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Prospects of Bone Marrow Mononuclear Cells and Mesenchymal Stem Cells for Treating Status Epilepticus and Chronic Epilepsy

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SUMMARY

Mononuclear cells (MNCs) and mesenchymal stem cells (MSCs) derived from the bone marrow and other sources have received significant attention as donor cells for treating various neurological disorders due to their robust neuroprotective and antiinflammatory effects. Moreover, it is relatively easy to procure these cells from both autogenic and allogenic sources. Currently, there is considerable interest in examining the usefulness of these cells for conditions such as status epilepticus (SE) and chronic epilepsy. A prolonged seizure activity in SE triggers neurodegeneration in the limbic brain areas, which elicits epileptogenesis and evolves into a chronic epileptic state. Because of their potential for providing neuroprotection, diminishing inflammation and curbing epileptogenesis, early intervention with MNCs or MSCs appears attractive for treating SE as such effects may restrain the development of chronic epilepsy typified by spontaneous seizures and learning and memory impairments. Delayed administration of these cells after SE may also be useful for easing spontaneous seizures and cognitive dysfunction in chronic epilepsy. This concise review evaluates the current knowledge and outlook pertaining to MNC and MSC therapies for SE and chronic epilepsy. In the first section, the behavior of these cells in animal models of SE and their efficacy to restrain neurodegeneration, inflammation and epileptogenesis are discussed. The competence of these cells for suppressing seizures and improving cognitive function in chronic epilepsy are conferred in the next section. The final segment ponders issues that need to be addressed to pave the way for clinical application of these cells for SE and chronic epilepsy. STEM CELLS *2015; 00:000–000*

INTRODUCTION

There are over 50 million patients with epilepsy in the world [1]. Although antiepileptic drugs (AEDs) are the mainstay of treatment, almost a third of these patients are refractory to such pharmacological intervention [2]. The patients with epilepsy can also present with status epilepticus (SE) manifested as prolonged seizures, which is a common neurological emergency and often resistant to treatment with AEDs. Moreover, AEDs merely provide symptomatic treatment without influencing the course of the disease. Currently available alternative options such as epilepsy surgery, ketogenic diet, deep brain or vagal nerve stimulation are either not feasible in all patients or only partially effective [3-6]. Thus, it is imperative to develop alternative therapeutic approaches that considerably modify the disease process and thereby thwart the evolution of SE into a chronic epileptic state. This understanding in recent years has led to a paradigm shift in research focus involving epilepsy therapeutics. Modern epilepsy research is more converged towards understanding the pathophysiology that has prompted considerable attention towards biotherapies. These include gene therapy and neural cell transplantation approaches [7], and more recently administration of mononuclear cells (MNCs) or mesenchymal stem cells (MSCs) derived from the bone marrow and other sources.

Numerous animal model studies have demonstrated that intracerebral gene and neural cell therapies in acute and chronic models of epilepsy have promise for providing neuroprotection, facilitating neural repair, inducing anti-seizure effects, delaying the time-course of epileptogenesis and thwarting/reducing the severity of chronic epilepsy [7-22]. Gene therapy appears to be beneficial for treating chronic refractory focal epilepsy and for restraining SE induced chronic epilepsy development [11,13]. Focal epilepsies, and in particular temporal lobe epilepsy (TLE), appear to be better candidates for gene therapy [14]. However, there are concerns that gene therapy approaches that alter the expression of a single gene may be offset by the modified expression of other endogenous genes, which may result in extensive modifications in synaptic, neuronal or circuit excitability [10]. Pertaining to intracerebral neural cell transplantation, studies have mostly focused on restraining the development of chronic epilepsy after SE or treating established chronic epilepsy. The donor neural cell types that are being critically examined in animal models of SE and chronic epilepsy include hippocampal precursor cells [12,22], neural stem cells (NSCs) [8,15,18], and gamma-amino butyric acid (GABA)-positive neuronal precursors [16-21]. The goals of these studies include the reconstruction of the disrupted circuitry [12,22], enhancement of the inhibitory neurotransmission in the epileptic areas through replacement of lost GABA-ergic interneurons [16-21] and addition of healthy astrocytes secreting anticonvulsant proteins and/or other trophic factors [8,15,18]. These approaches have yielded promising results so far, particularly in terms of reducing recurrent seizures, normalizing the host astrocytes that have become abnormal in epileptic areas, promoting neuroprotection and neural repair or improving cognitive and mood function [8,15-22].

