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REVIEW

Mesenchymal stem cells in the treatment of ischemic stroke: progress and possibilities

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Abstract: Stroke is a major cause of death and long-term disability in industrialized countries, and the only causal therapy for stroke comprises recombinant tissue plasminogen activator(rt-PA)-mediated recanalization of the occluded vessel. New experimental strategies focus on neuroregenerative approaches, among which the application of mesenchymal stem cells (MSCs) has gained increasing attention. MSCs, like other stem cells, have the capacity of unlimited self-renewal giving rise to differentiated cells from various cell lineages. Bone marrow (BM)-derived MSCs are the most frequently used MSC type in experimental stroke studies. Application of BM-derived MSCs and, in some studies, transplantation of MSCs from other tissue sources resulted in an improved functional recovery in experimental animals, although stroke volumes were not always affected by MSC transplantation. The underlying precise mechanisms of this phenomenon remain elusive, although MSC transplantation is considered to affect many diverse events, eg, by modulating the inflammatory milieu, stimulating endogenous neurogenesis and angiogenesis, and reducing glial scar formation. On the contrary, neuronal differentiation and integration of transplanted MSCs do not seem to affect stroke outcome significantly. On the basis of these preclinical studies, first clinical trials confirmed improved functional recovery in patients who had received BM-derived MSCs systemically, although the number of patients enrolled in these studies was low and there were no adequate control groups. In this review, we describe some fundamental biological characteristics of MSCs and further review some preclinical experimental studies, with special emphasis on BM-derived MSCs. We also review clinical trials in which MSCs have been used and conclude with a short outlook on the application of MSCs in stroke research.

Keywords: bone marrow, cerebral ischemia, mesenchymal stem cells, stroke

Introduction

Ischemic stroke is a major cause of death and the leading cause of long-term disability in industrialized countries. The only causal therapy for this devastating disease comprises local or systemic thrombolysis of the occluded vessel by using recombinant tissue plasminogen activator.[1–3] Thrombolytic therapy, however, is limited by a narrow time window. Only a few stroke patients receive this therapy.[4] Despite the complex pathophysiological mechanisms underlying ischemic stroke,[5,6] many experimental studies focused on manipulating various cell injury-inducing cascades. Although these studies reported neuroprotective effects of many drugs in animal models of ischemic stroke,[7–11] a successful translation from bench to bedside is still lacking.[12] Hence, the recent experimental focus has shifted from studies on acute neuroprotection toward neuroregenerative approaches with special emphasis on cell-based thera-

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pies. Precursor cells and stem cells of different origins have been studied thoroughly in experimental stroke models.[13–16]

Stem cells have unique characteristics as they are capable of unlimited self-renewal giving rise to mature cells from various lineages.[16] Based on their origin, stem cells are further classified as embryonic, fetal, and adult stem cells. Although embryonic stem cells and fetal stem cells are considered an attractive source for tissue engineering processes yielding beneficial effects after experimental stroke, their application is limited due to restricted availability, formation of teratomas, and ethical concerns.[13,17] Hence, adult stem cells or precursor cells have been thoroughly studied, and their neuroprotective potential after cerebral ischemia has been repeatedly shown.[15,16,18–21] Application of mesenchymal stem cells (MSCs) has become an interesting tool in stroke research as they offer broad therapeutic strategies, including clinically relevant autologous transplantation of bone marrow (BM)-derived MSCs.[22] In this review, we first describe some fundamental characteristics of MSCs with special emphasis on BM-derived MSCs. Thereafter, studies on MSCs in animal models of experimental ischemia will be reviewed, which will be followed by a critical review of MSC-based stroke therapy in patients. We conclude this review with a short outlook on the application of MSCs in ischemic stroke therapy.

Definition and biological characteristics of MSCs

Adult mammalian bone marrows contain a distinct but rare population of stem cells that are critically involved in hematopoiesis giving rise to differentiated cells from various cell lineages such as mesenchymal, neuronal, hepatic, or cardiac cells.[22–27] These cells are referred to as MSCs, marrow stromal cells, or mesenchymal stromal cells and have been studied intensively for more than 2 decades. However, a lack of common definition and an imprecise terminology have hampered the development of this field until recently.[28] For this review, we refer to these cells as mesenchymal stem cells or simply as MSCs. The defining characteristics of MSCs used by investigators are still inconsistent and occasionally induce confusion. Taking into account that isolation methods, expansion methods, and tissue sources differ between various studies, substantial differences between these cultivated MSCs cannot be excluded.[29] The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has suggested some fundamental standards to better define characteristics of MSCs.[29] This characterization of MSCs includes 1) cell adherence on plastic surfaces; 2) expression of CD105, CD73, and CD90 while lacking other surface markers such as CD45, CD34, or CD14; and 3) differentiation into osteoblasts, adipocytes, and chondroblasts in vitro.

