Cell and Tissue Research (2019) 375:563–574
https://doi.org/10.1007/s00441-018-2961-4

REVIEW

Myths, reality and future of mesenchymal stem cell therapy

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Received: 7 May 2018 / Accepted: 5 November 2018 / Published online: 20 November 2018

Abstract

Mesenchymal stem cell (MSC) therapy represents an alternative approach for tissue regeneration and inflammation control. In spite of a huge amount of preclinical data that has been accumulated on the therapeutic properties of MSCs, there are many conflicting results, possibly due to differences in the properties of MSCs obtained from different sources or underestimated mechanisms of MSC in vivo behavior. This review consolidates the in vivo effects of MSC therapy, discusses the fate of MSCs after intravascular and local delivery and proposes possible trends in MSC therapy.

Keywords Mesenchymal stem cells · Cell therapy · Exosomes · Liposomes · Diabetes mellitus

Introduction

The major characteristic of MSCs is their ability for self-renewal and differentiation into osteoblasts, chondrocytes and adipocytes (Dominici et al. 2006). The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy defines MSCs as plastic-adherent cells with the ability for tri-lineage differentiation characterized by the expression of CD73, CD90 and CD105 as positive and CD34, CD45, CD14, CD11b, CD79alpha, CD19 and HLA-DR as negative cell surface markers (Dominici et al. 2006; Lin et al. 2013, Galipeau and Krampera 2015, Galipeau et al. 2016). It is well known that this phenotype is not unique for MSCs. For example, CD73, an ecto-5′-nucleotidase converting adenosine monophosphate to adenosine, is also expressed by lymphocytes; endothelial, smooth muscle and epithelial cells; and fibroblasts (Galipeau and Krampera 2015; Galipeau et al. 2016; Samsonraj et al. 2017). CD90, a glycosylphosphatidylinositol-linked protein involved in cell adhesion, is found in endothelial cells, hematopoietic stem cells, lymphocytes, fibroblasts and neurons (Galipeau and Krampera 2015; Galipeau et al. 2016; Tamajusuku et al. 2006). CD105, a type I receptor for TGF-beta superfamily ligands, is expressed by monocytes, fibroblasts, chondrocytes and hematopoietic progenitor cells (Galipeau and Krampera 2015; Galipeau et al. 2016; Jurisic et al. 2010; Nassiri et al. 2011). CD34, a transmembrane protein sialomucin, is lost by many positive cells during in vitro cultivation (Galipeau and Krampera 2015; Galipeau et al. 2016; Stolzing et al. 2012; Lin et al. 2012). It is interesting that the STRO-1 antibody recognizing CD34 developed by Gronthos et al. in 1994 was considered for a while as the most reliable positive MSC marker (Gronthos et al. 1994, Lin et al. 2011a, b). Later, the same group generated a new STRO-3 antibody recognizing a minor portion of STRO-1+ cells (Gronthos et al. 2007). Based on STRO-3+ cell selection, Teva developed allogeneic bone marrow-derived MSCs called mesoblast (Rexlemestrocel-L, USA), which were used in clinical trials in 2014–2016 (See et al. 2011; Skyler et al. 2015).

Bone marrow-derived MSCs (BM-MSCs) are the cells most often used in clinical trials due to their easy standardization. There are several companies that have developed local medical agency-approved protocols to obtain either allogeneic or autologous BM-MSCs. Mesoblast or Rexlemestrocel-L (Teva Pharmaceutical Industries Ltd.) cells are BM-MSCs obtained from the BM of young volunteers, sorted using monoclonal antibody STRO-3, expanded up to 7–9 passages in vitro, then frozen and transported to the site of a trial. It has been shown that Rexlemestrocel-L cells demonstrated a higher proliferative and multilineage differentiative capacity than BM-MSCs obtained by plastic adhesion (See et al. 2011). Another type of standardized BM-MSC, Ixmyelocel-T (Aastrom Biosciences Inc.), is a combination of CD90+

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MSCs and filler CD45+ macrophage-like cells obtained from the autologous BM of patients by adhesion to plastic and in vitro expansion. The content of Ixmyelocel-T varies from patient to patient and each sample can contain from 3×10^7 to 30×10^7 cells with 5 to 55% MSCs (http://stemcellassays.com/2014/10/pipeline-ixmyelocel-t/) (Henry et al. 2016a, b). Consequently, each patient can receive from 1.5×10^6 to 165×10^6 MSCs during Ixmyelocel-T therapy, which raises the question as to how to compare the results of the treatment. Most often, MSCs are characterized by CD73, CD90 and CD105 expression (Gimble et al. 2007; Lin et al. 2011a, b; Mildmay-White and Khan 2017).

MSC therapy reached the stage of clinical trials based on multiple in vitro and in vivo animal studies. Evidently, hope for a new possibility to treat severe diseases sped up MSC application in clinics. Since 2010, more than 50 clinical trials have been conducted using MSC and other types of cell therapy; however, the efficiency of MSC therapy is rather low (Krueger et al. 2018; Fernández et al. 2018; Singh et al. 2016). Many questions, such as the biodistribution of MSCs, fate of injected cells, risk of microthrombosis due to MSC injection and some others, still need to be resolved.

