Therapeutic potential of intravenously administered human mesenchymal stromal cells

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Keywords
Mesenchymal stromal cells, cell therapy, intravenous, clinical application

Summary
Mesenchymal stem cells (MSC) represent a stem and progenitor cell population that has been shown to promote tissue recovery in preclinical and clinical studies. The study of MSC migration following systemic infusion of exogenous MSC is difficult. The challenges facing these efforts are due to a number of factors, including defining culture conditions for MSC, the phenotype of cultured MSC, the differences observed between cultured MSC and freshly isolated MSC. However, even if MSC populations consist of a mixture of stem and more committed multipotent progenitors, it remains probable that these cell populations are still useful in the clinic as discussed in this review.

Schlüsselwörter
Mesenchymale Stromazellen, Zelltherapie, intravenöse, klinische Anwendung

Zusammenfassung
Mesenchymale Stammzellen (MSC) gehören einer Stamm- und Progenitorzellpopulation an, die unterstützend bei Gewebeerneuerungen mitwirken, was sich in präklinischen und klinischen Studien gezeigt hat. Die Untersuchung des Migrationsverhaltens von MSC nach systemischer Infusion hat sich als schwierig herausgestellt. Die Herausforderungen bei diesen Bemühungen resultieren aus einer Vielzahl von Faktoren, wie die Definition der Kulturbedingungen für MSC, den Phänotyp der MSC, Unterschieden zwischen längere Zeit in Kultur expandierten MSC und frisch isolierten MSC sowie die umstrittene Frage, ob es sich überhaupt um eine einheitliche Zellpopulation handelt oder um eine Mischung von Stamm- und multipotenten Vorläuferzellen. Dennoch zeigen sich diese Zellen in zahlreichen Studien als vielversprechend für klinische Anwendungen, die in diesem Review diskutiert werden.

Keywords
● cardiovascular disease (76),
● Parkinson’s disease (62) and
● neurological disease (41).

The effectiveness of stem cell therapies in tissue regeneration in the clinic remains to be definitively shown in many of these examples. It is likely that further knowledge of the signals that mediate cellular growth, differentiation and migration are required for effective tissue regeneration. Several types of stem cells have been considered for such cell therapies, including (2, 15, 22, 49, 78, 102)
● bone marrow cells (BM),
● endothelial precursor cells (EPC),
● haematopoietic stem cells (HSC) and
● mesenchymal stem cells (MSC).

Mesenchymal stem cells
Definition and characterisation
Stem cells are defined as a population of primitive cells with capability of self-renewal and differentiation into multiple cell lineages (35). HSC and MSC are stem cell populations found within adult bone marrow (79). The bone marrow stroma was proposed to function as a structural support for the haematopoietic stem and progenitor cells in the bone marrow (26).

It was in the 1960s that Ernest A. McCulloch and James E. Till first revealed the clonal nature of haematopoietic stem cells as demonstrated by the spleen colony-forming assay (CFU-S) (10, 94). In the 1970s, MSC were first described by Friedenstein et al. (30) as an adherent, fibroblast-like population that could regenerate rudiments of normal bone in vivo (30, 31, 77). Friedenstein and colleagues reported an in vivo assay for examining the clono-

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The recent advances in biotechnology and understanding of regenerative medicine using the body’s own stem cells is now a clinical possibility as a novel therapeutic strategy to treat clinical diseases. A number of adult stem cell therapies have been explored as a means of treating diseases such as
● leukaemia (36),
● graft-versus-host disease (57, 58),
genic potential of stromal marrow cells (32, 34).

In this assay, stromal cells were referred to as colony-forming unit fibroblasts (CFU-F). Subsequent experiments revealed that marrow stromal cells possess self-renewal and multilineage differentiation capacity (3, 33, 77), features typical of stem cells. Consequently, many investigators started to refer to cultured stromal cells as MSC (20). In addition, MSC-like cells have been isolated from adipose tissue, epidermis, vessel wall, and cord blood (67). In the bone marrow, MSC represent a rare population, approximately 0.0001–0.001% of the nucleated cells.

Even though MSC are rare, they are readily enriched in culture by their preferential attachment to plastic surfaces (83) and can be easily expanded ex vivo.

