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REVIEW ARTICLE

Mesenchymal Stem Cell Delivery Routes and Fate

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MSC have been used in diverse animal disease models to investigate their regenerative capacity. Although the clinical outcome was often encouraging, the mode of action of the cells remains unresolved. Differentiation of MSC into cell types of their target organs was only rarely shown, with the exception of the musculoskeletal system. Thus, the effect of the cells on the clinical outcome in several disease models of tissue degeneration must be independent of trans-differentiation and caused by indirect or paracrine effects. Furthermore, tracking of the cells in vivo revealed that only a small proportion of the cells home and persists in the target sites, and that most of the cells are not detectable after 7～14 days post transplantation. It seems that MSC can deliver a profound clinical effect without trans-differentiation, without homing to target organs in significant numbers and despite the cell's disappearance within short periods of time. These finding also suggest that the full potency of MSC has not yet been exploited in the current applications. Here we will provide an overview of the different routes used for cell delivery and the fate of the cells after transplantation. The effects on clinical outcome are discussed with respect to the role cell entrapment in non-target organs may play for the observed clinical effects.

Keywords: Mesenchymal stem cell, Cell delivery, Distribution, Cell therapy

Mesenchymal stem cells (MSC) are self renewing cells with the ability to differentiate into several cell lineages. In addition to their stem cell characteristics, MSC are easily obtainable from many tissues and can be expanded in vitro without loss of potency.

Their differentiation into osteoblasts, chondrocytes and adipocytes has become a standard potency assessment for these cells and has defined their clinical applicability in musculoskeletal diseases. In vitro, MSC have also been differentiated into cells with phenotypic features of myocytes and cardiomyocytes, neurons, Schwann cells, endothelial cells and others (1, 2). These properties have raised hopes for the application of MSC in regenerative therapies beyond the repair of mesodermal tissues, including neurodegenerative disorders and cardiac degeneration. Beside MSC isolated and expanded from their originally described source, bone marrow, cells with characteristics of MSC were isolated and applied in clinical studies from adipose tissue, umbilical cord, cord blood and placenta. These tissues have the advantage that they are easily obtainable and higher initial numbers of MSC can be harvested. In a comparative study it was shown that the frequency of MSC in bone marrow is about 1 in 10^4 mononuclear cells, while it has been estimated in other tissues to be between less than 1 in cord blood and 5 in 10^4 cells in adipose tissue (3). For regenerative application, clinical cell delivery choice and homing prediction, it is of relevance to explore the in vivo location and function of MSC.

Defining the location of this rare cell type in situ is complicated by the lack of a single identifying marker for these cells, which are typically characterized by the co-expression of a range of surface markers, namely CD105 (Endoglin), CD90 (Thy-1), CD73 (NT5E), CD29 (integrin β1) and CD166 (activated leukocyte cell adhesion molecule). The molecular definition of MSC is further hampered by heterogeneity in cell culture. For example, we have identified various proportions of CD271 (p75NGFR) and nestin positive cells in different MSC isolates from bone marrow and from adipose tissues. These proportions

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fluctuated during cultivation. When CD271 positive MSC were selected and put back into culture, the proportion of CD271 negative MSC increased again within 2 passages (unpublished observations). This observation lends to the hypothesis that MSC are part of a dynamic fluctuation between a more differentiated cell type and a more stem like cell, at least in vitro. Mesenchymal precursor cells are co-localized during mammalian ontogenesis development with hematopoetic and heamangioblastic sites (4) and may be related to mesangioblasts (5). Recently, a perivascular identity of mesenchymal stem cells in vivo has been shown for several organs (6). Perivasular cells were shown to be of $CD146^+$, $CD34^-$, $CD45^-$, $CD56^-$, PDGF-Rβ and NG2 positive phenotype in skeletal muscle, pancreas, adipose tissue, and placenta. These cells expressed the typical MSC markers in vitro and in vivo and differentiated into myoblasts, osteoblasts, chondrocytes and adipocytes (6). The function of perivascular cells is not completely understood. Pericytes are part of the hematopoetic stem cell niche in bone marrow. Possible functions include the regulation of microvessel contractility (7), inhibition of endothelial cell division by TGF-β, and may, in some organs support endogenous regeneration. Nestin positive pericytes in brain vasculature may support neurogenesis, and in the testis, transiently nestin expressing pericytes are able to regenerate Leydig cells. In the absence of pericytes, blood vessels become hemorrhagic and hyperdilated, which leads to conditions such as edema, diabetic retinopathy, and even embryonic lethality (2, 6, 8). These are characteristics, and functions, that have also been ascribed to MSC and thus pericytes may be relevant for understanding the clinical effects of MSC.