Several studies suggest that MSCs have unique immune-modulating properties. MSCs have been recognized to evade immune recognition and decrease immune responses, properties that are essential in successful allogeneic transplantation medicine. It has been reported that MSCs do not induce a proliferative lymphocytic response,[30–32] a finding that might be related to the expression levels of major histocompatibility complex class II molecules.[28] Because the postulated immunoprivileged status of MSCs is essentially derived from in vitro experiments, the exact in vivo situation remains unclear. The extent of MSC-mediated immunosuppression in vitro seems to depend on the concentration levels of MSCs in culture; ie, when applied at low concentrations, MSCs induce immune responses rather than depressing them.[33] However, there is also some evidence suggesting that MSCs might not be suitable as "universal donor cells" for allogeneic transplantation. Allogeneic MSCs injected in mice have been reported to significantly increase the invasion of various immune-competent cells.[34]

MSCs are not only harbored within the BM of adult organisms but also found in various other tissues and compartments of both fetal and adult organisms, including blood, placenta, adipose tissue, skin, liver, and lung.[35–39] Although these cells share many similarities, some differences with regard to their differentiation profile and gene expression patterns still exist.[40] BM-derived MSCs are regarded to have the highest multilineage potential, which is a reason for their preferred use in experimental or therapeutic applications. We focus on reviewing recent studies on BM-derived MSCs in experimental stroke research in the next section.

MSCs in experimental stroke research

Neuroprotection after MSC treatment in experimental stroke research has been reported by many authors. However, questions as to the most appropriate application routes, transplantation timing, and mechanisms underlying MSC-mediated neuroprotection remain. Because the majority of MSC experiments in stroke research have been performed on BM-derived MSCs, we first focus on some fundamental studies using this cell type. We first review reports on systemic application of BM-derived MSCs and then the studies on

local application of MSCs and conclude this chapter with some examples of non-BM-derived MSCs.

Intravenous application of BM-derived MSCs

Although many studies have shown neuroprotection after the intravenous injection of BM-derived MSCs,[41–48] the underlying mechanisms are still elusive. This is in part due to differences in study designs. MSCs have the capacity to migrate into the ischemic lesion zone,[41] possibly via interaction between stromal cell-derived factor (SDF)-1 and its chemokine receptor-4,[46] where they act by two distinct mechanisms: 1) secretion of growth factors and 2) stimulation of angiogenesis within the peri-infarct zone. A study by Song et al[49] has suggested that MSCs also express and secrete brain natriuretic peptides (BNPs) among other growth factors. BNP, as a close homolog of the atrial natriuretic peptide (ANP), might therefore significantly help reduce the formation of postischemic edema as has been described for ANP before.[50] The biological relevance of BNP in cell-based stroke therapy, however, remains elusive because the study by Song et al was performed in vitro only. Growth factors that are secreted by the ischemic host tissue itself, such as vascular endothelial growth factor (VEGF) or epidermal growth factor (EGF), are also thought to be critically involved in MSC-mediated neuroprotection. For instance, Wakabayashi et al[47] observed that MSCs secrete insulin-like growth factor-1 followed by an enhanced expression of VEGF, EGF, and basic fibroblast growth factor within endogenous neural cells, which results in reduced infarct injury.[47] The systemic injection of MSCs was associated with direct antiapoptotic effects and modulation of inflammatory responses within the ischemic tissue resulting in reduced neural damage in the peri-infarct zone, where glial scar formation has been described to be reduced after MSC transplantation.[48,51] As MSC therapy results in enhanced levels of endogenous growth factors such as VEGF, MSCs have been reported to stimulate angiogenesis along the ischemic boundary zone via mechanisms involving enhanced expression of both endogenous VEGF and VEGF receptors.[52] Some studies have reported other mechanisms, such as the differentiation of transplanted cells into mature neural cells or the induction of endogenous neurogenesis by enhanced proliferation and differentiation rates of subventricular zone (SVZ)-derived neural precursor cells (NPCs).[43,51,53,54] Neuronal differentiation rates observed in these studies were, however, low and are therefore unlikely to substantially improve postischemic brain injury.[43,55]

