients.

There is mounting evidence that progression of ALS is related to inflammatory and immune responses. A recent study by Rentzos et al revealed that the levels of CD8 cytotoxic T-cells and natural killer T-cells were significantly higher and that the number of regulatory T-cells was significantly lower in the peripheral blood of ALS patients than in blood from normal controls [25]. Therefore, mesenchymal stem cell therapy might be able to modulate the host immune inflammatory response and extend the survival of ALS patients.

10. Neuroprogenitor cell therapy in ALS

$CD133^+$ stem cells have the ability to differentiate into multiple neural lineages. Recently, Martinez et al studied the effects of $CD133^+$ progenitor cells on survival in ALS patients. A total of 20 patients with ALS were randomized to a treatment group or a control group. The treatment group received a subcutaneous injection of 300 μg of filgrastim for 3 days to stimulate the overproduction of stem cells in bone marrow. $CD133^+$ stem cells were then separated from peripheral blood using magnetic bead separation. Patients then received $2.5–7.5 \times 10^5$ cells per 300 μL cerebrospinal fluid (CSF) by bilateral injection into the frontal motor cortex. They found that transplantation of $CD133^+$ progenitor cells resulted in a delay in disease progression and increased survival [26].

11. Stem cell therapy in ALS at the China Medical University

Mesenchymal stem cells can be derived from a number of tissue types, including adipose tissue, dental pulp, and umbilical cord blood. Our group is currently studying the effects of different types of stem cells in animal models of ALS to determine the type that is most effective at suppressing the immune and inflammatory responses and which can therefore be developed as an appropriate vector therapy.

Our preliminary data show that the effects of stem cells as treatment for neurodegenerative diseases may be due to their ability to secrete chemokines or their ability to regulate the immune response. We have found that transplantation of stem cells by cortical spinal tract injection prolongs the lifespan of mice with ALS by about 150 days. The results of immunohistochemical staining have revealed the presence of

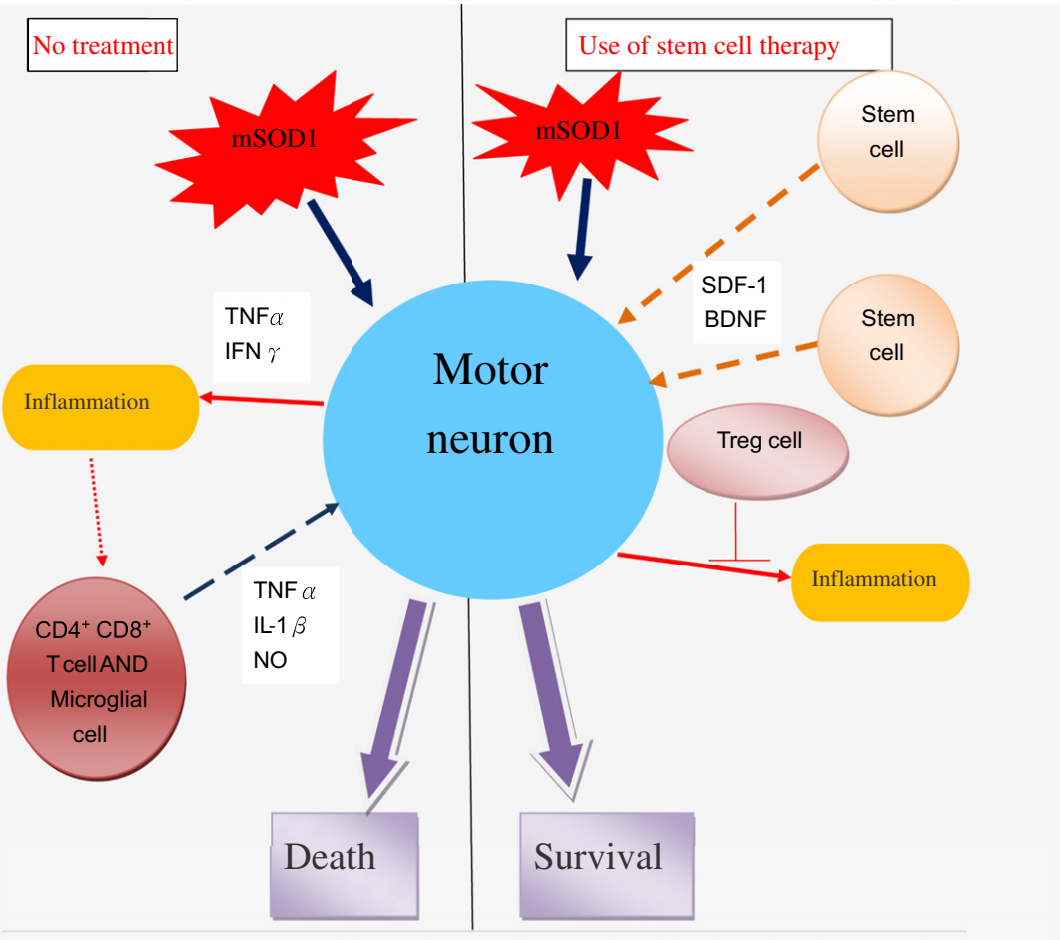

Fig. 1 – Hypothetical mechanisms governing the effectiveness of stem cell therapy as treatment for amyotrophic lateral sclerosis. Transplantation of stem cells results in increased levels of chemokines and trophic factors (SDF-1, BDNF, CXCR-4,), resulting in delayed progression of motor neuron disease. IFN = interferon; TNF = tumor necrosis factor; mSOD = mutant human superoxide dismutase; Treg = regulatory T-cell.

several types of stem cell in the lumbar spinal cord and evidence of increased levels of chemokines and trophic factors, such as stromal cell-derived factor-1 (SDF-1), Brain-derived neurotrophic factor (BDNF), and C-X-C chemokine receptor type 4 (CXCR-4). The findings support our hypothesis that transplanted stem cells are attracted to sites of injury by inflammatory signaling molecules (Fig. 1).

In the future, we plan to conduct preclinical trials to study the effects of stem cells that have been transfected with wildtype *SOD1* as well as the effects of other stem cell-based gene therapies on disease progression and survival in animal models of amyotrophic lateral sclerosis.

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