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Review

Mesenchymal stromal cells: cell-based therapies for traumatic central nervous system injuries

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Abstract

Traumatic central nervous system (CNS) injury often causes irreversible impairment, and new alternative therapies for the treatment of CNS injury and sequelae are expected to be developed. Recently, mesenchymal stromal cells (MSCs) have started being used as cell therapy for neurological disorders such as traumatic CNS injury based on their immunomodulatory, neuroprotective, and neurorestorative abilities. Based on the premise of basic research, numerous clinical trials using MSCs for the treatment of traumatic CNS injury have been performed, and the feasibility and efficacy of this therapy have been reported. In this review we aimed to shed light on the characteristics of MSCs and to discuss the basic and clinical research and recent progress in clinical studies using MSCs to treat various traumatic neurological injuries.

Keywords: Mesenchymal stromal cell; Traumatic central nervous system injury; Neurological disorders; Umbilical cord; Neurotrophic factors

1. Introduction

Traumatic central nervous system (CNS) injury often causes irreversible disorders. As for the mechanisms of the traumatic CNS injury, the initial injury is reported to cause necrosis and apoptosis of neural cells, followed by a secondary degeneration resulted from the apoptosis of undamaged neurons [1]. Basically there are two specific categories by which traumatic CNS injury occurs: traumatic brain injury (TBI) and spinal cord injury (SCI).

TBI and SCI are both caused by physical insults to the brain and spinal cord suddenly inflicted in situations such as traffic accidents, falls, and sporting activity. TBI is a primary cause of unexpected death and may induce serious sequalae [2]. On the other hand, SCI is also a severe traumatic insult of the CNS. Although there is a wide range in the occurrence rate, the total worldwide incidence of SCI has been reported to be 3.6–195.4 per million people [3]. These injuries trigger a neuroinflammatory reaction and disrupt neuroimmune communication, leading to serious deficits in sensorimotor functions; this may either lead to unexpected death or induce serious disabilities, motor and cognitive dysfunction [2]. TBI is also suspected to be a potential risk factor of neurodegenerative diseases, such as Alzheimer's disease [4], amyotrophic lateral sclerosis [5], and Parkinson's disease [6].

As for the treatment of traumatic CNS injuries, in addition to rehabilitative therapy, decompressive craniotomy, hyperosmolar treatment, and hypothermia therapy are performed to decrease intracranial pressure. However, these therapies are not perfect treatments in some cases. Moreover, most pharmacological trials that have been conducted and included glutamate antagonists, corticosteroids, free-radical scavengers, progesterone, have failed to demonstrate a significant clinical efficacy [7,8]. Therefore, new therapeutic alternatives for the treatment of CNS injury and sequelae are expected to be developed and clinical trials of these new therapeutics are urgent issues.

Recently, mesenchymal stromal cells (MSCs), also called mesenchymal stem cells, are used to treat various diseases, and researchers have been focusing on their immunomodulatory and neurotrophic abilities. MSCs have been reported to be effective in neurological disorder models such as traumatic CNS models; additionally, clinical trials using MSCs for the treatment of traumatic CNS injuries have already been demonstrated, which are summarized in the present review.

In this paper, we characterized MSCs, especially umbilical cord (UC)-derived MSCs, presented methods of isolation and cryopreservation, and discussed their efficacy and mechanisms of action in treating traumatic CNS injuries, as well as their application in clinical trials.

2. Methods to harvest and cryopreserve UC-MSCs

Following many experiments, MSCs have been reported to be able to be harvested from various tissues, including bone marrow (BM) [9], UC blood (UCB) [10], adipose tissue (AD) [11], dental pulp [12], periodontal ligament [13], tendon [14], skin [15], muscle [16], and UC [17].