Although there is no debate on the beneficial effect of an intravenous BM-derived MSC therapy in experimental stroke research, the most appropriate transplantation timing and the number of cells required for successful transplantation rates are still elusive. Hence, Omori et al[56] analyzed MSC-mediated effects on infarct injury and its dependence on both transplantation time and cell dosage. Although postischemic amelioration of brain injury was also observed in animals that received late transplantation of fewer MSCs, ie, a single dose of 1×10^6 cells, MSC therapy was most effective when given within 6 hours after stroke in dosages of 3×10^6 cells. Because no difference with regard to vessel density was observed between the treatment groups, the authors inferred that the early transplantation of more MSCs was beneficial due to immediate neuroprotection rather than the stimulation of postischemic angiogenesis. On the contrary, other studies described significantly improved poststroke recovery in animals that received MSC therapy 4 weeks after stroke,[46,57] albeit reduced infarct volumes were only observed in animals that had received MSC treatment within 7 days after stroke.[57] Komatsu et al[57] observed increased angiogenesis within the peri-infarct area of virtually all animals treated with MSCs, which – in their opinion – might explain the improved functional outcome of MSC-treated experimental groups. Different interpretations made on appropriate systemic MSC transplantation time points depend on the selection of the outcome. Although functional recovery is observed after late and early transplantation, reduction of infarct volume requires early transplantation. However, the assessment of functional recovery in the aforementioned studies was frequently restricted to simple neurological scoring missing a bunch of subtle and reliable motor coordination tests such as the cylinder test, rotarod test, or corner turn test.[58]

Among the aforementioned studies, a recent work by Zacharek et al[59] on the intravenous administration of BM-derived MSCs after transient focal cerebral ischemia showed that BM-derived MSCs proved to ameliorate poststroke functional outcome. The authors observed that MSCs derived from donor animals that had undergone stroke before MSC preparation were superior to MSCs derived from nonischemic animals. In other words, MSCs derived from ischemic rats enhanced angiogenesis, arterial density, and axonal regeneration and modulated growth factor expression patterns within the ischemic tissue of the recipient much more effectively than those derived from nonischemic animals. The mechanism by which ischemic lesions actually influence stem cell properties within the BM compartment

was, unfortunately, not analyzed in this study. Hence, further studies on stroke-mediated modulation of BM-derived MSCs and MSCs from other compartments such as the blood are urgently needed.

Other application routes of BM-derived MSCs

Among the application routes, intravenous injection of MSCs is the most studied application protocol in experimental stroke research. However, systemic application of MSCs results in poor neuronal differentiation rates of relatively low intracerebral cell numbers (see above), and studies focusing on neuroregeneration, ie, cell differentiation and integration of transplanted cells, might require higher local cell amounts. Alternatively, some studies analyzed effects of an intra-arterial injection of MSCs after stroke.[60–62] In line with intravenous transplantation studies, intra-arterial injection of BM-derived MSCs resulted in sustained and improved functional recovery of rodents. MSC-induced enhanced poststroke recovery was associated with increased axonal sprouting, remyelination, and synaptophysin expression, whereas glial scar formation and Nogo-A expression were reduced. Long-term beneficial effects were observed for as long as 1 year, and the MSC-mediated beneficial outcomes were found to be – at least in part – due to the abovementioned structural and molecular changes within the ischemic milieu.[60] MSC-mediated beneficial effects on stroke outcome in the latter study were already observed at 2 weeks after transplantation, when MSC-mediated neuroprotection might rather be a consequence of changes within the inflammatory milieu including paracrine secretion of local factors as has been described earlier for intravenous application routes. As such, the structural changes after intra-arterial MSC transplantation as observed at later time points might therefore only be one factor contributing to sustained neuroprotection, or this might be an epiphenomenon only.

Although intracerebral transplantation of BM-derived MSCs is of minor clinical relevance as compared with the intravenous application, local injections of MSCs might provide a higher number of cells, which is a prerequisite for further analysis of how MSCs induce postischemic neuroprotection. As has been described for other application routes, intracerebral transplantation of MSCs also resulted in improved functional recovery of recipient animals, with controversial results of MSC-mediated effects on infarct sizes.[63–65] Although neuronal differentiation of transplanted cells and stimulation of both proliferation and differentiation of SVZ-derived NPCs were observed, neuronal differentiation rates were low. Consequently, MSC-induced enhancement of endogenous neurogenesis is unlikely to significantly contribute to an enhanced poststroke recovery of recipient animals. On the other hand, MSC-mediated beneficial effects after intracerebral transplantation might rather be due to changes within the inflammatory ischemic tissue such as modulation of IL-10 expression.[64]