Thus, both gene and neural cell transplantation therapies have great promise for restraining the development of SE-induced epileptogenesis or treating established focal chronic epilepsies. However, these approaches may not be ideal for controlling acute SE that is resistant to AEDs. The limitation of gene and cell therapy for acute SE is often the affliction of seizure activity in multiple areas of the brain and the requirement for employing targeted transfection or transplantation in multiple affected areas. Delays in gene expression after intracerebral transfection or differentiation after intracerebral neural cell grafting are other issues that may affect the efficacy of these therapies for acute SE. Furthermore, application of gene or neural cell therapy as a pre-treatment strategy or autogenic neural cell grafting intervention early after SE is clinically impracticable. The use of allogenic stocks of neural cells generated through directed differentiation of human pluripotent stem cells (PSCs) may solve some of the above issues. However, such cells are currently not ready for clinical application because of their propensity to cause teratoma if contaminated with PSCs and long-term immunological complications [23]. From these perspectives, non-neural cell types such as MNCs or MSCs derived from the bone marrow and other sources have received considerable attention in the field of epilepsy therapeutics. It has been proposed that both MNCs and MSCs have the potential to restrain the development of chronic epilepsy when infused early after SE and modify the disease process with interventions occurring after the establishment of chronic epileptic state. Therefore, in this review, we critically discuss the prospects and limitations of MNC and MSC based therapies for SE-induced injury and chronic epilepsy, with an emphasis on possibilities for translating the bench research to bedside.

Basis for using MNCs and MSCs for Treating SE and Chronic Epilepsy

Both MNCs and MSCs derived from the bone marrow and other sources hold great promise for the treatment of a variety of diseases [24-34]. These cells also have minimal immunogenicity [24-26] and MSCs in particular, can be differentiated into multiple lineages and expanded easily in culture for multiple passages. There are many reasons for considering these cells as attractive for treating SE and epilepsy. To begin with, a multitude of studies have shown the efficacy of these cells to improve function in animal models of several neurological disorders such as multiple sclerosis, stroke, Alzheimer's disease and brain injury [27,28]. Although pre-

cise mechanisms that underlie beneficial effects have not been elucidated, potent anti-inflammatory effects of these cells have been demonstrated in multiple disease models [29-33]. Interestingly, several studies have shown that engrafting of infused MNCs/MSCs into the diseased brain is not a pre-requisite for obtaining functional recovery. Rather, a global modification of the immune system by these cells through potent anti-inflammatory and possibly other trophic effects are sufficient for affording neuroprotection and disease modification.

Moreover, MNCs and MSCs derived from the bone marrow and other sources have been shown as relatively safe to be used in humans [35-37]. Furthermore, unlike gene and neural cell therapy requiring injections/grafting into the site of injury or diseased brain loci, relatively non-invasive approaches can be employed to administer these cells. These cells are particularly amenable for dispensation through intravenous, intra-arterial, intraperitoneal, intrathecal or intranasal routes [38-41], which avoids any damage that can occur with direct injections of vectors or neural cells into diseased brain regions. Furthermore, these cells are easily accessible as donor cells because MNCs can be freshly harvested from the human bone marrow and the umbilical cord blood, and MSCs or MSC-like cells can also be expanded from fresh and frozen samples of several other tissues. For example, human adipose tissue derived stem cells (ASCs) are a great alternative source of MSCs, as they can be easily isolated from lipoaspirate (a byproduct of liposuction procedures) [42]. On the other hand, human dental-derived MSC-like cells obtained from a variety of dental tissues is another source of MSC-like cells displaying self-renewal, multilineage differentiation potential and immunomodulatory properties [43] .Furthermore, a large bank of MSC-like cells can also be obtained from several regions of the human umbilical cord, including the umbilical cord lining, the sub-endothelial layer, the perivascular zone and Wharton jelly [44]. Besides, huge amounts of MSCs can be obtained through human induced pluripotent stem cells (hiPSCs) [45]. Ability to obtain these cells from the bone marrow as well as from adipose, dental and umbilical cord tissues and hiPSCs particularly facilitates autogenic transplantation of these cells in patients, if found highly efficacious in animal models. There are also no ethical concerns regarding the use of MSCs.

Potential of MNCs and MSCs for Easing SE-induced Epileptogenesis

Status epilepticus (SE) is a time-critical emergency that requires prompt recognition and immediate treatment across all age groups [46-47]. Widely accepted definition of SE, including that adopted by the working group on SE of the Epilepsy Foundation of America is a 30-minute duration of seizures [48-49]. Seizure types in SE are defined as partial or generalized SE based on the international classification of seizure types and as defined by the International League Against Epilepsy (ILAE) [50]. Partial SE can be simple partial, complex partial and partial with secondary generalization. Simple partial SE refers to episodes where the patient maintains alertness and the ability to interact appropriately with the environment during partial seizure activity that lasts for 30 minutes or longer. Complex partial SE refers to episodes of partial seizures with confusion and amnesia for the ictus. On the other hand, partial seizures with secondary generalization represent an SE that initiates with partial onset seizures and subsequently becomes secondarily generalized, as per the criteria of ILAE. A prospective epidemiological SE study has revealed that 68% of SE patients displayed partial onset seizures and 32% exhibited generalized activity from the onset of SE [51]. While a brief single episode of seizure may not induce lasting changes in the brain, prolonged seizures or SE typically cause permanent circuitry changes in the brain [52-53]. Despite adequate treatment, SE has an overall mortality up to 30% and survivors have serious morbidities that includes developmental delays in children, cognitive impairments, chronic epilepsy and recurrent SE [51, 54-60]. The current standard essential treatment goal is to stop seizures using AEDs. However, SE is often refractory to initial two AEDs at recommended doses [61-62]. This is only a symptomatic treatment for arresting seizures but does not influence SE-induced changes such as epileptogenesis, which is a complex dynamic process that progressively alters the excitability of neurons, establishes critical aberrant circuitry, and likely involves intricate changes at network levels before the first spontaneous seizure occurs [63]. A multitude of epileptogenic changes ensue after an episode of SE, which evolve over a period of months, years or even decades and result in chronic epilepsy once they reach certain thresholds [64-66].