BM is considered the traditional source of MSCs, and the characteristics and application of BM-MSCs have been widely studied. However, BM isolation is an invasive and painful procedure that may cause hemorrhage, infection, and, in some cases, chronic pain [18]. Furthermore, BM-MSCs have been reported to show accelerated senescence following the donors age [19]. Also harvesting adipose in order to isolate AD-MSCs needs surgery and it accompanies invasiveness to some extent. Harvesting UCB doesn't need surgery, while UCB is harvested only when it is available with aseptic procedure, and UCB often cannot be collected cleanly depending on the condition of the delivery and the baby. On the other hand, the UC is often discarded as medical materials, therefore isolating UC is painless and noninvasive, and UC can always be collected unlike UCB. Furthermore UC-MSCs have multipotency properties comparable to those of MSCs derived from other tissues [13,20]. Furthermore, UC-MSCs have attracted attention from their immunomodulatory properties. They express human leukocyte antigen (HLA)-class I less than BM-MSCs [21] and are thus less immunogenic. For the purpose of treatment in the acute phase of TBI, it is considered difficult to prepare the patient for autologous BM- or AD-MSCs transplantation. Therefore, we are focusing on the UC as a major source of MSCs with an important potential for cell therapy, as they are suitable for allogeneic transplantation.

There are several protocols for isolating and culturing UC-MSCs. The improved explant method is generally used [22]; UC is minced into small fragments, and then seeded regularly on a tissue culture dish. Culture media is renewed every 3 days until fibroblast-like adherent cells reach 80–90% confluency. After that, cells are detached using trypsin. These cells are called passage 1 UC-MSCs, and this passage 1 MSCs are mass-cultured to passage 4 MSCs, which are usually destined for basic and clinical use.

For the treatment of CNS injury and sequelae, cryopreservation of MSCs is necessary, because cell therapy should be performed at any given time, either in the acute phase of injury or the chronic phase, when rehabilitation is attempted. Therefore, considering cell viability, cell therapeutics should generally be cryopreserved and thawed just before use. Moreover, long-term cryopreservation of MSCs is necessary, potentially because the same donor sample may be required for multiple times; thus this procedure should be further investigated in the future. Cryoprotectants are used to prevent damage in slow freezing [23]. Recently, we demonstrated the cryopreservation of UC tissue using a serum- and xeno origin-free cryoprotectant, STEM-CELLBANKER® (Zenoaq, Fukushima, Japan), and demonstrated that UC-MSCs cryopreserved retained phenotypes characteristic of MSCs, including immunosuppressive activity [24,25]. These MSCs cryopreserved in a tube can be used as cell therapeutics for patients in any phase of the traumatic CNS injury.

3. Characteristics of MSCs

The minimal criteria for defining MSCs are following: First, MSCs must be adherent cells when maintained in standard culture conditions. Second, MSCs need to express surface markers of MSCs: CD105, CD73, and CD90, but not CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR. Third, MSCs must differentiate into adipocytes, chondroblasts, and osteoblasts *in vitro* [26,27]. These criteria are common to all sources of MSCs.

The major properties of MSCs useful in the treatment of traumatic CNS injury are immunomodulation and neurotropism because most of traumatic CNS injury accompanies inflammation to some extent and neuronal damage. By the virtue of these properties, suppression of inflammation, neuroprotection and neurorestoration are enabled in the injured area of the CNS.

3.1 Immunomodulatory properties

Immunomodulatory effects are the most popular property of MSCs for clinical use [28,29]. The lack of HLA-class II in MSCs lead to prevention from recognition by CD4 positive helper T-cells [30]. In addition, MSCs can inhibit the proliferation of immune cells and their cytokine production [31–34]. The immunomodulation may be the result of MSCs releasing factors such as indoleamine 2,3-dioxygenase (IDO), prostaglandin E (PGE2), HLA-G5 modulating the functions of T cells [35], hepatocyte growth factor (HGF), and tumor growth factor beta 1 (TGF-β1) (Fig. 1) [36]. The immunosuppressive potential of MSCs is induced in the presence of inflammatory cytokines such as gamma interferon (IFNγ) and tumor necrosis factor alpha (TNFα) [37]. In contrast, with low levels of inflammatory cytokines, MSCs enhance T-cell activation and act as inducers of inflammation [38]. We also demonstrated that UC-MSCs cause inflammation with elevated expression of inflammatory cytokine such as interleukin 1 beta (IL-1β) in resting microglia in some lots [39]. These abilities of immunomodulation are also regulated via Toll-like receptors (TLRs) [40,41]. As for the difference between sources of MSCs in immunomodulatory properties, only a few studies directly compared the immunomodulatory potency of MSCs from different tissue sources, and the results revealed there is no significance in the immunomodulatory ability of MSCs from different sources [20]. Overall, this immunomodulatory property, regardless of the source of MSC, is thought to play a key role in the therapeutic effect of MSCs in traumatic CNS injuries.