Immune reaction to allogeneic cells in vivo

Multiple studies have discussed the low immunogenicity of MSCs, manifested by the lack of MHC class II (MHC II) and costimulatory molecules as well as low levels of MHC class I (MHC I) on their membranes (Humphrey 1986; Hunt et al. 1988). However, this is not unexpected, as MHC II and costimulatory molecules are expressed mostly by professional antigen presenting cells, such as dendritic cells, macrophages and B cells (Humphrey 1986). All other cell types (epithelial, endothelial and mesenchymal cells) do not express MHC II with minor exceptions, such as keratinocytes and none express costimulatory molecules sufficient to activate T cells. Expression of MHC I varies in MSCs obtained from different tissues, such as placental syncytial trophoblasts, chorion membrane cytotrophoblasts, fetal mesenchymal cells and maternal decidual cells (Hunt et al. 1988). The role of MHC molecules is relevant only if MSCs survive in the host organism for a long time. However, the fate of MSCs delivered by different routes is highly discussable.

The fate of MSCs after intravascular injection in intact recipients

Multiple studies conducted in humans, rabbits, dogs, rats, pigs and mice have demonstrated lung accumulation of intravenous (IV) injections of MSCs (Wang et al. 2015; Sood et al. 2015; Spriet et al. 2015; Turtzo et al. 2015; Meseguer-Olmo et al. 2017). Biodistribution studies are based on MSC labeling using either fluorescent dyes (Wang et al. 2015; Kim et al. 2015) or radioactive isotopes (Meseguer-Olmo et al. 2017; Spriet et al. 2015). Both approaches could only show the accumulation of the trackers associated with the cells, which can be live or dead. Moreover, tissue macrophages engulf the MSC debris and can be misrecognized as MSCs. IV injection of MSCs directs them to the lung capillaries, which have a diameter of 5–10 μm, while the average size of, for example, Wharton's Jelly MSCs, is 20 × 50 μm in the adhesive state (Fig. 1a) and 20 × 30 μm after trypsinization (Fig. 1b). Injection of 15-μm beads induced microthrombosis in mice (Evans et al. 2017). Leibacher et al. (2017) demonstrated quick disruption and apoptosis of BM-MSCs after IV injection into mice. Preincubation of MSCs in hyperosmolar buffer decreased the MSC size, delayed cell death and increased the frequency of traceable MSCs up to 24 h after transplantation (Leibacher et al. 2017). Our own experiments demonstrated that the major part of Wharton's Jelly-derived MSCs was trapped in the lung arterioles of rats (Fig. 1c), while only debris was found in the capillaries (Fig. 1d). Furlani et al. (2009) demonstrated 25–40% mortality in mice injected with different doses of MSCs. Ramot et al. (2010) demonstrated that palatal MSCs injected into mice induced multifocal thrombi in the pulmonary arteries. IV injection of MSCs into pigs induced lung atelectasis but not significant thrombosis (Mäkelä et al. 2015). At the same time, multiple papers have demonstrated the safety of IV injections of MSCs in humans (Glassberg et al. 2017; Packham et al. 2016; Skyler et al. 2015). In clinical applications, 100–300 million MSCs are usually injected via intravascular routes or locally (Glassberg et al. 2017, Packham et al. 2016, Skyler et al. 2015). As the vascular net is proportional to body mass, MSC number recalculated for mice (70 kg and 0.025 kg of weight for humans and mice accordingly) translates into 0.1 million cells per mouse, which is not sufficient for reliable detection. Consequently, the prothrombotic effect in humans is significantly lower and can be managed by the fibrinolytic system. Nevertheless, a large portion of MSCs will be disrupted due to their size and morphology, which explains why only low numbers of MSCs are registered in tissues (De Becker and Riet 2016).

Intraarterial (IA) injection of MSCs is characterized by whole-body biodistribution (Meseguer-Olmo et al. 2017; Spriet et al. 2015; Furlani et al. 2009; Kim et al. 2015; Khabbal et al. 2015; Ge et al. 2014), which is a result of post-cardiac blood flow. Furlani et al. (2009) investigated the kinetics of human MSCs after IA injection into SCID mice using intravital microscopy. The velocity of arterial blood decreased significantly 1 min after MSC injection and MSC entrapment in the capillaries led to 25–40% deaths in mice from pulmonary embolism, which depended on the number of cells injected (Furlani et al. 2009). Consequently, both IV and IA administration of MSCs in intact recipients should be