Usefulness of MNCs from the bone marrow or umbilical cord blood

Several studies have tested the efficacy of heterogeneous MNCs for controlling seizures when administered in the early phase after SE (Table 1). Costa-Ferro and associates were the first to suggest the therapeutic potential of bone marrow derived MNCs (BM-MNCs) for restraining SE-induced chronic epilepsy using a rat model [67].They injected rat/mouse BM-MNCs intravenously to rats at ~90 minutes after the induction of SE. Such treatment: (i) prevented the occurrence of stage V spontaneous recurrent seizures (SRS) in the early phase after SE; (ii) greatly reduced the frequency and duration of seizures in the chronic phase after SE; (iii) preserved long-term potentiation (LTP); and (iv) reduced the loss of neurons and gliosis in the hippocampus. These beneficial effects were associated with neither widespread engrafting of BM-MNCs into the hippocampus nor differentiation of engrafted cells into neurons or glia in the brain. Thus, neuroprotective and anti-inflammatory effects of BM-MNCs have likely eased epileptogenesis and chronic epilepsy in this study.

Indeed, a follow-up study using a mouse model of SE demonstrated the involvement of soluble factors produced by BM-MNCs in mediating antiinflammatory effects [68]. Mice treated with BM-MNCs or BM-MNC lysates after SE displayed diminished neuronal loss, reduced expression of genes encoding pro-inflammatory cytokines, and increased expression of genes encoding anti-inflammatory cytokines in the hippocampus. In addition, serum from these animals displayed reduced level of a pro-inflammatory cytokine (Tumor necrosis factor-alpha) and increased concentration of anti-inflammatory cytokines (interleukins 4 and 10). Furthermore, the expression of genes related to classic type-1 activation of microglia such as inducible nitric oxide synthase (iNOS) was reduced in animals receiving BM-MNCs or BM-MNC lysate. However, there are some issues that remain to be clarified in future studies. Since only behavioral seizures were measured, it was unclear whether electrographic seizures were also reduced in animals treated with BM-MNCs. Additionally, since BM-MNC cell suspension is a mixture of B-lymphocytes, T-lymphocytes and monocytes in different stages of maturation and progenitors such as hematopoietic stem cells, MSCs, endothelial progenitor cells and very small embryonic-like cells [69], it was unclear whether the beneficial effects observed were due to all BM-MNCs or other specialized progenitors such as MSCs. Another study using a rat model of SE showed that administration of MNCs from the human umbilical cord is also efficacious for providing hippocampus neuroprotection and reducing SRS in the chronic phase of epilepsy [70]. Collectively, these results imply that administration of MNCs early after SE is efficacious for restraining chronic epilepsy development, regardless of the source from which MNCs are derived.

Efficacy of purified MSCs from the bone marrow

The efficacy of administration of purified MSCs in the early phase after SE for restraining seizures has been examined (Table 2). In one of these studies, the neuroprotective effects of CD11b-, Sca1+, CD44+ MSCs isolated from the mouse bone marrow were first examined in a cell culture model [71]. They used a co-culture system in which mouse cortical neurons were cultured in direct contact with MSCs and then exposed to N-methyl-D-aspartate (NMDA). Such exposure in control sister cultures caused excitotoxicity due to NMDA receptor (NMDAR)-triggered calcium influx. However, co-culturing of cortical neurons with MSCs prior to NMDA exposure protected neurons against excitotoxic cell death. Neuroprotection was also observed when neurons were incubated with the MSC conditioned medium for 24 hours prior to NMDA treatment, which implied that MSC-secreted soluble factors mediated neuroprotection against NMDA. Furthermore, measurement of mRNA levels of Grin1, which encode the NR1 subunit of the NMDA receptor, showed that treatment of cortical neurons with NMDA increases Grin1 mRNA levels. Interestingly, cortical neurons pre-treated with MSC conditioned medium prior to NMDA exposure did not show this upregulation in Grin1, suggesting that MSCs have the ability to prevent the upregulation of NMDA receptor subunit expression. Studies on calcium fluxes using retinal ganglion cells revealed that MSC conditioned medium pre-treatment abolishes calcium increases that are typically seen in neurons with exposure to NMDA [71]. Microarray analysis showed that MSC treatment altered the gene expression pattern of cortical neurons to include non-neuronal and stem cell genes. This altered gene expression profile may have also promoted neuroprotection against glutamate toxicity [71].