3.2 Neurotrophic properties

MSCs have been reported to secrete heterogeneous lipid vesicles called extracellular vesicles (EVs), which act as mediators for inter-cell communication [42,43]. These EVs including exosomes secreted by MSCs are known to improve neuronal functions in models of neurological injury [43,44]. We have also demonstrated the amelioration

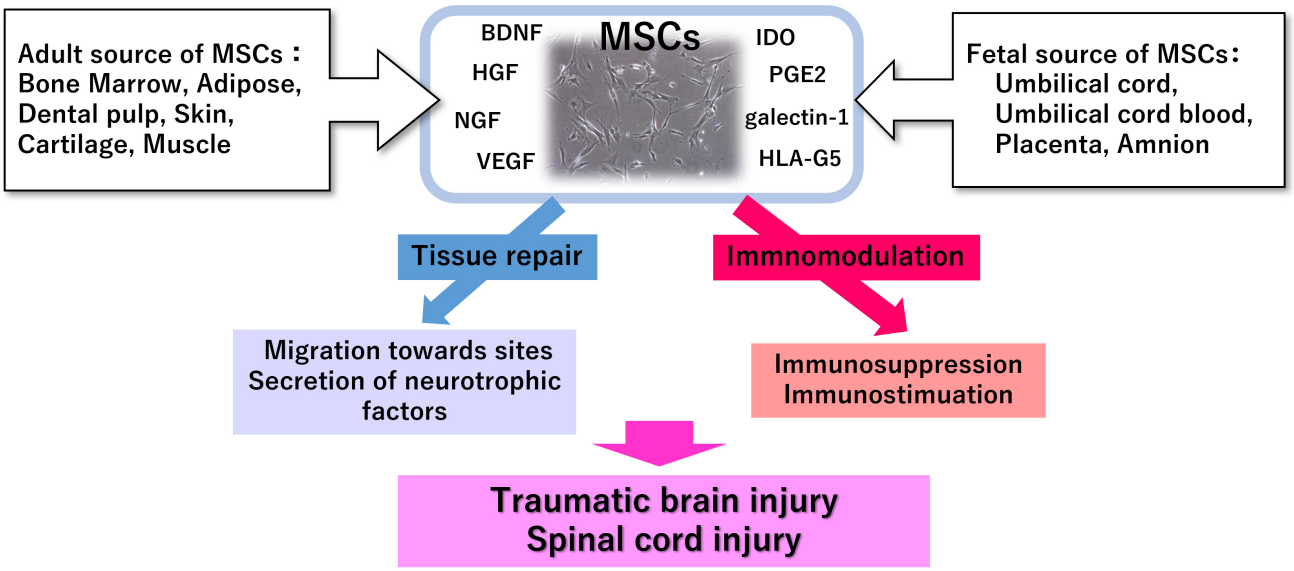

Fig. 1. Characteristics of MSCs and application for traumatic CNS injury. MSCs derived from various tissues secret several neurotrophic factors and immunomodulatory factors. These abilities of tissue repair and immunomodulation are expected as cell therapeutics for the treatment of traumatic CNS injury.

of neuronal injury followed by functional improvement in mice models after MSC administration, which was the result of trophic factors secretion rather than neuronal differentiation and eternal cell replacement by MSCs [45]. We also showed in an *in vitro* experiment that brain-derived neurotrophic factor (BDNF) and HGF secreted by UC-MSCs improved neuronal injury, as indicated by an increase in the immature neuron, neurite outgrowth, and cell proliferation, and also a reduction the number of neurons with apoptosis/necrosis [46]. These properties of neurogenesis and neuroprotection in MSCs could be the major mechanisms in treating traumatic CNS injuries. Furthermore, genetically modified MSCs are studied for treatment of traumatic CNS injury. The efficacy of genetically engineered MSC strongly expressing neurotrophic factors has been reported in TBI models [47].

