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CRITICAL REVIEW – INVITED COMMENTARY

Mesenchymal stem cell therapy for focal epilepsy: A systematic review of preclinical models and clinical studies

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Abstract

Drug-resistant epilepsy (DRE) is characterized by recurrent seizures despite appropriate treatment with antiseizure medication (ASM). Due to their regenerative and immunomodulatory potential, therapies with biologics such as mesenchymal stem cells (MSCs) offer a potential therapeutic benefit for structural causes of epilepsy, such as hippocampal sclerosis. In this article, we report a systematic review of the literature evaluating the preclinical and clinical studies of MSCs for DRE. Medline, Ovid EMBASE, Scopus, and the Cochrane Databases were searched electronically from their dates of inception to November 2021 using the following keywords: ((“mesenchymal”) AND (“stem cell”)) AND ((“epilepsy”) OR (“convulsion”) OR (“seizures”)). This review followed Preferred Reporting Items for Systemic Reviews and Meta-Analyses (PRISMA) guidelines. The initial query identified 488 studies representing 323 unique manuscripts. After application of selection criteria, 15 studies were included in this systematic review; 11 were preclinical studies and 4 were clinical studies. All preclinical studies were performed in rodents and all clinical studies were phase 1 trials. Thus far, therapy with MSCs appears to be safe for use in humans, as no severe adverse events related directly to the therapy were reported. Furthermore, MSC therapy appears to provide a statistically significant clinical benefit by reducing the seizure burden of patients, reducing the electrophysiological biomarkers of epilepsy, and improving their comorbidities, such as depression and anxiety. In addition, animal studies reveal that the therapy exerts its effect by reducing aberrant mossy fiber sprouting (reduce excitatory pathways) and increasing γ-aminobutyric acid (GABA)ergic interneurons (increase inhibitory pathways). Both preclinical and clinical studies have shown MSC therapy to be safe and preliminary effective, thus warranting further studies to investigate its therapeutic potential.

KEYWORDS
animal model, biologics, clinical trial, human study, mesenchymal stem cells

1 | INTRODUCTION

The International League Against Epilepsy (ILAE) task force defines epilepsy as a disease of the brain characterized by any of the following: (1) at least two unprovoked (or reflex) seizures occurring >24 h apart; (2) one unprovoked (or reflex) seizure and at least a 60% probability of further seizures, similar to the general recurrence risk after two unprovoked seizures; (3) diagnosis of an epilepsy syndrome.[1] The most common structural finding of temporal lobe epilepsy (TLE) in adults is hippocampal sclerosis.[2] This abnormality is the result of degeneration of γ-aminobutyric acid (GABA)ergic interneurons in this area. Normally these neurons secrete inhibitory neurotransmitters to stop the propagation of abnormal epileptiform activity resulting in a seizure.[3–5]

The mainstay of treatment for epilepsy is seizure control with antiseizure medication (ASM).[6–8] When two adequate trials of ASM fail to achieve sustained seizure freedom and both are well tolerated and appropriately chosen for an individual epilepsy syndrome, the disorder is referred to as drug-resistant epilepsy (DRE).[9] Patients with DRE with a seizure focus that can be localized to a specific area of the brain may be candidates for epilepsy surgery. The latter situation provides a window of opportunity for local delivery of alternative therapies.[10,11]

Recently, due to their regenerative potential, mesenchymal stem cells (MSCs) have arisen as a promising alternative to reinvigorate damaged tissue in multiple diseases, including epilepsy.[12–20] It is thought that these cells are able to repopulate and differentiate into the lost hippocampal interneurons of patients with hippocampal sclerosis and DRE, thus improving seizure outcome.[21–23] We systematically review the current literature for scientific documents involving preclinical models and human studies of MSC therapy for epilepsy.

Key points

- Mesenchymal stem cell (MSC) therapy in early phase trials showed no adverse events during treatment for drug-resistant epilepsy (DRE).
- Preliminary human studies on MSC therapy for DRE have suggested a clinical benefit on seizure control.
- Preliminary human studies on MSC therapy for DRE have suggested a clinical benefit on comorbidities (depression/anxiety).
- Further phase 2 clinical studies are warranted to explore this therapy.