Further investigation of the capability of MSCs for providing neuroprotection using an *in vivo* kainic acid (KA) model of glutamate excitotoxicity showed matching results [71]. Intravenous administration of EGFP+ MSCs at 24 hours after the induction of SE in a mouse model reduced neuronal damage, hypertrophy of GFAP+ astrocytes and activation of Iba-1+ microglia in the hippocampus. Since intravenously administered MSCs did not engraft into the injured hippocampus, it was clear that MSC-produced soluble factors bestowed neuroprotection. This is in agreement with the prevailing notion that MSC-mediated therapeutic benefits are not dependent upon their engraftment and integration into the affected organ [72]. Another study in a rat model examined the effects of intraperitoneal administration of human BM-derived MSCs an hour after SE. [73] The results showed considerable protection of principal neurons, reduced loss of GABA-ergic interneurons, normalization of pro-inflammatory cytokine levels, reduced concentration of myeloperoxidase and enhanced expression of genes encoding antiinflammatory cytokines in the hippocampus [73]. Nonetheless, these studies have one major caveat, which is the lack of assessment of the effects of MSC administration on the development of SRS after KA-induced SE. A recent study has examined the effects of intravenous administration of MSCs on SRS in a rat model of epilepsy however [74]. Cells were infused 24 or 36 hours after the first seizure induced by pilocarpine injection and behavioral SRS were monitored in the subsequent three weeks. Rats receiving MSCs after SE displayed ~66% reduction in behavioral SRS, in comparison to rats receiving PBS after SE. Taken together, the above studies suggest that inhibition of NMDA receptor subunit expression and glutamate-induced calcium fluxes by MSC-produced soluble factors likely underlie neuroprotection and restrained chronic epilepsy development after MSC administration.

Benefits of genetically altered MSCs

Several studies have also examined the usefulness of genetically altered MSCs for restraining seizures after SE (Table 2). Li and colleagues tested the effects of human MSCs engineered to release adenosine on the occurrence of seizures in a mouse model of SE [75]. Intrahippocampal grafting at 24 hours post-SE and evaluation at

three weeks after grafting via EEG recordings revealed reduced frequency and duration of SRS, in comparison to sham-grafted animals. Interestingly, an injection of selective adenosine-1 receptor antagonist reversed these beneficial effects, implying that paracrine augmentation of adenosine by grafted MSCs mediated seizure-suppressing effects. Histological analyses revealed surviving grafted MSCs in the infrahippocampal fissure at three weeks post-grafting. Thus, increased adenosine levels in the hippocampus mediated through grafting of human MSCs engineered to release adenosine can also reduce seizures after SE. This study also suggested that MSCs are useful as drug carriers or microfactories delivering drugs over protracted periods in the epileptic brain. Another recent study showed that blocking of Hes1 gene in bone marrow derived MSCs leads to differentiation of MSCs into neuron-like cells expressing the inhibitory neurotransmitter GABA in vitro [76]. Since the inhibitory GABA-ergic neurotransmission is reduced in the epileptic brain [77], this study examined the effects of intracerebroventricular grafting of Hes1 silenced MSCs on the suppression of SRS in a rat model of epilepsy. Grafting of MSCs within 2 hours after the induction of SE decreased mortality. At 1-3 weeks post-grafting, diminished epileptiform waves and discharges were seen with differentiation of some graft-derived cells into GABA+ cells in temporal lobe regions that are adjacent to parahippocampal cortical areas. However, graft-derived cells were absent at 4 weeks post-grafting, implying that both Hes1 silenced and naive MSCs may not survive for prolonged periods in the epileptic brain. Additionally, the overall effects on epileptiform waves mediated by Hes1 silenced MSCs and naive MSCs seemed quite similar in this study, which raises a question whether modification of MSCs into GABA-producing cells is required to obtain the beneficial effects. Long-term survival of MSCs is not a significant issue, if one-time grafting can modify the disease process permanently. However, the latter issue was not examined in this study.

Efficacy of MNCs and MSCs for Treating Chronic Epilepsy

Recurrent seizures that are refractory to two or more AEDs are known as drug-resistant epilepsy, which poses huge clinical, psychosocial and economic burden. As mentioned earlier, because of lack of efficient antiepileptogenic drug therapies for intractable epilepsy, alternative treatments such as gene and neural cell therapies are being developed using preclinical models of focal epilepsy (particularly TLE) with considerable success [7-22,78-80]. Since focal epilepsies such as TLE represent only a limited fraction of the overall epilepsy prevalence, alternative therapies that have minimal side effects and are also amenable for peripheral administration with least invasive procedures have immense value for treating multiple types of epilepsies, including hard to treat genetic epilepsies afflicting children.

A few studies have examined the efficacy of BM-MNCs or MSCs for treating chronic epilepsy (Table 3). In one of these studies, intravenous administration of EGFP+ mouse BM-MNCs into rats at 22 days post-SE reduced behavioral SRS in the subsequent two weeks [81]. Characterization of cognitive function using a water maze test further suggested amelioration of learning and memory impairments associated with chronic epilepsy in these rats. [81] In addition, the polymerase chain reaction analysis suggested the presence of EGFP+ BM-MNCs in the brain. [81] A follow-up study by the same group suggested that reduced neuron loss, diminished astrocyte hypertrophy, normalized expression of genes encoding pro-inflammatory cytokines, and increased expression of genes encoding antiinflammatory cytokines underlie the beneficial effects mediated by BM-MNCs in epileptic rats [82]. Additionally, this study has revealed that even a delayed administration of BM-MNCs after SE (i.e. at 10-month post-SE) is efficacious for reducing SRS, diminishing astrocyte hypertrophy, improving neurogenesis, and enhancing the expression of antiinflammatory cytokine genes in the hippocampus [82].