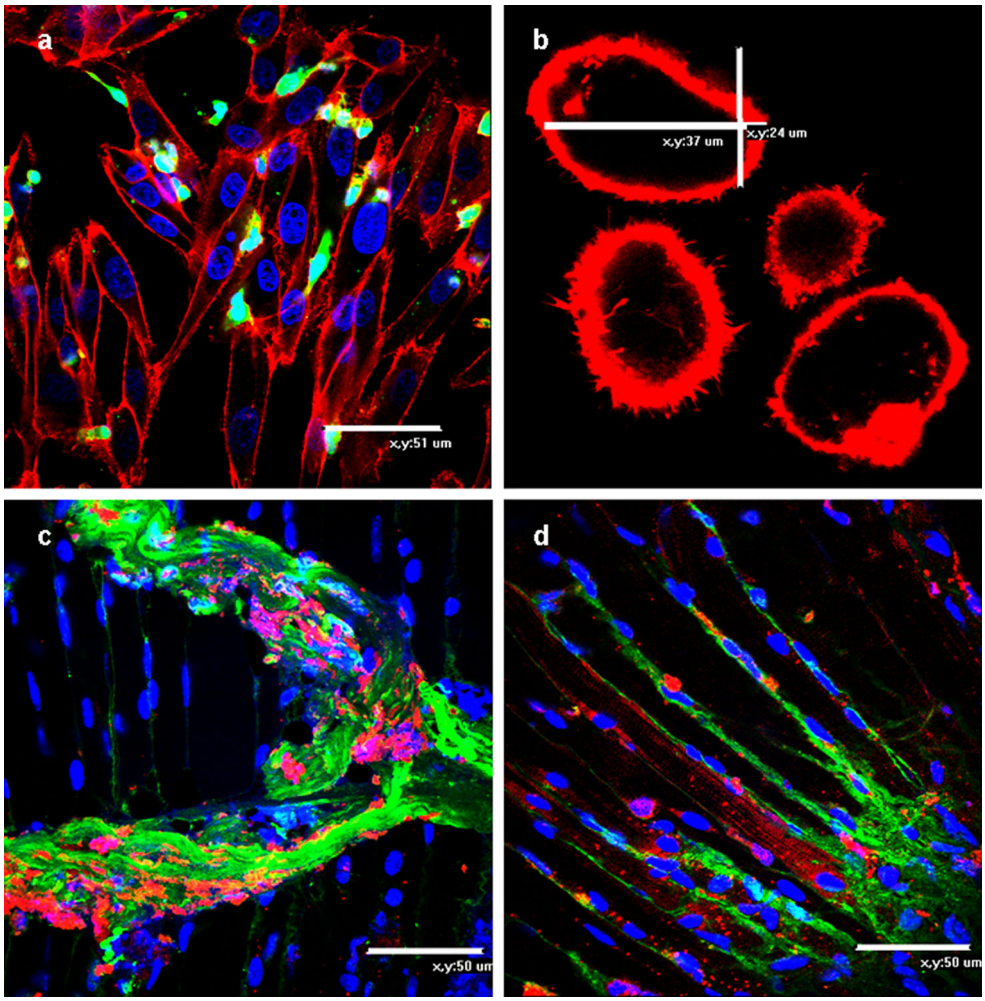

Fig. 1 Characterization of Wharton's jelly MSCs in vitro and in vivo. **a** Coculture of CD44 stained MSCs (red) and CFSE-stained peripheral blood lymphocytes (green). **b** MSCs trypsinized and loaded with PKH26 dye (red) for IV injection. **c** and **d** Major accumulation of MSCs in pulmonary artery (**c**) and cell debris in pulmonary capillaries (**d**) 4 h after IV injection in rats. Nuclei are stained with Hoechst 33342 (blue). Scale bars 50 μm

used cautiously. Treatment of MSCs by hyperosmolar buffer before intravascular injection (Leibacher et al. 2017), selection of MSC populations of small size (Ge et al. 2014), decreases in the number of injected cells (Furlani et al. 2009), shorter ex vivo MSC cultivation (Gleeson et al. 2015) and anticoagulant treatment (Moll et al. 2012) may help to avoid adverse effects.

Transmigration of MSCs: diapedesis, angiopellosis, or an inert event?

The therapeutic effects of MSCs are thought to be induced by two mechanisms: direct contact of MSCs with the surrounding cells or humoral factor production. In vitro studies of MSCs and immune cell interactions are focused on the analysis of MSC effects on lymphocyte proliferation, both naive and mitogen stimulated; apoptosis in T cells; T-helper (Th) 1/2/17 activation; dendritic cell differentiation; NK cytotoxicity; and T-regulatory (Treg) cell activation, all of which require direct contact between MSCs and lymphocytes (Bartholomew et al. 2002, Glennie et al. 2005, Akiyama et al. 2012, Aggarwal and Pittenger 2005, Beyth et al. 2005). This means that MSCs must migrate from the microcirculation to the tissue. The process of cell migration from the vessels to the inflamed tissue, called diapedesis, is well studied for polymorphonuclear neutrophils (PMNs) and lymphocytes. It occurs via a complex, multistep process starting with cell tethering, slow rolling, arrest, adhesion, crawling and transmigration (Brazil and Parkos 2016; Borregaard 2010; Walcheck et al. 1996). The average diameters of leukocytes and capillaries are 5–15 μm and 5–10 μm, respectively. This means that flowing leukocytes have to pass the capillaries one by one and in close proximity to the endothelial cells (Fig. 1c, d). This process is mediated by short contacts between P-selectin glycoprotein ligand 1 (PSGL1) on leukocytes and P/L-selectins on endothelial cells (Walcheck et al. 1996; Nourshargh and Alon 2014), leading to cell flattening and smooth rolling. The contact duration predetermines PMN activation. Liu et al. (2017a, b) demonstrated that the L-selectin mutant, which has increased duration of PMN bonding, also increased inflammatory injury and venous thrombi. Transmigration of leukocytes occurs in postcapillary venules,

which are larger in size (20–100 μm), where the rolling of leukocytes decreases. PMN rolling on endothelial P- and E-selectins stimulates signaling machinery that triggers bonding between leukocyte β1 integrins and their multiple ligands on endothelial cells. Both leukocyte activation and endothelial cell activation are coordinated by multiple intracellular and extracellular events (Brazil and Parkos 2016; Borregaard 2010; Walcheck et al. 1996; Nourshargh and Alon 2014).