4. Effects of MSCs in traumatic neurological injury models

The neuroinflammation accompanying the increase in reactive astrocytes and the activation of microglia following traumatic CNS injury has been reported as an important mechanism [48,49]. The trauma causes a multifaceted pathophysiological processes leading to the glial scar [49]. Increased reactive astrocytes are commonly observed in TBI. Astrocytes, through multiple bioactive factors, exert beneficial roles in TBI, including promotion and restriction of neurogenesis and synaptogenesis, modulation of neuroinflammation, and disruption and repair of the blood brain barrier (BBB) [50,51]. MSCs reportedly have the ability to reduce this reactive gliosis, leading to neurological amelioration [45,52]. Microglia are immune responder in the CNS that can switch to an activated microglia (M1) henotype secreting proinflammatory cytokines; conversely, they can turn to a resting microglia (M2) phenotype secreting anti-inflammatory cytokines and neurotrophic factors [53]. It is reported that after TBI, M2-microglia exert anti-inflammatory effects by releasing anti-inflammatory cytokines, resulting in tissue repair and regeneration facilitating phagocytosis [48]. Therefore, microglial polarization could be a target in treatment for TBI. We demonstrated that UC-MSCs exert immunomodulatory effects and change the phenotype of activated microglia to resting microglia, by suppressing the expression of IL-1β and phospho nuclear factor-kappa B (pNFκB) which plays a key role in inflammatory pathway in activated microglia, and changing their morphology from ameboid to ramified; this process is achieved by increasing active Rho guanosine triphosphatase (GTPase) through the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway which control the GTPase expression [39].

In the basic research using MSCs for traumatic CNS injury animal models, many reports have demonstrated the efficacy of MSCs and their secretomes (exosomes) for neurological improvement in traumatic CNS injury models. Kumar Mishra *et al.* [54] found an improvement in tissue, as well as functional behaviors in MSC-infused TBI mice. AD-MSCs-derived exosomes are reported to promote functional recovery, suppress neuroinflammation, reduce neuronal apoptosis, and increase neurogenesis in rats with TBI; this is achieved by suppressing microglia activation [55]. In addition, Xu *et al.* [47] reported that MSCs-derived exosomes promote a BDNF-mediated neurogenesis and inhibit apoptosis in rats with TBI. Exosomes from MSCs have also

shown potential in healing SCI in a rat model through inhibition of pericyte migration, which improved motor functioning and the structural integrity of the blood-spinal cord barrier [56]. Li *et al.* [57] demonstrated the inhibition of neuronal apoptosis via activation of the Wnt/beta-catenin signaling pathway by MSCs in an SCI model. The comparison of therapeutic effects of AD-MSCs, BM-MSCs, and cranial bone-derived MSCs on chronic SCI model rats have been reported [58]. As compared with AD- and BM-MSCs, cranial bone-derived MSCs highly expressed many neurotrophic factors which improved motor function in chronic SCI model. On the other hand, comparison of efficacy in BM-MSCs and UC-MSCs transplantations for SCI model showed that both MSCs reduced neuropathic pain and resulted in subsequent motor recovery after SCI, while survival rate and electrophysiological findings of UC-MSCs were significantly better than BM-MSCs [59]. These basic research data enable us to develop a protocol of clinical trials using MSCs for traumatic CNS injuries.

5. Clinical studies using MSCs for traumatic CNS injuries

Based on the mechanisms and efficacy suggested by the basic research mentioned above, many clinical trials using MSCs for traumatic CNS injury have been conducted [60]. Recent clinical trials using MSCs for traumatic CNS injuries are summarized in Table 1 (Ref. [60–72]). Regarding the origin of MSCs, BM, UC, UCB, and AD have all been widely used as sources. As for the administration route of MSCs, in most clinical studies, MSCs are administered by intrathecal injection (IT) or direct infusion into the injured sites. These clinical trials have mainly reported on the feasibility and efficacy of MSC therapies for neurological disorders, with no severe adverse events.

As for TBI, Wang *et al.* [61] demonstrated the results of a phase 2 clinical trial using UC-MSCs for patients with sequelae of TBI. In this study, UC-MSCs administration improved the patients' neurological function and self-care ability after 6 months. Moreover, Tian *et al.* [62] reported the clinical therapeutic effects and safety of autologous BM-MSCs therapy for TBI. The results showed improvement in the brain function, post-therapeutic improvements in consciousness, and motor functions. Additionally, they showed that the patients' age and the time between the injury and therapy influenced the outcomes of the cellular therapy; however, no correlation was found between the number of cell injections and therapeutic improvements. This last result is of great importance in deciding the protocol of administration.