2 | METHODS

2.1 | Search strategy

Our literature search strategy was framed using the Population, Intervention, Comparison, Outcome, and Study type (PICOS) model to develop relevant clinical questions formatted for systematic review.[24] These questions were: Do subjects with DRE (population) treated with MSCs either locally or systemically (intervention) show clinical and electroencephalographical improvement (outcomes) vs subjects treated with the current standard ASM (comparison) based on current preclinical and clinical studies of MSC therapy for epilepsy (study type). Medline, Ovid EMBASE, Scopus, and the Cochrane Databases were electronically searched from their dates of inception to November 2021. The following keywords were typed in the search box as stated: ((“mesenchymal”) AND (“stem cell”)) AND ((“epilepsy”) OR (“convulsion”) OR (“seizures”)). Our review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[25] We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

2.2 | Study selection

In accordance with the PRISMA guidelines, scientific publications were independently screened by two authors (A.R.F. and C.P.V.) through Endnote software for Mac (Version 9; Clarivate). No discrepancies between the screening authors were found. Duplicate records were automatically removed by the software and the remaining duplicate records were manually removed by the screening authors. All studies with at least one outcome of interest that included animal models of epilepsy or human subjects were screened when subjects received at least one application (either local or systemic) of naïve or engineered MSCs. Studies that used other types of stem cells or precursor cells, such as neural-induced pluripotent stem cells, were excluded. Abstracts, book chapters, and manuscripts not written in English were excluded. For duplicate studies with overlapping cohorts, the most complete report was included.

2.3 | Data collection and analysis

For preclinical studies, the animal strain, number of animals, model used, therapy used, MSC source of stem cells,

dosage, timing of delivery, method of delivery, and main findings were extracted from full texts, tables, and figures. For clinical studies, the number of subjects, study design, MSC source, dosage, timing of delivery, method of delivery, study endpoints, and main findings were extracted from full texts and tables. Due to the nature and heterogeneity of the data, a quantitative meta-analysis was not conducted; hence a quality assessment was not performed. Descriptive statistics were used to report the search results.

3 | RESULTS

3.1 | Search strategy

Our initial search (Figure 1) identified 488 manuscripts representing 323 unique scientific reports. After application of selection criteria to the title and abstract, 18 publications were selected for full-text analysis. Of the 18 publications, 15 met the inclusion criteria and were included in this article.[21–23,26–37] From the included 15 publications, 11 were preclinical models (Table 1) and 4 were clinical studies (one case report and three phase 1 clinical trials; Table 2).

3.2 | Preclinical study characteristics

All preclinical models (Table 1) were performed in rodents (eight rat and four mouse studies). A total of 416 small animals were included across studies. From the total, 139 animals (33.41%) received treatment with naïve MSCs, 81 (19.47%) with engineered MSCs, 113 (27.16%) received sham treatment, 75 (18.03%) served as controls, and 8 (1.92%) received treatment with ASM only. The models used included five (45.45%) intraperitoneal lithium-pilocarpine-induced status epilepticus (SE), two (18.18%) intraperitoneal pilocarpine-induced SE, three (27.27%) intrahippocampal kainic acid-induced SE, and one (9.09%) intraperitoneal pentylenetetrazole-induced chronic epilepsy model. From the 11 models; 6 (54.55%) used animal bone marrow-derived MSCs; whereas the remaining 5 (45.45%) used human-derived MSCs from bone marrow ($n = 2$), umbilical cord blood ($n = 2$), and a Wharton's jelly isolate ($n = 1$).

Cell dosage varied by method of delivery. From the 11 studies, 7 used stereotactic intrahippocampal injections with doses ranging from 4000 to 125 000 cells, 2 used an intrathecal (intraventricular) injection at a dose of 5 000 000 cells, and 3 used a systemic injection through a tail vein at a dose ranging from 1 000 000 to 3 000 000 cells (Figure 2).

3.3 | Clinical study characteristics

From the four included studies (Table 2), three were phase 1/pilot studies in adults with DRE and one was a pediatric case report. All three phase 1/pilot studies were open label and two of them were randomized. All studies had at least 1 year of follow-up; had a primary end point of safety, feasibility, and tolerability; and a secondary end point of efficacy and seizure burden reduction.