An extensive study of Dollet et al. (2016) concerning the MSC receptor repertoire demonstrated that in vitro expanded MSCs lack selectin ligands, which are needed to pass through the narrow capillaries and later transmigrate across the endothelial barrier. Multiple studies have demonstrated that inactivation of L-selectins completely abrogated PMN and lymphocyte transmigration (Liu et al. 2017a, b). Consequently, these results suggest that traffic in capillaries and binding to the endothelium are the critical weak point in the MSC engraftment process. At the same time, MSCs express a set of integrin molecules and matrix metalloproteinases needed to navigate in tissue (Dollet et al. 2016). Several studies tried to genetically or chemically modify MSCs to express adhesive molecules, such as L/P selectins and increase VLA-4 expression (Sackstein et al. 2008, Cui et al. 2017a, b). Teo et al. (2012) demonstrated, using in vitro coculture of BM-MSCs and endothelial cells, the ability of MSCs to migrate across TNFα-activated endothelium via paracellular (between endothelial cells) and transcellular (through an endothelial cell) diapedesis. MSC transmigration was not preceded by significant lateral migration and cell flattening and occurred much slower in comparison with PMNs. The authors concluded that MSCs transmigrate via some novel mechanism. The slow kinetics of MSC migration across endothelial cells found in vitro was supported in vivo in zebrafish experiments by intravital microscopy. Allen et al. (2017) demonstrated that human, rat and canine cardiac stem cells or adipose tissue-derived rat MSCs migrated via endothelium over hours, in comparison with leukocytes transmigrating over minutes. The authors called this process "angiopellosis"; it was characterized by slow dynamics and the absence of cell flattening, which is characteristic of leukocytes. The same slow kinetics of MSC transmigration was shown by Matsushita et al. (2011) in a coculture of bone marrow-derived MSCs and brain microvascular endothelial cell monolayers.

Slow kinetics and the absence of cell flattening of MSCs raise the question of whether angiopellosis involves cell-to-cell interaction or is an inert event observed for any corpusculated material. Injection of inert polymeric particles comparable in size with MSCs did not result in transmigration (Ruster et al. 2006). In contrast to polymeric particles, MSCs express several adhesion molecules, such as integrins and VLA-4 (Dollet et al. 2016; Teo et al. 2012; Ruster et al. 2006), which mediate weak adhesion to endothelial cells. MSC binding to the endothelial monolayer can result in a passive and slow "pushing-through" of MSCs across the endothelial cells forced by other flowing cells, a process called angiopellosis by Allen et al. (2017). Luk et al. (2016) tried to estimate the therapeutic effect of heat-inactivated cells. They generated MSCs that were unable to respond to inflammatory signals or secrete immunomodulatory factors but preserved their cellular integrity (i.e., adhesion molecules on their surfaces). Such cells showed the same biodistribution and persistence after infusion in mice with ischemic kidney injury (Luk et al. 2016). Moreover, heat-inactivated MSCs induced a response comparable to control MSCs, with an increase in IL-10 production and reductions in inflammation in mice. All these data support a passive model; however, adhesion-mediated bypass of the endothelial barrier by MSCs hints that the therapeutic effects can be passive and explained, possibly, by capillary damage, fibrinolytic system activation or local tissue hypoxia. Taken collectively, intravascular injection of MSCs inevitably leads to massive cell damage, while only a minor fraction can possibly transmigrate the endothelial barrier via a slow passive process mediated by integrins and VLA-4 interactions with endothelial cells.

The fate of MSCs after local injection

First, the results of IV injections of MSCs demonstrated low efficacy of therapy. In the attempts to improve it, many recent clinical trials have used local injections of MSCs, such as intramyocardial ones (Patel et al. 2016), or targeted delivery, such as into the pancreatic artery (See et al. 2011). In these settings, MSCs are injected directly into the target tissue, where they can be tracked up to 24 h without redistribution to other organs (Lebouvier et al. 2015; Meseguer-Olmo et al. 2017). Burk et al. (2016) tried to follow adipose-derived MSCs for 24 weeks after local injection in tendons of horses in a model of tendon disease. The authors used iron oxide and rhodamine-labeled cells traced by MRI and histology for the whole follow-up period of 24 weeks. The results demonstrated traces of labels in some cells (Burk et al. 2016); however, the histology showed that rhodamine was found in cells resembling tissue phagocytes, as the size and morphology of positive cell nuclei were the same as those of the surrounding cells (see Fig. 4 in Burk et al. 2016), while MSC nuclei were much larger (Fig. 1a). These data demonstrate that the most likely fate of MSCs after local injection was also their death via necrosis due to trauma and hypoxia or apoptosis induced by natural killer (NK) cells followed by macrophage engulfment.

