Adipose Tissue-derived Mesenchymal Stem Cells as a Strategy to Improve Recovery After Stroke

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Abstract

Introduction: Based on the positive results observed in experimental animal models, adipose tissue-derived mesenchymal stem cells (AD-MSCs) constitute a promising therapy for stroke treatment. However, several aspects need to be clarified to identify the optimal conditions for successful clinical translation.

Areas covered: This review focuses on AD-MSC treatment for ischemic and hemorrhagic stroke in experimental animal models. In addition, we will explore the optimization of treatment conditions including AD-MSC production, administration routes and therapeutic windows for their appropriate use in patients, and we will provide an update on clinical trials on this therapy.

Expert opinion: Compared with other cell types, AD-MSCs have been less investigated in stroke studies. Currently, experimental animal models have shown safety and efficacy with this treatment after stroke. Due to several advantages of AD-MSCs, such as their abundance and accessibility, they can be considered a promising strategy for use in patients. However, many questions are still to be resolved regarding their mechanisms of action, immune system modulation and the effects of AD-MSCs on all components of the brain that may be affected after ischemic and hemorrhagic strokes.

KEYWORDS: Adipose tissue, Hemorrhagic Stroke, Ischemic Stroke, Mesenchymal Stem Cells.

1. Introduction

Worldwide, stroke is the second highest cause of death and the leading cause of disability, [1] and its burden is increasing as a consequence of a longer life expectancy. Stroke can be classified into two types: ischemic (80-87%) and hemorrhagic (13-20%) [2]. Ischemic stroke occurs when the cerebral blood flow (CBF) decreases as a result of an obstruction, such as a thrombus or embolism. Currently, only IV recombinant tissue plasminogen activator (rtPA) is approved for the treatment of ischemic stroke; however, its narrow therapeutic window is just 4.5 hours from symptom onset, thus few stroke patients can benefit from this treatment. Hemorrhagic strokes are caused by the rupture of a brain blood vessel or by anomalies in the cerebral vascular structure. To date, hemostasis, management of blood pressure and intracranial pressure and surgical techniques are the therapeutic approaches for hemorrhagic strokes [3].

Cell-based therapy has emerged as a promising strategy for the treatment of ischemic and hemorrhagic stroke. This approach has demonstrated beneficial effects in improving functional outcome through mechanisms implicated in brain plasticity such as gliagenesis, oligodendrogenesis, remyelination, synaptic plasticity, axonal sprouting, and angiogenesis. Various cell types have been used: embryonic stem cells (ESC), neural stem cells (NSC), hematopoietic stem cells (HSC), mesenchymal stem cells (MSCs) and also induced pluripotent stem cells (iPS). Of all of them, MSCs are particularly good candidates for cell therapy after stroke as they are easy to obtain and due to their relatively low immunogenicity. Compared to other cell types, adipose tissue-derived mesenchymal stem cells (AD-MSCs) present several

advantages in clinical applications for neurological disorders [4]. AD-MSCs are derived from adipose tissue and thus are abundant, accessible and easy to obtain using lipoaspiration techniques. Moreover, they provide proliferation and differentiation potential [5] without adverse side effects [6-8] and they can be administered without ethical concerns. All of these advantages mean that AD-MSCs present a great opportunity for the treatment of diseases such as stroke.

In this review we focus on AD-MSC treatment for ischemic and hemorrhagic stroke in experimental animal models and we explore the optimization of this treatment and AD-MSC production, as well provide as an update on clinical trials on this therapy.

2. AD-MSC therapy in experimental animal models of ischemic stroke

Brain protection and repair responses are activated as a continuum soon after cerebral ischemia induction and both responses can be modulated by cell therapy [9]. The available data from experimental animal models confirm that AD-MSC administration induces improvements in functional recovery after ischemic stroke [7, 10-12]. This functional improvement in the animals is associated with a reduction in infarct volume [12] and interestingly, when a reduction in this parameter is not found [7, 11], a degree of tissue preservation is observed that includes a reduction in global cell death (both apoptosis and necrosis) and also a reduction in reactive gliosis [7]. More specifically, AD-MSC modulation of Bcl-2-mediated apoptosis signaling and proapoptotic caspase-12 expression has been described [13].