All four studies enrolled a total of 94 patients—89 (94.68%) were adults and 5 (5.32%) were pediatric—and

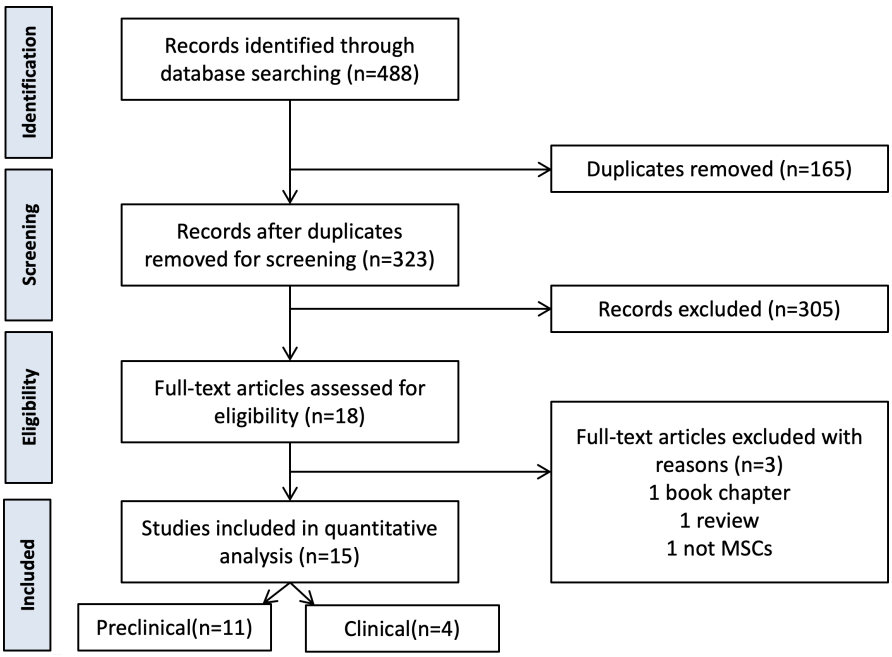

FIGURE 1 Preferred Reporting Items for Systemic Reviews and Meta-Analyses (PRISMA) flow chart illustrating the selection of articles included in this review

TABLE 1 Preclinical studies of MSC therapy for epilepsy

Author (year)	Study subjects (Total, group allocation)	Number of subjects per treatment group	Model and therapeutics	MSC source, dose, timing, and method of delivery	Main histopathological findings	Main clinical findings
Fukumura et al. (2018)[21]	Male Sprague-Dawley rats	29 total 10 naïve MSCs 11 sham 8 controls	Intraperitoneal lithium-pilocarpine–induced SE Naïve cells	Rat bone marrow, 1×10^6 cells, tail vein injection, 1 day after SE induction	MSCs reduced[a] aberrant mossy fiber sprouting in the dentate gyrus vs sham MSCs increased[a] the number of hippocampal GABAergic neurons (vs sham) to an amount similar to controls	MSCs decreased[a] seizure frequency and improved cognitive function vs. sham.
Huang et al. (2016)[23]	Male Sprague-Dawley rats	60 total 20 naïve MSCs 20 sham 20 controls	Intraperitoneal pilocarpine-induced SE Naïve cells	Human Wharton's jelly, 1×10^5 cells, bilateral intrahippocampal injection, 1 day after SE induction	MSCs reduced[a] brain edema and aberrant mossy fiber sprouting in the hippocampus vs sham Hippocampal volume and hippocampal neuron density were similar between the MSCs and control groups but greater[a] vs sham	MSCs reduced[a] the frecuency and duration of seizures vs. sham.
Kang et al. (2012)[27]	Wistar rats	16 total 8 naïve MSCs 8 sham	Intraperitoneal lithium-pilocarpine–induced SE Naïve cells	Rat bone marrow, 4×10^3 cells, right intrahippocampal injection, 30 days after SE induction	MSCs increased[a] adenosine A1 receptor in the temporal lobe and hippocampus and decreased[a] adenosine A2a receptor in the thalamus vs sham	MSCs reduced[a] the frequency and amplitude of epileptic discharges vs. sham.
Long et al. (2015)[29]	Male Sprague-Dawley rats	45 total 15 naïve MSCs 15 labeled MSCs 15 sham	Intraperitoneal lithium-pilocarpine–induced SE Naïve cells or Ultrasmall superparamagnetic iron oxide nanoparticles (USPIO)-labeled cells	Rat bone marrow, 5×10^6 cells, unilateral intraventricular injection, 2 h after SE induction	Labeled MSCs migrated toward the cortex and the hippocampus	Naïve and labelled MSCs reduced[a] the frequency of seizures vs. sham.
Long et al. (2013)[30]	Male Sprague-Dawley rats	64 total 20 naïve MSCs 20 engineered MSCs 20 sham 4 controls	Intraperitoneal lithium-pilocarpine–induced SE Naïve cells or genetically engineered cells (Hes1 gene silencing to promote GABAergic differentiation)	Rat bone marrow, 5×10^6 cells, unilateral intraventricular injection, 2 h after SE induction	Engineered MSCs differentiated into GABAergic neurons in the parahippocampal regions	Naïve and engineered MSCs reduced[a] seizure frequency and wave amplitude vs. sham.