The multipotency of MSC has now been convincingly confirmed in vivo and by the generation of a variety of terminally differentiated cell types in vitro, including osteocytes, chondrocytes, and adipocytes (16, 17, 61, 82). However, claims that MSC can differentiate into non-mesodermal lineages such as hepatocytes, neurons, and cardiomyocytes have only been shown under conditions in vitro and never in vivo (52, 63, 74, 79, 83, 106). MSC may also play a significant role in endogenous tissue repair (without the need for differentiation) by secretion of paracrine factors including cytokines and growth factors from the transplanted cells into the surrounding tissue (18, 19, 99).

The phenotypic characterisation of MSC generally relies on cell surface molecule detection using monoclonal antibody (mAb) staining and detection protocols (67, 79). However, no convincing unique markers have been found for MSC, although a combination of markers is characteristic of MSC. Freshly isolated MSC are so few in number that they are unlikely to be clinically useful. Most MSC isolation and expansion protocols rely on selection by adhesion to plastic and preferential expansion in culture. It is this cell population that most people refer to as MSC, even though they do not show true stem cell characteristics. For this reason, many now refer to ex-vivo expanded MSC as multipotent stem cells.

An attempt has been made to define cultured MSC using a limited number of cell surface markers. In particular, undifferentiated cultured MSC typically express CD29, SH2 (CD105), SH3 and SH4 (CD73), CD44, CD90, and CD166, but do not express common haematopoietic and endothelial markers such as CD11b, CD14, CD31, CD34, and CD45 (44). Another important property of MSC is their capacity to produce and secrete a large variety of soluble factors (21) that play important beneficial actions in tissue repair.

Cultured MSC are amenable to genetic manipulation using different vectors such as replication-deficient viral vectors. The over-expression of certain genes may then be used to enhance MSC function and survival or to deliver proteins of interest (28). For example, as mentioned, it has been shown that intramyocardial injection of MSC over-expressing the gene Akt, allows improved survival of MSC and consequently better myocardial protection after AMI (72).

Immunomodulatory properties

Until recently, it was assumed that MSC enjoyed immune privilege in allogeneic settings (55) neither exerting nor being subject to immunological reaction. However, Nauta et al. (70) showed that in a non-immunosuppressed host, allogeneic MSC may be eliminated, especially if gene-transfected (29). Human MSC suppress T- and B-cell lymphocyte proliferation in a mixed-lymphocyte-culture (MLC) or are induced by mitogens and antibodies in a dose-dependent manner (8, 14, 24, 27, 38, 50, 53, 55, 104).

The suppression is major histocompatibility complex (MHC) independent and is not reduced when MSC are separated from the lymphocytes in transwells, indicating that cell-cell contact is not required (14, 24, 27, 104). The mechanisms underlying the immunosuppressive effect remain to be fully elucidated.

When MSC are exposed to IFN-α, they express class II Ag but not costimulation molecules (96) suggesting that inhibition of T-cell response may be related to the induction of energy or apoptosis in cell-cell contact conditions. One study reported MSC-induced apoptosis of proliferating lymphocytes (81), however; most reports not only exclude the involvement of MSC-induced apoptosis of target cells but suggest that the arrest of apoptosis may be a major mechanism for MSC imparting a survival signal to immune cells as well as to other cells (14, 86).

Therapeutic role in haematopoietic regeneration

As shown already by the initial research of Friedenstein et al. (32) MSC, or more likely their progeny, play an important role in regulating the HSC niche and haematopoiesis. Additionally, the immune-privileged characteristic of MSC has led to their use in allogeneic haematopoietic stem cell transplantation. It is for these reasons that allogeneic MSC are a potential candidate to enhance the effectiveness of HSC transplantation. Lazarus and colleagues (1995) were the first to analyse the potential of MSC to support H SCT in a phase I clinical trial (54).

Since then it has been shown that MSC are not only safe but can also effectively increasing the rate of H SCT engraftment in both pre-clinical and clinical settings (6, 13, 42, 46, 51, 59, 65, 69, 73). The mechanism of this is currently unknown; discussed principles are the provision of growth factors and microenvironmental signal by the transplanted MSC.