Regarding repair and plasticity modulation by AD-MSCs, the levels of brain repair-associated markers implicated in neurogenesis and neuronal plasticity (Doublecortin (DCX); Neurofilament (NF), oligodendrogenesis (Olig-2), synaptic plasticity (synaptophisyn (SYP), and angiogenesis (vascular endothelial growth factor (VEGF) have been shown to be modulated after AD-MSC treatment [7, 12, 14]. In addition, augmented microvessel proliferation has been found after AD-MSC treatment in the brains of animals [15]. Unpublished data from our group suggest that AD-MSCs play a role in oligodendrogenesis and white matter fiber tract repair in experimental animals with subcortical stroke. These functions are of interest as most of

studies have not focused on the white matter component after stroke and the mechanisms of fiber tract injury [16].

Although the beneficial effects of AD-MSC therapy in experimental animal models of cerebral infarct are known, the mechanisms of action of AD-MSCs after stroke are unknown. At least three mechanisms have been proposed: (i) Transdifferentiation of the administered AD-MSCs at the injury site, (ii) AD-MSC-mediated immunomodulation, and (iii) stimulation of the brain repair response via the release of trophic factors and other signaling molecules by the AD-MSCs [8]. The potential contribution of each one of these mechanisms to functional recovery has had an impact on the experimental design of previous studies on animals, as we will discuss below.

Concerning the first mechanism, AD-MSCs can differentiate into various cell lineages [17, 18] and can express typical neural markers such as microtubule-associated protein (MAP-2) and glial acid fibrillary protein (GFAP) under differentiating conditions *in vitro* [12]. Early studies on experimental animals with stroke aimed to analyze the *in vivo* differentiation potential of AD-MSCs in the brain after intracerebral administration. Given the fact that only 2% of cells survived in the brain parenchyma, their integration into functional neural networks was not shown. For this reason, the authors attributed the functional recovery to cell-tissue interactions and AD-MSC-mediated trophic factor support [19]. It was proposed that AD-MSC cell homing in the brain was not required to elicit a good functional recovery [8, 20].

Subsequent studies analyzed the effects of AD-MSC administration using less invasive methods such as the IV route, confirming good results in terms of functional outcome [7, 12]. Differences in brain cell homing were found and some studies did not show cerebral implantation [7, 11], whereas other studies did observe cell implantation after IV administration when using repeated doses [12]. On the other hand, AD-MSC-mediated immunomodulation has been determined to be crucial for stroke recovery. *In vitro* studies show that AD-MSCs suppress the proliferation of activated blood mononuclear cells, modulating IL-6 and transforming growth factor-1 (TGF-1) levels [21]. In addition, levels of IL-18, toll-like receptor-4 (TLR-4) and plasminogen inhibitor PAI-1 have been shown to be reduced by AD-MSCs [12]. Previous studies have shown that mesenchymal stem cells (MSCs) are allocated in peripheral organs such as the spleen, lungs and kidney and are cleared through the kidney after IV administration [10, 22, 23]. Whether entrapment of AD-MSCs in peripheral organs might have effects on stroke recovery should be analyzed in future studies. Given the fact that the hypothalamic-pituitary-adrenal axis is a link between the brain and peripheral

organs and is known to be involved in immunomodulation [24], it would be interesting to analyze to what extent the peripheral allocation of cells is associated with a modulation of brain inflammation.

Regarding the release of trophic factors and other signaling molecules, *in vitro* studies have identified AD-MSC production of brain-derived neurotrophic factor (BDNF), VEGF [25] and TGF-b [26]. VEGF administration reduces infarct volume and brain edema after temporary middle cerebral artery occlusion (MCAO) [27]. Moreover, *in vitro* studies have shown that cell-free AD-MSC culture mediums enhance neuronal survival, reduce cell death and astrogliosis, enhance angiogenesis and improve functional recovery when administered to animals after stroke [26, 28].

In recent years, it has been reported that intercellular communication of MSCs is at least in part mediated by exosomes, which are small vesicles containing proteins and functional microRNAs and their use as a therapeutic strategy has been supported [29]. In fact, systemic administration of MSC-generated exosomes has been shown to induce recovery from stroke in animals [30]. Considering that there are various cell sources of MSC, such as the bone marrow, placenta or adipose tissue, it would be interesting to analyze possible differences in the therapeutic effect of exosomes derived from each of these sources. However, previous data suggest that there are no differences in efficacy when comparing IV administration of MSC derived from the bone marrow or adipose tissue [7]. As adipose tissue is especially abundant and easy to obtain, our future studies on experimental animals aim to analyze the potential of AD-MSC-derived exosomes after stroke.