TABLE 1 (Continued)

Author (year)	Study subjects (Total, group allocation)	Number of subjects per treatment group	Model and therapeutics	MSC source, dose, timing, and method of delivery	Main histopathological findings	Main clinical findings
Mohammed et al. (2014)[22]	Female Wistar rats	32 total 8 naïve MSCs 8 gabapentin 8 sham 8 controls	Intraperitoneal pentylenetetrazole-induced chronic epilepsy (13 injections total; 3/week) Naïve cells or gabapentin injection	Human umbilical cord blood, 1×10^6 cells, tail vein injection, after the 10th pentylenetetrazole injection	MSCs increased[a] GABA levels	MSCs reduced[a] epilepsy severity index as well as improved motor function and coordination vs. sham and vs. gabapentin.
Park et al. (2015)[31]	Male Sprague-Dawley rats	33 total 8 naïve MSCs 9 sham 9 positive controls 7 negative controls	Intraperitoneal lithium-pilocarpine–induced SE Naïve cells	Human umbilical cord blood, 5×10^5 cells, right hippocampal injection, 10 weeks after SE induction	MSCs increased[a] glucose metabolism in the hippocampus (measured through PET) vs sham No significant difference was seen in hippocampal volume vs sham	No significant difference was seen in seizure frequency vs. sham.
Salem et al. (2018)[33]	Male Sprague-Dawley rats	40 total 10 naïve MSCs intrahippocampal 10 naïve MSCs intravenous 10 sham 10 control	Intraperitoneal pilocarpine-induced SE Naïve cells	Rat bone marrow, 1×10^5 cells per side, bilateral intrahippocampal injection, or 3×10^6 cells, tail vein injection, 22 days after SE induction	MSCs reduced the histological, neurotransmitter, and inflammatory marker levels vs sham	Intrahippocampal injections were more effective than intravenous injections.
Ali et al (2017)[26]	Male C57B1/6 mice	67 total 30 naïve MSCs 28 engineered MSCs 9 controls	Intrahippocampal kainic acid–induced SE Engineered (IL-13) or naïve cells	Mouse bone marrow, 20×10^3 cells, intrahippocampal injection, 1 week before SE induction	IL-13 cells did not provide a neuroprotective microenvironment	IL-13 cells did not decrease the epileptic burden vs. naïve cells.
Li et al. (2009)[28]	Male C57B1/6 mice	12 total 6 engineered MSCs 6 sham	Intrahippocampal kainic acid–induced SE Adenosine-releasing MSCs	Human bone marrow, 1.25×10^5 cells, one-sided intrahippocampal injection, 1 day after SE induction	Not studied	Engineered MSCs reduced[a] frequency of spontaneous seizures vs. sham.

(Continues)

TABLE 1 (Continued)

Author (year)	Study subjects (Total, group allocation)	Number of subjects per treatment group	Model and therapeutics	MSC source, dose, timing, and method of delivery	Main histopathological findings	Main clinical findings
Ren et al. (2007)[32]	Male C57BL/6 mice (18 total, 12 engineered MSCs and 6 sham)	18 total 12 engineered MSCs 6 sham	Intrahippocampal kainic acid–induced SE Adenosine-releasing MSCs	Human bone marrow, 1.25×10^5 cells, hippocampal injection, 1 week before SE induction	Adenosine-releasing MSCs reduced[a] brain injury vs sham	Adenosine-releasing MSCs reduced[a] seizure frequency vs. sham.

AE, adverse events; ASM, antiseizure medications; EEG, electroencephalography; MSC, mesenchymal stem cells.
[a]Indicates a statistically significant result.

all studies used naïve MSCs as their therapy. Forty-nine patients (52.13%) received at least one dose of MSCs, 19 (20.21%) received a repeat course of MSCs, and 45 (47.87%) controls received standard of care with ASM only.