Therapeutic modulation of graft-versus-host disease

There have been many reports of the immunosuppressive ability and immunoprivileged properties of MSC (1, 8, 27, 50, 60). These properties have significant implications for their use in allogeneic haematopoietic stem cell transplantation (HSCT) and importantly, in the potential for modulating of graft-versus-host disease (GVHD). Additionally, MSC possess immunomodulatory properties and inhibit T-cell proliferation in vitro (55, 64, 80). Clinical studies to date have used several sources of MSC including autologous,
donor derived and third party (from an unrelated, unmatched donor) populations.

A direct immunosuppressive effect of MSC in vivo has been shown in a baboon model, in which infusion of ex vivo-expanded matched donor or third-party MSC delayed the time to rejection of histoincompatible skin grafts (8). The use of MSC as immunosuppressants in humans, as a corollary to the immunosuppressive effect of MSC in vitro and in preclinical animal models, has been suggested for the prevention and treatment of GVHD, in organ transplantation to prevent rejection, and in autoimmune disorders. Severe acute GVHD is associated with high mortality and is a major threat to successful HSCT and no effective therapy exists for severe corticosteroid-refractory acute GVHD (25, 88, 98).

The first case report showing that MSC could resolve treatment-refractory severe GVHD came from Sweden, where Le Blanc et al. (57) reported resolution of steroid-refractory severe gut and liver GVHD in a boy aged nine years with advanced acute lymphoblastic leukaemia (ALL) after infusion of maternal (i.e. haploidentical) MSC. Le Blanc et al. (56) reported the largest published trial, with 55 patients with severe, steroid-refractory GVHD undergoing MSC therapy in a phase II trial. Complete response was obtained in 30 of 55 patients, while nine patients showed some improvement in their GVHD. This was a promising result in patients with such a poor prognosis. This study predominantly used MSC from unrelated, HLA-mismatched donors, supporting a potential “off-the-shelf” therapeutic. A study by Kebrai et al. (47), utilising the commercial MSC Prochymal™ (Osiris Therapeutics Inc.) in combination with corticosteroids to treat de novo GVHD. They reported striking results, with 77% complete response and a 16% partial response from a total of 31 patients.

However, Osiris (www.osirisx.com) have reported preliminary results of their phase III clinical trial where the response to Prochymal was no different to placebo controls for either steroid-refractory treatment (35% treated vs. 30% in controls, n = 260) or first line therapy (45% treated vs. 46% in controls, n = 192) (5). This recent report highlights that while much enthusiasm has surrounded the effectiveness of MSC as a therapeutic for GVHD, much more research is required to validate these cells for therapy.

A difficulty with these clinical trials is that they have been initiated relatively recently and long-term follow up is unavailable. This is important as the effects of MSC on relapse are largely unknown. Ning et al. (71) reported a small study where 10 patients received a co-infusion of HLA identical sibling-matched HSCT and MSC. Only 1 of the 10 patients developed grade II-IV GVHD (compared to 8/15 historical controls). Relapse was higher in the MSC-treated patients (60%) compared to only 20% of matched controls. This suggests that whilst MSC may alter the course of GVHD, they may compromise GVL as a result of their suppression of T cells. Understanding how these cells mediate their immunomodulatory capacity may allow for further expansion of this therapeutic option to prevent and treat GVHD.

**MSC as a therapeutic tool**

**Inherited monogenic errors**

MSC transplantation holds promise as a cell therapy for genetic disorders of the mesenchyme. In their proof of concept trial, Horwitz et al. (45), performed allelo- geneic bone marrow transplantation on three children with severe osteogenesis imperfecta, a genetic disorder in which osteoblasts produce defective type I collagen resulting in osteopenia and severe bone deformities.

The investigators found evidence that marrow-derived osteoblasts precursors migrated to bone and enhanced bone formation in all three children. This study provided the first insight into the therapeutic potential of these stem cells. The clinicians in the first place used whole bone marrow, which in past has failed to yield successful mesenchymal engraftment (95), and later demonstrated that culture-expanded MSC can completely substitute for this, using autologous MSC which had been engineered to express a copy of the unmutated collagen IAI gene (43).