3. AD-MSC treatment in intracerebral hemorrhage animal models

Primary ICH accounts for 10–20% of all strokes, thus there are considerably fewer experimental studies analyzing new therapeutic approaches for the treatment of hemorrhagic compared to ischemic stroke. However, stem-cell therapy has shown promising results in some studies using hemorrhagic animal models. Several MSC sources are available for the treatment of this disease in basic studies and all have demonstrated good results. Results include increases endogenous neurogenesis at the level of the subventricular zone and decreased apoptosis in acute [31] and chronic [32, 33] experimental animal models of intracerebral hemorrhage. In addition, anti-inflammatory, antifibrotic, antioxidative paracrine, and regenerative effects were found after intraventricular hemorrhage in an experimental animal

model of newborn rats [34]. Moreover, various reports are accumulating evidence that AD-MSCs could be a novel therapeutic strategy for ICH. Previous experience in acute intraventricular transplantation of rat AD-MSCs showed not only functional recovery but also decreased cell apoptosis. Furthermore, AD-MSCs were able to differentiate into neuron-like and astrocyte-like cells around the hematoma. Finally, growth factor expression, particularly VEGF, was upregulated after using AD-MSCs [35]. Another study on acute intravenous human AD-MSC administration in a rat experimental model of intracerebral hemorrhage showed functional recovery by reducing acute perihematomal edema, inflammatory cell infiltration, glial thickness and apoptosis and inducing endothelial differentiation of AD-MSCs [36].

4. Optimization of AD-MSC treatment conditions

4.1. AD-MSC production for stroke

In order to obtain AD-MSCs for stroke patients, adipose tissue can be obtained from voluntary, healthy donors (allogeneic administration). The adipose tissue is subjected to collagenase treatment, and after several centrifugations the AD-MSCs are isolated. Cells are plated in flasks and when 90% confluence is reached, they are passaged to obtain the desired clinical doses. Purified cells are usually cryopreserved until needed for administration, thus when a stroke patient is ready to receive treatment, the cells are defrosted and cultured for at least one additional passage. Usually the process takes 3 to 6 passages and phenotyping, cytogenetics, safety and sterility testing is performed at every step. In general, once a stroke patient is ready to receive treatment, at least 7 days of AD-MSC preparation are needed before they are ready to be administered (**Figure 1A**).

Another option is to obtain the AD-MSCs from the stroke patient (autologous administration). With this type of administration, the total preparation time including collagenase treatment, plating, harvesting, phenotyping, cytogenetic and sterility testing is substantially increased. In this case there is no need for freezing, but the time for treatment preparation is usually around 4 weeks (**Figure 1B**).

In terms of costs, the procedure for obtaining AD-MSCs is expensive. AD-MSCs are extracted from the donor, isolated, planted, passaged, harvested, cryopreserved and tested before being administered to the patient. This treatment preparation is expensive because it requires specific sterile installations, equipment and material and trained, specialized personnel. However, if AD-MSC therapy is established as a conventional treatment in the future, the cost per patient could be significantly reduced. With this approach, AD-MSC preparation time would be approximately 1 day to harvest the cells and perform the quality, characterization and release testing (**Figure 1C**).

On the other hand, several aspects would need to be standardized to optimize the clinical setting for AD-MSC preparation. For example, it would be important to standardize the cell culture media, as some research groups use platelet lysate, Dulbecco's Modified Eagle Medium (DMEM), DMEM: F12 or Roswell Park Memorial Institute (RPMI) [37]. In addition, the usage (or not) of antibiotics and the type of vehicle to be used in AD-MSC administration must be standardized. Another important issue is to optimize injection procedures to avoid cell-adherence, and ready-to-use vials for administration should not be stored for more than 48 h. All of these aspects must be considered to improve AD-MSC production and to standardize the protocols for multicenter clinical trials.

In addition, to enhance the therapeutic potential, recent advances in AD-MSC culture have been made. These include the use of three-dimensional scaffolding [38], cell programming by using biodegradable polymeric nanoparticles [39], exposure to ischemia and oxidative stress *in vitro* [40] and the stimulation of cells by low-energy laser stimulation [41]. These advances are promising and need to be optimized for stroke by considering the specific needs and clinical conditions of stroke.