The patient in the case report by Dong et al. (2018)[34] received allogeneic umbilical cord derived MSCs in three treatment sessions: (1) 7×10^6 cells via intrathecal (IT) route and 5.6×10^6 cells intravenous (IV), (2) 1.625×10^7 cells IT and 3.6×10^6 cells IV, and (3) 2.05×10^7 cells IT. Patients (total $n = 22$, treated with MSCs $n = 10$) in the phase 1 study by Hlebokazov et al. (2017)[35] received autologous bone marrow–derived MSCs in one session via two different routes, $40–101 \times 10^6$ cells (mean $68.2 \pm 8.48 \times 10^6$) IV and $2.7–8 \times 10^6$ cells (mean $6.34 \pm 0.72 \times 10^6$) IT. All patients (total $n = 67$, treated with MSCs $n = 34$) in the second study by Hlebokazov et al. (2021)[36] received autologous bone marrow–derived MSCs in one session via two different routes: $1–1.5 \times 10^6$ cells/kg IV and 0.1×10^6 cells/kg IT; 14 patients included in the initial treatment group received a repeated course of MSC treatment 6 months after the initial course. Patients in the last included study ($n = 4$) by Milczarek et al. (2018)[37] received autologous bone marrow–derived MSCs in five different sessions, the first one at a dose of $0.38–1.72 \times 10^9$ IV and the next four sessions once every 12 weeks at a dose of $18.5–40 \times 10^6$ via IT injection.

4 | DISCUSSION

4.1 | Preclinical models of epilepsy and MSCs

4.1.1 | Animal models

Preclinical models provide insight into the pathophysiologic and therapeutic mechanisms of novel therapies. It is important to consider all animal data to establish safety and a potential therapeutic benefit before translating these therapies into the clinical stage.

The models created in small animals to represent chronic epilepsy consisted of a severe initial insult to the brain by inducing acute pharmacological status epilepticus (SE) with either an intraperitoneal injection of lithium and/or pilocarpine[21–23,27,29–31,33] or a hippocampal injection of kainic acid[26,28,32] and then terminating SE after 20–30 min with a benzodiazepine. This process creates a chronic injury to the brain producing chronic spontaneous recurrent seizures, which allows for an objective and measurable response to treatment.[21–23,26–33]

One of the main concerns using these models is that the severity of both the initial SE and the intensity of resultant chronic epilepsy tends to be highly variable and is therefore unpredictable. Inducing prolonged SE could lead to

TABLE 2 Clinical studies of MSC therapy for epilepsy

Author (year)	Study design (number of subjects)	Study end points	MSC source, dose, and method of delivery	Seizures at baseline and follow-up	Main findings
Dong et al. (2018)[34] ID: Not registered	Case report (1)	N/A	Allogeneic umbilical cord, three sessions: (1) 7×10^6 cells intrathecal and 5.6×10^6 cells intravenous, (2) 1.625×10^7 cells intrathecal and 3.6×10^6 cells intravenous, (3) 2.05×10^7 cells intrathecal	1 generalized seizure every 5 months	Patient showed EEG evidence of improvement (normalized), as well as clinical improvement in motor and language function.
Hlebokazov et al. (2017)[35] ID: NCT02497443	Phase 1, randomized, open label, 1 year follow up (22 adult subjects total, 12 controls treated with ASM & 10 treated with MSCs)	Primary: Safety and tolerability Secondary: Reduction of seizures	Autologous bone marrow, two injections: (1) $40–101 \times 10^6$ cells (mean $68.2 \pm 8.48 \times 10^6$) intravenous and (2) $2.7–8 \times 10^6$ cells (mean $6.34 \pm 0.72 \times 10^6$) intrathecal	Pre-treatment: 11 seizures per month (median) Post-treatment: 3 seizures per month (median)	No severe AEs in the intervention group. 1 patient had a mild headache after intrathecal injection that resolved spontaneously after 2 days. Laboratory values remained within normal ranges MSCs decreased[a] the number of monthly seizures, decreased[a] seizure severity score, and decreased[a] anxiety score vs. controls.
Hlebokazov et al. (2021)[36] ID: NCT02497443	Phase 1, randomized, open label, 1 year follow up (67 adults, 33 controls treated with ASM, 34 treated with MSCs)	Primary: Safety Secondary: Seizure frequency reduction	Autologous bone marrow, all intervention group received two injections: (1) $1–1.5 \times 10^6$ cells/kg intravenous and (2) 0.1×10^6 cells/kg intrathecal, 14 patients received a second treatment course 6 months after	Pre-treatment: 9.91 seizures per month (mean) Post-treatment: 3.67 seizures per month (mean)	No severe AEs after treatment MSCs decreased[a] average seizure count at 6 and 12 months vs. controls MSCs improved[a] EEG paroxysmal activity, decreased[a] anxiety and depression levels vs. controls Patients who received a second course of MSCs treatment showed a decreased[a] average seizure count at 12 months, and showed improved[a] EEG paroxysmal activity vs. single treatment group.
Milczarek et al. (2018)[37] ID: Not registered	Phase 1, non-randomized, open label, 2-year follow up (4 pediatric patients total)	Primary: Safety and feasibility Secondary: Efficacy	Autologous bone marrow, all patients received 5 cell implantations: (1) Bone marrow nucleated cells intrathecal and intravenous MSCs $0.38–1.72 \times 10^9$, (2–5) intrathecal MSCs $18.5–40 \times 10^6$ cells once every 12 weeks	Patient 1: 20–40 seizures per week to occasional fever seizures Patient 2: 30–60 seizures per week to 1 per week Patient 3: 14–21 seizures per week to 1 per week Patient 4: 10–40 seizures per week to 7 per week	No severe AEs observed, although all children showed transient mild hyperthermia (38°C) after implantation MSCs reduced number of seizures and partially normalized EEG patterns.