As for the clinical trials involving MSC treatment for SCI, Vaquero *et al.* [64] reported that patients who were administered BM-MSCs showed clinical improvements in sensitivity, motor power, spasticity, neuropathic pain, sexual function, and/or sphincter dysfunction, regardless of the level/degree of injury, age, or time elapsed since the SCI. They also showed the efficacy and feasibility of administration of repeated doses of BM-MSCs [65]. Ten patients with incomplete SCI received administrations of autologous BM-MSCs at 1, 4, 7 and 10 months; all of them showed some extent of improvement in sensitivity and motor function. Interestingly, after three administrations of MSCs, the mean values of BDNF, glial-derived neurotrophic factor, ciliary neurotrophic factor, and neurotrophin 3/4 slightly increased compared with basal levels. Hur *et al.* [68] showed the effects and feasibility of autologous AD-MSCs transplantation in patients with SCI. Over the 8-month follow-up, the patients who received AD-MSCs did not experience any serious adverse events, and several patients showed mild improvements in neurological function. Transplanting collagen scaffolds with human UC-MSCs has also been reported to have therapeutic potential as a treatment for SCI. Collagen scaffolds with human UC-MSCs were transplanted directly into the injury site, and the recovery of sensory and motor functions was observed in both patients [66]. Oh *et al.* [67] reported on the injection of autologous BM-MSCs into the intramedullary area and subdural space, and concluded that this single MSCs application was safe, despite having a very weak therapeutic effect compared to multiple MSC injections. These clinical trials reporting the efficacy and feasibility of MSCs encourage us to conduct further large-scale clinical studies using MSCs for traumatic CNS injury.

6. Challenges and perspectives

In clinical studies, many sources of MSCs are used, which makes it difficult to have a consensus about the appropriate protocol for the treatment of traumatic CNS injuries. Additionally, variations in culture media, isolation methods, cell counts methods, the number of passages, donor age, delivery methods and finally host receptibility might further blur the potential differences among MSCs therapeutic effects. MSCs feature numerous advantages and one of them is low tumorigenesis risks [73,74]. However, the possibility of tumorigenesis in MSCs are always discussed. MSCs have been reported to possess the properties of both tumor suppression and promotion [75–77]. On the other hand, EVs including exosome secreted from MSCs have no risk of tumorigenesis. Genetically engineered MSCs are also attracting attention for treatment of traumatic CNS injury. By using genetically engineered MSC, which strongly expresses neurotrophic factors, are reported to be effective for TBI at the basic experimental stage [47]. These genetically modified MSCs also have the potential to expand the possibilities of treating traumatic CNS injury. These secretome of MSCs and also genetically engineered MSCs might be future possible therapeutics for treatment of traumatic CNS injuries. Furthermore, since MSCs are used to treat skin injuries with wound healing [78], MSCs may also be effective in skin and soft tissue trauma in traumatic CNS injury. In the future, all injured

Table 1. Recent clinical trials using MSCs for traumatic CNS injuries (modified reference) [60].