Another study examined the effects of implantation of autologous MSCs labeled with paramagnetic iron oxide particles (PIOP) into the right hippocampus in rats, a month after the induction of SE [83]. Tracking of graft-derived cells at 1 and 3 months post-grafting using magnetic resonance imaging (MRI) showed migration of implanted cells towards the corpus callosum and the ependyma lining the lateral ventricles. Measurements using EEG performed 15 days and 3 months after grafting showed significant reductions in the frequency and amplitude of epileptiform discharges. Rats receiving MSCs also exhibited survival of graft-derived cells at 3 months post-grafting. There was also an improved ratio of adenosine 1 receptor (A1R) and adenosine 2a receptor (A2aR) at 3 months post-grafting, in comparison to progressive reductions in the density of A1Rs seen between 1 and 6 months post-SE in animals receiving no grafts. This finding suggested that adenosine receptors play an important role in chronic epilepsy development and MSC administration can normalize this alteration in adenosine receptors, likely through sustained release of adenosine. While these results are interesting, there are some limitations in this study. These include the lack of quantification of critical parameters such as adenosine levels, the extent of inflammation, all SRS using long-term EEG recordings and graft derived cells and their phenotypes. Furthermore, engrafting of cells was not confirmed with immunohistochemical methods. Hence, it was unclear whether PIOP+ elements observed with MRI represented the surviving injected cells or macrophages that engulfed PIOP from dead grafted cells or the fusion of host cells and PIOP labeled grafted cells.

Are MNC or MSC Therapies for Epileptic Conditions Ready for Clinic?

From the discussion of studies performed in animal models of epilepsy, it appears that both MNCs and MSCs are efficacious for restraining SE-induced chronic epilepsy when treated early after SE, and for easing SRS and cognitive dysfunction when administered after the establishment of chronic epilepsy. However, there are several issues that remain to be addressed prior to considering the clinical application of MNC or MSC therapy for a variety of epileptic conditions. The foremost issue is that, the exact mode of action or the underlying mechanism by which these cells restrain SRS and improve cognitive function are mostly unknown though global antiinflammatory effects and modification of glutamate receptors have been suggested in some studies. While a precise knowledge on mechanisms is not a pre-requisite for proceeding with clinical trials as long as beneficial effects are consistently seen and the procedure is safe, knowing modes of action would allow further improvement of the treatment procedure through the use of appropriate cells, the most reliable route of administration and the best time-window of intervention for maximal efficacy. The possible mechanisms by which MNCs and MSCs likely exert beneficial effects when administered after SE or in chronic epilepsy are proposed and illustrated in Figure 1, which are based on studies performed using these cells in different disease models. [34] Conditions such as SE or recurrent seizures are typically associated with hippocampus injury. This can increase concentrations of pro-inflammatory cytokines and release damage-associated molecular pattern molecules (DAMPs) in the brain and the circulating blood. When MNCs or MSCs are administered peripherally, they get trapped first in organs such as lungs, liver, spleen and lymph nodes, where they get activated and release microvesicles and paracrine anti-inflammatory factors including the tumor necrosis factor-inducible gene 6 protein **(**TSG-6) and stanniocalcin-1 into the blood stream [34]. These vesicles and factors then cross the blood brain barrier, mediate neuroprotection and disease modification through antiinflammatory and other unknown mechanisms (Fig. 1). It is also possible that a small fraction of peripherally administered MSCs directly engraft into the brain and facilitate similar favorable effects through paracrine signaling mechanisms (Fig. 1).

In epilepsy studies discussed in this review, an anti-inflammatory effect was evidenced through reduced hypertrophy of astrocytes, diminished numbers of activated microglia, normalization of the expression of genes encoding pro-inflammatory cytokines, enhanced expression of genes encoding antiinflammatory cytokines and reduced pro-inflammatory cytokines in the serum. These antiinflammatory effects are particularly relevant for treating SE or chronic epilepsy as the role of immunity and inflammation is considered an integral part of the pathogenic processes associated with seizures in refractory epilepsy [84]. The current immunotherapy medications for epilepsy include administration of antiinflammatory and immunomodulatory agents such as corticosteroids, adrenocorticotropic hormone, immunoglobulins, plasmapheresis and monoclonal antibodies that are used currently for other disorders associated with inflammation [84]. Since many of these medications have significant side effects, MNC or MSC administration appears more attractive for clinical trials in multiple epileptic conditions as an antiinflammatory and immunomodulation therapy [85]. However, the next major issue is to identify sources of these cells that are clinically practicable and safe. Autogenic BM-MNC and MSC administrations have been considered to be safe for many disease conditions and are also clinically practicable for conditions such as refractory chronic epilepsy. However, urgent autologous cell therapy may not be feasible for emergency conditions such as SE when a patient is requiring intubation in the emergency room. Such conditions may employ delayed administration of autologous MNCs/MSCs as a treatment to restrain epileptogenesis after the initial precipitating injury. The use of allogenic cells from pre-banked stocks is another option as MNCs or MSCs can be harvested and banked from multiple sources such as bone marrow, lipoaspirate of liposuction procedures, and umbilical cord and dental tissues as well as from hiPSCs [42-45]. Another advantage of using MNCs or MSCs is that immunosuppression may not be required even when allogenic cells are administered, if the primary goal is to obtain an instant disease modification effect. Nevertheless, in conditions where the long term survival of administered bone marrow cells are desired (e.g. when they were employed as drug carriers or microfactories delivering drugs over protracted periods), immunosuppression may be critical to prolong their survival in host tissues. Empirical studies in disease models would be needed in the future to determine the optimal protocol however. Furthermore, long-term studies to identify potential safety hazards, including the potential risk of tumors from karyotypically abnormal cells, or developmentally reprogrammed or regressed cells after prolonged culture would be helpful.