One critical aspect for MSC transplantation and subsequent therapeutic efficacy is the delivery method. The optimal cell delivery technique should provide the most regenerative benefit with the lowest side effects. The present analysis of the used delivery techniques and the associated outcomes in terms of cell fate in animal models may provide information on the mode of action of the cells. The most common routes of MSC transplantation outside tissue engineering-based methods are by intravenous or intra-arterial infusion, or by direct intra-tissue injection. The observed results of these routes are explored.

Intravenous injection of MSC

The most convenient mode of MSC transplantation is the intravenous route. This delivery route in almost all cases let to a distribution of MSC mostly in lungs, but also in spleen, liver, bone marrow, thymus, kidney, skin (9), and to tumors (10-12). These sites of MSC engraftment do not appear significantly different between healthy animals and disease models in most studies. The dynamics of cell fate are somehow different between studies and models used. In a mouse model, cells traced 48 hours after intravenous transplantation were mostly detected in the lung, liver, intestine, skin and bone marrow. About 5～10% of the injected cells remained in the spleen, while no cells were detectable in lymph nodes (13, 14).

In general, it does not seem to be of importance for the cells destinations whether these are autologous/syngeneic, allogeneic or even xenogenic. In a mouse study, the tissue distribution and degree of engraftment was directly compared between syngeneic and allogenic MSC after tail vein or portal vein injection. After tail vein injection, more cells were found in lymphoid tissues 24 hours after injection, 12～19% of the cells were found in spleen, 4～5% in lymph nodes, versus 5～7% and 1～2%, respectively, after portal vein injection. Engraftment levels in bone marrow and thymus were not different between the injection routes. Portal vein injection had a benefit over tail vein injection for liver engraftment, where cells were detectable after 1 and 7 days. Very few cells were detectable in the lungs after portal vein injection, in contrast to tail vein injection. After 7 and 21 days, cells were almost undetectable in all tissues. When the same injection routes were compared in a heart transplantation mouse model, the amount and distribution of cells did not change and was very low in all organs. No difference was found between the syngeneic and allogenic origin (15).

The survival or ability to detect the cells in the different homing organs was relatively short, but vary at a low level between different studies. Less than 0.01% of intravenously injected human cells were detectable after 4 weeks in the lungs of mice (16). A total body count of labeled MSC by bioluminescence showed a decrease from the intensity measured at 10～15 minutes post intravenous infusion to about 60% after 24 hours and less than 10% after 72 hours. No cells were detectable after 7 days in the lungs, spleen, liver and kidney in an acute kidney injury model in the same study (17). Interestingly, the apoptosis rate of detected MSC was less than 1%, making apoptosis an unlikely cause for the cell's disappearance.

Engraftment in the lungs, and in the other major target organs, is a very rapid event, cells can be detected already seconds or minutes after intravenous transplantation (17, 18). The cause for this entrapment in the lungs is likely a combination of mechanical and physiological conditions and may be due to the small capillary size, the large capil-

lary network and the strong adhesion properties of MSC. Lung entrapment may cause unwanted side effects such as embolism, at least in small animal models. Only few MSC transmigrate within 24 hours through the endothelial barrier to reside in the perivascular space, but without further differentiation. Transmigrated cells were able to divide and to cause osteogenic differentiation with features of osteosarcoma in the lung of immune-compromised mice. Some of the cells also formed calcium deposits within the capillaries, confirming the intravascular entrapment. The osteogenic and hyperplastic process was only observed when murine MSC were used in this mouse model, not with human MSC. It was shown that the murine cells, unlike human MSC, can acquire chromosomal abnormalities in culture and may thus be prone to aberrant growth (16). It would be interesting to see whether MSC entrapped in the lungs, or the other primary MSC target organs lung and spleen acquire a pericytic phenotype. This could be implied from a study where 29% of human adipose derived MSC injected into rat mesenteries acquired a perivascular phenotype in vivo MSC (19).