The mechanisms of immune reactions to allogeneic cells are well known and depend on two major consequent events: lysis by NK cells or activated cytotoxic CD8+ T cells. Low numbers of transplanted cells are killed by NK cells and eliminated by resident macrophages without adaptive immunity activation. In case of high numbers, such as in organ

transplants, both innate immunity and adaptive immunity are activated, invariably leading to transplant rejection in the absence of immunosuppressive therapy (Lin and Gill 2016). Transplant rejection results from NK cell and cytotoxic T cell activation tightly regulated by a balance between activating and inhibitory signals via immunoreceptor tyrosine-based activation motifs (ITAM) or inhibition motifs (ITIM) in intracellular domains of cell receptors (Barrow and Trowsdale 2006). NK cells use 14 germ-line encoded killer cell immunoglobulin-like receptors (KIRs), six of which contain ITAM and are activating receptors (aKIRs), while the 8 others contain ITIM and are inhibitory (iKIRs) ones (Barrow and Trowsdale 2006). Binding of iKIR to self MHC I results in an ignoring of activating signals (Fig. 2a). Decreased expression of MHC I by self cells induced by various factors (infection, trauma, malignization, etc.) leads to predominant aKIR signaling and cell killing, which is called the "missing self hypothesis" (Ljunggren and Karre 1990). Mismatch in iKIR/MHC I binding (Fig. 2b, circle) will lead to NK cell activation (Rajalingam 2012). However, activation of NK cells depends not only on the level of inhibitory signals but also on the strength of activating ones. Usually, a decrease in MHC I expression manifests as cell stress and induction of aKIR ligands, a phenomenon called the "induced-self" signal. Today, aKIR ligands are not well characterized but they are associated with the same stress conditions as described above. A balance between stimulatory and inhibitory signals also regulates the activation of T cells (Daëron et al. 1995). Not only allogeneic but also autologous MSCs injected into solid organs, such as the pancreas or heart, are likely to be killed by NK cells, as during injection, the tissue is disrupted, which leads to alarmin synthesis and NK cell activation.

Most clinical trials demonstrate that MSC treatment is rarely associated with adaptive immune responses, such as anti-HLA antibody generation (Packham et al. 2016; Skyler et al. 2015). Consequently, the major mechanism of MSC elimination can be killing by NK cells. The results of in vitro experiments on the effect of MSCs on NK cells are controversial. Several recent studies demonstrated that BM-MSCs stimulated NK cells for enhanced IL-12/IL-18-induced interferon-gamma secretion (Thomas et al. 2014; Cui et al. 2016). Similar results were obtained by Liu et al. (2017a, b), who demonstrated a significant increase in the number and activation of hepatic NK cells relative to the control in mice that received BM-MSCs via intrahepatic injection. Of note, MSCs were detected in the liver 24 h after injection and, at later time points, were eliminated without redistribution. Contrary to these results, Valencia et al. (2016) detected significantly reduced NK cell cytotoxic activity, which was associated with MSC susceptibility to NK cell-mediated lysis. A decrease in NK cell activity and simultaneous MSC lysis can simply manifest as an exhaustion of NK cell function due to MSC killing, which is limited by the number of effector-target contacts (Gallois et al. 2015; Schafer et al. 2015).

Both basic mechanisms of tissue homeostasis and many experimental data demonstrate that MSCs do not survive for a long time after injection. Nevertheless, there are numerous clinical trials demonstrating some efficacy of MSC treatment. There must be some explanations for the mechanisms of MSC activity. To obtain insight into these mechanisms, the results of clinical trials must be considered in detail.

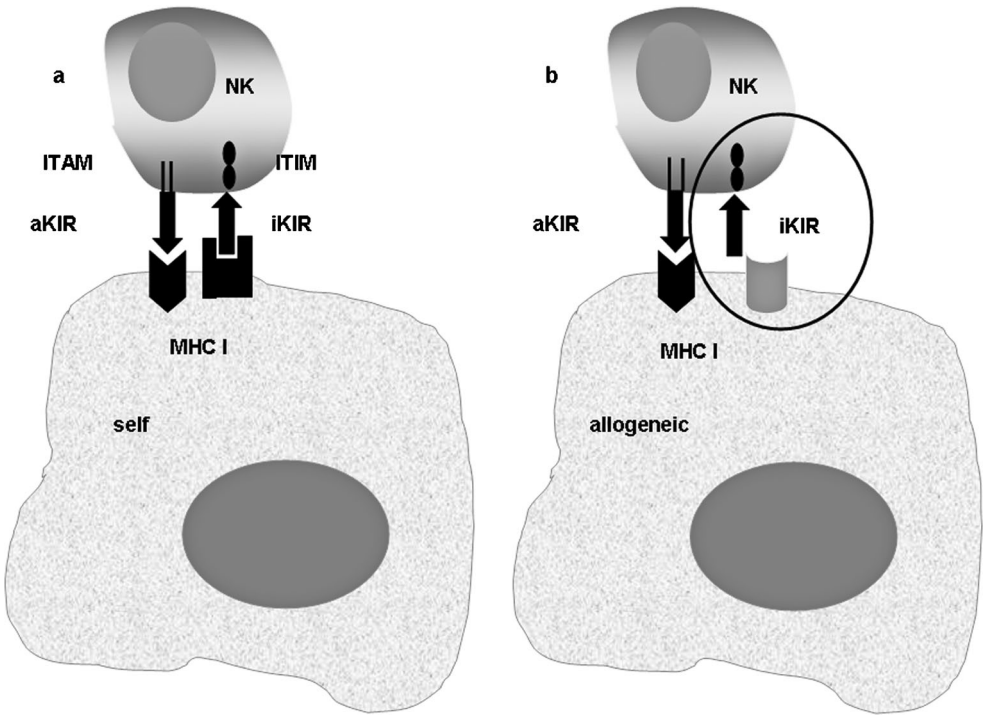

Fig. 2 Mechanisms of NK cell activation. All NK cells bear two types of killer immunoglobulin-like receptors (KIRs); one type (iKIR) transduces inhibitory signals via the ITIM motif upon binding self MHC class I molecules (**a**), while the other (aKIR) transduces activating signals via the ITAM motif upon binding various targets. NK cell activation depends on the balance of activating and inhibitory signals. When MHC I/iKIR is mismatched (**b**, circle), activating signals dominate and NK cells become "licensed" to kill this target