Moreover, it is imperative to identify the best route for administration of MNCs or MSCs for epileptic conditions. Animal model studies in epilepsy have so far used intravenous, intracerebral or intraperitoneal routes of administration and have shown some efficacy with all of these approaches [67,68,70,71,73-76,80-83]. Nonetheless, exploring the efficacy of additional routes may be important, since studies in other neurological models have shown that administration of these cells through intranasal routes are also efficacious. Besides, in an animal model of stroke, intra-arterial administration of MNCs has shown greater efficacy for reducing brain damage possibly because of targeting of infused MNCs into injured areas [86]. Yet, it remains to be seen whether such targeting of cells into the injured brain areas would be more efficacious for restraining seizures in epilepsy since the effects seem to be mediated main-

ly through antiinflammatory activity via modulation of the entire immune system rather than specifically targeting inflammation in the brain. Also, cell dose and cell size are important aspects to consider particularly for the intra-arterial delivery of cells, as administration of higher doses of cells or larger cells (e.g. MSCs) can decrease cerebral blood flow and cause embolic events and lesions in the brain, which may result in functional deficits [87]. However, intra-arterial delivery of cells can be performed safely without infarcts if appropriate protocols (e.g. microneedle technique) are followed [88]. Thus, head-to-head comparisons of the efficacy of different routes of administration of MNCs and MSCs using SE and epilepsy models in future studies would be helpful. If administration of cells through intranasal routes result in functional benefits that are comparable to that obtained with intravenous, intra-arterial or intraperitoneal routes of administration, clinical application could utilize intranasal route, as dispensation through this route likely has minimal side effects and is also amenable for repeated administration if found efficacious for treating the disease.

Furthermore, the most suitable time-window for intervention with these cells for maximal efficacy, especially for conditions such as SE, need to be ascertained. Additionally, detailed analyses of long-term effects of both single and repeated administration of these cells on SRS are needed using chronic video-EEG recordings, as most studies performed so far have either recorded only behavioral seizures or used EEG recordings for very short periods following one-time administration. Since soluble factors from these cells have been shown to modulate NMDA receptor subunit expression in neurons, it may be necessary to examine whether repeated administration would have adverse effects on learning and memory function. Besides, as only focal epilepsy models have been used for testing the efficacy of these cells so far, mechanistic studies in other epilepsy prototypes including models of genetic epilepsies afflicting children are urgently needed. Currently, there are no ongoing clinical trials using MNCs or MSCs for SE or other epileptic conditions. However, additional preclinical studies addressing the various issues discussed above would likely pave the way for clinical translation of this approach within the next five years.

CONCLUSIONS

Early intervention with BM-MNCs or MSCs has shown considerable promise for restraining SE-induced chronic epilepsy in several animal prototypes. Similarly, delayed intervention with BM-MNCs or MSCs after SE has shown efficacy for ameliorating SRS and cognitive dysfunction associated with chronic epilepsy. The simplicity of procuring these cells from both autogenic and allogenic sources, ability to obtain functional benefits with a relatively less invasive route of administration and no immunosuppression, relative lack of serious adverse outcomes and suitability to use in all etiologies of SE or refractory epilepsies make them attractive for clinical application. Such clinical application may provide a feasible and practical way for in situ immunomodulation, neuroprotection and possibly anti-epileptogenesis in diseases like medically refractory status epilepticus and inoperable pharmacoresistant epilepsies.

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AUTHOR CONTRIBUTIONS

S.A.: Design, collection, assembly and interpretation of information; manuscript writing; and final approval of manuscript; A.K.S.: Conception and design; collection, assembly and interpretation of information; manuscript writing; and final approval of manuscript.

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Figure 1. Proposed mechanism of action of mesenchymal stem cells when administered after status epilepticus (SE) or chronic epilepsy. Conditions such as SE or recurrent seizures cause hippocampal injury, which up-regulates pro-inflammatory cytokine levels and releases damage-associated molecular pattern molecules (DAMPs) into the brain and the circulating blood. When MSCs are administered peripherally, most cells get trapped in lungs, liver, spleen and lymph nodes, where they undergo activation and start to release microvesicles and paracrine factors into the blood stream. These molecules cross the blood brain barrier to facilitate neuroprotection and brain repair. It is also likely that minority of peripherally administered MSCs engraft directly into the brain and promote beneficial effects.