Entrapment of MSC in capillaries does not appear to be a general phenomenon. When MSC were injected in the femoral artery in a skeletal muscle injury model, a very large proportion of these also accumulated in the lung, indicating that MSC can pass the peripheral capillaries (20). Whether capillaries need to exert certain features to attract and keep MSC is not clear. However, the amount of MSC in the lungs was significantly reduced by the application of the vasodilator sodium nitroprusside before cell transplantation (18). This finding may argue for a rather mechanical mechanism for cell trapping in the lung. It remains to be investigated whether the reduced entrapment also leads to an increased cell homing to the target organ, and subsequently to improved clinical outcomes.

Improving target site engraftment

After systemic injection intravenously for myocardial regeneration, very few cells engraft in the infracted heart (21, 22). There is also little difference in the accumulation of cells in lung, spleen or liver when the cells were administered by direct intramuscular or by intravenous injection (22). Direct injection should have the advantage of precise localization of the cells, despite their invasive properties. On the other hand it has been shown that regardless of the delivery route, only 1～5% of delivered cells engraft within the target site for regeneration. Only 3.5% of all injected cells were retained in the heart after 6 weeks. No cells were detected in the brain. The number of cells in the target tissue may thus be increased by increasing the number of injections, volume of injection or increasing the cell concentration in the injected volume (21, 23, 24).

Intravascular cell delivery routes require that the cells reach their target site, remain there and are able to migrate through the endothelial layers. Stem cells have the capacity to migrate and need in addition to initiate a sequential process of recognition and firm adhesion. On the molecular level chemokines activate integrins required for endothelial attachment and subsequent transendothelial migration. It was shown that once attached, about half of the MSC crossed the endothelium within 30～60 minutes, before transmigration ceased (25). Short-term tissue homing of mesenchymal stem cells into spleen and bone marrow after intervenous injection in mice is influenced by the age of the recipient and cell senescence. This age dependency was not observed for lung and liver homing 20～24 hours post injection. Over-expression of CXCR4 alone did only increase the bone marrow homing of mesenchymal stem cells in animals that had received prior irradiation (26). Another study on irradiated mice showed 6～12% of intravenously injected autologous and allogeneic MSC in the recipients bone marrow at day 10, when their number rapidly declined. MSC were also not detected in pancreas, lungs, spleen and liver at day 10. However, in this diabetic model the disease was effectively reversed due to repression of beta-cell specific T-cell responses (27).

A further way to increase engraftment of MSC into the target tissues instead of their capillary entrapment in the lung and other organs is the phenotypic modification of the cells before engraftment. Exposure to a bone microenvironment, or to TGF-β1 before systemic transplantation of MSC increased engraftment in various tissues, and extended the restricted survival of the cells in skeletal tissues to more than 28 days (28). Along this line, when we induced a neural phenotype in MSC by exposure to a neuronal differentiation medium and injected the cells intravenously in a cerebral stroke animal, the number of cells and their survival in the brain increased significantly when compared to naive MSC (unpublished observation).

Using a different cell delivery route may have some effect on their distribution, but rarely on long term engraftment of the cells, or on clinical outcome. A comparative analysis of Intra arterial and intravenous injection of MSC in a model of acute kidney injury (AKI) and in healthy mice showed distinctive patterns of distribution, but no long term engraftment. While cells accumulated in the

kidneys after intra-arterial injection in AKI animals, in healthy animals a diffuse distribution was found by bioluminescence or iron nanoparticle imaging (17, 29). Signal intensities of labeled cells declined within the 4 days of observation. Most of the cells reaching the kidney remained in the glomerular capillaries, only very few cells were found in the interstitial tissue. The majority of the cells injected into the jugular vein again ended up in the lungs (17).

Without previous modification, the number of MSC that reach the target site is variably low. Only 150.000 of the 5×10^6 intravenously injected MSC were detectable in the infarcted heart one month after injection (30), while another study found only 1% of the intravenously injected cells in the heart (9). MSC that were injected into the femoral artery were detectable for less than 3 days in the lung and only for about 7 days in the injured skeletal muscle (20). Studies performed in pigs found MSC in the infarcted and peri-infarcted area of the heart after intravenous injection even 1 or 3 months later, respectively (31, 32). In both studies infarct size reduction and improvement of left ventricular function were shown. In two other studies this long survival was not confirmed, instead MSC were detected after intravenous injection in the lung, spleen, liver and bone marrow, but not in the heart after myocardial infarction in pigs at 2 weeks post injection (33, 21). In contrast, direct injection into the infarct area, and intra-coronary injection resulted in engraftment in the heart in these studies.