Diabetes mellitus

Type 1 diabetes mellitus (T1D) is a metabolic disorder characterized by loss of the insulin-producing beta cells in pancreatic islets, leading to insulin deficiency. T2D is characterized by the defective responsiveness of body tissues to insulin and decreased insulin production. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state and death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers and damage to the eyes. MSCs are used to treat long-term complications and the targets of therapy can be to control the level of glycated hemoglobin (HbA1c) < 7% (normal level < 6%); to improve glomerular filtration; to increase C-peptide and insulin areas under the curve; and to reduce fasting glycemia and daily insulin requirements.

IV delivery of MSCs in T2D

Two studies used Rexlemestrocel-L to estimate the safety and tolerability of MSC therapy in T2D. In a randomized, placebo-controlled dose escalation study, Packham et al. (2016) investigated the effect of 100–300 million MSCs via IV injection on the glomerular filtration rate in 20 T2D patients. No patients developed anti-HLA antibodies or severe adverse effects (SAEs). Only a trend of stabilizing or improving glomerular filtration was found. The second pilot study, conducted in 61 T2D patients by Skyler et al. (2015), used 25–150 million MSCs via IV injection, aiming to control HbA1c (< 7%). There were neither anti-HLA antibodies nor SAEs detected. The clinical target was achieved in 33% of the high-dose MSC group vs 0% in the placebo group.

Local delivery of MSCs in T1D and T2D

There was low efficacy of IV therapy initiated clinical trials with targeted delivery of MSCs to the diseased organ. In a randomized placebo-controlled study, Bhansali et al. (2014) compared the effects of autologous BM-MSCs or mononuclear cells (BM-MNCs) injected into the pancreatic artery of 21 T2D patients; each patient received either $1.0–1.4 \times 10^9$ BM-MNCs or $0.8–2.3 \times 10^7$ BM-MSCs. No changes in HbA1c level were found, while a reduction in insulin requirement by ≥ 50% from the baseline and a C-peptide area increase were found in both groups compared to controls. Better results were obtained by Cai et al. (2016) in the pilot randomized controlled open-label study in 21 T1D patients. A mixture of allogeneic umbilical cord MSCs (10^6/kg) and autologous BM-MNCs (10^8/kg) was injected into the pancreatic artery. C-peptide and insulin areas increased 106% and 50%, respectively, while HbA1c, fasting glycemia and daily insulin requirements decreased 13%, 24% and 29%, respectively, in treated patients.

Powell et al. (2012) studied the effect of autologous BM-MSCs in 72 patients with critical limb ischemia related to peripheral arterial disease with or without T2D. MSCs were administered via intramuscular (i.m.) injection in 20 sites. The treatment led to a decreased number of major treatment failure events, while amputation-free survival was not statistically different between groups. The trial conducted by Lu et al. (2011) compared the efficacies of i.m. treatment with BM-MSC or BM-MNC injections in the legs of patients with diabetic critical limb ischemia and foot ulcers. The therapy led to minor therapeutic effects, such as faster ulcer healing, while there was no significant difference between groups in terms of pain relief and amputation. Meta analysis of 7 clinical trials which included 359 patients with critical lower limb ischaemia demonstrated that the efficacy of MSC therapy remains to be confirmed (Wahid et al. 2018).

In our opinion, the most interesting conclusion from the studies of Bhansali et al. (2014) and Lu et al. (2011) is an equal efficacy of BM-MNC and BM-MSC treatments, showing that MSCs are likely to be dispensable and can be replaced by BM-MNCs, which are obtained much easier and delivered at less expense and risk.

Cardiomyopathy

Another example of MSC application is cardiomyopathy, a group of diseases that affect the heart muscle. It can be categorized into ischemic cardiomyopathy (IC) and nonischemic cardiomyopathy (NIC). Clinical benefits of MSC therapy are estimated by the change in left ventricular ejection fraction (LVEF) and left ventricular end-diastolic diameter (LVEDd); New York Heart Association (NYHA) class; Minnesota Living with Heart Failure Questionnaire scores (MLHFQ); myocardial perfusion; 6-min walk distance; and Kansas City Cardiomyopathy clinical and functional status scores.