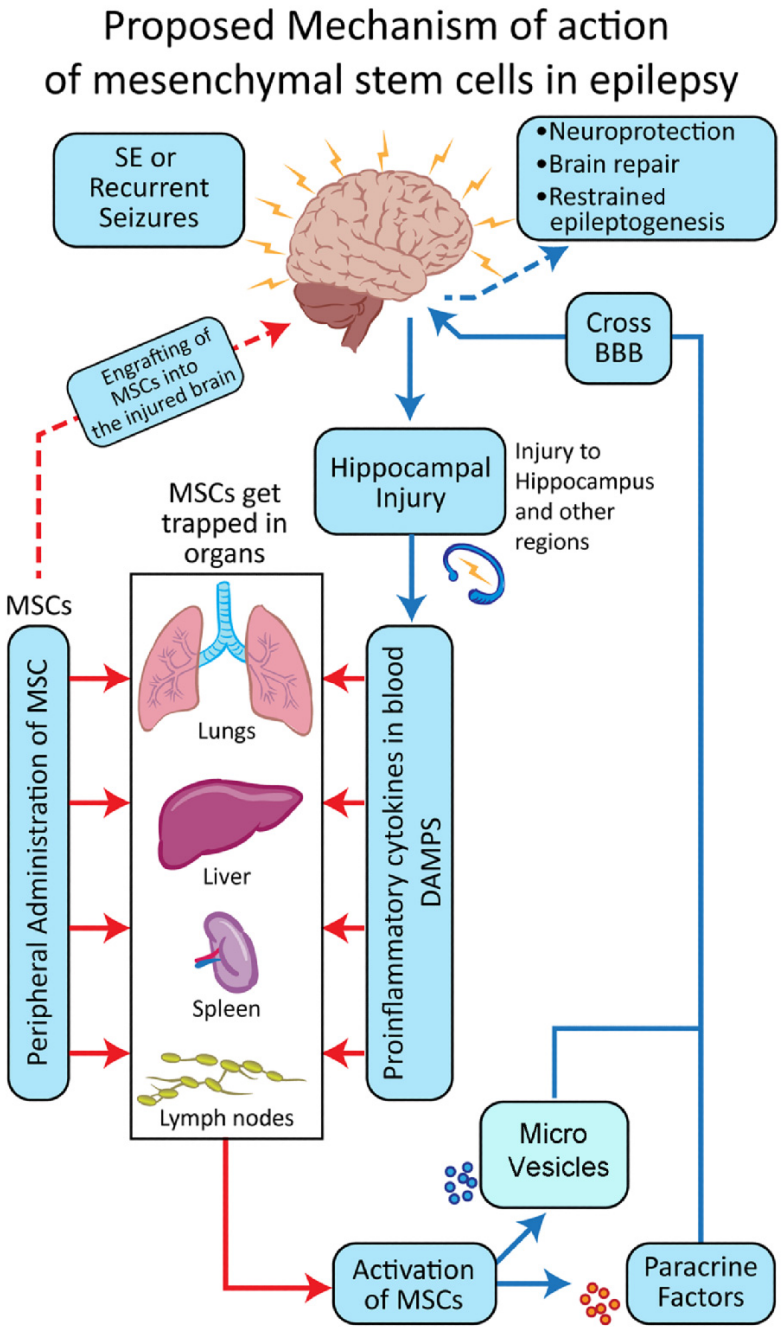

Table 1 Studies on the effects of early administration of bone marrow or umbilical cord derived mononuclear cells (MNCs) after status epilepticus

Author	Type and characteristics of animal model used	Timing of intervention with cells after insult	Type of cells infused and route of administration	Outcome measures examined	Major findings
Costa-Ferro et al., 2010[67]	Rat model of SE, induced through intraperitoneal administration of lithium chloride and pilocarpine.	90 minutes after status epilepticus (SE) induction and seizure termination.	Bone marrow mononuclear cells (BM-MNCs) from EGFP transgenic mice. Intravenous administration (tail vein injection).	Video monitoring between post-SE days 15-22 and 110-117. Analysis of long-term potentiation (LTP) in hippocampus slices. Histology	No seizures in the early phase after SE and reduced seizures in the chronic phase. Protective effects on LTP. Decreased neurodegeneration. Engrafting of some BM-MNCs into the hippocampus and cortex.
Leal et al., 2014[68]	Mouse model of SE, induced through intraperitoneal administration pilocarpine.	3 hours after the onset of SE.	Bone marrow derived mononuclear cells (BM-MNCs) from EGFP transgenic mice. Injections into the retro-orbital plexus.	Histology Analyses of cytokines and their gene expression at 4 hours to 7 days after BM-MNC administration.	Some CD11b+ BM-MNCs were found in perivascular areas (at 4 hours) and brain parenchyma (at 8 hours) but declined dramatically by 24 hours post-grafting. Reduced neuronal loss in the hippocampus. Reduced expression of pro-inflammatory cytokines. Increased expression of antiinflammatory cytokines.
Costa-Ferro et al., 2014[70]	Rat model of SE, induced through intraperitoneal administration of lithium chloride and pilocarpine.	Immediately after the induction of SE.	Human umbilical cord blood derived MNCs. Intravenous administration.	Analyses of behavioral spontaneous seizures. Histology	Reduced frequency and duration of spontaneous seizures at 15-300 days post-SE. Reduced neuronal loss in the hippocampus.