[a]Indicates a statistically significant result.

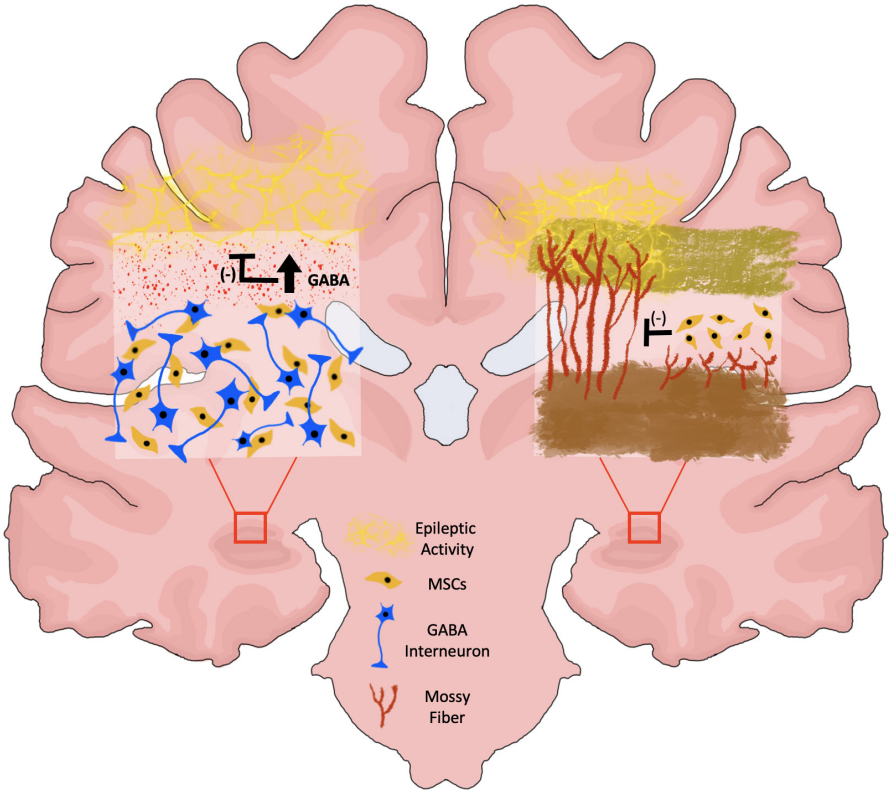

FIGURE 2 Illustration showing the pathophysiological mechanisms within the hippocampus of mesenchymal stem cell (MSC) treatment for epilepsy. (Left) MSCs increase the γ-aminobutyric acid (GABA)ergic interneuron density, thus increasing the release of inhibiting neurotransmitters, which interrupt propagation of epileptiform activity. (Right) MSCs inhibit mossy fiber sprouting, a phenomenon known to contribute to the development of seizures