IV delivery of MSCs

A single-blind, placebo-controlled, randomized trial conducted by Butler et al. (2017) studied the effect of IV injection of 1.5×10^6/kg allogeneic BM-MSCs in 22 NIC patients. The authors showed that the change in LVEF and ventricular volumes was not significant in comparison with controls; however, the treatment was safe and associated with some improvements in health status and functional capacity (Butler et al. 2017). Another study conducted by Bartolucci et al. (2017) estimated the effect of allogeneic umbilical cord-derived MSCs after IV injection of 10^7/kg MSCs in patients with heart failure and reduced ejection fraction. The therapy was safe and no SAEs or alloantibodies were registered; improvements in LVEF, functional status and quality of life were observed in treated patients. However, at study completion, groups did not differ in mortality and heart failure admissions. Other trials arrived at similar conclusions (Florea et al. 2017).

Local delivery of MSCs

MSC infusion by the IV route offers several advantages over local or targeted delivery. MSCs manufactured in standard conditions, such as Rexlemestrocel-L, Ixmyelocel-T, or Cellistem cells, can be administered at any healthcare facility. Intracardial delivery requires a cardiac catheterization laboratory, which is costly. Nevertheless, many more trials employ local MSC therapy in the hope of higher efficacy.

Patel et al. (2016) treated 126 patients with heart failure due to IC with Ixmyelocel-T administered intramyocardially. The treatment resulted in a 37% reduction in cardiac events (deaths, admission to hospital and number of clinic visits) compared to placebo. Henry et al. (2016a, b) compared the effects of intramyocardially injected Ixmyelocel-T in IC vs NIC patients. A trend of improvement in 6-min walk distances and MLHFQ scores was found only in IC patients. Rather unexpected results were obtained by Hare et al., who compared allogeneic vs autologous BM-MSCs in 37 NIC patients. The patients received 10^8 MSCs via intramyocardial injections in 10 left ventricular sites. The 12-month SAE incidence was 28% with allogeneic MSCs vs 64% with autologous ones. The ejection fraction, 6-min walk test and MLHFQ scores were improved in the allogeneic MSC group compared to the autologous MSC group (Hare et al. 2017). It is not clear why allogeneic MSCs were more effective and induced fewer SAEs. The presented data demonstrate that the efficacy of intramyocardial MSC delivery varies from trial to trial.

It is of interest to understand if BM-MNCs could be as effective as BM-MSCs, as was shown for diabetic patients. Xiao et al. (2017) conducted a randomized study on the efficacy of BM-MSCs and BM-MNCs in 37 NIC patients. LVEF and myocardial perfusion improved significantly in both groups compared to the control. Taylor et al. (2016) tried to determine which BM-MNC fraction was responsible for a favorable outcome in 78 IC patients. Autologous BM-MNCs were phenotyped and injected transendocardially. The authors showed that LVEF, LVESV and VO_2 max significantly improved in 22% of patients at 6-month follow-up. The improvement was associated with increased numbers of B cells and macrophages in BM-MNC samples.

Taken collectively, BM-MSC IV treatment is not sufficiently effective (Butler et al. 2017; Bartolucci et al. 2017; Florea et al. 2017); BM-MSC intracardial delivery demonstrates better therapeutic effects in ischemic compared to nonischemic cardiomyopathy and the effect is still marginally described as a "reduction in cardiac events" and "a trend of improvement in 6-minute walk distance and MLHFQ score". At the same time, the effects of BM-MNCs and BM-MSCs are likely to be comparable for both diabetes and cardiomyopathy (Bhansali et al. 2014; Lu et al. 2011; Xiao et al. 2017; Taylor et al. 2016), as the improvements were found only in of 22–37% patients.

From MSCs to MSC-derived exosomes

The beneficial effects of MSCs were originally attributed to their capacity to differentiate into specialized cell types; however, presented evidence suggests that MSCs do not have a chance to differentiate in the host. The modern concept attributes the beneficial effects of MSC therapy to paracrine or autocrine factors, which are released as exosomes (Monsel et al. 2016). Paracrine/autocrine signaling is a form of cell communication via humoral factors over a relatively short distance, as opposed to endocrine factors. Exosomes are membrane vesicles 30 to 100 nm in diameter secreted by cells (Oshima et al. 2002). Exosomes can contain up to 20,000 molecules, such as proteins, RNA, or DNA. Evidently, each exosome can contain different sets of molecules. Multiple groups consider MSC-derived exosomes as a replacement for MSC therapy (Huang et al. 2017; Cui et al. 2017a, b).

MSC preparation requires cell isolation and a relatively long cultivation; some preparations, such as Rexlemestrocel-L, require cell sorting and Ixmyelocel-T must be characterized using flow cytometry. The isolation and detection of exosomes are the next level of complexity and, consequently, the next level of cost. Exosomes are obtained by differential ultracentrifugation followed by microfiltration or size-exclusion chromatography, making exosome preparation even more costly. Keeping in mind that exosomes are membrane vesicles of 30 to 100 nm diameter, it would be much easier to produce liposomes loaded with relevant proteins. The question is which exosome components are responsible for the beneficial effects of MSC therapy.

Preclinical studies of MSC-derived exosomes

Currently, there are no ongoing clinical exosome trials. Multiple preclinical models have demonstrated a comparable or higher efficacy of exosomes. Huang et al. (2017) studied the effect of MSC exosomes in a rat model of spinal cord injury. Systemic administration of exosomes attenuated lesion size, improved functional recovery and significantly promoted angiogenesis. Cui et al. (2017a, b) demonstrated that adipose-derived MSC exosomes injected into rats with induced myocardial ischemia reduced myocardial infarction and decreased the serum levels of creatine kinase-myocardial band, lactate dehydrogenase and cardiac troponin I. Wang et al. (2017) demonstrated alleviated cartilage destruction and matrix degradation after intra-articular injection of either embryonic MSCs or their exosomes in a mouse model of osteoarthritis. Similar efficacy was shown for MSC exosomes by other groups (Zhu et al. 2017; Drommelschmidt et al. 2017; Nong et al. 2016; Ophelders et al. 2016; Hu et al. 2015; Akyurekli et al. 2015).