4.2. Type of administration, delivery route and therapeutic window

As mentioned earlier, AD-MSC administration can be autologous (AD-MSCs from the same patient) or allogeneic (AD-MSC derived from healthy donors). The immunomodulatory and immunoprivileged properties of AD-MSC are of value for their allogeneic application, as it has been shown that AD-MSCs have low MHC Class I expression and lack MHC Class II molecules and costimulatory molecules CD40, CD80 y CD86 [42]. In addition, class I molecules may activate alloreactive T cells, but with the absence of costimulatory molecules, a secondary

Another important aspect of cell therapy is the route of administration. As previously described, a number of delivery routes have been used in experimental stroke models, including intraventricular, intrastriatal, intra-cerebral, intra-arterial and intravenous. In terms of AD-MSCs, few studies have directly compared delivery routes. Du et al compared the therapeutic effect of the three most commonly used administration routes (intravenous, intra-arterial or intra-ventricular) of AD-MSCs in a rat stroke model. Their findings suggest that compared with intra-ventricular delivery, intravascular injection allows higher dose injections with fewer invasions and it appears to be optimal with regard to therapeutic efficacy, safety and feasibility [44]. In this respect, the intravenous administration of AD-MSCs may prove to be safer and less invasive for stem cell therapy in stroke patients.

On the other hand, soon after blood flow reduction, the entire ischemic cascade is initiated and protection and repair mechanisms are activated as a continuum [45] to reduce brain damage. This cascade is why time is relevant for therapeutic interventions. Various time points are considered, depending on the expected mechanism of action: early vs. late phase administration (**Figure 2**). However, we must consider that in all conditions, stem cell therapy could help widen the therapeutic window of opportunities for stroke patients, which is currently limited to 4.5 h for rtPA.

By administering AD-MSCs as early as possible, both protection and repair mechanisms could be modulated. In our previous experience with rodents, IV administration of AD-MSCs at 30 min after stroke showed significant improvement in functional recovery scales even at 24 h and was maintained at 14 days after stroke. Moreover, we observed higher levels of brain repair-associated markers implicated in neurogenesis and neuronal plasticity (NF), oligodendrogenesis (Olig-2), synaptic plasticity (SYP), and angiogenesis (VEGF) after AD-MSC treatment [7, 10]. Other authors have also obtained good results when administering the cells at 24 h after the induction of stroke [12]. Although time point relationships in animals versus humans are a subject of debate, the beneficial effects of early administration (30 min, 24 h, etc.) are attributed to a modulation of both protection and early repair processes.

On the other hand, a number of studies in animals have also shown positive effects on brain repair stimulation (neurogenesis and angiogenesis) when administering the cells during the post-acute/chronic phase (late phase administration) [46]. Cell administration up to 1

month after stroke showed functional recovery, reduced scar thickness, and an increased number of proliferating cells and oligodendrocyte precursor cells along the subventricular zone in the ipsilateral hemisphere [47]. However, in another study, late phase administration (1 month) did not show a functional outcome [48]. Considering that all of these studies were performed with bone marrow derived MSCs, it would be interesting to analyze this approach with AD-MSCs in future studies.

5. Road to the clinic

Cell therapy has emerged as a novel strategy for the treatment of a many diseases. MSCs have been used in several clinical contexts such as ischemic stroke, cardiomyopathy, myocardial infarction, Crohn's disease, and graft versus host disease, as reflected in a systematic review and meta-analysis [49]. A total of 87 clinical trials with AD-MSCs have been performed worldwide [37]. However, few studies have evaluated the safety of the administration of AD-MSCs after ischemic stroke. One study is a pilot clinical trial, phase IIa, to evaluate the safety of a single IV administration of allogeneic AD-MSCs or placebo (or vehicle) administered in the first 2 weeks after the onset of stroke symptoms (NCT01678534). The other clinical trial is in phase I/II and assesses the safety and effects of autologous AD-MSCs via internal carotid artery and IV administration (NCT01453829) [49]. These two clinical trials are designed to demonstrate the safety of AD-MSC administration, but more studies are needed that permit the inclusion of more patients, in order to assess the efficacy of AD-MSC treatment.