Transplantation of non-BM-derived MSCs in experimental stroke models

Although BM-derived MSCs have been thoroughly characterized in vitro and used for experimental stroke research, data on the application of MSCs derived from sources other than BM are still scarce. Nevertheless, a significant number of studies analyzed the therapeutic potential of non-BM-derived MSCs originating from different sources, such as embryonic stem cells, blood, placenta, or adipose tissue.[66–69] As has been described for BM-derived cells, systemic application of these cells yielded significantly improved functional outcome after stroke in each experimental condition. The majority of studies, however, lack a systematic in vivo comparison between the cell type analyzed and BM-derived MSCs that is a "therapeutic gold standard". Furthermore, the quality of the study is often limited to descriptive data implying two parameters: the extent of tissue injury and the extent of functional impairment. Therefore, the therapeutic potential of these MSCs as compared with BM-derived MSCs in experimental stroke research cannot be sufficiently assessed. As a consequence, further studies with emphasis on systematic comparison between different cell types in vivo and their modulating characteristics within the ischemic milieu are required.

Transplantation of MSCs in stroke patients

While considering the abovementioned beneficial effects of BM-derived MSCs on postischemic recovery in rodent models, only limited data are available on translational approaches into the clinic.[70,71] In 2005, Bang et al[70] described a successful autologous transplantation of BM-derived MSCs in patients suffering from severe stroke. In that study, eligibility for enrolment in the study was defined by the National Institutes of Health Stroke Scale as at least 7.[70] The authors observed improved modified Rankin scores and higher Barthel indexes in patients who had systemically received MSCs during an observation period

of 1 year. Although this is an interesting observation, some critical aspects have to be addressed. Out of 30 patients, only five patients received MSC therapy, leaving the treatment group small in comparison with the control group. Furthermore, study blindness was restricted to an initial observation period, ie, during the first week after stroke, which was followed by an allocation of patients to the experimental groups on day 7 after stroke. Treatment procedures themselves were, however, not blinded. MSC transplantation was late with a first injection between weeks 4 and 5 and an additional injection between weeks 7 and 9 after the onset of symptoms. Although autologous MSC transplantation requires time-consuming ex vivo cell culture expansion before the injection of cells, transplantation timing seems to be late, taking into account the reduced infarct sizes after early transplantation time points in rodent stroke models as described earlier. MSC-induced beneficial modulation in stroke treatment seems to decline with increasing cell passage numbers in rodents.[72] The aforementioned clinical trial was succeeded by a 5-year follow-up study[71] due to several reasons, including safety concerns as to the use of fetal calf serum and fetal bovine serum in cell culture.[73] The authors observed sustained improved functional outcome in patients treated with MSCs for as long as 5 years with no significant side effects and no changes in mortality rates as compared with control patients. Beneficial outcome in patients treated with MSCs was associated with enhanced serum levels of SDF-1, which might be regarded as an epiphenomenon. Because MSCs induce endogenous neurogenesis in animal stroke models, the authors further studied an ischemic involvement of the lateral border of the SVZ and correlated the extent of injury of this region with the functional outcome of patients. Thus, the authors described a correlation between reduced ischemic injury of the lateral border of the SVZ and improved functional outcome of patients treated with MSCs. Whether the reduced involvement of the SVZ within the treatment group was due to genuine stimulation of endogenous neurogenesis by transplanted cells or it is due to MSC-mediated secretion of trophic factors followed by subsequent neuroprotection of residing cells remained unclear. Nevertheless, the promising results of systemic MSC transplantation in stroke patients warrants further preclinical studies aiming at increasing our understanding of how MSCs induce poststroke protection so that further clinical trials might benefit from these findings, resulting in improved study designs in the future.

Conclusion

There is no doubt that MSCs from different tissue sources enhance functional recovery in experimental stroke research models, even when applied at later time points regardless of application routes. There is also some evidence that early transplantation is required for the reduction of infarct size. The mechanisms underlying these observations are, however, not yet fully understood. Therefore, more sophisticated preclinical studies involving systematic analysis of the properties of MSCs from different tissues are needed. Special emphasis should also be put on ex vivo cell culture expansion and on how these procedures alter the biological properties of the cells to be transplanted. Depending on the outcome of these studies, clinical trials should not only be restricted to BM-derived MSCs but also be performed using MSCs from other sources such as blood, skin, or adipose tissue.

Disclosure

The authors declare that they have no conflict of interest.

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