animal death, thus increasing the initial number of animals required to establish study end points with enough power to identify a statistical difference.[21,23,26,29–31] The severity of the chronic epilepsy is then classified from 1 to 5 using the Racine scale.[38,39] The scale is considered as follows: Class (1) orofacial automatisms, class (2) head nodding, class (3) partial body clonus without rearing, class (4) partial body clonus with rearing, and (5) generalized seizure with falling.[38,39] Models of chronic epilepsy utilize animals whose severity is greater than class 3. Because a high Racine classification is needed, the initial insult to develop the disorder may be severe enough to cause the animal to expire. Hence, to allow for these losses, a larger number of animals needs to be considered in the study design.[21,23,29–31] This specific selection in studies is due to a highly variable response to treatment through the different severity classes, where a more favorable response is seen with the less-severe classes.[22]

4.1.2 | MSC source, dosage, and delivery method

The majority of animal models have used bone marrow–derived MSCs due to their uncomplicated harvesting method.[21,26–30,32,33] The two main methods of delivery are either systemically through an intravenous injection,[21,22] or locally through a hippocampal injection.[23,26–28,31–33] Intrathecal injection was also shown to be beneficial.[29,30] Furthermore, Salem et al. (2017)[33] showed that local intrahippocampal injections achieved a more effective response compared to intravenous injection.

Long et al. (2015)[29] labeled their MSCs with ultrasmall, superparamagnetic iron oxide nanoparticles to be able to track their migration through magnetic resonance imaging (MRI). The researchers found that these cells migrate toward epilepsy-damaged areas such as the hippocampus and the cortex, with a persistent signal identified 2 months after initial delivery.[29]

4.1.3 | Therapeutic targets

A frequent histopathological feature of TLE is an aberrant organization of the dentate gyrus in the hippocampal formation. Specifically, there is an aberrant extension of the axonal bodies of the granule cells in the middle layer of the dentate gyrus, a process known as mossy fiber sprouting.[40–42] In a normal brain, these processes synapse with mossy cells in the hilus and the CA_3 pyramidal neurons. It is theorized that injury to this area interrupts these connections and causes granule cells in the dentate gyrus to sprout and innervate the excitatory inner molecular layer of the hippocampus, hence resulting in an abnormal excitatory circuit that promotes epileptogenicity.[43,44] Small animal studies evaluating the therapeutic effects of MSCs have found statistically significant histological evidence that aberrant mossy fiber sprouting is reduced, promoting a reduction in epileptogenesis.[21,23]

Hippocampal sclerosis is characterized by a reduction in the number of GABAergic interneurons.[3–5] Normally, these neurons have an inhibitory function and halt the propagation of epileptiform activity throughout the hippocampus that would otherwise potentially provoke a seizure.[3–5] Animal models in rodents have shown that MSCs have the potential to differentiate into GABAergic interneurons, and a statistically significant increase in their number within the hippocampus.[21–23] A study performed by Long et al. (2013)[30] successfully explored genetically modifying MSCs to silence the Hes1 gene, whose function is to inhibit GABAergic differentiation, thereby inducing MSC differentiation into GABAergic interneurons. Histological analysis of animals treated with MSCs have also shown a statistically significant increase in hippocampal volume compared to sham treatment.[21,23]

4.1.4 | Clinical benefits

Animal models of epilepsy treated with MSCs show a statistically significant clinical response compared to control animals. Several studies have found that MSCs reduce the frequency, duration, and severity of spontaneous seizures.[21–23,27–30,32] Some studies have investigated the electrophysiology of their subjects through electrocorticography (ECoG) or electroencephalography (EEG), showing a reduction in the frequency and the amplitude of seizures.[27,30] MSCs also seem to have a positive effect on the cognitive ability of animals, with a statistically significant improvement in their cognitive capabilities and returning it to a normal level.[21,22]

Macroscopically, damaged brain parenchyma from epileptic animal models have been shown to benefit from MSCs.[32] Huang et al. (2016) showed that reduced brain edema was present on MRI in rats treated with MSCs. In addition, Park et al. (2015) evaluated the metabolic activity of the hippocampus through PET scanning and demonstrated increased glucose metabolism in animals treated with MSCs.[31]

4.2 | Clinical studies of epilepsy and MSCs

4.2.1 | Study design

Because novel therapeutic studies involving treatment with MSCs for epilepsy are scarce and in the early stages of development,[34–37] our literature search yielded only four phase 1 clinical studies,[34–37] one as a case report[34]; all of the reports had the primary objective of feasibility and safety with a secondary objective to evaluate efficacy.[34–37] In addition, all four studies included a follow-up period of at least 1 year to provide support for their objective to measure efficacy.[34–37] The two largest studies were performed by the same group with a similar therapeutic regimen, although without overlapping patient populations.[35,36]