Regarding hemorrhagic stroke clinical trials, there are considerably fewer studies analyzing stem cell therapy for its treatment and none are using adipose tissue as a source of MSCs. However, other sources of MSCs have shown promising results for treatment in some clinical trials of hemorrhagic stroke. Autologous intracerebral transplantation of bone marrow/derived MSCs is being tested in a clinical trial to assess their safety and feasibility in patients with chronic hemorrhagic stroke (NCT01714167). Moreover, the safety of repeated IV injections of MSC derived from the umbilical cord blood will be assessed (NCT02283879). Furthermore, a phase I clinical trial on the safety and dose determination of umbilical cord blood cells will be evaluated in premature infants with intraventricular hemorrhage (NCT02274428) [49]. Even if no clinical trial is analyzing the effects of AD-MSCs in hemorrhagic stroke patients, existing trials are opening the path to prove whether MSCs could be safe as treatment for this kind of

stroke. If these clinical trials demonstrate safety and feasibility in patients with cerebral hemorrhage with these sources of MSCs, new clinical trials could be initiated with AD-MSC treatment to confirm these results.

To date there is no evidence of negative results in terms of safety after AD-MSC administration; however it is important to consider that AD-MSCs are important contributors to the development and progression of previously existing tumors [50-52].

6. Conclusion

Experimental evidence suggests that AD-MSC administration can enhance recovery from ischemic and hemorrhage stroke in animal models. However, many questions are still to be resolved regarding cell production, routes of administration, the therapeutic window, mechanisms of action, and immune-modulation to improve AD-MSC treatment. From the clinical point of view, it is necessary to demonstrate the safety and efficacy of these cells in stroke patients.

7. Expert Opinion

As the global population ages, the incidence of stroke is increasing and continues to be a public health problem. There is a clear need for better ways to improve functional and cognitive outcomes after stroke. AD-MSC therapy is a promising approach that has shown good results in experimental animal models. However, the mechanisms of action are not yet fully understood. It is important to highlight the relevance of studying the mechanisms of AD-MSC therapy and its effects on all components of the brain that may be affected after ischemic and hemorrhagic stroke. For decades, research on stem cell therapy has focused on the effects of this treatment on gray matter repair (neurogenesis, angiogenesis, sinaptogenesis, astrogeneisis, vasculogenesis). More recently, a few translational studies are beginning to highlight the importance of considering the white matter component for repair after stroke [16]. Not only are ≤25% of ischemic strokes in humans subcortical or lacunar and confined to white matter regions, but cortical infarcts also produce white matter injury. The high frequency of this damage motivates research into the mechanisms underlying the repair of damaged white matter (axons and myelin) after a stroke [53, 54].

Of the mechanisms attributed to AD-MSC function, functional recovery seems to be mediated by immunomodulation and trophic factor release. In our experience, the administration of BDNF (a trophic factor secreted by AD-MSCs) results in better functional outcomes, oligodendrogenesis, remyelination and fiber connectivity, as observed in rats subjected to subcortical damage in ischemic stroke [55]. Further studies to analyze the effects of additional factors released by AD-MSCs are warranted, to better understand their mechanisms of action.

As mentioned earlier, the administration of AD-MSCs can be autologous or allogeneic. To treat patients during the acute phase of stroke, allogeneic administration would be the ideal route to reduce the time needed for cell expansion and production. Allogeneic AD-MSC administration is promising, as experimental evidence in animal models of stroke has shown enhanced recovery.

One of the most important issues in cell therapy studies in stroke is the time to AD-MSC administration. Immediately after stroke, a natural repair response is activated including both protection and repair processes that continuously communicate to reduce the injury. By administering the AD-MSCs as early as possible, these mechanisms could be modulated. For this reason, in our previous experimental animal studies, AD-MSC were administered as soon as 30 minutes after stroke. Unfortunately, human clinical conditions are quite different because the situations of stroke patients cannot be predicted. Having cryopreserved cells poses a technical problem regarding the time of administration, because to improve their viability, AD-MSCs must be thawed and cultured for at least one week prior to administration. If cell therapy were established as the usual treatment, it would be possible to continuously produce AD-MSCs and avoid the need to freeze the cells, reducing by at least one week the time that is now needed for cell preparation.