Reference	Traumatic CNS injuries	Source of MSCs	Number of patients		Mean age, year	Administration route	Cell dose	Number of administration	Results	Adverse events
			Experimental	Control						
Wang *et al.*, 2013 [61]	TBI	UC	20	20	27.5 ± 9.4 28.6 ± 10.1	IT	6.0×10^7 cells	4	Comprehensive functional recovery and improvement in the ADL after 6 months	Mild dizziness, headache
Tian *et al.*, 2013 [62]	TBI	BM	97	0	21.1 35.3	IT	$3.0–5.0 \times 10^6$ cells	1	Improvement of consciousness and motor function after 14 days	No
Xiao *et al*., 2018 [63]	SCI	UCB	2	0	28 30	Transplantation into the lesion with collagen scaffolds	4×10^7 cells	1	Improvement of motor function after 3, 6, 12 months Improvement of sensory function after 2, 4, 12 months	No
Vaquero *et al*., 2019 [64]	SCI	BM	11	0	44.91 (28–62)	IT	$3 \times 100 \times 10^6$ cells	3	Motor, sensory and bladder-bowel functional improvement after 4, 7, 10 months	No
Vaquero *et al*., 2017 [65]	SCI	BM	10	0	42.2	IT	30×10^6 cells at 3-months interval	4	Motor, sensory and bladder-bowel functional improvement after 3, 6, 9, 12 months	Head ache
Satti *et al*., 2016 [66]	SCI	BM	9	0	31.6 (24–38)	IT	1.2×10^6 /kg at 4 weeks interval	2/3	Safety assessment only	No
Oh *et al*., 2016 [67]	SCI	BM	16	0	40.9 (18–65)	Direct injection into the lesion + IT	1.6×10^7 cells 3.2×10^7 cells	1	Very weak therapeutic efficacy after 6 months	Sensory deterioration, muscle rigidity, tingling sense
Hur *et al*., 2016 [68]	SCI	AD	14	0	41.9	IT	$3 \times 3 \times 10^7$	3	Improvement of function after 8 months	Nausea, head ache, vomit
Mendonça *et al*., 2014 [69]	SCI	BM	14	0	35.7 (23–61)	Direct injection into the lesion	5×10^6 cells/cm^3 per lesion volume	1	Motor, sensory and bladder-bowel functional improvement after 6 months	Pain, cerebrospinal fluid leak
Cheng *et al*., 2014 [70]	SCI	UC	10	34	35.3 (19–57)	Direct injection into the lesion	$2 \times 2 \times 10^7$ cells	2	Motor, sensory and bladder functional improvement after 6 months Superior efficacy than that of rehabilitation therapy	Radiating neuralgia
Dai *et al*., 2013 [71]	SCI	BM	20	20	22–54	Direct injection into the lesion	20×10^6 cells	1	Improvement of motor, sensory and bladder function after 6 months	Fever, headache, pain
Karamouzian *et al.*, 2012 [72]	SCI	BM	11	20	33.2 (23–48)	IT	$0.7–1.2 \times 10^6$ cells	1	Possible efficacy in the motor and sensory function	No

sites might be treated by MSCs in patients with traumatic CNS injury.

7. Conclusions

Recent clinical trials indicate that the use of MSCs as a new cell therapy is expected to be effective in combination with conventional rehabilitation and other medication.

Regarding allogeneity, autologous transplantation might be desirable when considering the possibility of rejection of the host, but this completely depends on the MSCs source. Isolation of autologous BM- or AD-MSCs in adults with traumatic CNS injury is possible, while autologous UC- or UCB-MSCs in adults is very difficult because this would have required cryopreservation of these cells decades ago. Although it is related to the timing of preparation and administration of MSCs. Previous studies have mainly used MSCs for the purpose of treating sequelae and recovering function after the acute phase. In anticipation of the immunomodulatory effect, administration of MSCs immediately after injury should also be considered.

As for the administration route, many clinical trials have opted for IT or direct infusion into injured sites because of the existence of the BBB, which would make it difficult for the intravenously injected MSCs to migrate to the injured CNS sites. In our study, most of the intravenously injected UC-MSCs were trapped in the lungs and could not reach the injury site in the brain [45].

Traumatic CNS injuries have multiple causes and their pathogenesis involves multiple factors, therefore cell therapeutics that have immunomodulatory functions and secrete neurotrophic factors might be more suitable candidates for CNS injury therapeutics.

The prognosis of patients with traumatic CNS injury remains abysmal, with a high mortality rate. It would be necessary to further investigate the appropriate protocol for MSC administration, and large-scale clinical studies on using MSCs to treat traumatic CNS injury will extend the possibility of MSCs therapy in the future.

Abbreviations

CNS, central nervous system; TBI, traumatic brain injury; SCI, spinal cord injury; MSCs, mesenchymal stromal cells; UC, umbilical cord; UC-MSCs, umbilical cord-derived mesenchymal stromal cells; BM, bone marrow; UCB, umbilical cord blood; AD, adipose tissue; IDO, indoleamine 2,3-dioxygenase; PGE2, prostaglandin E2; TGF-β1, tumor growth factor beta 1; IFNγ, gamma interferon; TNFα, tumor necrosis factor alpha; TLR, Toll-like receptor; HLA, human leukocyte antigen; IL-1β, interleukin 1 beta; pNFκB, phospho nuclear factor-kappa B; GTPase, guanosine triphosphatase; Akt, protein kinase B; HGF, hepatocyte growth factor; EV, extracellular vesicle; BDNF, brain-derived neurotrophic factor; BBB, blood brain barrier; PI3K, phosphoinositide 3-kinase; IT, intrathecal injection.

Author contributions

Conception and design of the study—TM, KS and TNI. Acquisition of data—TM and KS. Drafting or revising the manuscript—All authors. All authors have approved the final article.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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