4.2.2 | Safety and efficacy of MSCs for epilepsy

The first study by Hlebokazov et al. (2017)[35] was a randomized, open label, phase 1 study involving a total of 22 patients with DRE: 12 received standard of care with ASMs serving as controls and 10 received treatment with MSCs. Patients received two injections within the same day, one IV and one IT at the level of the lumbar spine. For their primary end point, they reported no severe adverse events (AEs) associated with MSC treatment.[35] However, 1 of 10 of their patients reported a mild headache after the intrathecal injection that resolved spontaneously after 2 days. Their secondary analysis seemed to show a statistically significant decrease in number of spontaneous seizures, decreased seizure severity scores, and decreased anxiety levels when compared to the control group.

Following up on their pilot study, Hlebokazov et al. (2021)[36] performed a larger randomized, open label, phase 1 study involving a total of 67 patients with DRE: 33 received standard of care with ASMs, serving as controls, and 34 received treatment with MSCs following the same treatment protocol. However, they sought to investigate the impact of repeat stem cell application by repeating the initial treatment course after 6 months in 14 patients. In agreement with prior work, they found no severe AEs related to treatment with MSCs.[36] For their secondary end point they suggested a decreased average seizure count with additional EEG evidence of this reduction.[36] Moreover, the patients who received a repeated treatment course appeared to show further clinical and EEG improvement compared to their counterparts.[36] In addition, depression and anxiety levels of their patients treated with MSCs appeared to be reduced compared to their controls.[36]

A small non-randomized, open label, pilot study in four pediatric patients by Milczarek et al. (2017) reported no severe AEs. However, all subjects developed a transient mild hyperthermia (38°C) immediately after MSC injection. For their secondary objective, there appeared to be a reduced number of seizures and partially normalized EEG paroxysmal patters in all four patients. The last study published by Dong et al. (2018)[34] was a case report of a pediatric patient with cerebral palsy who received three treatment courses of MSCs. They reported no AEs related

to the therapy along with EEG and clinical evidence suggesting improvement in both epilepsy and neurological function.

4.3 | Strengths and limitations

This study has inherent strengths and limitations. To the best of our knowledge, this is the first systematic review evaluating both preclinical and clinical models of MSC therapy for epilepsy. There is a risk of bias during the selection process, although two blinded authors screened all available manuscripts to reduce this bias. In this case, no discrepancies in manuscript selection were found between the two authors. However, in case of any discrepancies, we suggest that the two screening authors re-review the full texts of those manuscripts to carefully select which ones meet the selection criteria. Although we attempted to make the search as comprehensible as possible, caution needs to be taken as the literature search may have been limited by the methodology. Future meta studies on this topic should include indexed keywords, author-provided keywords, and synonyms in addition to the main terms. On the other hand, the inclusion of Scopus database provides a broader search because non-medical manuscripts are also included. There is also a risk of bias due to the nature of the included studies, as they are all in preclinical and early stages of clinical development, there are small sample sizes, and there is high variability between dosages and routes of administration. Because all included studies are in the early stages, they were not powered to explore the efficacy of treatment. Careful interpretation needs to be made of these results as they are prone to result in false positives. This systematic review was not pre-registered in PROSPERO as it was initially queried for another purpose. However, we encourage all authors working on systematic reviews and meta-analyses to pre-register their protocol to ensure high-quality scientific publications and to avoid duplicated efforts. However, we provide a contemporary up-to-date review of the literature on this topic.

5 | CONCLUSION

The treatment of epilepsy with MSCs in humans is supported by preliminary studies and thus far appears devoid of major AEs. MSC administration is still in the early stages of therapeutic development; however, available reports suggest potential improvement in the electroclinical profile of patients with DRE. Although the precise dosage and route of administration for MSC is yet to be determined, improvement in comorbidities such as anxiety and depression are an important aspect to consider in future trials. The use of MSC holds promise for patients with DRE, and phase II trials appear warranted.

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CONFLICT OF INTEREST

EHM serves on an advisory board and receives consulting fees from Boston Scientific Corp. Neither of the remaining authors have any conflict of interest to disclose.

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