Another aspect to highlight regarding the time to AD-MSC administration is the patient status after stroke. Having shown that AD-MSC administration induces a modulation of the immune system response, depending on patient status, immune system response-mediated AD-MSCs also could present a problem of infection control. Preclinical experimental models of stroke are developed in conditions of infection control, because the animals are isolated and include healthy controls. However the patients participating in a clinical trial are not in the same conditions and they can experience infections before or after suffering a stroke. A patient with an infection should not be subjected to an immune system modulation by any

treatment such as AD-MSC administration. This aspect must be analyzed in future studies on experimental animals and patients.

   Currently, only a few studies with a limited number of patients are ongoing using AD-MSCs. In order to demonstrate their efficacy in stroke patients, an investment is needed in developing multicenter clinical trials that include a high number of patients. The results would be of help in optimizing treatment parameters and cell production.

Declaration of interest

Acknowledgments

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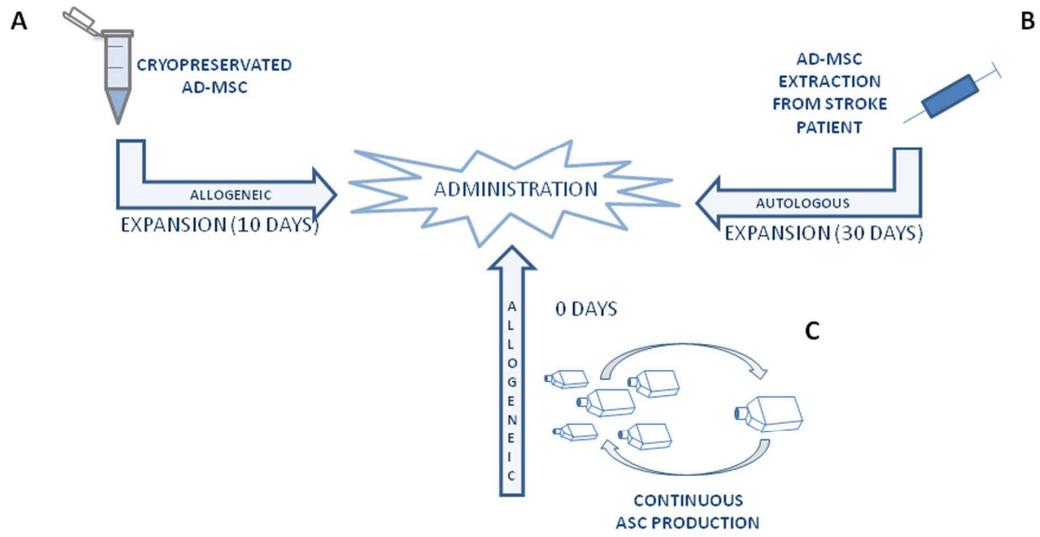
Article highlights

- AD-MSCs are a promising therapy for ischemic and hemorrhagic stroke treatment.

- Many questions are still to be resolved regarding cell production, the route of administration, the therapeutic window, and mechanisms of action and immune modulation.

- AD-MSC production must be optimized according to the desired time to administration in stroke patients.

- It is necessary to evaluate the safety of AD-MSCs in stroke patients.

Legend of figures

Figure 1. Representation of alternatives to AD-MSC-derived treatment production and the time required to obtain them. A) AD-MSCs obtained from a voluntary healthy donor take 1 week for their preparation prior to administration **B**) Autologous sources need 4 weeks for preparation. **C**) Continuous production of AD-MSCs would mean 1 day would be needed prior to administration.

Figure 2. Therapeutic strategies mediated by various time points of AD-MSC administration: early or late. Brain protection and repair mechanisms are activated as a continuum after stroke. Both mechanisms are enhanced after early AD-MSC administration, while late AD-MSC administration only enhances brain repair mechanisms. The handicap of early administration is the difficulty in terms of obtaining an adequate number of AD-MSCs.

Figure 1

Figure 2

AD-MSC-mediated BRAIN PROTECTION

AD-MSC-mediated BRAIN REPAIR

Hours/Days   Weeks   Months   Years

EARLY AD-MSC ADMINISTRATION   LATE PHASE AD-MSC ADMINISTRATION

Brain protection and repair are enhanced   Effects limited to brain repair

Difficult to obtain sufficent amounts of AD-MSC   Easy to obtain sufficent amounts of AD-MSC