The three children suffering from severe osteogenesis imperfecta received a standard marrow ablative chemotherapy regimen followed by marrow infusion from either completely or partially HLA-matched siblings. Two of the three patients had complete haematopoietic engraftment, and the third had mixed chimerism. After bone marrow transplantation, osteoblasts culture-expanded from iliac bone of the first two patients were found to contain 1.5–2% donor cells; cells from the third child could not be grown.

Clinical improvement was achieved in each child, with decreased numbers of osteocytes, increased osteoblasts lining the bony matrix and evidence of new lamellar bone formation.

Therefore, MSC have curative potential in single molecular genetic defects.

The clinical values of MSC in other genetic defects has still to be evaluated. Candidate diseases are, for example, hepatic storage diseases, or defects in the blood coagulation system.

**Cardiovascular disease**

Whole bone marrow and MSC, which are available from a variety of sources, are currently leading the field in cell-based therapies for cardiac disorders (4, 11, 23, 66, 84). In this regard, it remains highly controversial whether cell-based therapies improve cardiac function by engraftment and differentiation (12, 76, 87) or by releasing paracrine factors (7, 39, 40, 68, 105). Quevedo et al. (85) addressed this issue by demonstrating long-term MSC survival and engraftment of bone marrow-derived adult MSC in chronically injured myocardium after transendocardial injections in pigs.

However, so far there has been no definitive proof of MSC differentiation into cardiac constituents in the infarcted hearts in animals or humans (39, 75, 101). It therefore remains to be proven what role, if any, MSC will play in cellular cardiomyoplasty (1).
Bone marrow and adipose tissue are widely available, but have to be collected by standardised and tightly controlled procedures to fulfil GMP criteria.

**Genomic stability of clinically applied MSC**

Another major safety concern for clinical use is the genomic stability of MSC. This includes the risk of cell transformation and the knowledge about regulation of senescence in MSC. Use of immortalised MSC transduced by human telomerase reverse transcriptase revealed that transformation of human MSC is a long, multistep process involving the deletion of p16ink4a (93). A few studies have shown cultured human MSC with chromosome abnormalities that finally transform (89, 90). However, these results were retracted because they were related to contamination by exogenous cancer cell lines (37, 103).

Using karyotype and comparative genomic hybridisation, other authors have shown the genetic stability of human MSC (unlike mouse MSC) during a long culture process. Furthermore, it has been shown that clinical grade-cultured human MSC, regardless of the presence of aneuploidy (mainly duplication of chromosomes 5 and 8), reaches senescence and the cells are never transformed (100).

Up to date, the different production processes have been effective and based on phenotypic analysis and differentiation potential, a first set of simple controls have been defined. However, controls of the final product should be providing precise data on efficacy and safety. The next challenge will be to develop production processes that reach good manufacturing practice goals and to define more accurate control methods of culturing MSC (92).

**Conclusions, outlook**

The successful application of MSC as cellular therapy may improve function and reduce morbidity. There are several clinical trials being performed worldwide to examine the systemic administration of MSC to treat a variety of diseases and tissue defects.

Despite general excitement and promising results, there is a major lack of understanding of how MSC target specific tissues.

The balance between the beneficial effects from locally engrafted MSC versus systemic effects from secreted paracrine factors that diffuse into target tissues is unclear.

Progress into whether MSC mobilise and home under steady-state conditions is stifled by the difficulties in identifying and isolating native MSC. Most studies utilise culture-expanded MSC that do not express many of the cell-adhesion or chemokine receptors that are responsible for the homing of leukocytes and HSC.

Furthermore, tracking of MSC after local transplantation or systemic infusion has relied on techniques that have inherent disadvantages, including indirect methodology, significant manipulation of the host biology or use of exogenous MSC source.

Accumulating evidence suggests that MSC have a significantly larger role in regulating wound healing and inflammatory diseases that previously thought. Given the number of diseases that could potentially benefit from MSC administration and the desire for minimally invasive therapies, systemic infusion of MSC that can promote tissue regeneration and immunosuppressive effects represents an attractive therapeutic approach.

It is likely that the number of potential therapeutic applications of MSC and their efficiency and efficacy will continue to grow as the fundamental biology that is responsible for the MSC regenerative properties and migration responses continues to be elucidated.

**References**

K. Kollar; E. Seifried; R. Henschler: Mesenchymal stromal cells