Table 2 Studies on the effects of early administration of normal mesenchymal stem cells (MSCs) or genetically engineered MSCs after status epilepticus

Author	Type and characteristics of animal model used	Timing of intervention with cells after insult	Type of cells infused and route of administration	Outcome measures examined	Major findings
Voulgari-Kokota et al., 2012[71]	Mouse model of SE, induced through intraperitoneal injection of kainic acid.	24 hours after status epilepticus (SE).	Mouse MSCs expressing EGFP. Intravenous treatment.	Histopathology at 7-days post-grafting.	No signs of engrafting of MSCs into the brain. Reduced neuronal loss and diminished activation of astrocytes and microglia.
Abdanipour et al., 2011[74]	Rat model of SE, induced through intraperitoneal administration of pilocarpine.	24 or 36 hours after the first seizure.	Autologous MSCs. Intravenous treatment.	Measurement of behavioral seizures for 3 weeks post-grafting.	66% reduction in behavioral seizures.
Shetty et al., 2014[73]	Rat model of SE, induced through graded intraperitoneal injections of kainic acid.	An hour after the induction of SE.	Human bone marrow derived MSCs. Intraperitoneal administration.	Neurodegeneration and neuroinflammation in the hippocampus.	Protection of principal neurons. Reduced loss of GABA-ergic interneurons. Normal levels of pro-inflammatory cytokines. Reduced concentration of myeloperoxidase. Enhanced expression of genes encoding antiinflammatory cytokines. Reduced numbers of ED-1+ activated microglia.
Li et al., 2009[75]	Mouse model of hippocampus CA3 lesion, induced through microinjection of kainic acid into the amygdaloid nucleus.	24 hours after SE.	Human MSCs (engineered to release adenosine). Implanted stereotactically into the infra-hippocampal fissure	16 hours of continuous electroencephalographic (EEG) recordings (3 weeks after grafting). Histology	Significant reduction in seizure intensity with reversal of effect after adenosine 1 receptor (A1R) antagonist. Grafted cells survived and were restricted to the implanted infrahippocampal fissure.

Long et al., 2013[76]	Rat model of SE, induced through intraperitoneal injections of lithium chloride and pilocarpine.	2 hours after the induction of SE.	MSCs expanded from rat bone marrow engineered to suppress Hes1 gene. Implanted stereotactically into the right lateral ventricle.	Behavioral observation and EEG monitoring. Survival Histology	Decreased mortality, reduced epileptiform waves and EEG bursts in grafted animals. Smaller fraction of graft-derived cells gave rise to NeuN+ and GAD-67+ cells in parahippocampal cortical areas at 7-14 days post-grafting. No neuronal differentiation of graft-derived cells was seen in the hippocampus.

Table 3 Effects of administration of bone marrow derived mononuclear cells (BM-MNCs) or mesenchymal stem cells (MSCs) in chronic epilepsy

Author	Type and characteristics of animal model used	Timing of intervention with cells after insult	Type of cells infused and route of administration	Outcome measures examined	Major findings
Venturin et al, 2011[81]	Rat model of SE, induced through intraperitoneal administration of lithium chloride and pilocarpine.	22 days after status epilepticus (SE).	BM-MNCs from EGFP mice. Intravenous treatment (tail vein injection).	Video monitoring for 2 weeks after cell treatment. Behavioral analysis using a water maze test.	Significant reduction in seizures. Improved learning and memory function.
Costa-Ferro et al., 2012[82]	Rat model of SE, induced through intraperitoneal injection of lithium chloride and pilocarpine.	22 days post-SE (Group A). 10 months after SE (Group B).	BM-MNCs from EGFP transgenic mice. Intravenous treatment (tail vein injection).	Video monitoring for a week after cell treatment on 22 days post-SE. Video monitoring for 8 weeks after cell treatment at 10 months post-SE. Histology	Group A: 62-65% reduction in seizures. Reduced hippocampal neurodegeneration and astrocyte hypertrophy, normalization of pro-inflammatory cytokine gene expression and increased expression of antiinflammatory cytokine gene expression. Group B; 62-97% reduction in seizures Reduced astrocyte hypertrophy, increased neurogenesis and increased expression of antiinflammatory cytokine gene expression.
Huicong et al., 2013[83]	Rat model of SE, induced through intraperitoneal injection of lithium chloride and pilocarpine.	One month after SE.	MSCs from rat bone marrow labeled with paramagnetic iron oxide particles (PIOPs) and implanted directly into the right hippocampus.	MRI at 1 and 3 months post-grafting. EEG at 15 days and at 3 months after SE. Survival Histology	Injected MSCs moved towards midline of the brain. Significant decrease in sharp waves. Normalization of adenosine A1 and 2A receptors ratio in the hippocampus.