Preclinical studies of MSC-conditioned medium

Exosome cargo is likely to reflect the MSC secretome and differs from soluble MSC-conditioned medium (CM) by the lipid membrane, which encapsulates proteins. Timmers et al. (2007) studied the effect of MSC-CM in a porcine model of ischemia and reperfusion injury. Both IV and intramyocardial MSC-CM treatment significantly reduced infarct size and improved systolic and diastolic cardiac performance. Fractionation studies revealed that only a fraction of 100–220 nm provided cardioprotection. This fraction evidently corresponds to exosomes.

Secretome analysis of MSC-CM identified a panel of pro- and anti-inflammatory cytokines, such as TGFβ1; interleukins (ILs) 1β, 6, 8, 9, 10, 12, 13, 17, 18 and 27; IFN-γ; TNFα; MIP-1α; extracellular matrix; and cell adhesion proteins, chemokines, matrix proteases and their inhibitors, as well as many others (Kalinina et al. 2015; Vizoso et al. 2017). Not all paracrine factors filling exosomes mediate a therapeutic effect. Some proteins can be dispensable, while others can diminish the effect of the functional factors. A detailed analysis of individual activity and their combinations encapsulated in a liposomal delivery system can lead to the production of a more efficient and standardized therapy.

Conclusions

There are several important problems that should be resolved before efficient clinical application of MSCs. First, multiple preclinical studies have raised a question of MSC intravascular therapy safety. IV or IA MSC injection in small animals results in dose-dependent lung atelectasis and thrombosis. At the same time, most clinical studies do not register severe adverse effects. This contradiction is a result of different numbers of MSCs injected into animals and humans. The average number of MSCs in clinical trials is up to 10^7/kg, while more than 10^8/kg is used in animal experiments, which is needed for a reliable identification of cells or therapeutic effects. The second problem is the inability of intravascular injected MSCs to efficiently transmigrate across the endothelial barrier, which is a result of the lack of P/L-selectin expression in MSCs as well as of proper intracellular machinery needed to bypass capillaries smaller in diameter than the MSC size. Consequently, the efficacy of IV or IA MSC therapy is rather low. Possible mechanisms of MSC efficacy are likely to be connected to the recovery of damaged capillaries, fibrinolytic system activation, local tissue hypoxia and chemokine and cytokine production by host damaged cells. The role of humoral factors produced by MSCs after IV/IA injection could be marginal. Of interest, the effect of intravascular injection of BM-MNCs was comparable to that of BM-MSCs. Evidently, MNCs express P/L-selectins, safely circulate and can transmigrate across the endothelial barrier. However, BM-MNCs migrate into the spleen and bones (Mäkelä et al. 2015) to the place of their maturation, orchestrated by chemokine regulation. Consequently, MSCs and MNCs differ in multiple parameters, including prothrombotic effects, the set of humoral factors they produce and biodistribution routes. The question of why the effects of MNC and MSC therapy are comparable awaits resolution.

Recent clinical studies with local MSC injections have demonstrated that clinicians have become aware of these two major problems and are now trying to deliver MSCs directly into damaged organs. However, the efficacy of local therapy did not increase much. The efficacy was still rather low, often described as a “trend” and found only in 20–30% of patients. The reason for local MSC therapy failure is probably an inability of MSCs to survive in the tissue for a long time due to cell and tissue damage, leading to cell necrosis and local activity of resident innate immunity cells, such as NK cells, which induce apoptosis in transplanted cells. This appears to be true not only for allogeneic MSCs but also for autologous MSCs, as injected cells disrupt the normal architecture of the organ, resulting in cell stress and alarmin synthesis.

Modest results of local therapy shifted the scientific community to the study of humoral components of MSCs. It was found out that only some forms of humoral factors, named exosomes or microvesicles, demonstrate effects comparable to MSC whole-cell therapy. To the best of our knowledge, there are no ongoing clinical trials studying the effect of exosomes. One of the main obstacles is the complicated process of exosome production, which makes exosome-based therapy even more expensive. In addition, preclinical studies demonstrate a comparable efficacy of exosomes and MSC therapy.

The exosomal pool contains hundreds of humoral factors, part of which can be responsible for the therapeutic effect, while others may diminish or even abrogate it. Moreover, membrane lipids per se can also be involved in the therapeutic action. In our opinion, the future perspective for preclinical studies is not to develop the best standardized protocol for exosome production (Pachler et al. 2017) but to study which, if any, soluble factors play a role. Unfortunately, in vitro studies are not of much help in this direction. Development of a liposomal format of therapeutic exosomal content will possibly result in an effective, standardized, safe and relatively inexpensive preparation.

Funding information This work was supported by the Russian Science Foundation grant no. 14-25-00179.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement on the welfare of animals All procedures involving animals were in accordance with the ethical standards of the institution.

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