Successful recruitment of MSC from the systemic circulation to the area of need is probably effected by chemotactic stimuli. These may change over time and thus the window of therapeutic cell application may be limited. For stromal cell derived factor 1 (SDF-1), maximal expression was observed after 24 hours in acute myocardial infarction (MI) (34). Indeed, recruitment to the heart was highest when cells were injected at day 1 post MI, and for functional improvement cells had to be injected within the first four days post MI. On the other hand, in many studies an effect of intravenously injected cells was seen when they were injected shortly after MI. For example, when MSC were injected 3 hours post MI, only about 150.000 of the 5 Mio injected cells could be detected in the rat heart after 4 weeks, yet improved ventricular function and increased angiogenesis was observed (29). When cells were injected into the left ventricular cavity cell uptake in the infracted area and also in the spleen increased about fourfold at 4 hours post infusion. The cell uptake in the MI area was also about 2 fold higher than in sham animals, but there was no difference whether MSC were transplanted 2 or 14 days post MI. In any case, cells were still detectable after 7 days in the MI area (9, 22). Cell delivery before a critical time may be thus one cause for the low integration of intravenously delivered MSC in the heart, but it probably does not have a strong effect on the clinical outcome.

Possible benefits of MSC engraftment in non-target organs

Entrapment of MSC in the lung may have adverse consequences. Entrapment of the cells in spleen and liver, however, may elicit beneficial results. For example, CD3 lymphocytes were upregulated in the spleen and lymph nodes upon intravenous MSC transplantation and a shift in the T-cell phenotypes towards a tolerogenic status were observed. The ratio of regulatory T-cells to cytotoxic $CD8^+$ T-cells was also elevated, in addition to a THl to TH2 polarization implied by cytokine expression shifts in recipient splenocytes (13, 14). These findings suggest that MSC engraftment in the spleen changes the proportions of different T-lymphocyte subsets and may trigger one important mechanism by which grafted MSCs suppress potentially destructive immune responses.

In a mouse model of heart transplantation it was shown that portal vein injection of MSC was more efficient that intravenous injection in prolonging survival. MSC injection was only effective in extending survival when injected before transplantation, thus a protolerogenic milieu was essential (15). This might be reached by an increased MSC number in the liver when compared to intravenous cell transplantation, where at least two cell injections were necessary to have the same increase in graft survival. In contrast to spleen, contact between donor MSC and the host immune system in the liver may promote the observed immune inactivation. A role for Kuppfer cells in this context is likely since their blockage prevents tolerance induction (35). By increasing the number of MSC in the liver by portal vein injection, this privileged organ for tolerance induction may be exploited for a stronger immune-suppressive effect in cell therapy.

Safety of systemic cell delivery in clinical studies

Most human clinical studies with MSC were performed for graft versus host disease (GvHD). Injection of in vitro expanded allogeneic MSC to alleviate GvHD did not show adverse effects and improvement of outcome (36). No side effects related to engraftment or entrapment of injected MSC in non target organs were found in case study on

stroke and multiple systems atrophy patients and (37, 38). In a different setting, the engraftment of hematopoetic stem cells improved by co-transplantation of MSC, although the latter do not engraft in the bone marrow itself and prompt hematopoetic recovery was achieved in several studies in breast cancer patients with no adverse effects (39). First results of a clinical trial of MSC transplantation for cardiac repair showed that allogeneic MSCs taken from young donors and intravenously injected improved heart and lung function significantly. In the same study arrhythmic events were four times less frequent than in the control group (40). The intravenous delivery route in this setting seems to show efficacy. In comparison, the injection of high or low dosage MSC directly into the damaged myocardium still let the cells to migrate to extracardiac organs, especially to the spleen (41). At the same time, although the delivery track was visible, cells were rarely detected in this study at the injury site.

In conclusion, the systemic intravenous delivery route for MSC shows clinical efficacy in several preclinical models and in preliminary clinical settings. This efficacy seems to be independent of the cell source and its autologous or allogeneic characteristics. The positive clinical outcome occurs independently of long term engraftment of the MSC in the actual target site, and is perhaps rather due to interactions with non-target tissues such as spleen and liver. Although mostly focused on studies in cardiac-schemia, similar conclusions can be drawn from analysis of neurodegenerative diseases such as stroke (42). In addition to clinical benefit, possible entrapment of the cells in the lungs does not cause adverse events. It needs to be explored whether the effects of systemic and targeted delivery can be differentiated and their specific advantages used for improvement of regenerative efficacy.

Potential Conflict of Interest

The author has no conflicting financial interest.

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